EFFECT OF PARTICLE SIZE ON XYLOSE, GLUCOSE AND ARABINOSE PRODUCTION FROM OIL PALM TRUNK

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JUDUL : EFFECT OF PARTICLE SIZE ON XYLOSE, GLUCOSE AND ARABINOSE PRODUCTION FROM OIL PALM TRUNK

SESI PENGAJIAN : 2012/2013

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CHAPTER 1

INTRODUCTION

1.1 Background of Proposed Study

Oil palm trunks (OPT) are a possible lignocellulosic source (Young *et. al.,* 2011). According to Jung *et. al.,* (2011), approximately 4.49 million hectares in Malaysia are used oil for palm agriculture and around 15.2 million tonnes of OPT are generated yearly. The residue of oil palm trunk in Malaysia makes it as the most abundant biomass resources. In order to reduce the numbers, the residues are burned in the field which causes major air pollution (Najafpour *et. al.,* 2007). Hydrolysis of the lignocellulosic of OPT can produce xylose, glucose and arabinose (Lim *et. al.,* 1996; Chin *et. al.,* 2011). However most the research only studied on the hydrolysate product. The generated solid waste (cake) is not analysed for further study. Therefore, the abundant of biomass is still a problem to the environment. Thus, in order to maximise the production, through delignification, acid pre-treatment and enzymatic hydrolysis, the solid waste (cake) containing cellulose and lignin will be converted to glucose. This method helps reduce the waste, decrease the excessive of oil palm trunks (OPT) and save the environment.

1.2 Problem Statement

The chemical composition of the OPT is cellulose 41.2%, hemicelluloses 34.4%, lignin 17.1%, ash 3.4% and ethanol soluble 2.3% (Robert *et. al.*, 2008). Acid pre-treatment of oil palm trunk (OPT) recover cellulose. Then, cellulose will be converted to glucose. Because of this, lignin and hemicelluloses will be generated approximately 51.5% as the liquid waste. The waste eventually pollutes the environment.

Previous studies recover hemicellulose from the biomass through acid hydrolysis producing xylose, glucose and arabinose (Lim *e.t al.*, 1996; Chin *et. al.*, 2011). These studies generated 48.3% solid waste which consists of cellulose and lignin. Therefore, abundant of waste is still being generated either from acid-pretreatment or acid hydrolysis.

1.3 Research Objectives

- To study the effect of different particle size of oil palm trunk (OPT) on the production of xylose, glucose and arabinose.

- To determine the maximum yield of xylose, glucose and arabinose from the oil palm trunk (OPT).

1.4 Research Questions

- What is the effect of particle size to the production of xylose, glucose and arabinose?

- What is the maximum yield of glucose, xylose and arabinose from the oil palm trunk (OPT)?

1.5 Scope of Study

Pre-treatment of oil palm trunk (OPT) was crushed in a high speed rotary cutting mill and sundried for a day. The resulting sample was sieved to obtained different particle size. The particle size consists of 160, 200, 315, 630 and 800 µm sieves by using vibrator sieve shaker. Acid hydrolysis process was carried out in an autoclave at constant temperature 120^0C for 30 to 60 minutes. The acid used for the process was sulphuric acid. For delignification process, the NaOH was used to pre-break the line of the lignin and cellulose.

Acid pre-treatment was done before the enzymatic hydrolysis. It involves the removal of lignin content using peracetic acid (PAA). Then the enzymatic method was done. Commercial cellulolytic enzymes (cellulase and cellobiase) act as catalysts to convert cellulose to glucose. The anaylsis of production of xylose, glucose and arabinose from the liquid waste was done by High Performance Liquid Chromatoghraphy (HPLC) with the column used was RCM Monosaccharide.

Meanwhile analysis production of the glucose from the enzymatic hyrolysis was done by YIS Biochemistry Analyser.

1.6 Significance of Study

- Acid hydrolysis of hemicelluloses and cellulose hydrolysis by enzyme from oil palm trunk (OPT) was studied in this research in order to reduce liquid or solid waste generated from the process.

- Liquid waste filtered after acid hydrolysis was treated to produce xylose, glucose and arabinose from hemicelluloses (Lavarack *et. al.*, 2001; Najafpour *et. al.,* 2007).

- Solid waste filtered after acid hydrolysis was treated to produce glucose. Thus, this research will only generate 17.1 % lignin as the liquid waste. The minimum amount of waste can reduce the pollution and more environmental friendly.

CHAPTER 2

LITERATURE REVIEW

2.1 Monosaccharides

Monosaccharides are one type of sugars. They are small components that belong to the class of carbohydrates. Monosaccharides are the simplest carbohydrates and are categorized according to either aldehyde or ketone derivatives. Since they are the simplest carbohydrates, monosaccharides cannot be hydrolyzed to smaller carbohydrates. A monosaccharides has just one ring instead of disaccharides has two and polysaccharides has many rings. Figure 2.1 shows type of carbohydrates consists of monosaccharides, disaccharides and polysaccharides.

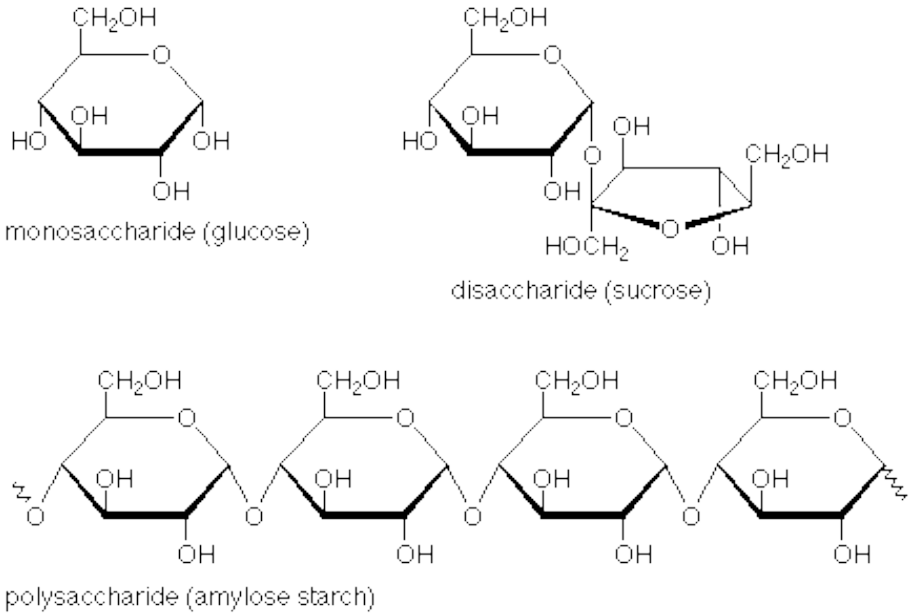

Figure 2.1 Type of carbohydrates

(Source : Sjoman, *et. al.*, 2008)

Monosaccharides can be differentiated based on their three different characteristicswhich are carbonyl group placement, the number of carbon atoms contained and its chiral handedness. Simple sugars that have aldehyde as the carbonyl group are called aldose. Meanwhile carbonyl group of ketone attached to the monosaccharides is named ketose.

2.1.1 Xylose

According to Sjoman *et. al.*, (2008), xylose is a pentose sugar and it has molecular structure as $C_5H_{10}O_5$. They normally hydrolyzed from xylan like wood, rice husk, corn stalk, wheat straw and flax straw. Pretreatment of complex structure

of lignocellulosic biomass can produce xylose. It can be done either by chemical or biological hydrolysis method.

Xylose can be appears to be a major component of hemicellulose in biomass and agricultural waste residue (David *et. al.*, 2003). It can be used as a raw material (substrate) for production of many varieties of compounds by chemical and biological processes (Wymn, 1994). Usually, lignocellulosic biomass will be easily released xylose by hydrolysates of samples with acid catalysts (Martin *et. al.*, 2002). Figure 2.2 describe the structure of xylose.

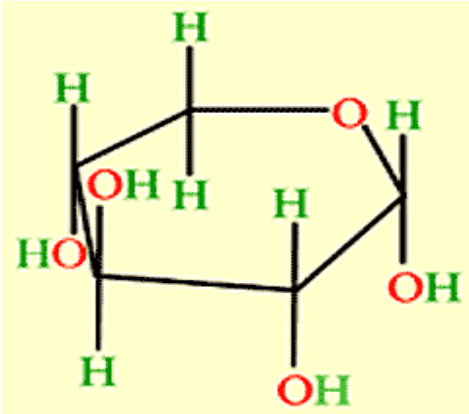

Figure 2.2 Structure of Xylose

(Source: David *et. al.*, 2003)

2.1.2 Glucose

Glucose is a simple monosaccharide sugar which is a very essential carbohydrate in biology. Glucose ($C_6H_{12}O$) contains six carbon atoms, one of which is part of an aldehyde group Thus, glucose is an aldohexose. According to Gailliot *et.*

al., (2007), glucose is one essential fuel for the brain. The brain's activities depend greatly on glucose for energy. The major function of glucose in a organism (prokaryotes or eukaryotes) is to provide immediate energy to the cell (Gibss *et. al.*, 2012). These cells convert this molecule to energy with its organelles (mitochondria), organs of the cell.

Cellular respiration usually starts with glucose in both prokaryotes and eukaryotes. It is also one of the main products of photosynthesis (Gibbs *et. al.*, 2012). Glucose is commonly available in the form of a white powder or as a solid crystal, called dextrose. It can also be dissolved in water as an aqueous solution, glucose syrups. Its solubility level is very high (McMurry, 1988). Figure 2.3 below shows the structure of glucose.

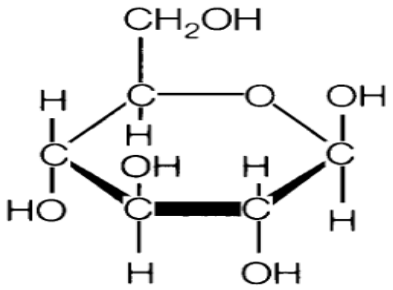

Figure 2.3 Structure of Glucose

(Source: Gibbs *et. al.*, 2012)

2.1.3 Arabinose

Arabinose is a simple monosaccharide sugar consists of five carbon atom. The functional group that attached to this simple monosaccharide is aldehyde.

According to Sanchez *et. al.,* (2010), in certain agricultural biomass and hardwoods contain a large fraction of pentose sugars such as D-xylose and L-arabinose. The molecular structure of arabinose is $C_5H_{10}O_5$. The characteristics of the arabinose are white crystalline and water soluble solid.

It usually used as the culture medium in bacteriology (Sanchez *et. al.*, 2010). It naturally occurred in an L-form. Combination between undigested sucrose and L-arabinose produce a short chain fatty acid and its acts in the same way to dietry fiber. Thus, it has huge points as a sweetener and food additive. This advantages help in regulating blood sugar and maintaining good health. Figure 2.4 is the structure of arabinose.

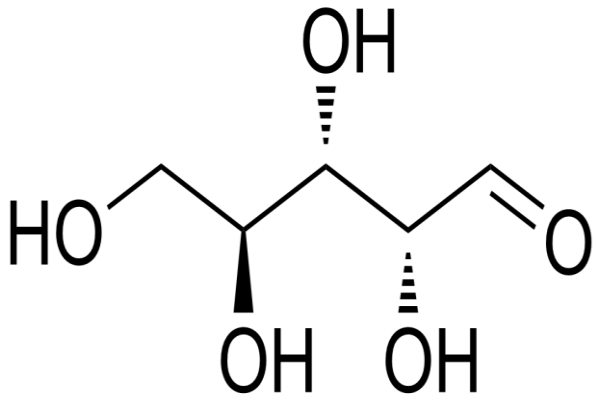

Figure 2.4 Structure of Arabinose

(Source: Sanchez *et. al.*, 2010)

2.2 Application of Monosaccharide

2.2.1 Application of Xylose

Currently, industry such as liquid fuel production, food industry and pharmaceutical industry are examples of industries that are attracted to used xylose as a part of their production. According to Howard *et. al.,* (2003), xylose can also be further processed to generate furfural and xylitol which has wide application in industries. Figure 2.5 below shows the general in lignocelluloses bioconversion into value added products.

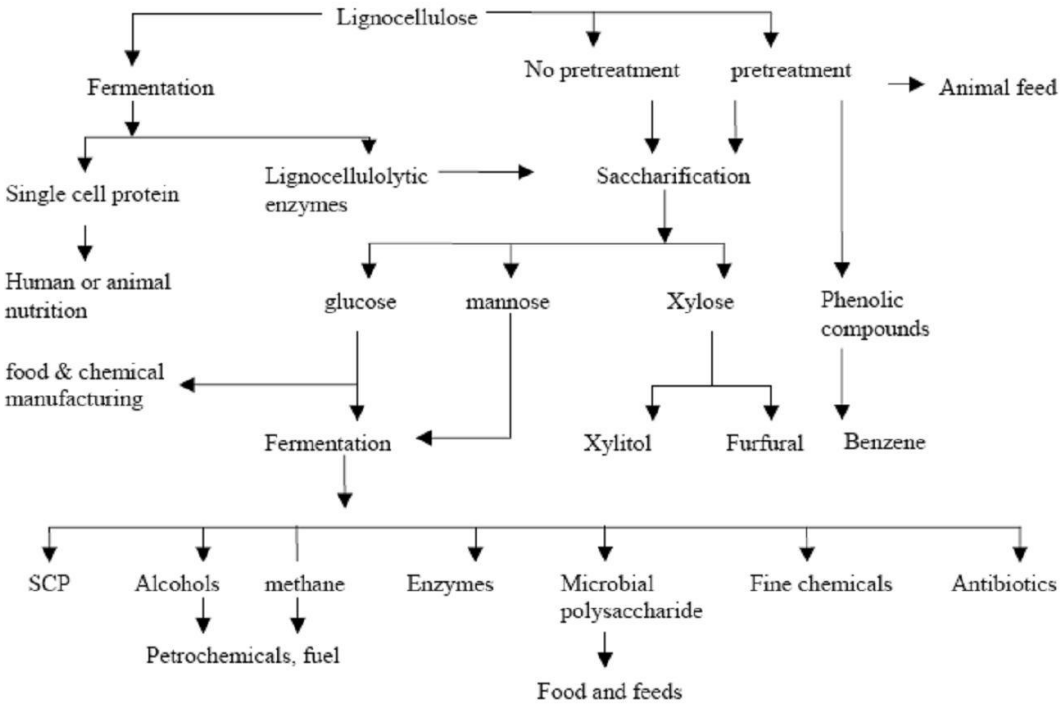

Figure 2.5 General process in lignocellulose bioconversion into value-added bioproducts

(Source: Herzog *et. al.*,2012)

2.2.1.1 Liquid Fuel Indusries

A great number of energy sources such as heat, liquid fuels and electricity can be generated through the biomass. Conversion of biomass into liquid or gaseous fuels can be used to generate electricity as well as the coal usage. Since three decades ago, biomass holds a huge prospective as a source of renewable energy. According to one of the research of Herzog *et. al.*, (2012), this trending is due to the price of oil and gases continue to fluctuate. Other than that, greenhouse gas emission reductions canbe controlled (Taherzadeh, 2007). Although combustion process of biomass still producing CO_2, but they result in zero net emissions of greenhouse gasses (Miskam *et. al.*, 2004).

According to Taherzadeh (2007), further treatments of hemicellulose produce fuel ethanol. Ethanol becomes an essential product in the fuel market. The market is predicted to achieve 100 billion liters in 2015. (Licht, 2006). Figure 2.6 shows general flow of ethanol production from biomass.

$$hemicellulose \xrightarrow{hydrolysis} pentose\ and\ hexoses \xrightarrow{fermentation} ethanol$$

Figure 2.6 General flow of ethanol production from biomass

(Source: Taherzadeh, 2007)

Pretreatment of hemicelluloses by diluted acid hydrolysis or enzymatic hydrolysis generated pentose and hexoses sugar. Further utilization of those sugars by fermentation produced fuel ethanol. *Saccharmoyces Cerevisiae* (Jung *et. al.*, 2011), is one of the yeast that have the capability for the cost efficient ethanolic

fermentation. Optimization time and pH has been studied by Chin *et. al.,* (2010), and the maximum ethanol yield and productivity (Sanchez *et. al.,* 2010) can be obtained using 33.2^0C at pH 5.3.

2.2.1.2 Xylitol Production

Xylitol is a five carbon sugar alcohol commonly occurs in nature. According to Winkelhausen (1998), xylitol is a normal intermediate in human metabolism. One of the advantages of xylitol is they can act as an alternative sweetener especially for non-insulin dependent diabetics. Xylitol has low glycemic index and 40% fewer calories than sugar. Thus, it is more preferable sweetener for diabetics' diet. According to Silva *et. al.,* (2012), it is metabolize through the insulin independent pathway in the body and consume leisurely from the intestine so that alteration of blood glucose in the body will occur. Currently, due to its high sweetening influence, it is broadly used in chewing gums industry.

In addition, xylitol has high demand in odontological and pharmaceutical industry. According to Zhang *et. al.,* (2012), it can inhibit the metabolism of dental carries formation and demineralization of tooth enamel (Silva *et. al.,* 2012). It also acts as anticariogenicity, and tooth rehardening properties. Since xylitol can decrease the potential of tooth decay (Makinen, 2000), and reduce plaque growth causing by acid creating in mouth, it is usually formulated in the tooth paste. It can protect people's mouth and teeth if the product is applied daily.

Due to these advantages of xylitol, xylose from complex structure of lignocellulosic biomass is essential to pretreat by chemical or biological hydrolysis method. It can be generated by hydrogenation catalytic of xylose or be produced by some microorganisms (yeast) as a natural metabolic intermediate (Chen *et. al.,* 2010). Figure 2.7 describe the production of xylitol by biotechnology process.

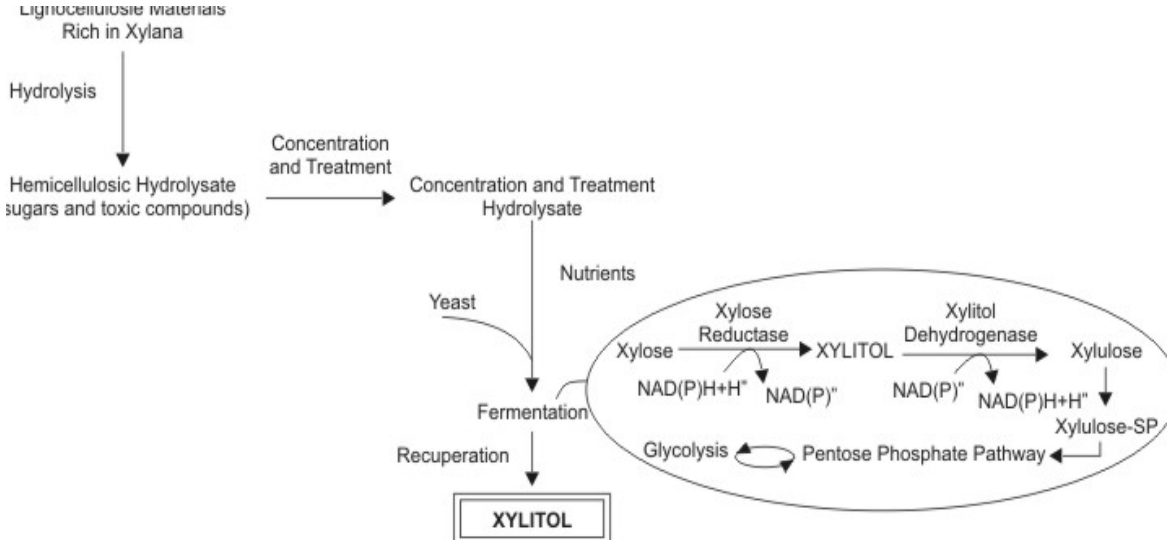

Figure 2.7 Xylitol Production by Biotechnology Process\

(Source: Makinen, 2000)

2.2.2 Application of Glucose

Glucose has existed many years ago. It had been revealed by the writings of Moorish about 1100s ago. It has many functions in life such as primary sources of energy and precursors in food industry. Glucose is a word derived from a Greek word glycos that has meaning of sweet. It is fundamental that a person checks their glucose level in order to avoid diseases called diabetes and live healthy life. Glucose interacts with the endocrine and digestive systems. Currently, glucose had been

discovered as value substrate used in almost all industrial for its unique characteristics. It has mainly used in the manufacture of a numeral of products in food industries, pharmaceuticals and fermentation industries.

2.2.2.1 Glucose as Main Sources of Energy

Glucose is a major nutrient for cells. In the cellular respiration process, cells extract the energy kept in glucose molecules (Campbell, 2002). During digestion, glucose is one of the three dietary monosaccharides that easily absorbed into the blood stream. It also functions as a metabolic intermediate because of its most conformationals stable among others. With respects to the characteristic of low tendency, glucose tends to reduce the function of many enzymes. The carbon skeletons also provided as raw material for the synthesis of other types of small organic molecules such as amino acids and fatty acids.

