EFFECT OF SAWDUST SPECIES AND PARTICLE SIZE ON XYLOSE PRODUCTION

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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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JANUARY 2012

DECLARATION

I declare that this thesis entitled *"Effect of sawdust species and particle size on xylose production"* 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.

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Name : Suharti Binti Md Ishak

Date : 25 January 2012

Special dedication to my beloved father, Md Ishak Bin Sabran, my mother, Siti Eishah Binti Setu, families, and friends, who gave me everlasting inspiration, never ending encouragements and priceless support towards the success of this research.

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ABSTRACT

Xylose is a pentose sugar and commonly hydrolyzed from lignocellulosic material. This study was carried out to identify the species and particle size on xylose production was investigated. Three types of hardwood species (*Meranti, Keruing and Resak*) and five differences particle size of sawdust (800, 615, 400, 315 and 200 µm) were used in the production of xylose. Hydrolysis method by using diluted sulfuric acid was employed. The sawdust was reacted with diluted sulfuric acid to degraded the hemicellulose from lignocellulosic structure. The higher xylose concentration that produced was 32.12 g/l. The overall results indicated that the sawdust species and particle size were *Keruing* and 400µm respectively exhibited the highest concentration of xylose due to the acid hydrolysis effects. From the Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM), the structure of treated sawdust was change. The maximum xylose production from sawdust was produce at 3.24% sulfuric acid concentration and reaction time 60 minutes.

ABSTRAK

Xylosa adalah gula pentosa dan biasanya dihidrolisiskan daripada sisa buangan tumbuh-tumbuhan. Kajian ini dijalankan bertujuan untuk mengenalpasti spesis dan saiz habuk kayu yang terbaik dalam penghasilan xylosa telah dikaji. Tiga jenis spesis kayu keras (*Meranti*, *Keruing* dan *Resak*) dan lima saiz habuk kayu (800, 615, 400, 315 dan 200 μm) digunakan dalam penghasilan xylosa. Kaedah hidrolisis dengan menggunakan asid sulfurik cair telah bertindak balas dengan habuk kayu bagi menyingkirkan hemisellulosa daripada ikatan struktur selulosa. Kepekatan xylosa yang maksima terhasil adalah 32.12 g/L. Keputusan keseluruhan menunjukkan bahawa spesis dan saiz habuk kayu yang terbaik dalam penghasilan xylosa yang tinggi adalah *Keruing* dan 400μm. Keputusan analisa daripada Spektrum Infra Merah (FTIR) dan imbasan Mikroskopi Elektron (SEM) mununjukkan perubahan struktur dan permukaan sisa habuk kayu selepas proses hidrolisis. Penghasilan maksimum xylosa daripada habuk kayu adalah pada kepekatan asid 3.24% dan masa prarawatan 40 minit.

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LIST OF ABBREVIATIONS & SYMBOLS

%	-	Percentage
wt%	-	Weight percent
g	-	Gram
kg	-	Kilogram
ml	-	Milliliter
µl	-	Microliter
CO_2	-	Carbon Dioxide
H_2O	-	Water
^oC	-	Degree Celsius
FTIR	-	Fourier Transform Infrared Spectroscopy
HPLC	-	High Liquid Performance Chromatography
OPF	-	Oil Palm Fronds
RMW	-	Red Meranti Wood
SEM	-	Scanning Electron Microscope

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF RESEARCH

There are four main sources of lignicellolosic materials which are forest biomass, herbaceous grass, agriculture residue and municipal waste. The production of lignocellulosic materials is increasing throughout the year in the peninsular Malaysia and the economical disposal of them is a serious problem to the sawmill industries. Bioconversion and hydrolysis of the lignocellulosic waste materials residue can produce a valuable product since it is renewable, widespread and cheap sources of raw material in nature and it is can become an environment friendly option to reducing generated of waste (Kuhad and Singh, 1993). Because of that, most of the industries increasing towards to reuse and recycle back the agro-industrial by-products to produce renewable product. It is commonly used as fuel in manufacturing plants and local utilities and chemicals or food ingredient production (Musatto *et al*., 2006).

The majority of lignocellulosic materials are constructed from three major polymeric components which are cellulose, hemicellulose and lignin (Gabrielii *et al*., 2000). Since xylose is present in small amounts in fruits and vegetables, the extraction from these sources is uneconomical. Commonly, chemical reduction of xylose is expensive because extensive purification and separation steps are necessary (Parajo *et al*., 1996). However, there are others lignocellulosic waste material like wood, rice husk, corn stalk, wheat straw and flax straw that treated as a waste in the industry. There are present high amount of xylose compared to fruit and vegetables (Sjoman *et al*., 2008).

In this research, hardwood species of sawdust is being used as a raw material in the production of xylose. Sawdust is a residue from the sawmill industries that usually treated as a waste. However, it is a heterogeneous material composed primarily of cellulose, hemicellulose, and lignin (Bludworth *et al*., 1993). The cell wall polysaccharides in the sawdust can degraded into their corresponding constituents by hydrolytic procedures either hydrothermal process, enzymatic process or acidic process. On hydrolysis, cellulose yields glucose and the non-cellulosic polysaccharides yields xylose, mannose, galactose and arabinose as well as acetic and hydroxycinnamic acids (Musatto *et al*., 2006).

1.2 PROBLEM STATEMENT

Nowadays, in Agro-industrial and plantation of timber industry, there is produced high value of hardwood saw mill residue, which is currently treated as solid waste (Nirdosha *et al*., 2009). In practice this residue is burned in indicators which may be causes of environmental pollution problems in nearly localities and offers limited value to the industry (Rahman *et al*., 2006). Furthermore, saw mill residue uses is still limited. Basically, it is used as animal feed or simply as landfills (Musatto *et al*., 2006).

By considering this scenario, an alternative practice should be considered by the sawmill industry to commercialize the residue from hardwood species to recycle back without causing environmental pollution and produce valuable product. This practice will requires less energy, and diminishes pollutants in industrial effluents, as well as being more economically advantageous due to its reduced costs.

According to the prior research on the hydrolysis of saw mill residue, it is only focus on the softwood species such as corn, rice husk, sugarcane baggase and so on. However, there are lacked of studies regarding production of xylose from hardwood species of sawdust. The information regarding this sugar monomer that produced from the hardwood species of sawdust is also hardly available.

1.3 RESEARCH OBJECTIVE

- The main objective is :-
    - To produce maximum yield of xylose from three types of sawdust and differences particle size.

- The specific objective are :-
    - To determine the amount of xylose production in the difference species and particle size of hardwood.
    - To determine the effect of species and particle size in xylose production by qualitative and quantitative analysis.
    - To determine the best type of species and particle size of sawdust on xylose production.

1.4 SCOPE OF RESEARCH

- Focus on the production of xylose from hardwood species of sawdust (*Meranti*, *Keruing* and *Resak*) that are collected from saw mill industry at Gambang and Kuantan Pahang.
- Study the effect of five differences particle size of sawdust in xylose production (0.80, 0.63, 0.40, 0.315 and 0.20 mm).
- The qualitative analysis involve is Scanning Electron Microscope (SEM) and Fourier Transform Infrared Spectroscopy (FTIR).
- The quantitative analysis involved is High Performance Liquid Chromatography (HPLC) and Kappa Number method.

1.5 SIGNIFICANCE OF RESEARCH

Nowadays, there is great political and social pressure to reduce the pollution arising in industrial activities. Almost all developed and underdeveloped countries are trying to alter this reality by modifying their processes so that their residues can be recycled to produce more valuable product (Musatto *et al.,* 2006). Since wood sawmill residue is an economic and widespread resource in Pahang, Malaysia, processing of difference type of sugar monomer is the first successful commercial application with several research initiatives underway to find uses of sawmill residue (Nirdosha *et al.*, 2009).

Xylose is one of the sugar monomer that can be produced from hardwood species of sawdust. However, there are lacks of studies regarding the production of xylose from hardwood species of sawdust. Most of the prior studies are only focusing on the softwood species like rice straw, sugarcane baggase and sago trunk cortex. Other than that, there are no studies regarding the particle size and sawdust species of hardwood in the xylose production. Hence, this research is come out to focusing on the studies of the effect of particle size and sawdust species of hardwood in xylose production.