According to Campbell (2002), one main common metabolism that degrades the sugar glucose to carbon dioxide and water is cellular respiration. This process release energy stored in sugar and makes that energy available for cellular works. Figure 2.8 shows the general process of cellular respiration.

$$organic\ compunds\ (sugar) + \ oxygen \ \rightarrow carbon\ dioxide + water + energy$$

Figure 2.8 General process of cellular respiration

(Source: Campbell, 2002)

2.2.2.2 Glucose in Food Industries

Enzymatic hydrolysis of biomass produces commercial glucose (Zhang *et. al.*, 2012). There are many crops that can be used as the raw material of starch such as maize, sago, rice and sugar cane. Glucose is broadly used in the production of table syrups, jellies, chewing gums and candies for most food industries. One of the examples of glucose that mainly used in the food industry is dextrose. According to Tate and Lyle (2012), dextrose are term derive from D-glucose. It is a crystalline sugar used as a nutritive sweetener which approximately 70% as sweet as sugar.

Furthermore, most of the bakery products use glucose syrup that gives optimum sweetness (Hull, 2010). It also helps in preventing of crystallization and keeps the preparation of fresh and long half life of the ice cream. Besides a better and smooth texture ice creams are develops. Figure 2.9 below describe the percentage of value contribution of biscuit categories.

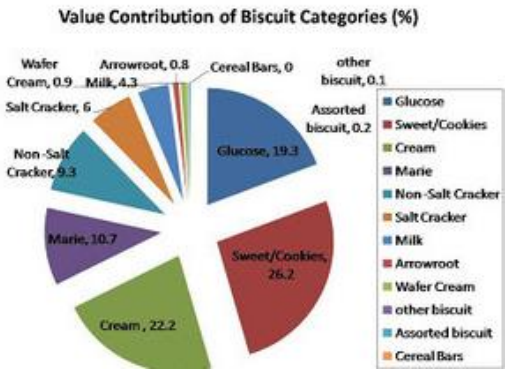

Figure 2.9 Value contribution of biscuit categories (%)

(Source: Hull, 2010)

2.2.2.3 Glucose in Pharmaceutical industry

According to Bhosale *et. al.,* (1996), some of medicine such as syrup cough contains a mixture of glycerol and glucose. It is a valuable vehicle either for the cough syrups or vitamin based tonics. One of the examples of cough syrup containing glucose is Buttercup Blackcurrant flavour cough syrup. Figure 2.10 is a Buttercup Blackcurrant cough syrup.

Figure 2.10 Buttercup blackcurrant cough syrup

(Source: Bhosale *et. al.,* 1996)

2.2.3 Application of Arabinose

Other than complex lignocellulosic material, arabinose also exists in mucus, pectin acid, bacterial polysaccharides and oher indicans (Saha, 2000). Role of arabinose is similar to the role of xylose. As a non calorie compound sweetener, it is a good cure to disease such as obesity, high blood sugar and diabetes. According to Shandong Futaste Co, US FDA and Japan approved the arabinose to be used as a safe food additive for it low calorie characteristic.

According to Bauer *et. al.,* (2008), arabinose is highly demand in the pharmaceutical industry. Most of the non- prescription drugs and nutritional supplements used arabinose as the main raw material especially for anti obesity drugs. The role of arabinose as the low-calorie sweetener can be seen by its ability of inhibition of sucrose into glucose fructose, absorption inhibitor and storage of excess dietary sugar as fat making. The inhibition helps to slow down the release of sugar from processed food and sweets thus stabilise the blood sugar levels. This advantage helps to maintain a more normalised sugar release and reduce the sugar spikes for better insulin availability.

Other than that, arabinose can be used for the preparation of bacterial culture medium. Arabinose is important for the pGLO system in which if arabinose is present in the nutrient medium, bacteria will take it up (Bauer *et. al.,* 2008). That arabinose will interacts directly with araC protein which is bound to the DNA. The araC proetein will alter their shape cause by the interaction and helps the RNA polymerase to bind the PBAD promoter thus; GFP (green fluorescent protein) will be transcribed. Figure 2.11 describe overall process of GFD production with the presence of arabinose.

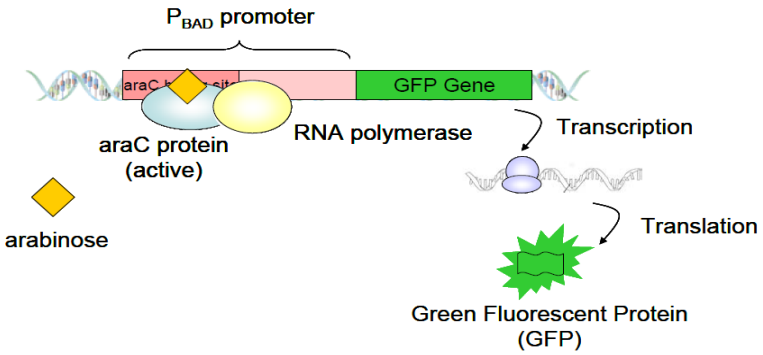

Figure 2.11 General Process of GFD Production with the presence of Arabinose (Source: Campbell, 2002)

2.3 Sources of Monosaccharides

Carbohydrates can be consumed either naturally or commercially. Direct consumption of xylose, glucose and arabinose are mostly can be obtained from fruits and vegetables (Marin, 2010). Deficiency of those sugars especially glucose increase the potential of hypoglycaemia disease. Hypoglycaemia or known as low blood sugar is a commonly viewed problem. It happened when the amount of sugar inside the human body decrease to the value below 70mg/L. Right amount of sugar intake either in a natural way or commercial way should be considered to lead a healthy life.

2.3.1 Natural Sources of Monosaccharides

2.3.1.1 Xylose

According to Bird (2012), xylose mainly found in guava, pears, wild berries, kelp and spinach. This article is supported by Marin *et. al.,* (2010) that stated aloe vera, cabbage and corn in addition of those natural dietary sources of xylose sugar. Table 2.1 below shows some of the example of natural dietary of xylose.

Table 2.1 Some of example of natural dietary of xylose

Fruit	Herbs	Seeds	Vegetables
Guava	Aloe vera	Psyllium Seeds	Corn
Pears	Echinacea		Broccoli
Blackberries	Boswellia		Spinach
Loganberries			Eggplant
Raspberries			Beans

(Source: Bird, 2012)

Since overall nutrient content of such ripened fruits and sugar easily decrease during the processing and storage, it is desirable to consume fully ripened fresh fruit and vegetables for maximum nutrient content intake especially monosaccharides. According to Bravo *et. al.,* (2009), ripe cranberries are detected with highest amount of xylose which is 320 pg for 1.0 g of fresh weight.

2.3.1.2 Glucose

Glucose usually found as a monomer of more complex carbohydrates such as sucrose and fructose. It is seldom found in food in its sole molecular form (Teller, 2011). Such examples of natural dietary sources of glucose are bread, pasta, cereals and rice. According to one article of Husseini (2011), in order of the cells to used that sugar as the core sources of energy, the digestive system acts by breaking down the complex carbohydrates into many molecules of glucose. Table 2.2 below describe the food that contains glucose.

Table 2.2 Examples of Food contains Glucose

Example	Fruits	Vegetables	Others
1	Dates	Corn	Potato
2	Apricots	Squash	Milk
3	Apples	Zucchini	Honey
4	Tomatoes	Legumes	Pasta
5	Grapes	Onion	Yogurt

(Source: Teller, 2011)

Among of these foods, grapes are especially rich in glucose. They are also known as the grape sugar. Meanwhile, honey contains 38% of glucose for its composition. According to Cook and Samman (1996), glucose is the outcome of the breakdown of glycogen stores in the liver and muscles. Figure 2.12 is the sugar grapes and honey that contains high composition of glucose.

Figure 2.12 Grapes and honey natural sources high in glucose

(Source: Cook and Samman, 1996)

There is a different between glucose intakes for the animals compared to humans. In animals, liver and kidneys are places for the non-carbohydrates intermediate synthesize the glucose (Manach *et. al.*, 2006). Instead of plant, they not use glucose but they generate the glucose from their photosynthesis process.

2.3.1.3 Arabinose

The natural dietary sources of arabinose are not common as the natural sources of glucose and xylose. According to Shaw (2008), arabinose level riches in certain fruits such as apples and pears. Figure 2.13 are the example of fruits that contain high levels of arabinose sugar.

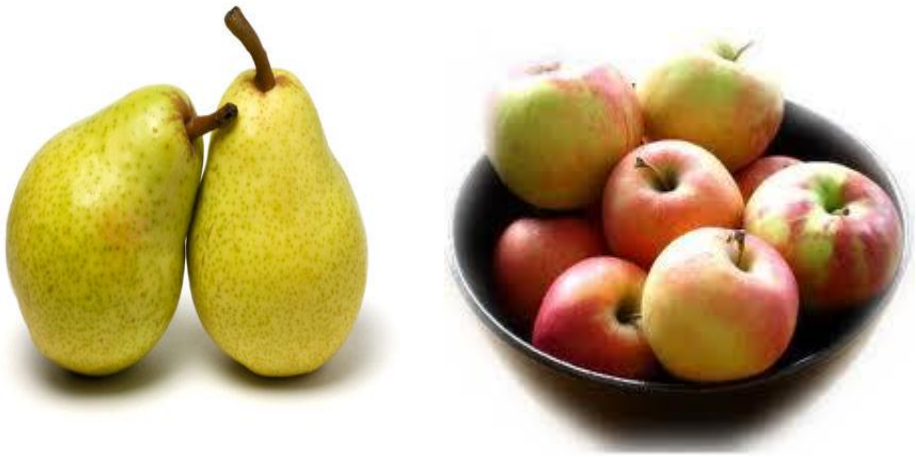

Figure 2.13 Examples of fruits rich in arabinose sugar

(Source: Shaw, 2008)

Instead of fruits, they also can arise from dysbiotic overgrowth of yeast. Table 2.3 shows some examples of arabinose sugar inside some foods that easily consume by humans (Ahmed and Labavitch, 1980).

Table 2.3 Examples of arabinose sugar from natural dietary sources

No	Sources
1	Wheat
2	Rice
3	Beans
4	Oats
5	Barley

(Source: Ahmed and Labavitch, 1980)

Although the pentose sugars found in most insoluble fibres or roughage, those fibres are uncomplicated for the consumption of human body to pass through the digestive tract. Deficiency of arabinose sugars mostly may be a problematic for children that cause the elevated urinary levels of arabinose.

2.3.2 Synthesis Process

Agricutural, municipal waste and forest residue are the example of widespread renewable resources in Malaysia. They are universally known as lignocelluloses biomass. The advantages of this lignocelluloses biomass are firstly they have great potential of renewable, well-known, and an economical source of the residue that can be used as a raw substance to generate profitable products such as bio fuel, and added value bio product by the process of bioconversion. Normally, lignocellulosic biomass are composed from four differences sources (Table 2.4) which are forest biomass, agricultural residue, herbaceous grass and municipal waste.

Table 2.4 Sources of lignocellulosic material

Specification	Source	Example	References
Forest biomass	Wood	• Hardwood	Bludworth *et. al.,* 1993
		• Softwood	
	Residue	• Bark	
		• Sawdust	
Agricultural residue	Food crop	• Corn	Sjoman *et. al.,* 2008
		• Stover	
		• Wheat straw	
	Non-food crops	• Cotton stalk	Najafpour *et. al.,* 2007
		• Sugarcane bagasse	Wong *et. al.,* 2011
		• Oil palm trunk	
Herbaceous grass	Grass	• Switch grass	Karimi *et. al.,* 2006
		• Bermuda grass	

2.3.2.1 Oil Palm Trunk

Oil palm trunks (OPT) are a possible lignocellulosic source from palm oil mills. Presently, as much as 4.49 million hectares in Malaysia are used oil palm cultivation and around 15.2 million tonnes of OPT are generated annually. The residue of oil palm trunk in Malaysia makes it as the most abundant biomass resources. It has been believed that oil palm trunks yielded about 24-32% hydrolysable sugars (Najafpour *et. al.*, 2007). Each species has different chemical composition and different structure of trunk. Oil palm trunk is monocotyledons trees that consist of vascular bundle and parenchyma tissue. Table 1 shows chemical composition of lignocellulosic biomass samples. The lignin composition of oil palm trunk (OPT) is the lowest compare to the other samples (Table 2.5). Figure 2.14 are the abundant of oil palm trunk biomass in Malaysia.

Figure 2.14 Abundant of Oil Palm Trunk in Malaysia

(Source: Najafpour *et. al.*, 2007)

Table 2.5 Chemical Composition of Lignocellulosic Biomass

Species	Lignin composition (%)	Holocellulose composition (%)	Cellulose composition (%)
Oil palm trunk (OPT)	18.4	78.5	47.5
Sawdust of rubber wood	27.6	88.2	55.6
Sawdust mixed hardwood	32.4	77.3	55.7

(Source: Chin *et. al.*, 2011)

After 20-25 years, replanting of plants need to be done as a result of declining yield the replanting generated about 80.4 tonnes of dry biomass. Thus, the abundant of oil palm trunk (OPT) usually being left after on the plantation site or burned. Burning the residue causes of oil palm trunk (OPT) causes environmental problems (Wong *et. al.*, 2011). Table 2.6 exhibits estimation of new planting and the residue of the oil palm based on the Malaysian Palm Oil Board (2005). Until year 2020, total measure areas are increasing even though the new planting is decreasing.

Table 2.6 Estimation of oil palm from year 2005 until 2020

Year	New Planting (ha)	Immature Areas (ha)	Mature areas (ha)	Total Planted areas (ha)
2005	135 000	578 000	3 592 000	4 170 000
2010	46 000	130 000	4 389 000	4 522 000
2015	63 000	291 000	4 616 000	4 907 000
2020	1 000	74 000	4 841 000	4 915 000

(Source: Abdullah, 2005)

Many researches study of the production of xylose, glucose and arabinose from parts of the oil palm not only its trunks, but included the oil palm empty fruit brunch and oil palm fronds. Table 2.7 shows the summary of the parts of oil palm being study to generate xylose, glucose and arabinose.

Table 2.7 Summary of parts of oil palm being used to produce monosaccharides

Title	Raw Material	Product	References
Optimization studies on acid hydrolysis of oil palm empty fruit brunch fiber for production of xylose.	Oil palm empty fruit brunch	Xylose	Rahman *et. al.*, 2006
Production of glucose from oil plam trunk and sawdust of rubberwood and mixed hardwood	Oil palm trunk (OPT)	Glucose	Chin *et. al.*, 2011
Ethanol production from oil palm trunks treated with aqueous ammonia and cellulase	Oil palm trunks	Glucose	Jung *et. al.*, 2011
Production of xylose from oil palm empty fruit bunch fiber using sulphuric acid	Oil palm empty fruit bunch fiber (OPEFB)	Xylose	Rahman *et. al.*, 2005
Enzymatic hydrolysis of oil palm empty fruit bunch using immobilized cellulase enzyme	Oil palm empty fruit bunch	Glucose	Alkhatib *et. al.*, 2011

2.3.2.2 Agricutural Residue and Waste

One type of fine raw materials that enable producing D-xylose is rice straw. According to Karimi *et. al.,* (2006), the three main compositions of the rice straw structure are cellulose (32–47%), hemicellulose (19–27%) and lignin (5–24%). In hemicelluloses, pentose sugars are dominant monosaccharides. One of the study by the Zhang *et. al.,* (2010), xylose is the most important sugar followed by arabinose and hexoses. Chemical or enzymatic hydrolysis of xylan generates D-xylose as the main product (Saha *et. al.,* 2005; Yu *et. al.,* 2008).

Figure 2.15 Rice Straw Lignocellulosic Material

(Source: Zhang *et. al.,* 2010)

Table 2.8 shows the summary of hemicelluloses, cellulose and lignin that could be converted to xylose, glucose and arabinose by the chemical or enzymatic hydrolysis.

Table 2.8 The contents of cellulose, hemicelluloses, and lignin in common agricultural residue and waste

Lignicellulosic materials	Cellulose (%)	Hemicelluloses (%)	Lignin (%)
Hardwood stems	40-55	24-40	18-25
Softwood stems	45-50	25-35	25-35
Nut shells	25-30	25-30	30-40
Corn cobs	45	35	15
Grasses	25-40	35-50	10-30
Paper	85-99	0	0-15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15-20	80-85	0
Cotton seed hairs	80-95	5-20	0
Newspaper	40-55	25-40	18-30
Waste papers from chemical pulps	60-70	10-20	5-10
Primary wastewater solids	8-15	NA	24-29
Swine waste	6.0	28	NA
Solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
Coastal Bermuda grass	25	35.7	6.4
Switch grass	45	31.4	12.0

(Source: Sun and Cheng, 2002)

2.3.2.3 Hard wood Species

The most frequent and well known species of hardwood in Malaysia is Red *Meranti* wood (RMW). A lot of growth of Red Meranti wood in peninsular and east of Malaysia make it most preferable to be process into marketable product by the main sawmill. Thus, abundant quantity of sawdust waste generated from those plant. Since it is a lignocellulosic biomass, the composition of the wood consists of three major component of biopolymer which is cellulose, hemicellulose and lignin.

It is estimated that 29% of xylan which is a sugar polymer that built from the pentose sugar xylose contains inside the RMW biomass. However, the usage of hardwood species is still restricted in Malaysia. According to the Rafiqul *et. al.,* (2011), the hard wood species normally are used as the animal feed, fuel in manufacturing plants local utilities in a big scale. Table 2.9 are the summary of hardwood species that used in xylose, glucose and arabinose production as the raw material. Figure 2.16 shows the abundant of Red Meranti wood biomass.

Figure 2.16 Red Meranti Wood Biomass

(Source: Raiqul *et. al.*, 2011)

Table 2.9 Summary of hardwood species raw material that used in xylose production

Title	Raw Material	Product	References
Design of process parameters for the production of xylose from wood sawdust	Red *Meranti* wood	Xylose	Rafiqul *et. al.*, 2011
Generation of xylose solutions from Eucalyptus 32lobules wood by autohydrolysis-posthydrolysis process: posthydrolysis kinetics	Eucalyptus 32lobules wood	Xylose	Garrote *et. al.*, 2001
Optimization of acid hydrolysis from the hemicellulosic fraction of Eucalyptus grandis residue using response surface methodology	Eucalyptus grandis residue	Xylose	Eliana *et. al.*,2007
Production of glucose from oil palm trunk and sawdust of rubberwood and mixed hardwood	Mixed hardwood sawdust	Glucose	Chin et. al., 2011

2.4 Process of Monosaccharides Production

The successful of biochemical conversion from lignocellulosic biomass is highly depending on the pretreatment steps. Degradation of lignocellulosic biomass into a bio product such as monosaccharides could be done by three ways which is physical process, chemical process and enzymatic process. According to Harmsen *et. al.,* (2010) the choices of the suitable pretreatment depends on the economic consideration, environmental affect and mostly the aim of the biomass pretreatment itself.

Pretreatment by physical process alone could not produce the sugars. It should be continue with the chemical hydrolysis or enzymatic hydrolysis. Both chemical and enzymatic hydrolysis has the advantages and disadvantages although the generation of xylose, glucose and arabinose can be achieved.

2.4.1 Physical Pre treatment

Physical pretreatment are treatment that not involve the chemical, enzyme or microbes use. It is usually a first treatment that can enhance the lignin removal. According to Gupta and Polach (1985), physical pretreatment can be divided into two parts. The first part is the removal of obvious contamination and secondly the reduction size of the sample. This is the reason for the most bio conversion, the first steps involve are the physical pretreatment that includes grinding, screening and drying.

As stated by Young *et. al.,* (2011), the lignocellulosic material was crushed in a high speed rotary cutting mill. Prior to further pretreatment, reducing the size and increasing the surface area are done by crushing of the samples. Other than increasing the surface area and volume ratio, reduction of particle size also ease the material handling.

2.4.2 Chemical Pretreatment

2.4.2.1 Diluted Acid Hydrolysis

The most common chemical pretratment that applied to the bioconversion of lignocellulosic materials are weak acid hydrolysis and strong acid hydrolysis (Harmsen *et. al.,* 2010). Production of xylose from hemicelluloses through the commonly used acid hydrolysis is an efficient and cheap method. As stated by Najafpour *et. al.,* (2007), acid hydrolysis is a direct hydrolysis method and this single stage acid hydrolysis is considered as simple reaction.

According to Najafpour *et. al.,* (2007), compared to the concentrated acid hydrolysis, a single stage hydrolysis with diluted acid (less than 5% acid concentration) was used generally. Acid act catalyst in acid hydrolysis mainly degrades the hemicelluloses to generate xylose without altering the cellulose and lignin component. There are disadvantages of acid hydrolysis method involving the further treatment. Acid can be corrosive, therefore neutralisation results in the development of solid waste.

This method is more suitable for lignocellulosic biomass with low lignin composition because sturdy inhibitors can happen if the hemicelluloses sugars were further degraded to furfural and hydroxymethyl furfural. According to Gomez *et. al.,* (2003), dilute acid pre-treatment widely studies because it's effectively solubilise hemicelluloses into the monomeric sugar (arabinose, galactose, glucose, mannose and xylose).

Diluted acid hydrolysis of lignocellulosic material mostly generates xylose from hemicelulose in liquid state with controlled conditions. Instead of degeneration of hemicelluloses, the cellulose and lignin structure are unaltered and remained as a solid phase. According to one of study of Parajo *et. al.,* (1998), hemicelluloses are easily hydrolyzed during the process due to its amorphous brunched structure compared to crystalline nature of cellulose which need rigorous treatment condition.

The crucial fact is that the most important parameter affecting yield of sugar is the concentration of acid (Harmsen *et. al.,* 2010) Maximum yield of xylose froduction from lignocellulosic biomass conversion can be achieved via higher temperature condition within in a short time. However, contamination with the existence of furufural, hydroxyl-methyl furfural and soluble derivatives would occur if high temperature parameter is applied.

After the pre-treatment, the solid material was separated by centrifugation and the filtrate (hemicelluloses fraction) was analysed for solubilised sugars which is xylose, glucose and arabinose (Roberto *et. al.,* 2003).

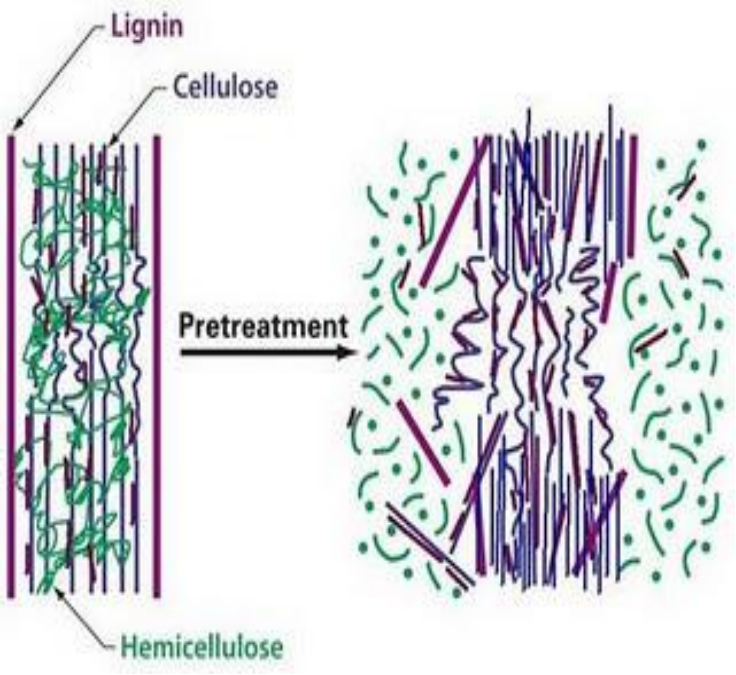

Figure 2.17 Lignocellulosic material structure during acid hydrolysis process

(Source: Harmsen *et. al.*, 2010)

2.4.2.2 First Stage Pre treatment

Normally, first stage pre treatment involves the removing of lignin composition in biomass material greatly. According to Zhao *et. al.*, (2008), peracetic acid is an effective and efficient chemical to degrade the lignin content successfully. Removal of lignin is necessary since it is undesirable towards the bioconversion marketable product. It fills the spaces between cellulose, hemicelluloses and pectin and acts as support to the wood structure (Ralph, 2010). Its characteristic which is implementation of destructive enzyme through the cell wall allows the protection of trees against degradation. Table 2.10 is the summary of chemical pretreatment that used to degrade the lignocellulosic biomass.

Table 2.10 Summary of chemical pretreatment method of lignocellulosic biomass

Title	Raw Material	Method	Product	References
Production of glucose from oil palm trunk and sawdust of rubberwood and mixed hardwood	Oil palm trunk Rubberwood Mixed hardwood	Acid hydrolysis (concentrated sulphuric acid)	Glucose	Chin *et. al.*, 2011
Acid hydrolysis of pretreated palm oil lignocelllulosic wastes	Palm oil empty fruit bunch	Acid hydrolysis (hydrochloric acid)	Reducing sugar	Najafpour *et. al.*, 2007
Production of xylitol from concentrated wood hydrolysates by *Bebaryomyces hansenii* : Effect of the initial cell concentration	Eucalyptus lobules wood chips	Acid hydrolysis (Sulfuric acid) and fermentation	Xylitol	Parajo *et. al.*, 1996
Design of process parameters for the production of xylose from wood sawdust	Red *Meranti* wood	Acid hydrolysis (Sulfuric acid)	Xylose	Rafiqul *et. al.*, 2011
Optimization studies on acid hydrolysis of oil palm empty fruit bunch fiber for production of xylose	Palm oil empty fruit bunch	Acid hydrolysis (Sulfuric acid)	Xylose	Rahman *et. al.*, 2011
The acid hydrolysis of sugarcane bagasse hemicelluloses to produce xylose, glucose and other products	Sugarcane bagasse	Acid hydrolysis (dilute sulphuric acid and hydrochloric acid)	Xylose Glucose	Lavarack *et. al.*, 2002

2.4.3 Enzymatic Hydrolysis

Proteolytic enzymes hydrolysate protein more gently than acid, does not required high temperature and usually target specific peptide bonds. According to Alkhatib *et. al.,* (2011), low catalytic efficiency and instability of enzymes were considered as a barrier in an improvement of large scale operations and usage. Therefore, the use of cellulase enzyme is needed in enzymatic hydrolysis. In the chemical method, the hydrolysis is catalyzed by an acid while cellulolytic enzymes reacted with the lignocellulosic biomass for the enzymatic hydrolysis (cellulase and cellobiase) act as catalysts (Martin *et. al.*, 2002).

Presently, enzymatic hydrolysis has attracted growing attention as an alternative to acid hydrolysis. It can be performed under milder reaction conditions which is pH around 5 and temperature less than 50^0C with lower energy consumption and lower environmental impact. In addition, it does not present corrosion problems, and gives high yield of pure glucose with low formation of by-products that is favorable for the hydrolysate use in bioconversion processes (Liao *et. al.,* 2005).

Enzymatic hydrolysis is carried out by highly specific cellulase enzyme (Begiun and Aubert, 1994) and attacking the cellulose materials. The overall process (Figure 2.18) can be described as offensive of ß-14-endoglucanase towards the low crsytallinity region in the cellulose fiber. This will create cellobiohydrolase a free chain ends. Further degradation will occur by removing cellobiose units from the free chain ends. The cellobiase will further hydrolyze by the cellobiose to produce glucose (Prasad *et. al.*, 2007; Sun and Cheng, 2002; Cao and Tan, 2002).

$$cellulose \xrightarrow{b-1,4\ glucane} cellobiose \xrightarrow{b-glucosidase} glucose$$

Figure 2.18 Overall process of enzymatic hydrolysis

(Source: Prasad *et. al.*, 2007)

The summary of research studies that use enzymatic hydrolysis to break down the lignocellulosic biomass and produce xylose, glucose and arabinose are shown in Table 2.11.

Table 2.11 Summary of enzymatic hydrolysis method of lignocellulosic materials

Title	Raw Material	Method	Product	References
Enzymatic hydrolysis of oil plam empty fruit bunch using immobilized cellulase enzyme	Oil palm empty fruit bunch	Enzymatic hydrolysis (Cellulase)	Reducing sugar	Alkhatib *et. al.,* 2011
Ethanol production from oil palm trunks treated with aqueous ammonia and cellulase	Oil Palm trunk	Enzymatic hydrolysis (cellulase)	Glucose	Jung *et. al.*, 20011
Effect of mixing on enzymatic hydrolysis of cardboard waste: Saccharification yield and subsequent of the solid residue using a pressure filter	Cardboard Paper	Enzymatic hydrolysis (hemicullulase and cellulase)	Glucose	Kinnarinen *et. al.,* 2011
Radiation-Induced decomposition and enzymatic hydrolysis of cellulose	Plant	Enzymatic hydrolysis (cellulase)	Glucose	John Wiley, 1978

2.5 Reason for the Implementation of Various Hydrolysis Method towards the Lignocellulosic Biomass

Pretreatment by dilute acid hydrolysis is the first step generates the xylose, glucose and arabinose from lignocellulosic biomass (oil palm trunks) in this research. According to Chin *et. al.,* (2011), the acid used is sulphuric acid (H_2SO_4). Most of the study stated that concentration of sulphuric acid below 4wt% is widely used since it is cheap and effective. Mixtures of biomass with dilute sulphuric acid hydrolyse hemicelluloses to xylose and other monosaccharides. Instead of acid hydrolysis, filtration of the material is done and this process leaves the cellulose inside the remaining solid waste (cake). Thus, low yield of xylose production is generated with only one step of hydrolysis. The advantages and disadvantages of dilute acid hydrolysis are described in Table 2.12.

Table 2.12 Advantages and disadvantages of dilute acid hydrolysis

No	Advantages	Disadvantages
1	Powerful agents for hemicellulose hydrolysis	Toxic
2	Improved cellulose hydrolysis	Corrosive
3	High yield xylose production	Expensive
4	Low production yield of glucose and arabinose	Hazardous

(Source: Agbor *et. al.,* 2011)

After the acid hydrolysis method, the degradation of lignocellulosic material is continued with the biological treatment (enzymatic hydrolysis). Enzymatic hydrolysis is a safe and environmentally friendly method (Jung *et. al.,* 2011).

Nowadays, the method is increasingly being advocated for its low energy requirement of lignin removal. Removal of the solid waste from the acid hydrolysis to the environment is considered as the abundant of biomass. Therefore, combination of both dilute acid hydrolysis and enzymatic hydrolysis maximise the production of monosaccharides. The solid waste (cake) made up of cellulose be converted to glucose and only lignin composition is being throw. This method helps reduce the waste, decrease the excessive of oil palm trunks (OPT) and save environment.

2.6 Parameters involve in Monosaccharides Production

2.6.1 Effect of Particle Size

Literature review on the Najafpour *et. al.*, (2007) stated that small fine particle of lignocellulosic using a grinding mill may increase the surface area and reduce the diffusion problem related to the reactant involved. The effect of solid particle size was investigated for the size of 250, 500, 710 and 1000 microns. The present result (Table 2.13) indicated the reduction of size to submicron scale enhanced remarkably the glucose yield (Yeh *et. al.,* 2009).

Table 2.13 Effect of particle size on the production of glucose.

Particle Size (μm)	Glucose Concentration (g/L)
250	16
500	15.5
710	15
1000	14

(Source: Yeh *et. al.,* 2009)

Particle size gives high impacts towards the lignocellulosic biomass hydrolysis. As stated by Yeh *et. al.*, (2009), total surface area of the lignocellulosic material increases with the size reduction of the biomass. This scenario helps diminish the mass and heat transfer limitations during the hydrolysis. Other than that, smaller particle size improved the dilute acid hydrolysis of hemicelluloses since affinity between cellulose and enzyme could be enhanced.

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Table 2.14 Summary of effect of particle size towards the hydrolysis of lignocellulosic biomass

Title	Raw Material	Method	Product	References
Effect of Particle size on the rate of enzymatic hydrolysis of cellulose	Microcrystalline cotton cellulose	Enzymatic hydrolysis (Cellulase)	Glucose	Yeh *et. al.,* 2009
Impact of impregnation time and chip size on sugar yield n pretreatment of softwood for ethanol production	Softwood	Dilute acid pretreament Enzymatic hydrolysis	Glucose Mannose	Monavari *et. al.,* 2009
Effects of physical and chemical pretreatments on xylose and glucose production from oil palm press fibre	Oil palm press fibre	Hydrolysis Saccharification	Xylose Glucose	Aziz *et. al.,* 2002

2.6.2 Effect of Solid Liquid Ratio

The solid liquid ratio (SLR) was studied by Lavarack *et. al.,* (2002), where as the solid to liquid ratio (SLR) were reduced, the rate of decomposition of xylose would also reduced. This is because the hydrolysate was diluted as the xylose produced. The lowest solid liquid ratio (SLR) resulted in minimum sugar concentration. According to Jung *et. al,*. (2011), two different solid liquid ratios (SLR) of 1:6 and 1:12 were studied. At 1:12 ratio, enzymatic digestibilities increased from 70% to 83%.

Table 2.15 Enzymatic digestibility at temperature 60^0C.

Solid Liquid Ratio (SLR)	Enzymatic Digestibilty (%)
1:6	70
1:12	83

(Source: Jung *et. al.*, 2011)

As stated by Betancur and Pereira (2010), during the pretreatment process, high xylose concentrations and yield of hydrolysis is obtained by applied a high concentration of sulphuric acid. An ideal condition should be constructed to get yield of hydrolysis with low levels of inhibitors. Nowadays, the extremely low acid condition has been applied mostly for hemicelluloses hydrolysis (Kim *et. al.,* 2001) since high acid concentration cause the formation of furfural with further degradation of hemicelluloses.

2.6.3 Effect of Temperature

Temperature did play an important part on the enzymatic hydrolysis process. It can be supported by Najafpour *et. al.,* (2007) that shown by the kinetic study that generation rate of monomer sugar reaction affected by the temperature. Based on the theory of enzyme, sufficient energy is required in order to achieve a good orientation of collision. Action of enzyme is greatly influenced by the temperature. Therefore, collision and binding of enzyme with the substrate active site is necessary for the conversion of product from substrate.

Nevertheless, too much heat and too low temperature highly affect the production of monosaccharides from the cellulose material (Vecken and Hamelers, 1999). Enzymes need optimum temperature for them to become active and avoid denaturation. According to the previous research the optimum temperature of high yield of glucose production was carried out at 50^0C. Above the temperature of 50^0C, denaturation occurs (Figure 2.19) and ruthlessly diminished the catalytic activities.

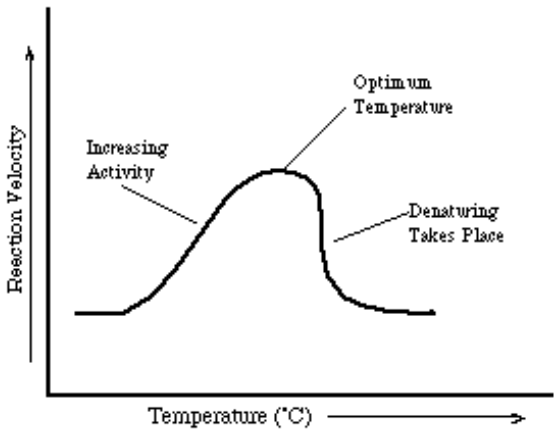


Figure 2.19 Effect of temperature on reaction rate

(Source: Vecken and Hamelers, 1999)

2.6.4 Effect of Species

According to Chandra *et. al.,* (2007), different composition of cellulose, hemicelluloses and lignin enclose inside the different type of lignocellulosic biomass. In general plant biomass has 40-50% cellulose. Some of the plants such as cotton and hemp bast-fibre made up or 80% cellulose, 20-40% hemicelluloses and 20-30% lignin by weight (Mckendry, 2002). This difference composition generates different yield hydrolysis of the lignocellulosic materials.

This complexity of the specified biomass related to the affiliation between the carbohydrates and its structural components. The features that make the biomass hard to deal are (Mosier *et. al.,* 2005) firstly the lignin protection, porosity, crystallinity and lastly the degree of polymerization of cellulose.

Roughly 90% of dry weights of most plants are made up of cellulose, hemicelluloses, lignin and pectin (Table 2.16). The lignin acts as the protective barrier towards the destruction of plant cell for the conversion of bio fuels (which is further hydrolysis of monosaccharides) by the bacteria and fungi. The selection of plant species depends on the added value product and bio conversion.

Table 2.16 Composition of lignocellulosic biomass

Lignicellulosic materials	Cellulose (%)	Hemicelluloses (%)	Lignin (%)
Hardwood stems	40-55	24-40	18-25
Softwood stems	45-50	25-35	25-35
Nut shells	25-30	25-30	30-40
Corn cobs	45	35	15
Grasses	25-40	35-50	10-30

(Source: Sun and Cheng, 2009)

2.7 Analytical Method of Monosaccharides Production

2.7.1 Qualitative Analysis

2.7.1.1 High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) is highly versatile because it has capability to separate a chemical compounds in the mixture compare with others type of chromatographic. It used the chromatographic separation technique to isolate the compound from mixture for purify, quantify and identify the single component in the mixture. The separation of component occur when the component dissolve in the solvent and travel relatively different through the column with the pressure exist in the mobile phase. This chromatographic commonly can operate with different types of stationary phase such as in solid and liquid phase.

The relative affinities of the mixture between compound and solvent make it travel with different rates. The mixture with higher affinity travels slowly toward stationary phase and vice-versa for lower affinity. Prolonged of contact between the

solute and stationary phase determine the amount of resolution. Small particle sizes of stationary phase used in column chromatography give the larger surface area and the separation occur are more effective. Table 2.17 shows the summary of the HPLC method from the previous studies.

Table 2.17 Summary of HPLC method

Title	Column	Mobile Phase	Detect	Flow rate	Temperature	References
Optimization studies on acid hydrolysis of oil palm empty fruit brunch fiber for production of xylose	SUPELCOSIL LC-NH_2	Aqueous acetonitrile (75%)	RI	1.5 ml/min	50^{o}C	Rahman *et. al.,* 2006
Optimization of xylose production from sago trunk cortex by acid hydrolysis	Inertsil NH_2	Deionized water and acetonitrile	RI	0.5 ml/min	40^{o}C	Nurul Lina *et. al.,* 2011
Effect of autohydrolysis and enzymatic treatment on oil palm (Elaeis guineensis Jacq.) frond fibres for xylose and xylooligosaccharides production	Sugar-pak I	0.1 mM CaEDTA	RI	0.6 mL/min	90^{o}C	Hanim *et. al.,* 2011
Generation of xylose solution from Eucalyptus globulus wood by autohydrolysis-posthydrolysis process: posthydrolysis	Aminex HPX 87H	1.8 x 10^{-}4 M H_2SO_4	RI and DAD	0.6 ml/min	45^{o}C	Garrote *et. al.,* 2001
A study on the consecutive preparation of D-xylose and pure superfine silica from rice husk	Aminex HPX-87H	0.01N H_2SO_4	RI	0.6 mL/min	65^{o}C	Zhang *et. al.,* 2010

2.7.1.2 Biochemistry Analyzer

A biochemistry analyzer is equipment that designed to analyze and measure different chemicals and characteristics of various biological samples quickly. There are many types of biochemistry analyzer and mostly used in life sciences laboratories and for medical use. According to Mock *et. al.,* (1995), the usual roles of biochemistry analyzer in a life sciences laboratory are determination of the structure and function of biomolecules including nucleic acid and enzymes.

The components inside the common biochemistry analysers are an automated sampler holder and charger, calibration systems, automated dilutors, temperature controlled analyzer chamber and an output device with computer communications. One of the methods to introduce the sample into the analyzer is inserting tubes into circular carousels that rotate for the sample availability. This automation of testing procedure has reduced the time consuming tasks from hours or days to a few minutes depending on the samples (Tothill, 2001).

Nowadays, a multiple of different automated biochemistry equipments (Figure 2.20) are available. There are constructed depending on the wide different application in the laboratory. One of the roles of instruments that have been upgrade is measuring of the concentration and determination of characteristics of specific substance in a speedy ways with minimum amount of operator involvement (Tothill, 2001). This description make the biochemistry analyzer is much easier and safe to use for the analysis works.

Figure 2.20 Typical biochemistry analyzer

(Source: Tothill, 2011)

2.7.2 Qualitative Analysis

2.7.2.1 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is an instrument that uses for qualitative analysis in the recognizing types of functional groups. It is beneficial for recognizing functional group either organic or inorganic compounds (Van *et. al.,* 2006). Commonly, it is suitable for the analysis of solids, liquids and gasses. Molecular bond is vibrant at different frequencies reliant on the elements and the types of bonds. The frequencies where it can be vibrated are different for each given bond. The wavelength of the light immersed is characteristics of the chemical bond in the spectrum. Figure 2.21 shows the principle of the FTIR.