CHAPTER 2

LITERATURE REVIEW

2.1 OVERVIEW OF LIGNOCELLULOSIC BIOMASS

Malaysia is well known for its widespread renewable resources of agriculture, municipal waste and forest residue. It is commonly known as lignocelluloses biomass which highly potential to renewable, widespread, and a cheap source of the residue that can be used as a raw material to become marketable products such as biofuel, bioenergy, and added value biomolecules by using bioconversion process. Lignocellulosic materials are commonly come from four differences sources (Table 2.1) which are forest biomass, agricultural residue, herbaceous grass and municipal waste.

Table 2.1: Sources of Lignocellulosic material

Specification	Source	Example
Forest biomass	Wood	• Hardwood • Softwood
	Residue	• Bark • Thinning • Sawdust • Pruning
Agricultural residue	Food crop	• Corn • Stover • Kernel fibers • Wheat straw
	Non-food crops	• Cotton stalk • Sugarcane bagasse
Herbaceous grass	Grass	• Switch grass • Bermuda grass • Rye straw
Municipal waste	Residential source	• Waste paper • Waste food
	Non-residential	• Paper mill • Sludge waste paper & board

2.2 STRUCTURE OF LIGNOCELLULOSIC BIOMASS

Lignocellulose is a structural material in cell wall of plant that surrounded by a polysaccharide which provides support, strength and shape to the plant. It is commonly found in roots, stalks and leaves (Sierra *et al*., 2006). It is complex internal structure and mainly composed of three major components which are cellulose, hemicelluloses and lignin. The component of the lignocellulosic biomass is shown in Figure 2.1. In the structure, Lignin provides structural function as a matrix in which cellulose and hemicellulose is embedded. While, cellulose obtained the crystalline fibrous structure and it appears to become the core of the complex. Then, Hemicellulose is positioned in between the microfibrils and macrofibrils of cellulose. The structure of the lignocellulose structure is shown in the Figure 2.2.