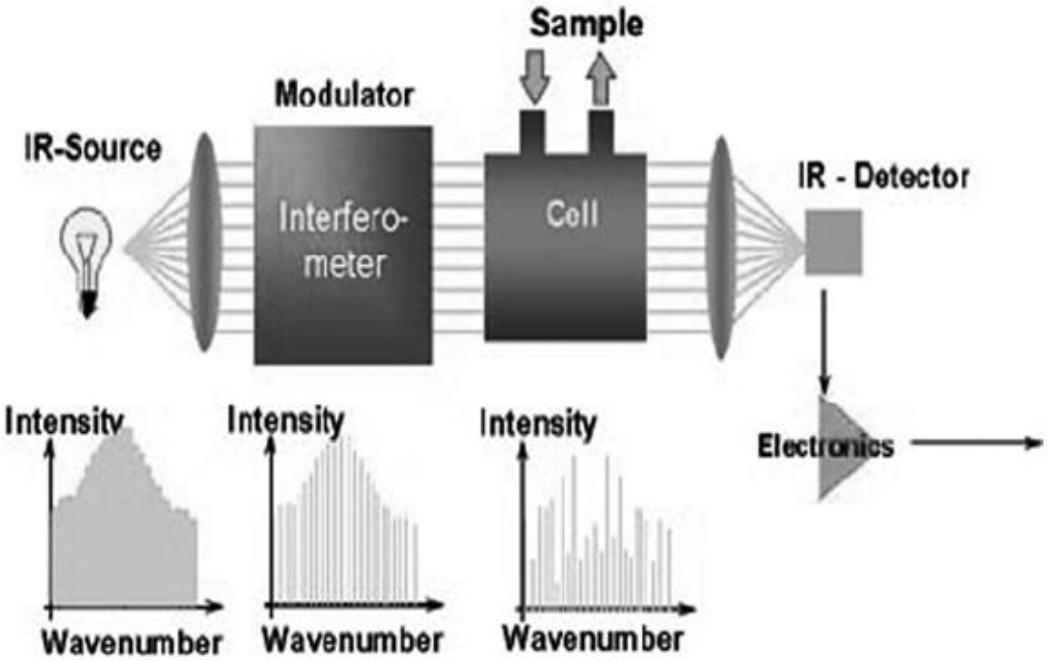


Figure 2.21 Principle of fourier transform infrared spectroscopy (FTIR)

(Source: Van *et. al.,* 2006)

2.7.2.2 Scanning Electron Microscope (SEM)

Scanning Electron Microscope (SEM) is a device that used electron microscope to form an image. A specimen can be focus at one time with higher resolution and closely spaced specimen can be magnified with higher levels. The electron beams that produce by the electron gun at top of the microscope follow a vertical path through the microscope in vacuum condition (Ralph, 2010). The beam travels over electromagnetic fields and lenses, which emphasis the beam down to the sample. Electrons and X-rays are evicted from the sample, once the beam hits the sample. X-rays, backscattered electrons, and secondary electrons are collected by detectors and it converts them into a signal that is sent to a screen like to a television

screen and creates the final image. Figure 2.22 shows the principle of the Scanning Electron Microscope (SEM).

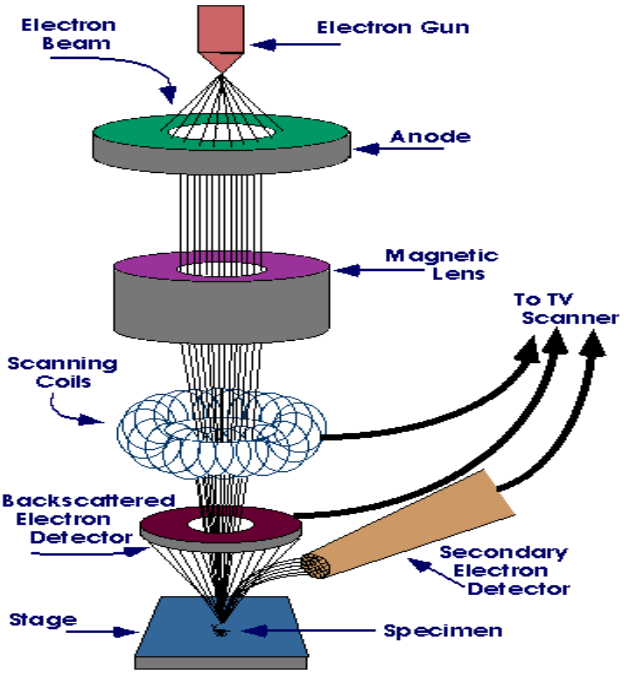


Figure 2.22 Principle of scanning electron microscope (SEM)

(Source: Ralph, 2010)

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Introduction

In order to achieve the objective of the research, the raw material used must from the biomass. This research involved a few stages of pretreatment of the biomass. Figure 3.1 shows the flowchart of the whole pretreatment procedure.

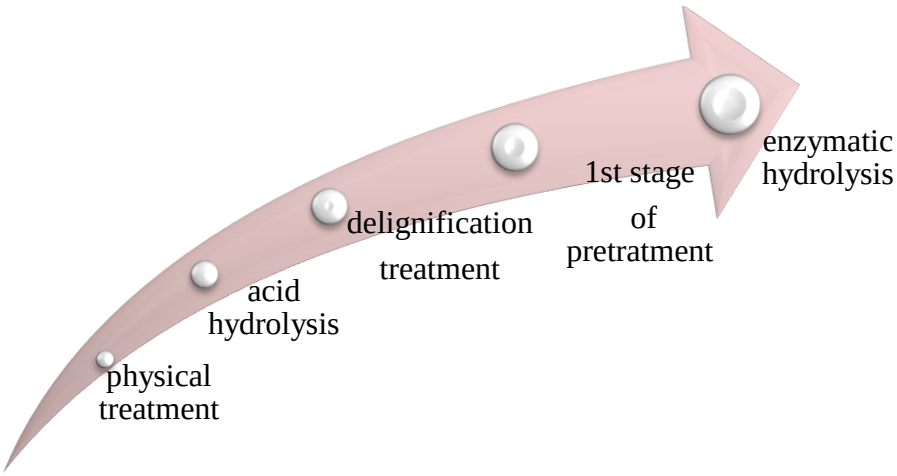


Figure 3.1 Overall pretreatment process

3.2 Pre-treatment of Raw Material

The lignocellulosic biomass selected in this study was oil palm trunk (OPT). The oil palm trunk (OPT) was obtained from Felda Jengka, Pahang. Due to large size of oil palm trunk collected, it was cut into small pieces using chain saw at the saw mill industry at Gambang, Pahang. Dusts generated from this cutting process. Then, they were collected for sorting process.

The resulting sample was sieved into five different sizes which are 160, 200, 315, 630 and 800 µm sieves by using vibrator sieve shaker at 10rpm for 30 minutes repeatedly. The sample then was sun dried for a week to remove moisture content of the sample. The removal of moisture content was detected by weighing the sample every day until the weight of the sample become constant. Finally the sample was stored at room temperature for further used. Figure 3.2 shows the summary physical pretreatment of raw material.

Figure 3.2 Summary of physical pretreatment of oil palm trunk

3.3 Acid Hydrolysis

Sun dried oil palm trunk (OPT) with particle size of 160µm was filled in 500 ml beaker. Then it was mixed with 3.24% w/w H_2SO_4 solution in a 1:8 solid liquid ratio (Wong *et. al.,* 2011). The slurries were stirred using magnetic stirrer for 15 minutes at room temperature until completely mixed. The hydrolysis process was carried out in an autoclave (Hiclave HVE-50, Hirayama, Japan) (Wong *et. al.,* 2011) at constant temperature 120^0C for 40 minutes (Mohamed *et. al.,* 2011).

The flasks were cooled at room temperature for desired time after completing the process. Then, the insoluble solid were separated from aqueous solution by filtration. Filtration process was done using a filter paper (Whatman No1). The hemicellulosic hydrolysate was obtained at the filtrate at pH 1.12. The remaining cakes were contained cellulose and lignin (Musatto *et. al.,* 2005). The filter cake remained in the filter paper was oven dry at 60^0C for 3 hours (Azmalisa *et. al.,* 2010). Figure 3.3 describe the overall acid hydrolysis process.

sample measurement for
acid hydrolysis process

addition of 3.24% H_2SO_4 into the beaker
with ratio 1:8 of the sample

sample before autoclave with
temperature 120^0C for 40 minutes

sample after acid hydrolysis process

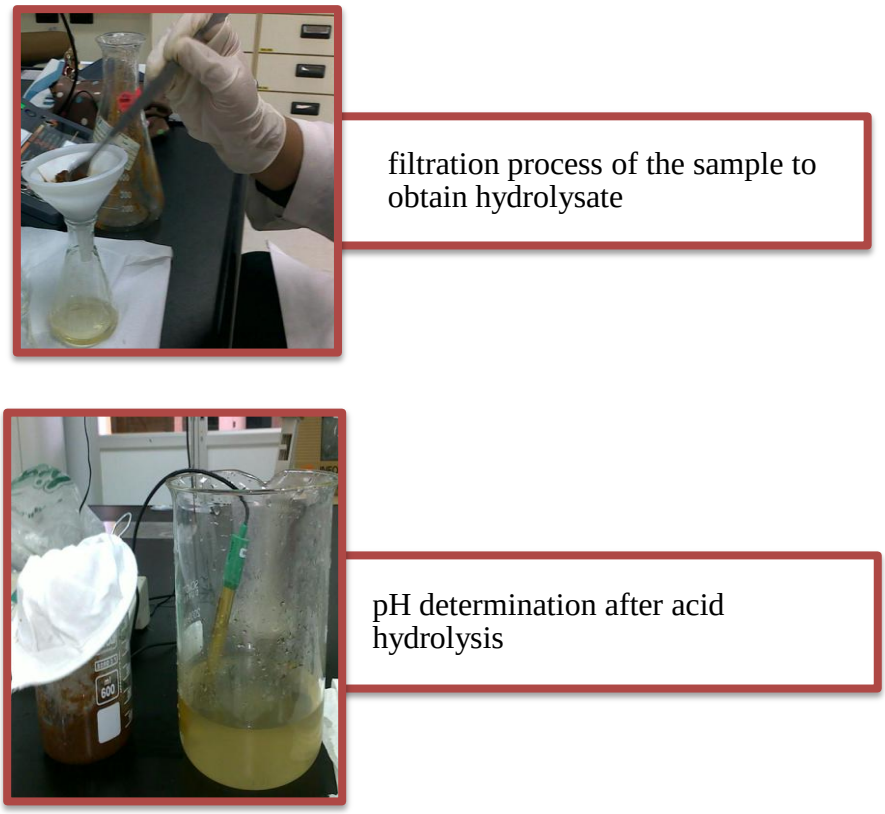


Figure 3.3 Overall acid hydrolysis process

3.4 Neutralisation

Prior to the HPLC analysis, the acidic filtrate solution was neutralized with calcium hydroxide (CaOH) to achieve pH around 6 to 8 (Chin *et. al.*, 2011). The calcium hydroxide was added gradually and the solution was stirred by using magnetic stirrer for 15 minutes. Then, the pH reading of the stirred solution was checked until it reaches an average pH 6.5. Calcium hydroxide was added more if the solution did not reach the neutral solution. The $CaSO_4$ precipitate form was removed by filtration (Figure 3.4). The filtrate (neutral sugar solution) was stored at 4^0C and analysed for production of xylose, glucose and arabinose (Bludworth *et. al.*, 1993). Figure 3.5 shows the process flow of the neutralisation process.

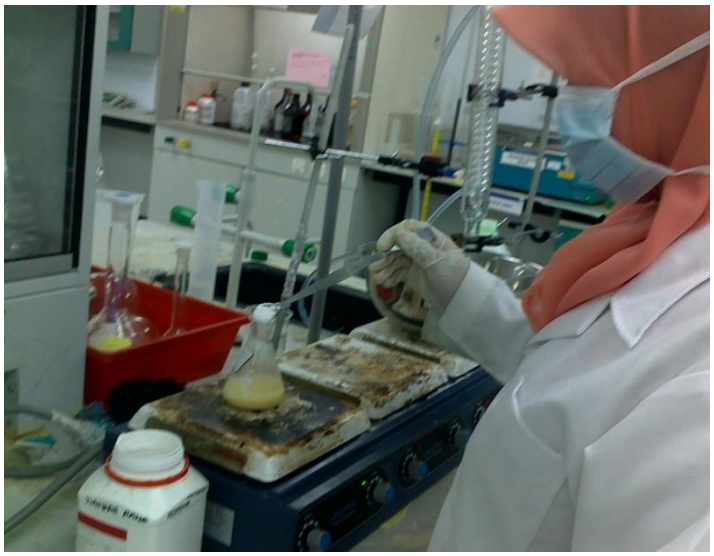


Figure 3.4 Neutralisation process using calcium hydroxide

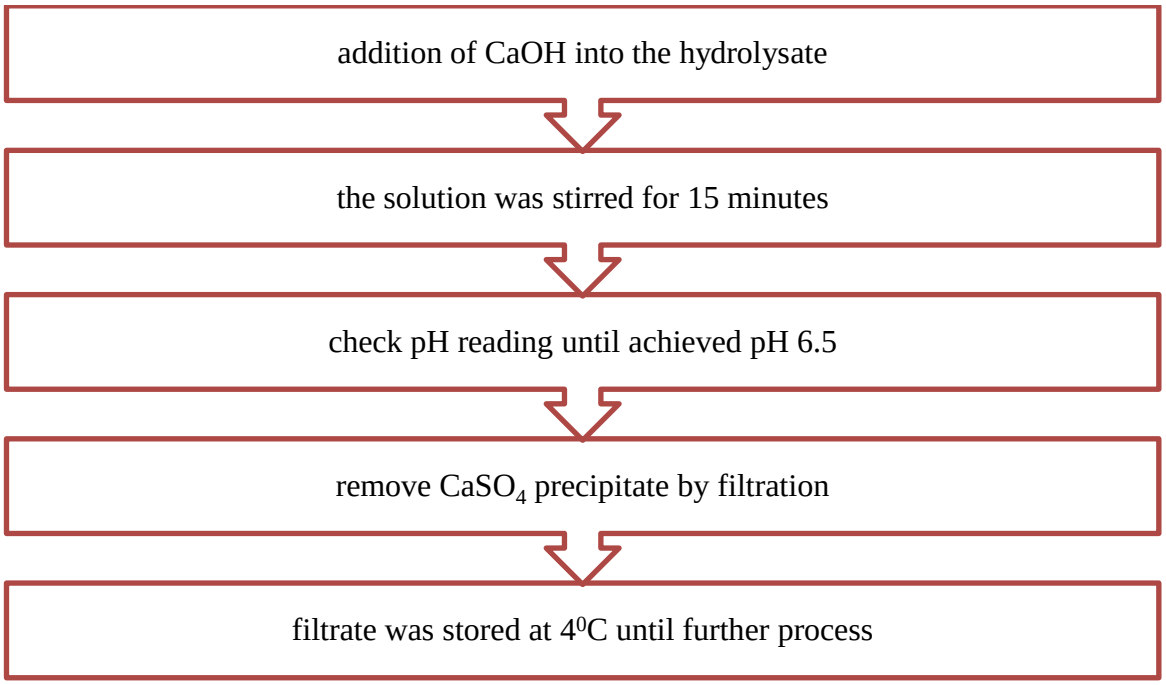


Figure 3.5 Process flow of neutralisation

3.5 Analysis by High Performance Liquid Chromatography (HPLC)

Xylose, glucose and arabinose were determined by High Perfomance Liquid Chromatoghraphy (HPLC) with a RI (Agilent, and 1200 series) as the detector of the concentration of the component (Azmalisa *et. al.*, 2010) (Figure 3.6). The column that used was RCM Monosaccharide, 7.8mm x 300mm column aluted at 80^0C using water as mobile phase with 0.6ml/min flow rate, injection volume 10µL and a retention time was 20 minutes for each sample (Lina *et. al.*, 2011).

Before run the HPLC analysis, the column was washed first by purging process for 3 hours. The standard solutions that consist of xylose, glucose and arabinose were diluted in five different concentrations. The standard solutions and samples were filter by using 0.45 µm membrane filter before transferring into the HPLC vial. Figure 3.7 describe the summary of HPLC analysis.

Figure 3.6 High performance liquid chromatoghraphy analysis

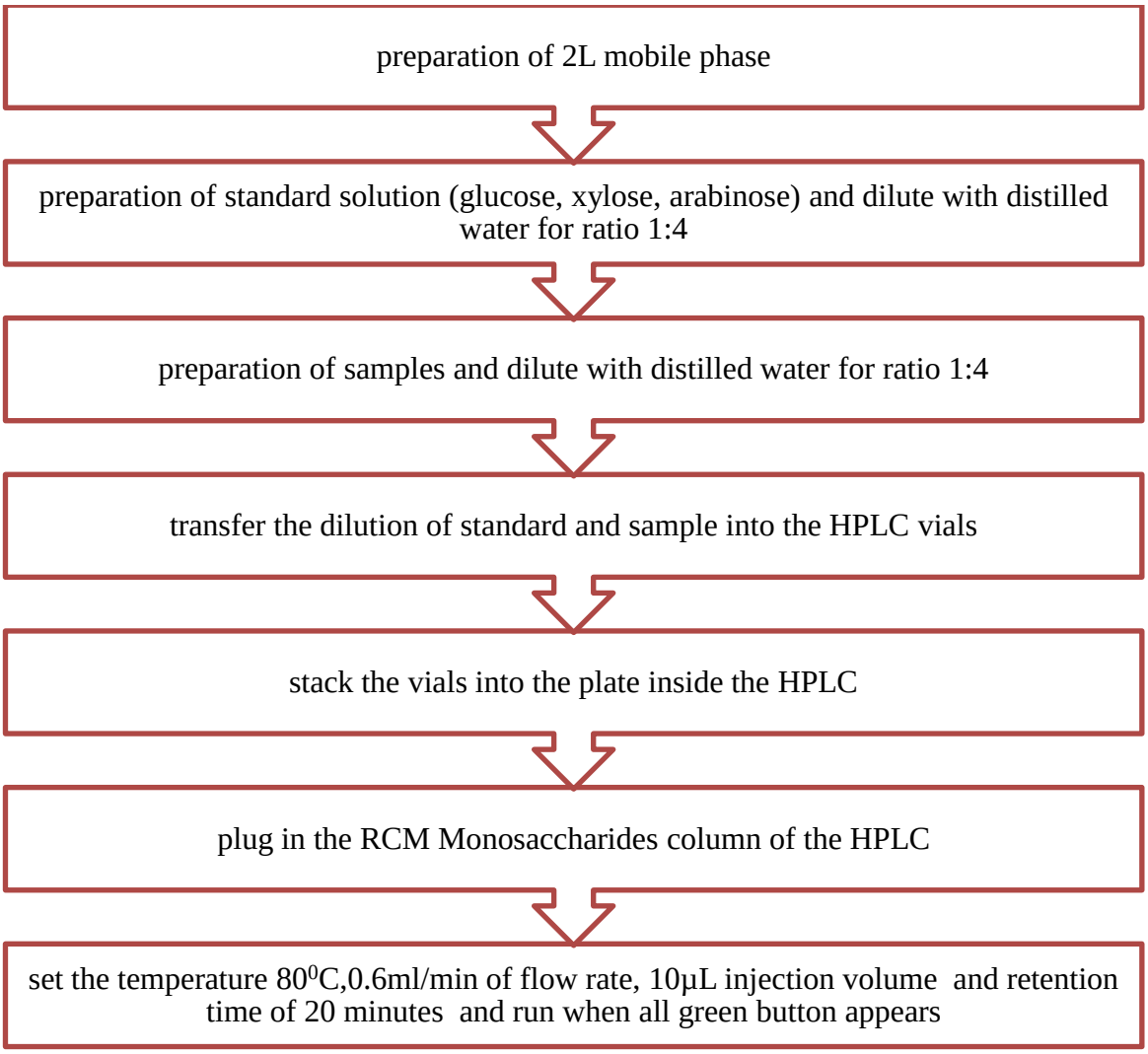


Figure 3.7 Summary of HPLC analysis

3.6 Delignification

In delignification process, remaining filter cake was reacted with sodium hydroxide (NaOH). 18% w/w of NaOH was used. All the experiments were done using 500mL Erlenmeyer flask. The NaOH was used to pre-break the line of the lignin and cellulose (Brodeur *et. al.*, 2011). The samples were loaded with the ratio of 1:6. This method was done using oil bath shaker. The temperature was set 110^{0}C with duration time of 90 minutes. According to Zhao *et. al.*, (2008), this condition

will recover the maximum of the cellulose recovery and also enhance the probability of enzymatic hydrolysis.

After that, the samples were washed using hot de-ionized water until the washed water become pH 7 or nearby. If the pH reading not reached the neutral reading, keep washed the sample with the hot de-ionized water. Then, filtration process was done to filter the filtrate and kept the cake. The samples (cake) were dried in oven at 60^0C for 24 hours and kept until further process. Figure 3.8 shows the overall delignification of oil palm trunk.

preparation of 18%w/w NaOH

soaked the OPT dust in NaOH solution using 6:1 (L:S) with temperature 1100C for 90 MInutes in oil bath shaker

neutralisation process using hot de-ionised water

pH measurement after neutralisation process

Figure 3.8 Overall process of delignification

3.7 Acid Pre-treatment (First stage)

According to Zhao *et. al.,* (2008), the next treatment was done to remove lignin content using peracetic acid (PAA). Peracetic acid (PAA) was proven as a chemical that can remove lignin content in biomass materials greatly. Peracetic acid was prepared by self before proceed with the treatment (Figure 3.9). Preparation of peracetic acid was involved with 30% (v/w) hydrogen peroxide and 70% (v/w) acetic acid. The initial volume ratio of acetic acid and hydrogen peroxide was 1:5. The mixing of hydrogen peroxide and acetic acid using 500mL conical flask was done in a fume hood due to their strong smell.

The reaction was operated for 24 hours (Zhao *et. al.,* 2008). In this research, according to Zhao *et. al.,* (2008), 50% (v/w) of peracetic acid was used and operated at 80^0C for 90 minutes with solid liquid ratio 1:6. The samples then were washed using hot de-ionized water until the washed water become pH 7 or nearby. The samples were kept washed if the pH reading not approaching pH 7 or nearby. After that, filtration step was done to separate between the filtrate and the cake. The samples (cakes) were dried in oven at 60^0C for 24 hours and keep until further process. Figure 3.10 describe the overall first stage acid pretreatment.

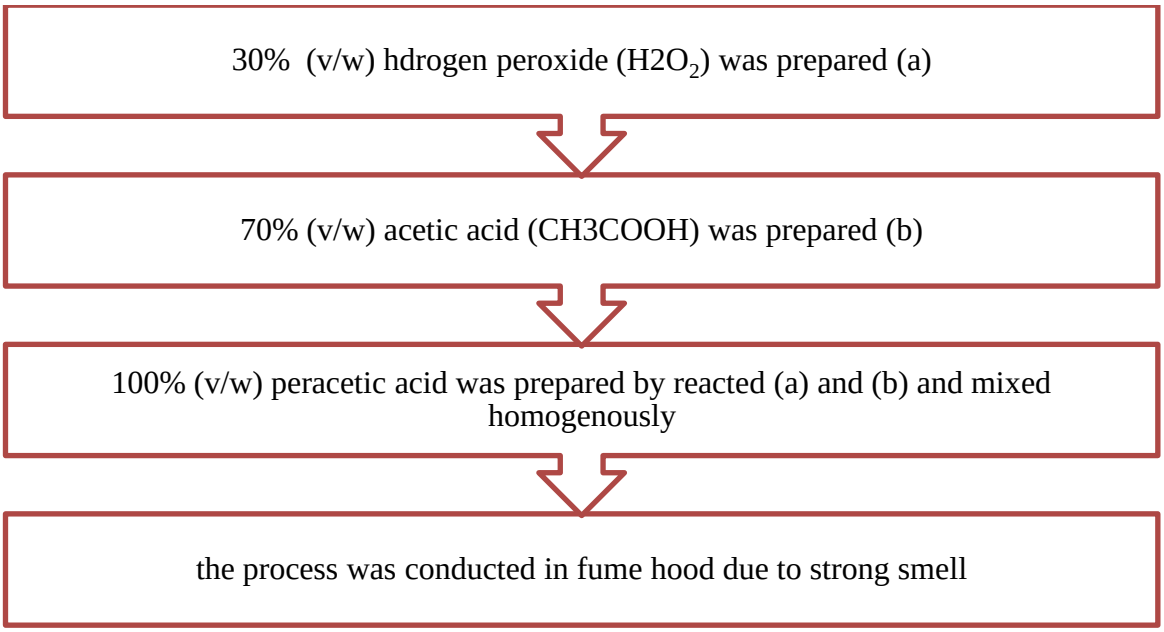


Figure 3.9 Preparation of peracetic acid (CH_3COOOH)

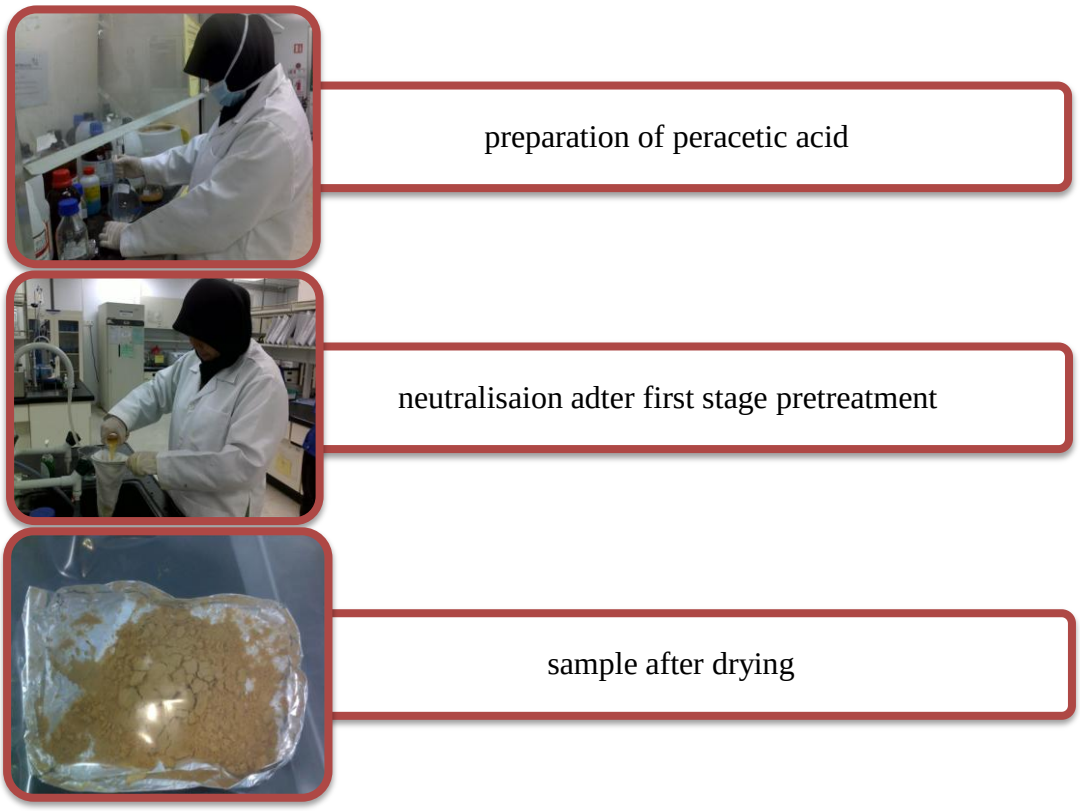


Figure 3.10 Overall first stage pretreatment

3.8 Enzymatic Hydrolysis

Then, the sample treated will undergo enzymatic hydrolysis. Enzymatic hydrolysis was used as a process to degrade cellulose fibres to glucose due to the environmental friendly factors. It was carried out in 500mL Erlenmeyer flask with 100mL working volume of sodium citrate buffer solution (Yeh *et. al.,* 2009). The 100mL volume of sodium citrate buffer was loaded with five grams sample. According to previous research, the high yield glucose production was carried out at 50^0C for 24 hours using sodium citrate buffer solution. Two types of enzyme used which were Cellulase from *Trichoderma Reesei* and Cellobiase from *Aspergillus Nig*er have been purchased from Sigma Aldrich (M) Sdn Bhd.

The preparation of sodium citrate buffer was done by self (Figure 3.11). 0.2M of acetic acid and 0.2M of sodium acetate was prepared as for the buffer solution. Then, the chemical prepared was mixed to make it homogenously reacted. 0.02% of the sodium azide was added into the solution to inhibit the bacteria and microorganisms. After that, the pH was checked by using pH meter to get the desire reading (pH 5). Lastly the solution was autoclaved at 121^0C for 15 minutes and kept in the fridge (4^0C) until further process.

100mL of buffer solution react with 100µL of enzyme mixture (cellulase and cellobiase). 100µL of enzyme mixture was based from 50 µL of cellulose and 50 µL of cellobiase. 5 ml of samples were taken every one hour for duration of twelve hours for analysing step. The samples were boiled with 90^0C for 15 minutes in water bath shaker to cut the enzyme reaction. Then, the samples were centrifuged at 4^0C

for 10 minutes with 10000rpm. The supernatant obtained was used for analysis of glucose. Figure 3.12 shows the summary of enzymatic hydrolysis.

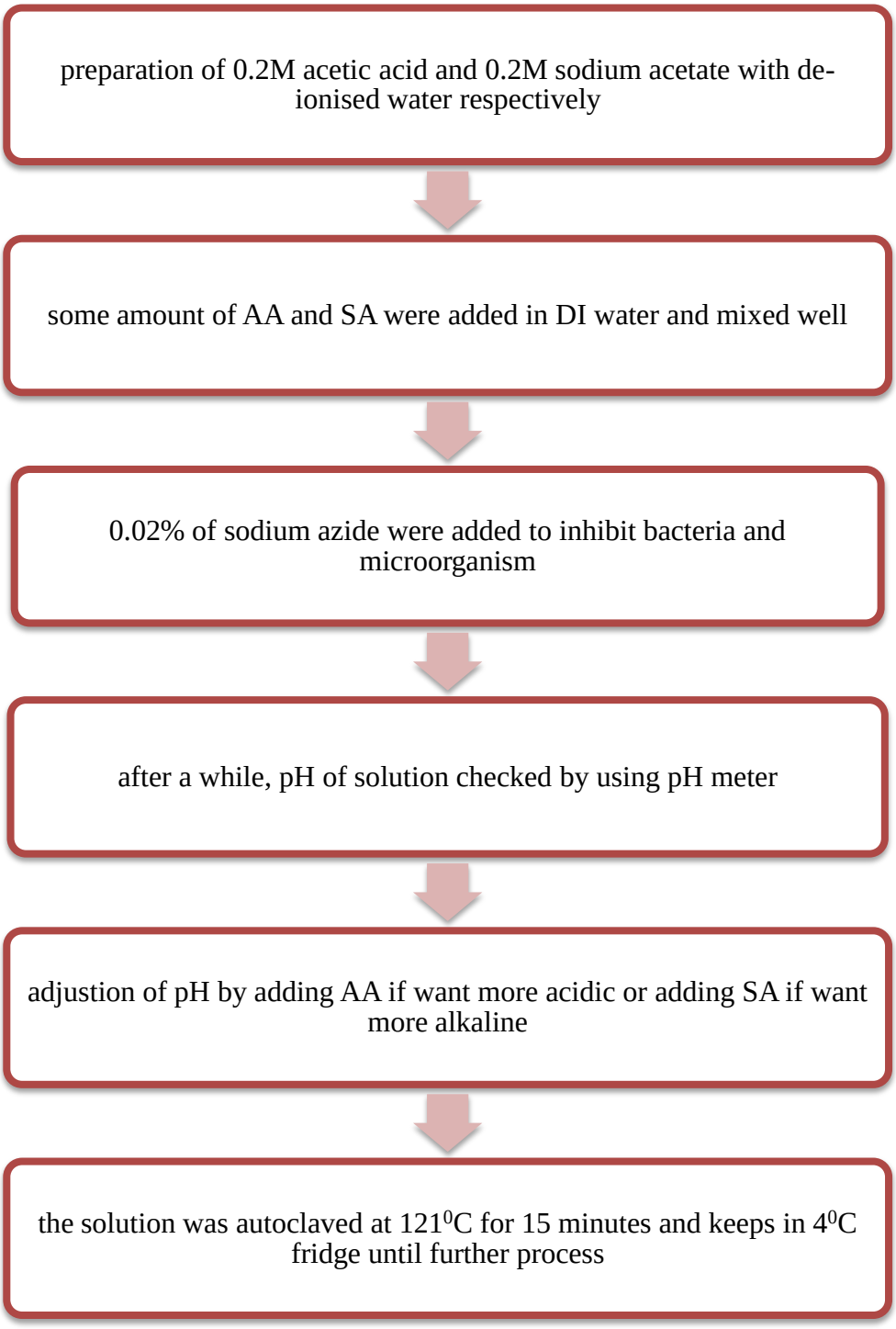


Figure 3.11 Preparation of sodium acetate buffer solution

preparation of cellulose (OPT)

addition of sodium acetate buffer soution and enzyme to the cellulose (100 mL buffer solution react with 100 µm of enzyme mixture)

incubation process with temperature 50^0C and every one hours, 5 mL of sample was taken until duration time of 12 hours.

the samples were boiled (90^0C) in the water bath shaker to cut the enzyme reaction

the samples were centrifuged at 4^0C for 10 minutes (10000rpm) and the supernatant were keep in fridge at 4^0C for further analysis

Figure 3.12 Summary of enzymatic hydrolysis

3.9 Anaylsis of Glucose by Glucose Analyser

After the enzymatic hydrolysis, the filtrate obtained from the filtration was used for the analysis of glucose (Figure 3.13). The filtrate was measured using glucose analyser. The samples were introduced by inserting tubes into circular carousels that rotate for the sample availability (Tothill, 2001). The glucose was detected by YSI Biochemistry Analyser.

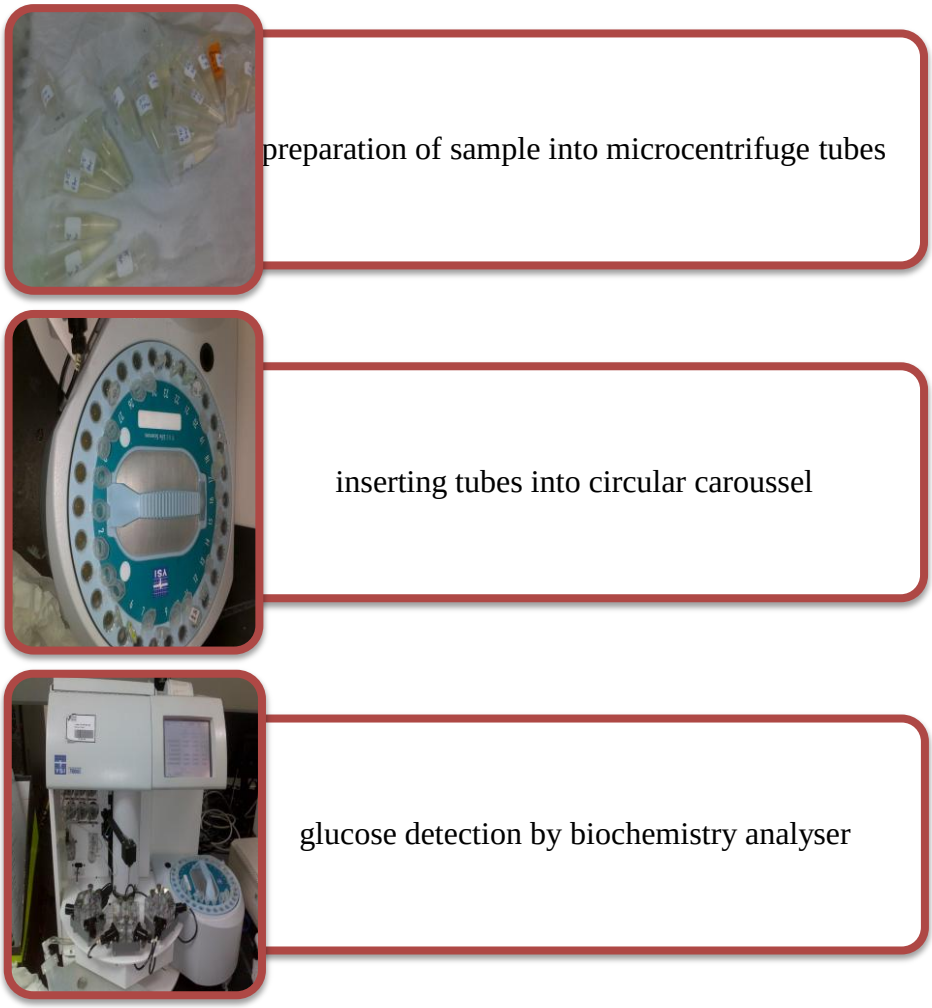


Figure 3.13 Overall flow of glucose detection

3.10 Scanning Electron Microscope (SEM)

The surface morphology of the treated and untreated oil palm trunk dusts were examined by a scanning electron microscope (EVO 50) (Figure 3.10). The samples were sputter with platinum and observed under SEM since the sample are not conductor type. The micrographs were taken at magnification 500, 800, 1000 and 1500 (Muna'im *et. al.*, 2011).

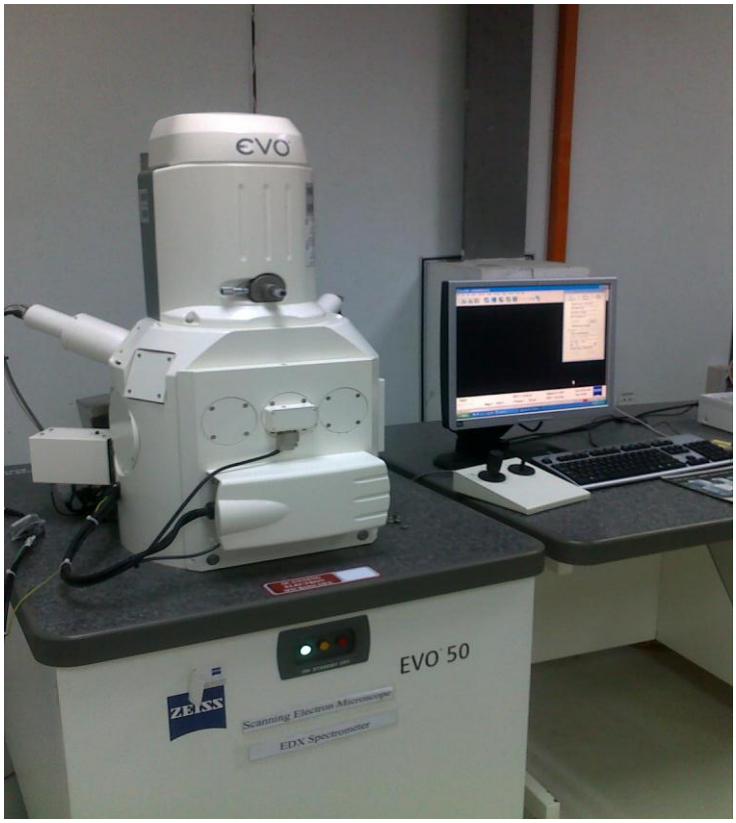


Figure 3.14 Scanning electron microscope (EVO 50)

3.11 Fourier Transform Infrared Spectroscopy (FTIR)

OMNIC ESP software (Thermo Nicolet Instrument Corporation, Madison, WI) (Figure 3.15) was used to recorded the infrared spectra of the cellulose and treated oil palm trunk powder. Cellulose obtained from laboratory (store) and treated oil palm trunk dusts were prepared before analyzed with FTIR. According to Muna'im *et. al.,* (2011), FTIR was used to determine functional groups linked in the surface of untreated and treated sawdust.

Figure 3.15 Fourier transform infrared spectroscopy (FTIR)

3.12 Summary of Methodology

Figure 3.16 shows the overall process of xylose, glucose and arabinose production from oil palm trunk.

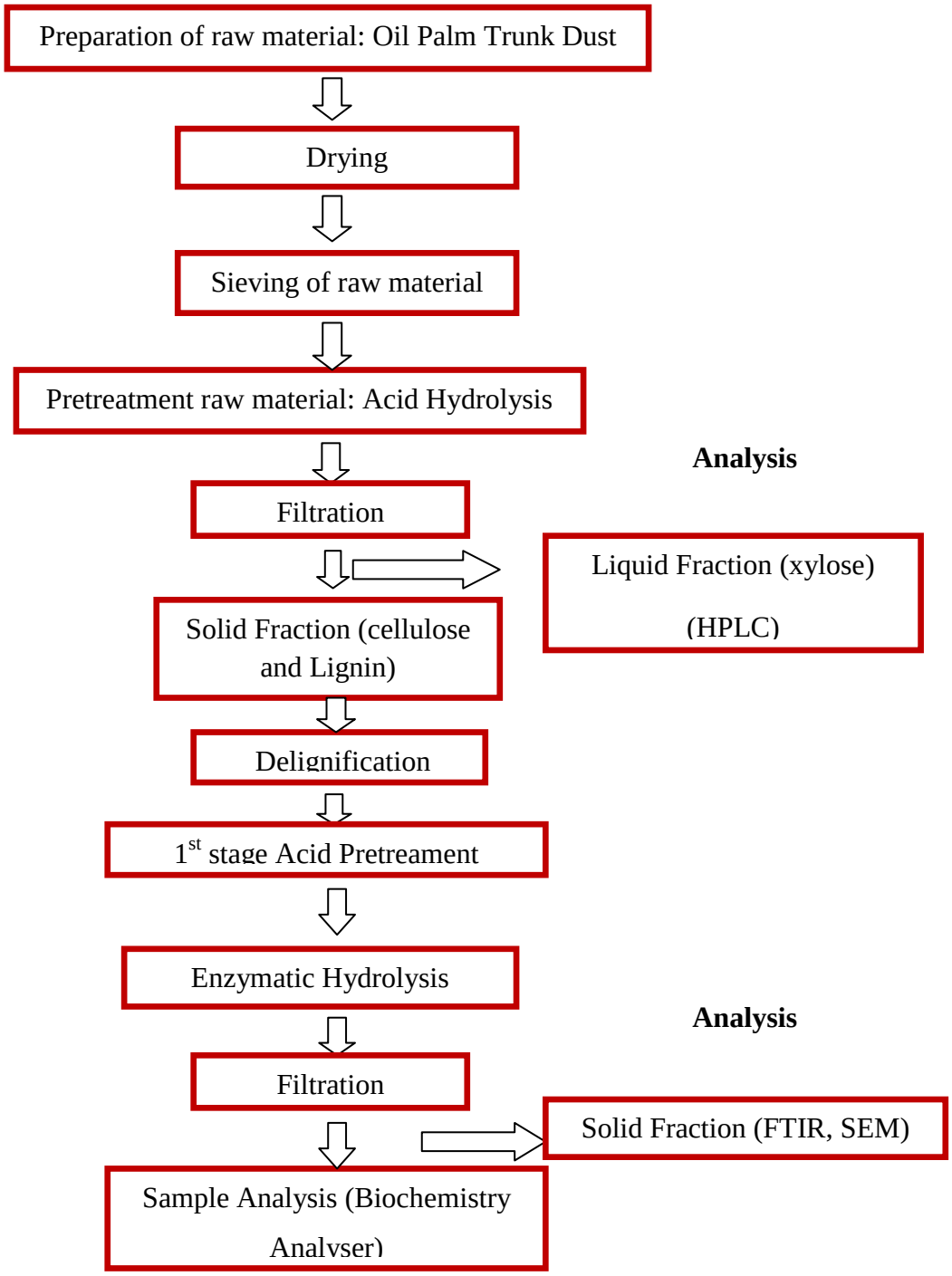


Figure 3.16 A process for monosaccharide production

CHAPTER 4

RESULT AND DISCUSSION

4.1 Introduction

In this chapter, the results gained from the experiment were discussed. The experiment was performed to study the effect of particle size of oil palm trunk on monosaccharides (xylose, glucose and arabinose) production. In order to achieve the research objectives, degradation of hemicellulose by acid hydrolysis was come out to obtain the xylose from the structure of oil palm trunk. Instead of acid hydrolysis, the maximum glucose production was generated by the enzymatic hydrolysis. Crystalline cellulose has lower accessibility and solubility compared to the open branches structure in hemicellulose (Parajo *et al.,* 1998). The quantitative analysis (HPLC and Biochemistry Analyzer) and qualitative analysis (FTIR and SEM) was done to find out the optimum xylose, glucose and arabinose production with difference particle size of oil palm trunk.

4.2 Effect of Particle Size on Monosaccharies Production during Acid Hydrolysis Process

Differences stage of particle size ranging from 160 to 800 µm of oil palm trunk were selected to determine the maximize amount of xylose and by-product (glucose, galactose, maltose and arabinose) production from the acid hydrolysis method.

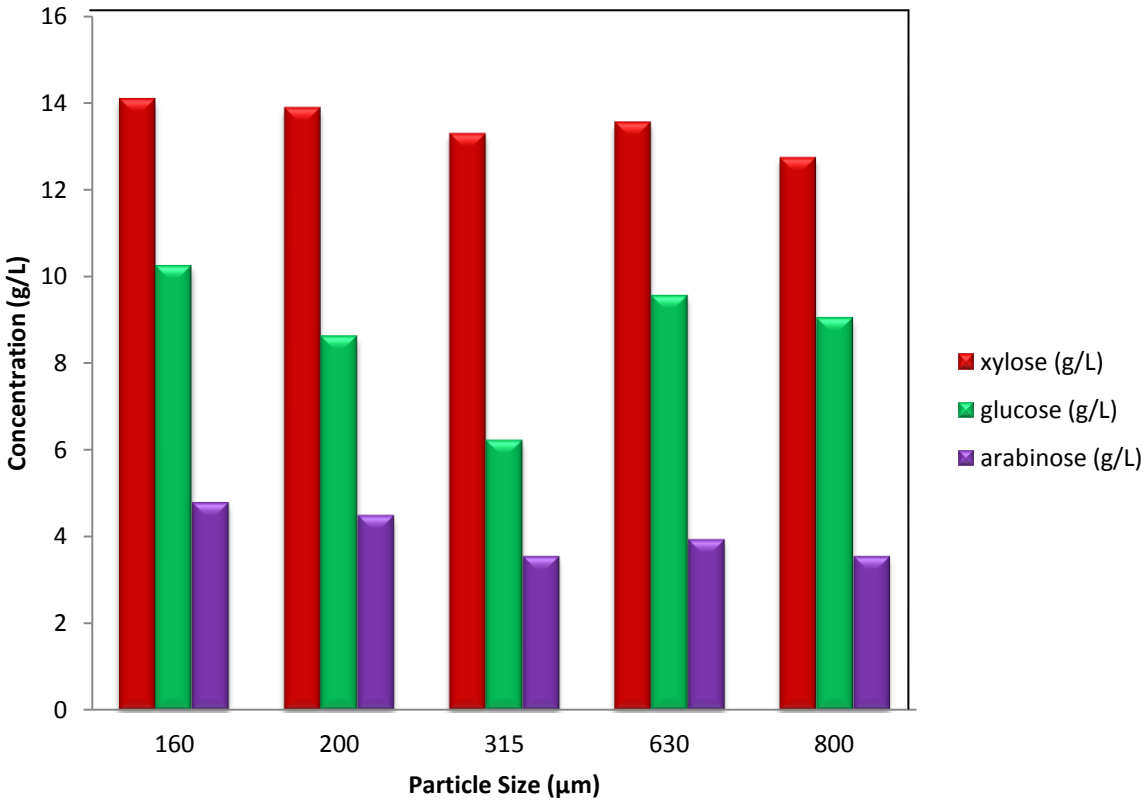


Figure 4.1 Production of monosaccharides (g/l) based on particle size

Figure 4.1 represents the results obtained from the High Performance Liquid Chromatography (HPLC) analysis of monosaccharides (xylose, glucose and arabinose) contents from different range of particle size. From the result, the highest concentration of xylose, glucose and arabinose generated from the lignocellulosic

biomass (OPT) were 14.082 g/L, 10.233 g/L and 4.761 g/L respectively at the particle size of 160 µm.

The conversing yield for xylose, glucose and arabinose were calculated base on the composition of hemicelluloses and cellulose on dry basis (Table 4.1). Since generation of xylose, glucose and arabinose were the highest at particle size 160 µm, thus the highest conversing yield produced were 32.7%, 19.87% and 11.07% respectively.

Table 4.1 Composition of oil palm trunk on dry basis

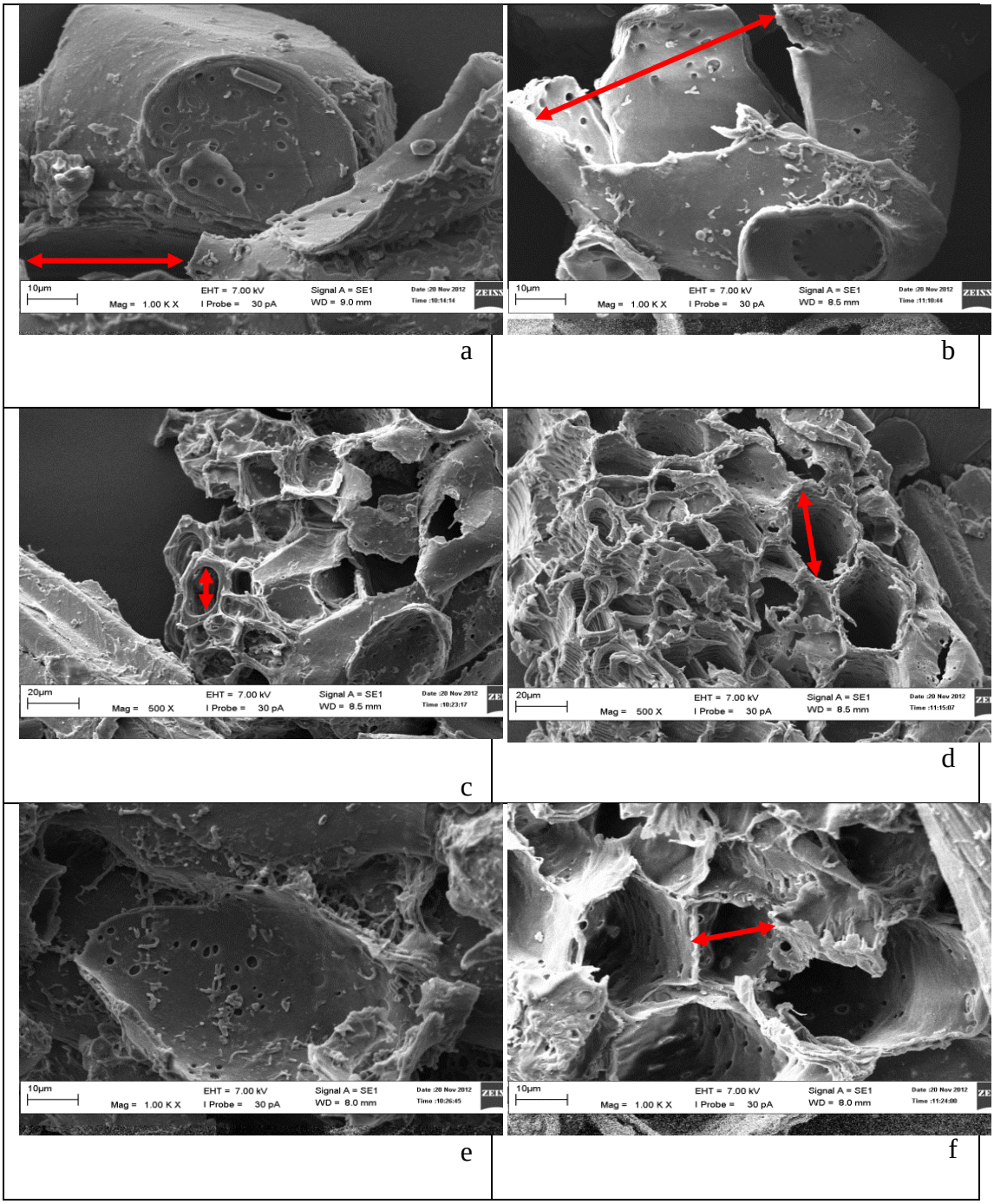
Main fraction	**Percent (%)**
Cellulose	41.2
Hemicellulose	34.4
Lignin	17.1
Others	7.3

(Source: Robert *et al.,* 2008)

The highest concentration of monosaccharides generated was at the smallest particle size (160 µm). This is because smallest particle size leads to the increasing of surface area. Moreover, the contact between reactant (sulphuric acid) and surface area was much easier compared to the largest particle size. In addition, the diffusion problems related to the reactant were reduced with decreasing particle size.

This pattern of results can be supported by SEM micrograph (Figure 4.2) and FTIR spectrum (Figure 4.3). In SEM micrograph, it has been observed that surface morphology of untreated and treated oil palm trunk composites were totally varies. Meanwhile, for the FTIR spectrum the formation of functional group also differs by

comparing between commercial cellulose and treated (acid hydrolysis) oil palm trunk.



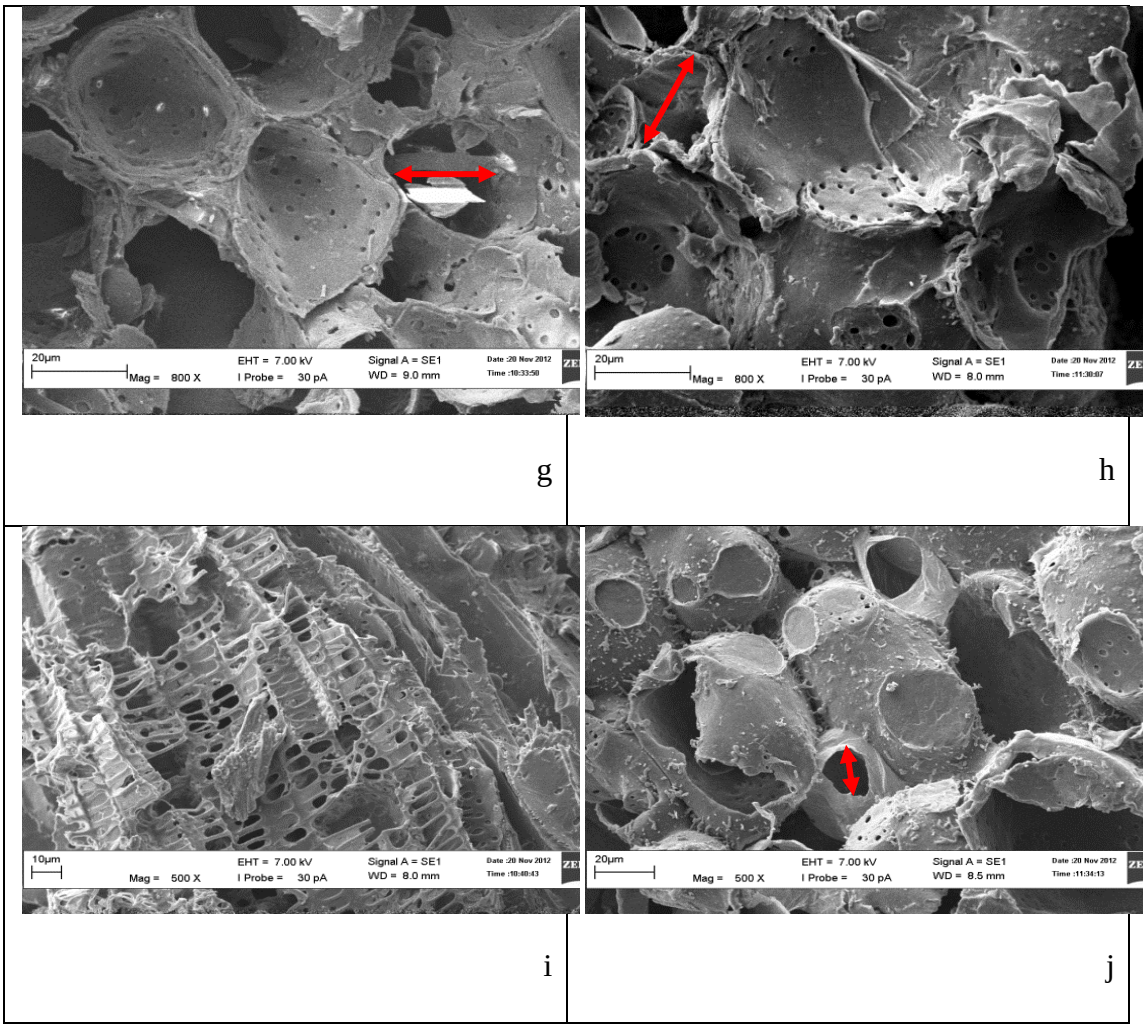
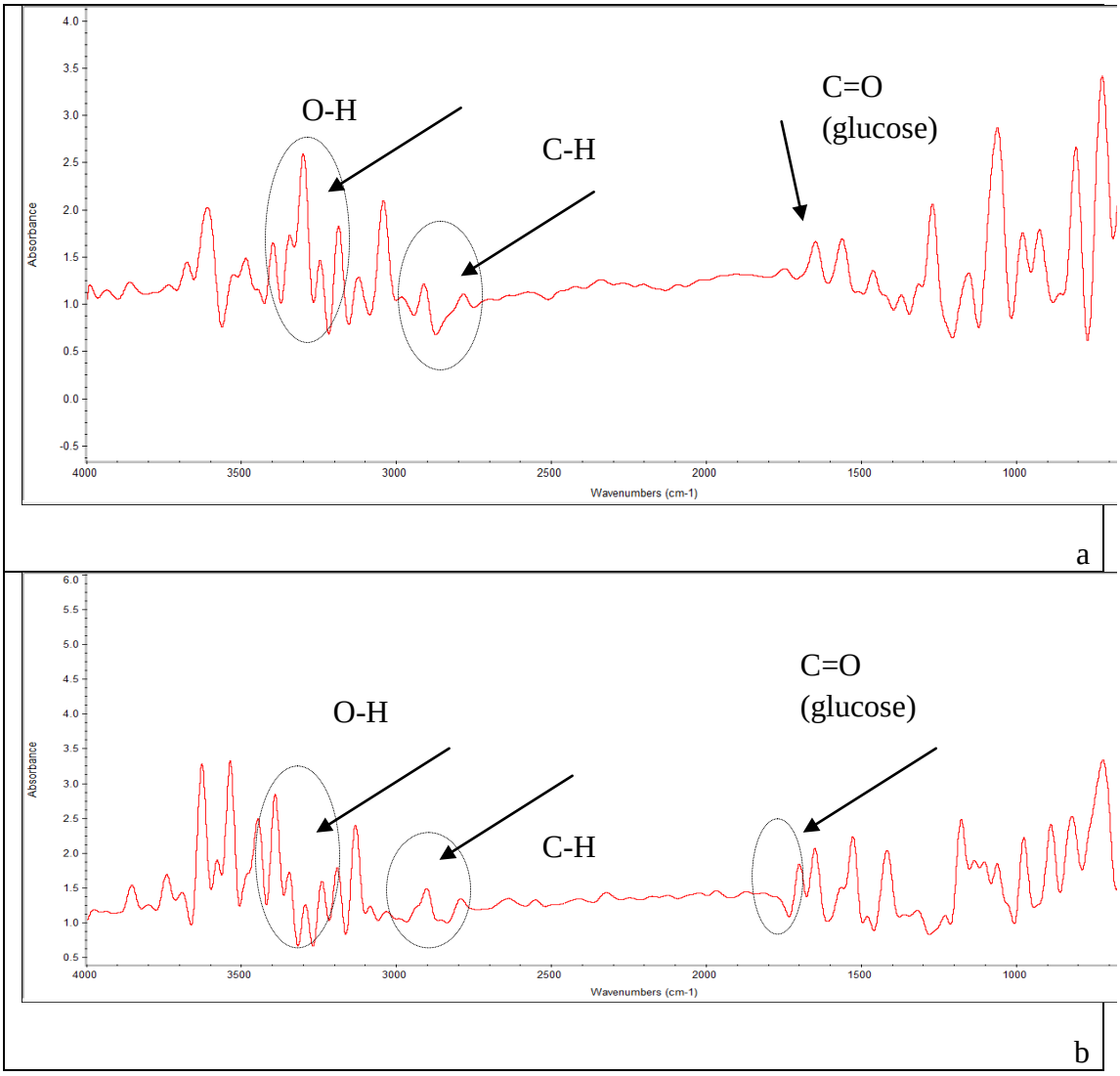
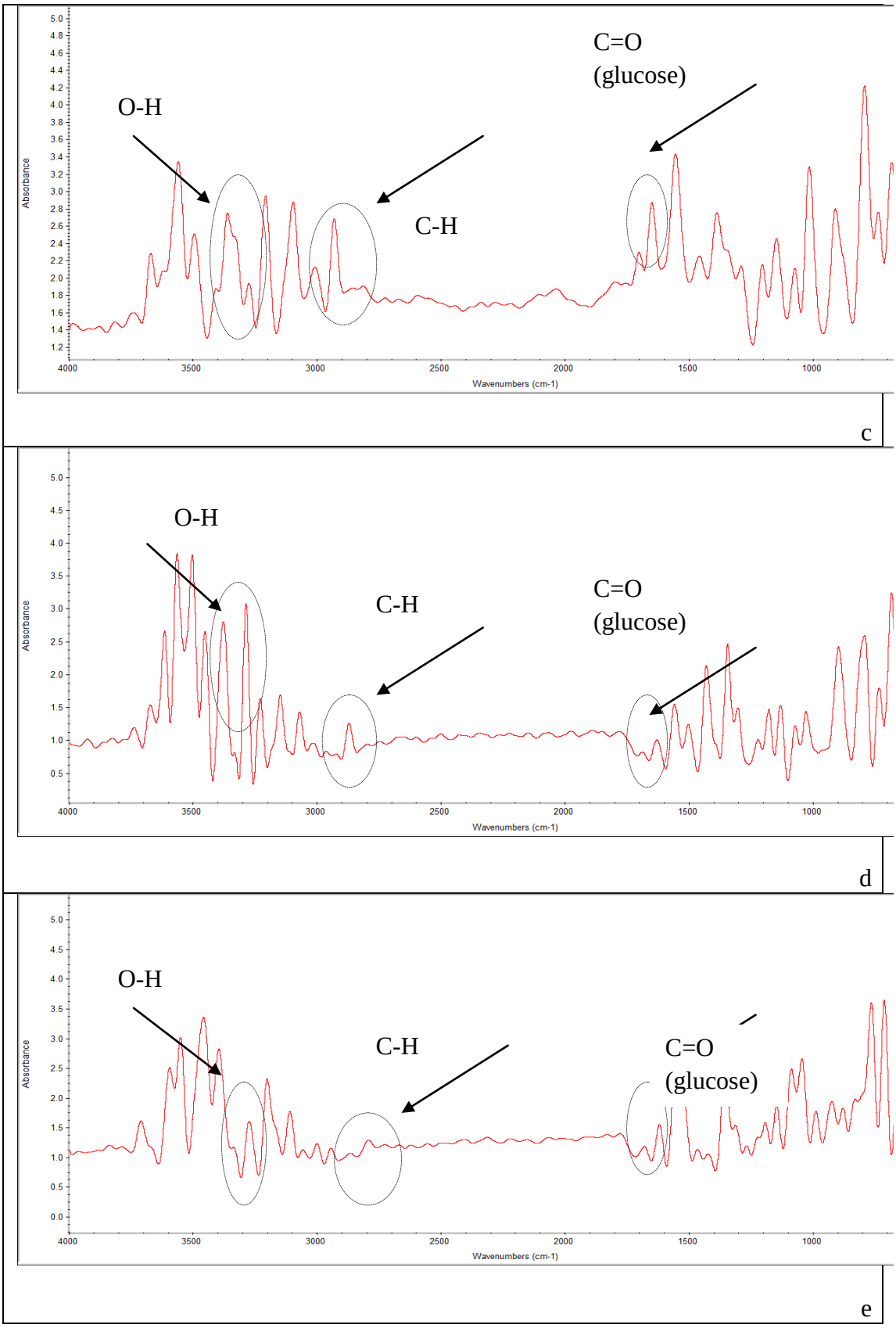


Figure 4.2 a) SEM micrograph of untreated OPT at 160 µm. b) SEM micrograph of treated OPT at 160 µm. c) SEM micrograph of untreated OPT at 200 µm. d) SEM micrograph of treated OPT at 200 µm. e) SEM micrograph of untreated OPT at 315 µm. f) SEM micrograph of treated OPT at 315 µm. g) SEM micrograph of untreated OPT at 630 µm. h) SEM micrograph of treated OPT at 630 µm. i) SEM micrograph of untreated OPT at 800 µm. j) SEM micrograph of treated OPT at 800 µm

Since highest production of xylose, glucose and arabinose were produced at particle size 160 µm, the hole appeared was larger compared to the other particle size (Figure 4.2). The diameter of hole before treated (Figure 4.2a) with 3.42% H_2SO_4 was 3 cm and after treatment (Figure 4.2b) the diameter became 5 cm. This obvious difference in diameter of hole showed that the particle size give effects on the

degradation of hemicelluloses and cellulose of lignocellulosic oil palm trunk. Next, at particle size 200 µm, the diameter before treatment (Figure 4.2c) was 0.9 cm and after treatment (Figure 4.2d) was 1.8 cm. The smallest diameter of hole generated after treatment (Figure 4.2j) was 0.6 cm at particle size of 800 µm. The lowest production of monosaccharides was also at 800 µm of particle size. This result proves that the smallest particle size give highest monosaccharides production since less degradation of hemicelluloses and cellulose occurred at larger particle size.




O-H
C-H
C=O
(glucose)
Absorbance
Wavenumbers (cm-1)
c
O-H
C-H
C=O
(glucose)
Absorbance
Wavenumbers (cm-1)
d
O-H
C-H
C=O
(glucose)
Absorbance
Wavenumbers (cm-1)
e

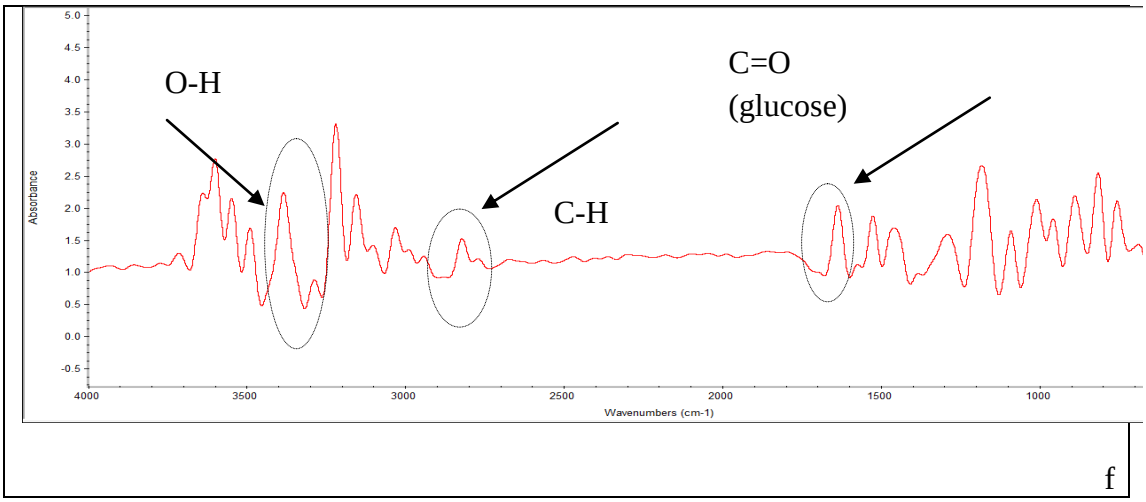


Figure 4.3 a) FTIR spectrum of commercial Cellullose. b) FTIR spectrum of treated OPT at 160 µm. c) FTIR spectrum of treated OPT at 200 µm.d) FTIR spectrum of treated OPT at 315 µm. e) FTIR spectrum of treated OPT at 630 µm. f) FTIR spectrum of treated OPT at 800 µm.

Commercial cellulose is a long chain made up from glucose monomer. Glucose has hydroxyl bond (O-H), C=O bonds, and C-H bonds. From the FTIR spectra results, comparison between commercial cellulose (Figure 4.3a) and treated OPT at particle size 160 µm (Figure 4.3b) shows that no absorption bonds produce for C=O groups of ketone and decreasing of hydroxyl group (O-H) at particle size 160 µm (Figure 4.3b) since strong degradation of hemicellulose occurred for xylose generation. The peaks for C=O bond, O-H bond and C-H bond at particle size 160 µm were lower than peaks at particle size 200 µm, 315 µm, 630 µm and 800 µm since highest degradation of functional group occurred at the 160 µm particle size.

FTIR spetra results also show many impurities appeared at 500-1000 cm^{-1} due to C-X bonds of the lignocellulosic OPT. The obvious functional group that missing from the lignocellulosic oil palm trunk was C=O bonds for ketone. This proved that degradation of hemicelluloses to xylose occurred during acid hydrolysis

process. Peak of absorption bond produce for C-H bonds and O-H bonds (Figure 4.3 b, c, d, e, and f) were higher than commercial cellulose due to no treatment for lignin was done before the acid hydrolysis process.

According to Najafpour *et al.,* (2007), highest concentration of xylose was successfully obtained 16 g/L at 250 µm particle size of empty fruit bunch. Highina *et al.,* (2012) also reported that glucose production from wheat straw was 0.8 g/L at particle size less than 300 µm. Highest glucose concentration was produced at submicron particle size (Yeh *et al.,* 2009). Those studies described that highest production of monosaccharides were generated at the smallest particle size of lignocellulosic material.

4.2 Effect of Particle Size on Glucose Production during Enzymatic Hydrolysis

Solid wastes from the acid hydrolysis undergo the enzymatic hydrolysis. Glucose production from the enzymatic hydrolysis was analyzed using YSI Biochemistry Analyser. Figure 4.3 shows the concentration of glucose generated from the hydrolysis process.

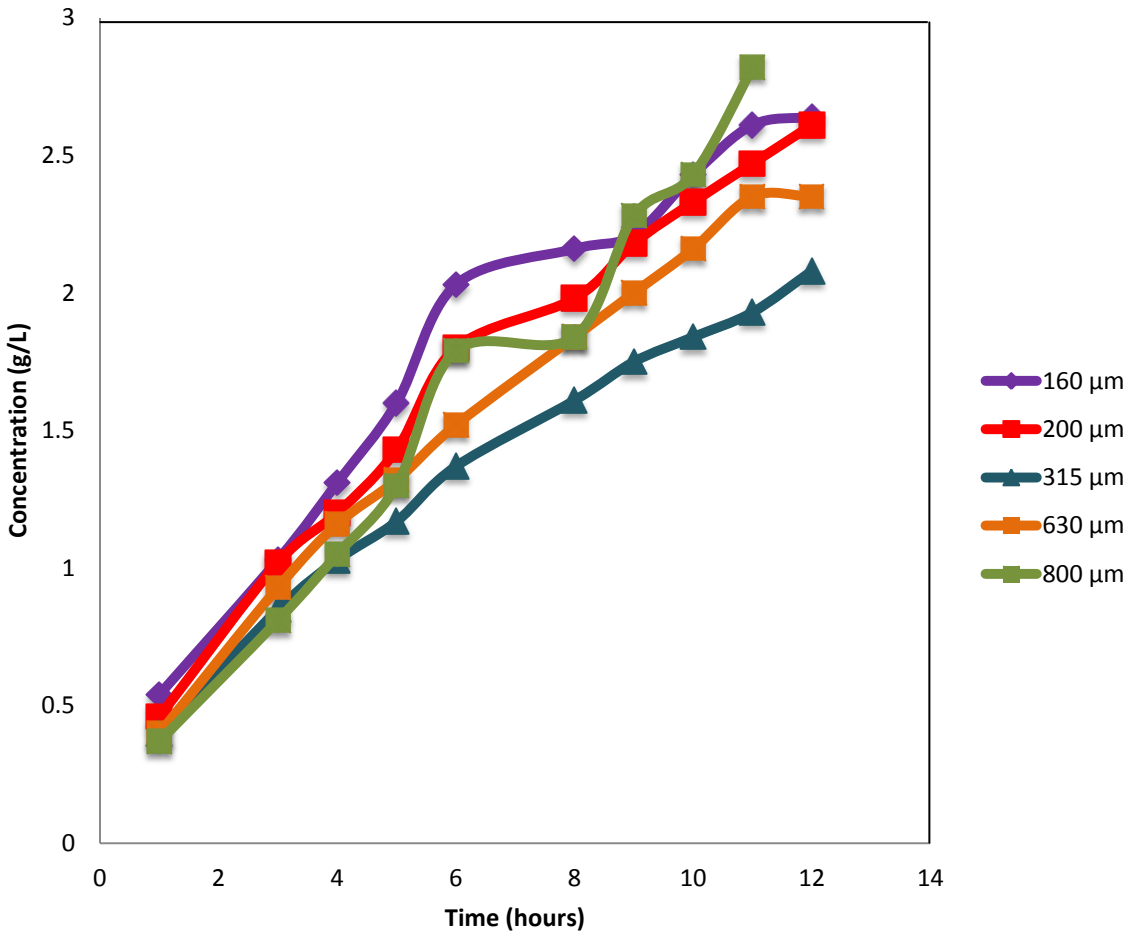


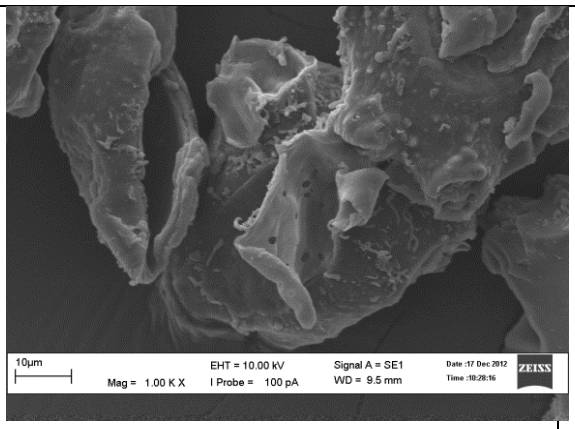
Figure 4.4 Glucose Production (g/L) based on Particle Size

From the results, the highest glucose concentration generated was 2.82 g/L at 800 µm particle size. At 160 µm, 200 µm, 315 µm, 630 µm and 800 µm particle size,

the percent gradient of each line were 18.9%, 18.95%, 14.52%, 17.63% and 23.66% respectively. It was clearly seen that highest glucose production was at 800 µm. Moreover, the conversing yield of glucose for 160 µm, 200 µm, 315 µm, 630 µm and 800 µm particle sizes were 8.8%, 8.7%, 6.9%, 7.8% and 9.4%. However, when combining the glucose got from acid hydrolysis and enzymatic hydrolysis, the highest glucose concentration was 12.873 g/L at 160 µm particle size. The glucose concentration got was approximated with the value of highest xylose concentration; 14.082 g/L that obtained from acid hydrolysis method.

From the results, although the solid waste residue was feasible and economical to produce glucose, however, there are no significant differences among this particles size in producing glucose. The particle size gave no significant on glucose production because the modification of the interfacial energy may give effect on the transfer of enzymes and sugar.

This result can be supported by the SEM micrograph (Figure 4.5). In SEM micrograph, it has been observed that surface morphology before and after treated oil palm trunk composites were totally varies.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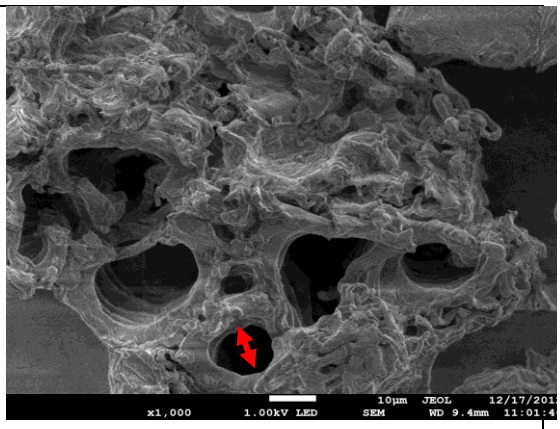
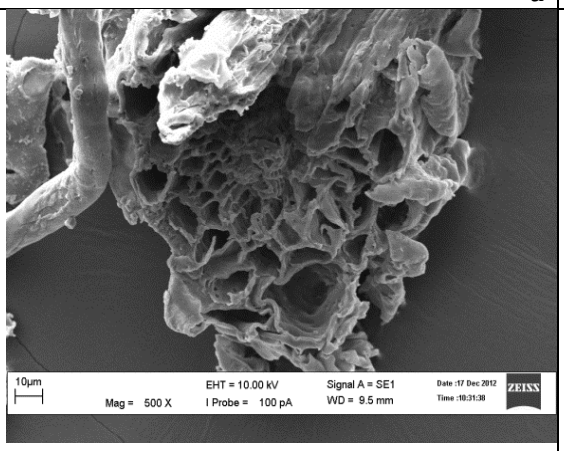
EHT = 10.00 kV
Signal A = SE1
Mag = 1.00 K X
I Probe = 100 pA
WD = 9.5 mm
ZEISS

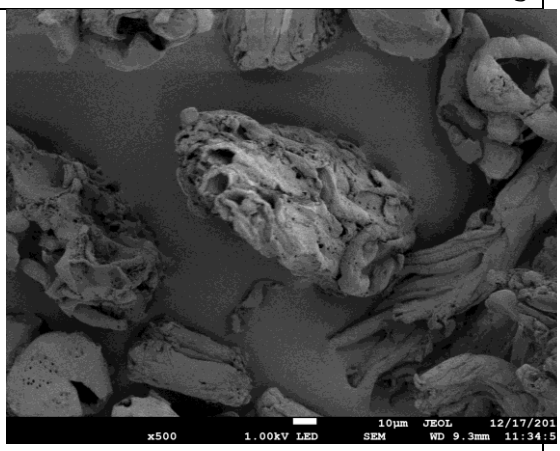
a


x1,000
1.00kV LED
10µm JEOL
SEM
WD 9.4mm

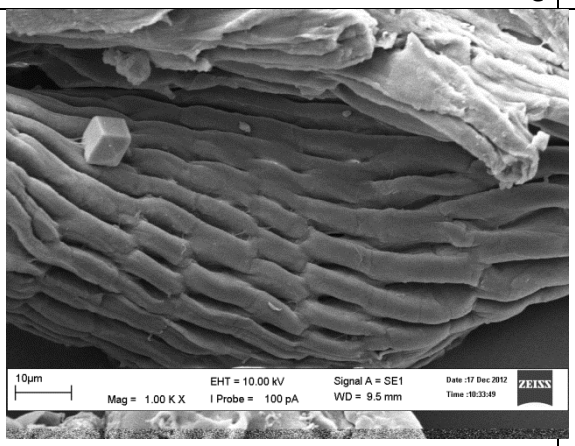
b


10µm
EHT = 10.00 kV
Signal A = SE1
Mag = 500 X
I Probe = 100 pA
WD = 9.5 mm
ZEISS

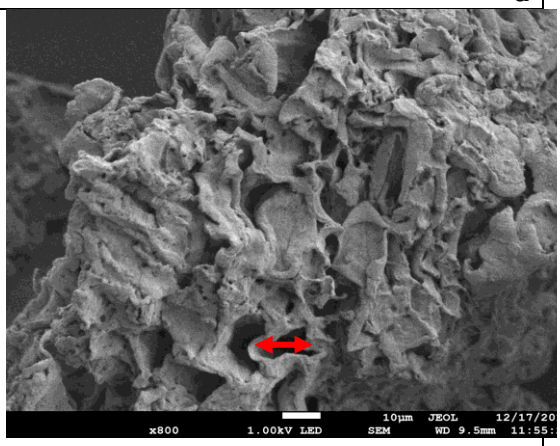
c


x500
1.00kV LED
10µm JEOL
SEM
WD 9.3mm

d


10µm
EHT = 10.00 kV
Signal A = SE1
Mag = 1.00 K X
I Probe = 100 pA
WD = 9.5 mm
ZEISS

e


x800
1.00kV LED
10µm JEOL
SEM
WD 9.5mm

f

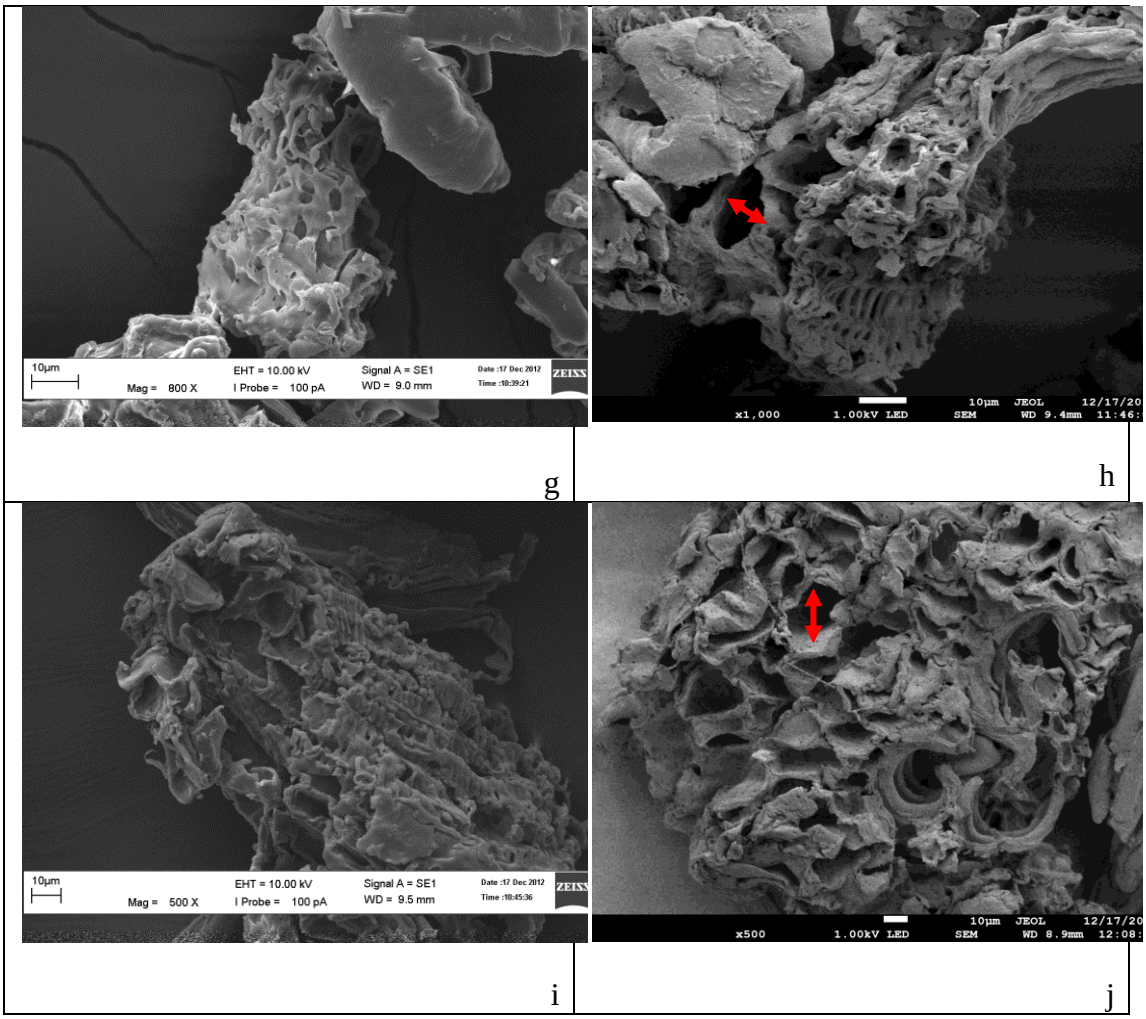


Figure 4.5 a) SEM micrograph of untreated OPT at 160 µm. b) SEM micrograph of treated OPT at 160 µm. c) SEM micrograph of untreated OPT at 200 µm. d) SEM micrograph of treated OPT at 200 µm. e) SEM micrograph of untreated OPT at 315 µm. f) SEM micrograph of treated OPT at 315 µm. g) SEM micrograph of untreated OPT at 630 µm. h) SEM micrograph of treated OPT at 630 µm. i) SEM micrograph of untreated OPT at 800 µm. j) SEM micrograph of treated OPT at 800 µm

Hole was obtained in the treated oil palm trunk structure. The hole was obtained after the treatment due to the degradation of cellulose from the enzymatic hydrolysis process. When the glucose was come out from the oil palm trunk structure, the hole was present in the structure. The amount of hollow that presents is dependent on the amount of glucose production. From the Figure 4.5i (particle size

800 µm) the holes were smaller and not visible as compared to the holes at Figure 4.5j. The diameter after enzymatic hydrolysis at particle size 800 µm was 0.7 cm. The diameter was the largest compared to the other diameter of particle sizes since highest glucose production for enzymatic hydrolysis generated at 800 µm particle size. This was due to strong degradation of cellulose to generate glucose. Since particle size gave no significant in glucose production, the holes appeared were quite similar in number.

It has been reported that particle sizes below than 530 µm gave no increase in glucose production (Wen *et al.*, 2004). According to Duff and Murray (1996); Sun and Cheng (2002), this scenario was due to the impact on the transfer of enzymes and sugar between the solid substrate and bulk solution cause by the alteration of the interfacial energy.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Pretreatment of lignocellulosic material of oil palm trunk capable in generates xylose, glucose anda arabinose. First hydrolysis of oil palm trunk dusts was carried out with dilute sulfuric acid (3.24%) at the temperature 121°C for 40 minutes to obtain high concentration of monosaccharides. The yield of monosaccharide obtained was depended on particle size. From the experiment, the highest xylose, glucose and arabinose produced were 14.092 g/L, 10.233 g/L and 4.761 g/L respectively at 160µm particle size. Next, solid wastes from first hydrolysis were continued with enzymatic hydrolysis to produce more glucose concentration. The hydrolysis involved cellulase, cellobiase and sodium acetate buffer that done at temperature 50^0C for 12 hours. However, the yield of glucose was not depended on particle size. The highest glucose generated was 2.82 g/L at 800µm particle size. Overall, the highest glucose production was 12.837 g/L which is from acid hydrolysis and enzymatic hydrolysis at 160µm particle size..

5.2 Recommendation

In order to enhance the quality of xylose, glucose and arabinose production, some recommendations are required to make sure this study more effectively:

i. Certain steps which is first stage acid pretreatment and delignification should be remove in order to prevent glucose loss before enzymatic hydrolysis
ii. Optimum condition for enzymatic hydrolysis should be identified by doing the enzymatic activity
iii. Purification method should be done in order to obtain high purity of xylose, glucose and arabinose.

EFFECT OF PARTICLE SIZE ON XYLOSE, GLUCOSE AND ARABINOSE PRODUCTION FROM OIL PALM TRUNK

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A thesis submitted in fulfillment
of the requirements for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
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FEBRUARY 2013

SUPERVISOR DECLARATION

I hereby declare that we have checked this thesis and in my 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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STUDENT DECLARATION

I declare that this thesis entitled *"Effect of particle size on xylose, glucose and arabinose production from oil palm trunk"* is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : ……

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Special dedication to my beloved father, Hj Razib Bin Dollah, my mother, Hjh Khalidah Binti Musa, families, and friends, who gave me everlasting inspiration, never ending encouragements and priceless support towards the success of this research.

ACKNOWLEDGEMENT

In the name of Allah, the most Grateful, the most Merciful

Alhamdulillah, with the successful completion of my Undergraduate Research Project, I would like to thank to Allah S.W.T because of His blessing and giving me strength throughout finishing my research.

I would like to take this opportunity to express my sincere thanks and appreciation to my dearest supervisor, Associate Professor Dr Mimi Sakinah binti Abdul Munaim for her guidance, inspiration, patient, encouragement, critics and support in completing my final year project.

Also not forgotten to all FKKSA lab assistants that help me so much to deal with the laboratory machines and handling all of the chemicals required along this investigation and FKKSA Lab, UMP which provides me with all the facilities which is needed for my project. I feel thankful for all instruments provide here.

Last but not least, exclusive thanks to all of my beloved families, my mother, my father, sister and brothers for everything that they have sacrifices for the sake of my study and my future. I am very grateful to have family members that very supportive and understanding especially my mother and my beloved father. Thank you so much. To all my colleagues and friends, thanks for the support and the knowledge that we had shared together.

ABSTRACT

Oil palm trunks are one of the potential lignocellulosic sources for monosaccharides production. Acid hydrolysis and enzymatic hydrolysis method were combined to obtain higher yield of monosaccharides. Therefore, this paper was designed to study the effect of particle size of oil palm trunk (OPT) on the production of xylose, glucose and arabinose. In acid hydrolysis, 3.24% sulphuric acid (H_2SO_4) was used at temperature of 121°C for 40 minutes to degrade the hemicelluloses thus generates xylose. The filtrate was analysed using High Performance Liquid Chromatography (HPLC).The result showed that the highest xylose, glucose and arabinose generated were 14.092 g/L, 10.233 g/L and 4.761 g/L respectively at 160µm particle size. Solid wastes from acid hydrolysis were degraded by cellulotic enzyme to produce glucose and the hydrolysate was analysed using YSI Biochemistry Analyser. The highest glucose produced was 2.82 g/L at 800µm particle size. Overall, the highest glucose production was 12.873 g/L at 160µm particle size. As the conclusion, combination of both methods helps in increasing the glucose production and reduces the abundant waste of biomass. From this research, it is recommended that purification method should be done in order to obtain high purity of xylose, glucose and arabinose

ABSTRAK

Batang kelapa sawit adalah salah satu sumber lignoselulosik yang berpotensi untuk penghasilan monosakarida. Kaedah hidrolisis asid dan hidrolisis enzim telah digabungkan untuk memperolehi pengeluaran monosakarida yang lebih tinggi. Oleh itu, tujuan kajian ini dijalankan adalah untuk mengkaji kesan saiz zarah batang kelapa sawit (OPT) terhadap pengeluaran glukosa, xilosa, dan arabinosa. Hidrolisis asid melibatkan penggunaan 3.24% asid sulfurik (H_2SO_4) pada suhu 121°C selama 40 minit untuk merungkaikan ikatan hemiselulosa dan menghasilkan xilosa. Cecair turasan daripada hidirolisis asid dianalisa menggunakan sistem kromatografi (HPLC). Keputusan kajian menunjukkan bahawa penghasilan xilosa, glukosa dan arabinosa yang tertinggi adalah masing-masing sebanyak 14.092 g /L, 10.233 g /L dan 4.761 g /L pada saiz zarah 160µm. Sisa pepejal daripada hidrolisis asid diuraikan oleh enzim selulotik untuk menghasilkan glukosa. Hidrolisat daripada hidrolisis enzim dianalisa menggunakan YSI Biokimia Analisa. Penghasilan glukosa yang tertinggi dihasilkan adalah sebanyak 2.82 g / L pada saiz zarah 800µm. Secara keseluruhannya, penghasilan glukosa yang tertinggi adalah sebanyak 12.873 g/L pada saiz zarah 160µm. Sebagai kesimpulan, gabungan dua kaedah hidrolisis membantu dalam meningkatkan penghasilan glukosa dan mengurangkan lebihan sisa biojisim. Daripada kajian ini, adalah dicadangkan bahawa kaedah penulenan boleh dijalankan untuk mendapatkan ketulenan xilosa, glukosa dan arabinosa yang tinggi.

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micrograph of untreated OPT at 800 µm. j) SEM micrograph of treated OPT at 800 µm

LIST OF ABBREVIATIONS

FTIR	Fourier Transform Infrared Spectroscopy
GFP	Green Flouresent Protein
HPLC	High Liquid Performance Chromatography
L	Liquid
OPT	Oil Palm Trunks
PAA	Peracetic Acid
RMW	Red Meranti Wood
S	Solid
SEM	Scanning Electron Microscope

LIST OF SYMBOLS

g	Gram
kg	Kilogram
ml	Milliliter
µl	Microliter
H_2O	Water
ºC	Degree Celsius
cm^{-1}	Per Centimeter
g	Grams
g/L	Grams per liters
kg	Kilogram
M	Molarity (moles/ liters)
mm	Millimeter
µm	Micrometer
mol/dm^3	Moles/ decimeter Cubed
rpm	Rotation per minute
v/w	Volume per weight
w/w	Weight per weight
%	Percentage

APPENDIX A

PREPATION OF ACID SOLUTION

A) 2M Sulfuric Acid (H_2SO_4)

$$M = \frac{SG \times Purity \times 1000}{Mw}$$

Where,

M = molarity @ concentration of stock H2SO4
SG = specific gravity of H2SO4 = 1.84
Purity = percentage of stock H2SO4 = 96% = 0.96
MW = molecular weight H2SO4, g/mol = 98.08 g/mol

Molarity of H2SO4 needed from stock solution is as follow:

$$M = \frac{1.84 \times 0.96 \times 1000}{98.08}$$

$$= 18.01 \text{ M}$$

Thus, using equation:

$M_1V_1 = M_2V_2$
(2M)(1000L) = (18.01M) V_2
V2 = 106.6 L = **106.6 mL stock H2SO4** is needed to be dilute with 1000 L distilled water to obtained 0.04M H2SO4.

APPENDIX B

STANDARD STOCK SOLUTION OF HPLC

1) Xylose standard stock solution (100 g/l) : 10 ml

1 g of xylose was diluted in 10 ml H_2O

Composition (g/l)	Stock solution (µL)	Water (µL)
1	10	990
2	20	980
4	40	960
8	80	920
16	160	840

2) Glucose standard stock solution (20 g/l) : 50 ml

2 g of Glucose was diluted in 100 ml H_2O

Composition (g/l)	Stock solution (µL)	Water (µL)
0.5	25	975
1	50	950
2	100	900
4	200	800
8	400	600

3) Arabinose standard stock solution (20 g/l) : 50 ml

0.2 g of Arabinose was diluted in 10 ml H_2O

Composition (g/l)	Stock solution (µL)	Water (µL)
0.1	10	1990
0.5	25	975
1	50	950
2	100	900
4	200	800

APPENDIX C

HIGH PERFORMANCE LIQUID CHROMATOGHRAPHY (HPLC) RESULTS

REFERENCES

Abdul Muna'im M.M.I., Hamdan S., Rezaur M.R. & Saiful M.I. (2011). Treated Tropical Wood Sawdust-polyprolylene Polymer Composite: Mechanical and Morphological Study, *Journal of Biometerials and Nanobiotechnology,* 2, 435-444

Abdullah, R. (2003). Price Prospects for Selected Oils and Fats in 2005: Malaysian Palm Oil Board.

Alkhatib, M., Abd, R.M., Alam, M.Z., & Saleh, H.M. (2011). Enzymatic Hydrolysis of Oil Palm Empty Fruit Bunch using Immobilized Cellulase Enzyme. *Journal of Biotechnology*, 10, 18811-18815.

Arenas, T.L., Rathi, P., Jimenez, E.R., & Cruz, M.S. (2010). Factors Affecting The Acid Pre-treatment of Lignocellulosic Biomass: *Batch and Continuous Process. 20th European Symposium on Computer Aided Process Engineering.*

Azmalisa, T., Asma, I.W., Zulkafli, H., & Norazwina, Z. (2010). Optimization of Glucose Production from Oil Palm Trunk via Enzymatic Hydrolysis. *The 13th Asia Pacific Confederation of Chemical Engineering Congress.*

Betancur, G.J.V & Pereira, N. J. (2010). Sugarcane Bagasse as Feedstock for Second Generation Generation Ethanol Production from Part 1: Diluted Acid Pre treatment Optimisation. *Electronic Journal of Biotechnology*, 13, 3.

Bird, D. (2012). Natural Dietary Sources. Which Foods contain Glyconutrients? Aloe Vera (*Barbadensis*)- The Medicine.

Bludworth, J. & F. Carl Knopf. (1993). Reactive Extraction of Lignin from Wood using Supercritical Ammonia-water Mixtures. *Journal of Supercritical Fluids,* 6, 249-254.

Bravo, L. (2009). Polyphenols: Chemistry, Dietary Sources, Metabolism and Nutirtional Significance. *Nutrition Reviews*, 56, 11.

Brodeur, G., Yau, E., Badal, K., Collier, J., Ramachandran, K.B., & Ramakrishnan, S. (2011). Chemical and Physicochemical Pre-treatment of Lignocellulosic Biomas: A Review. *Journal of Enzyme Research*, 1-17.

Charles E. Wyman. (1994). Ethanol from Lignocellulosic Biomass: Technology, Economics and Opportunities, *Bioresource Technology* 50, 3-16.

Chin, K.L., H'ng, P.S., Wong, L.J., Tey, B.T., & Paridah, M.T. (2011). Production of Glucose from Oil Palm Trunk and Sawdust of Rubber wood and Mixed Hardwood. *Journal of Applied Energy*, 88, 4222-4228.

Cook, N.C. & Samman, S. (1996). Flavonoids-Chemistry, Metabolism, Cardio Protective Effects and Dietary Sources. *Journal of Nutritional Biochemistry*, 7, 2.

Gailliot et al., (2007). Self-control Relies on Glucose as a Limited Energy Source: Willpower is more than a Metaphor. *Journal of Personality and Social Psycology*, 92, 235-336.

Gao et al., (2010). Hemicelluloses and Auxiliary Enyme for Improved Conversion of Lignocellulosic Biomass into Monosaccharides. *Biotechnology for Fuels*.

Gibb, M. & Cynkin, M.A. (2012). Conversion of Xylan, D-Xylose and Lignocellulosic Biomass into Furfural using $AlCl_3$ as Catalyst in Ionic Liquid. *Bioresources Technology*.

Harmsen et al., (2010). Literature Review of Physical and Chemical Pre treatment Processes for Lignocellulosic Biomass. *Food and Biobased Research*.

Herzog et al., (2012). Renewable Energy Sources. Encyclopedia of Life Support System.

Jung, Y.H., Kim, I.J., Kim, J.J., Oh, K.K., Han, J.I., Choi, I.G., & Kim, K.H. (2009). Ethanol Production from Oil Palm Trunks Treated with Aqueous Ammonia and Cellulase. *Journal of Bioresources Technology*, 102, 7307-7312.

Lavarack, B.P., Griffin, G.J., & Rodman, D. (2002). The Acid Hydrolysis of Sugarcane Bagasse Hemicellulose to Produce Xylose, Arabinose. Glucose and Other Products. *Journal of Biomass and Bioenergy*, 23, 367-380.

Lievonen, S.M., Laaksonen, T.J. & Roos, Y.H. (2002). Nonenzymatic Browning in Food Models in the Vicinity of the Glass Transition: Effect of Fructose, Glucose and Xylose as Reducing Sugar. *Agricultural Food Chemistry*, 50, 24.

Makinen, K.K., Soderling, E. (2006). A Quantitative Study of Mannitol, Sorbitol, Xylitol and Xylose in Wild Berries and Commerial Fruits. *Journal of Food Science.*

Martin, P.A.W., Blackburnsmall, M., Schroder, R.F.W., Matsuo, K., Li, B.W. (2002). Stabilisation of *Cucurbitacin E-Glycoside*, a Feeding Stimulant for Diabrotocite Veetles, Extracted from Bitter Hawkesbury Watermelon. *Journal of Insect Science*, 20.

Misson, M., Haron, R., Kamaroddin, M.F.A., & Amin, N.A.S. (2009). Pretreatment of Empty Fruit Bunch for Production of Chemicals via Catalytic Pyrolysis. *Bioresource Technology*, 100, 2867-2873.

Mohamad, N.L., Kamal, S.M.M., & Abdullah, A.G.L. (2011). Optimization of Xylose Production from Sago Trunk Cortex by Acid Hydrolysis. *African Journal of Food Science and Technology*, 2(5), 102-108.

Mussatto, S.I., Dragone, G., Fernandes, M., Milagres, A.M.F., & Roberto, I.C. (2008). The Effect of Agitation Speed, Enzyme Loading and Substrate Concentration on Enzymatic Hydrolysis of Cellulose from Brewer's Spent Grain. *Journal of Cellulose*, 15, 711-721.

Najafpour, G., Ideris, A., Salmanpour, S., & Norouzi, M. (2007). Acid Hydrolysis of Pretreated Palm Oil Lignocellulosic Wastes. *Journal of IJE Transactions B: Applications*, 20, 147-154.

Parajo J.C., Dominguez H. & Dominguez J.M. (1995). Production of Xylitol from Raw Wood Hydrolysate by Debaryomeyces hanseii NRRL Y-7426. *Bioprocess Eng*. 13, 125-131.

Radillo et al., (2011). Fermentable Sugars from Lupinus Rotundiflorus Biomass by Concentrated HCl Hydrolysis. *Bioresources*, 6.

Rahman, S.H.A., Choudhury, J.P., Ahmad, A.L., & Kamaruddin, A.H. (2007). Optimization Studies on Acid Hydrolysis of Oil Palm Empty Fruit Bunch Fiber for Production of Xylose. *Bioresource Technology*, 98, 554-559.

Sanchez et al., (2010). Improved Xylose and Arabinose Uitlization by an Industrial Recombinant *Saccharomyces Cerevisiae* strain using evolutionary Engineering. *Biotechnology for Fuels*, 3.

Silva et al., (2010). D-Xylitol: Fermentative Production, Application and Commercialization.

Sjoman, E., Manttari, M., Nystrom, M., Koivikko, H., & Heikkila, H. (2008). Xylose recovery by nanofiltration from different hemicellulose hydrolyzate feeds, *J. Membr. Sci.* 310, 268–277.

Soha, B. C. (2000). α- L- Arabinofuranosidases: Biochemistry, Molecular Biology and Application in Biotechnology. *Biotechnology Advance*, 18,5.

Taherzadeh, M.J., & Karimi, K. (2007). Acid-based Hydrolysis Processes for ethanol from Lignocellulosic Materials: A Review. *Journal of Bioresources*, 2, 422-499.

Thanapimmetha et al., (2011). Chemical and Microbial Hydrolysis of Sweet Sorghum Bagasse for Ethanol Production. *Bioenergy Technology*.

Tothill, I.E. (2001). Biosensors Developments and Potential Application in the Agricultural Diagnosis Sector. *Computers and Electronics in Agriculture*, 30, 1-3.

Winkelhausen, E., & Kluzmanova, S. (1998). Microbial Conversion of D-Xylose to Xylitol. *Journal of Fermentation and Bioengineering*, 86, 1-14.

Wong, L.J., H'ng, P.S., Chin, K.L., Tor, E.S., Tan, S.E., Tey, B.T., & Maminski, M. (2011). Oil Palm (*Elais Guineensis*) Trunk as a Resource of Starch and Other Sugars. *Journal of Applied Sciences*, 16, 3053-3057.

Yang et al., (2012). The Optimization of Formic Acid Hydrolysis of Xylose in Furfural Production. *Carbohydrate Research*, 357, 53-61.

Yeh, A., Huang, Y. C., & Chen, S.H. (2009). Effect of Particle size on The Rate of Enzymatic Hydrolysis of Cellulose. *Journal of Carbohydrate Polymer*, 79, 192-199.

Zhang et al., (2012). Xylitol Production from D-Xylose and Horticultural Waste hemicellulosic Hydrolysate by a New Isolate of *Candida Athensensis* SB18. *Bioresources Technology*, 105, 134-141.

Zheng, Y., Pan, Z., & Zhang, R. (2009). Overview of Biomass Pre-treatment for Cellulosic Ethanol Production. *Journal of Agricultural and Biological Engineering*, 2, 51-69.