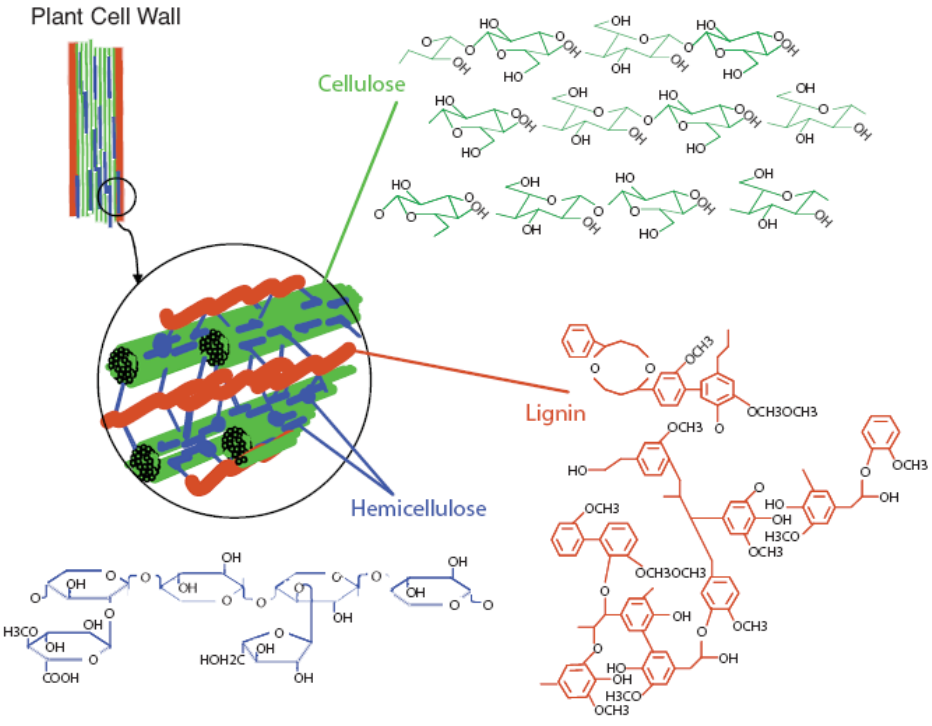

Figure 2.1: Component of lignocellulosic biomass

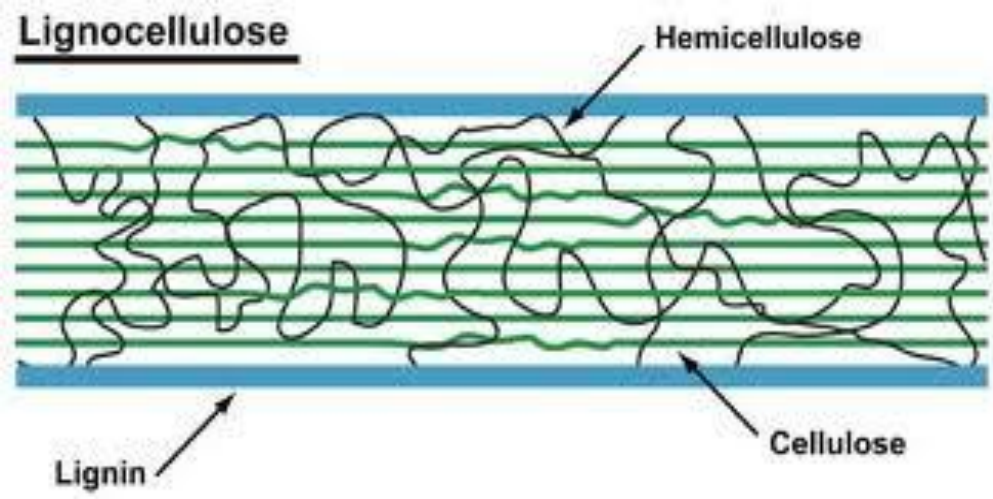

Figure 2.2: Structure of lignocellulose biomass

Furthermore, The structure of lignocellulose also consist of small amount of other component like water, protein and other compounds which do not participate significantly in the forming of lignocellulose structure. However, the content of the main components of the lignocellulose biomass in the nature are vary in the range shown in the Table 2.2.

Table 2.2: The contents of cellulose, hemicelluloses, and lignin in common agricultural residue and waste (Sun and Cheng, 2002)

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

2.2.1 Lignin

Lignin is the most abundant natural non-carbohydrate organic compound located in lignocellulosic biomass structure. It is form by polymer of aromatic subunits with complex molecule and contains highly branches polymer that usually derived from phenyl propane units linked. There are three types of phenyl propionic alcohols that usually exist as monomers of lignin which are coniferyl alcohol, sinapyl alcohol, and coumaryl alcohol. Each of this monomer has an aromatic ring with different substituents (Mussato *et al*., 2010). The linking of monomers that consisting a variety of bonds is shown in Figure 2.3.

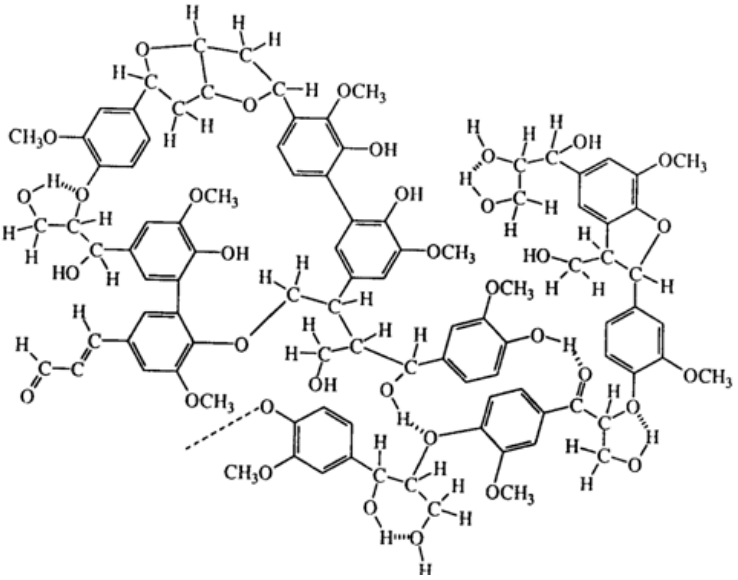

Figure 2.3: Structure of a section of a lignin polymer

In the lignocellulose, lignin is closely bound to cellulose and hemicellulose and able to form covalent bonds with hemicellulose. Due to the existing bond, it will provide structural rigidity and prevention of swelling of lignocelluloses.

2.2.2 Cellulose

Cellulose is predominant polymer in lignocellulosic biomass followed by hemicellulose and lignin. According one of the studies from Howard *et al.(*2003) shows that the cellulose composition in the hardwood and softwood species are higher than hemicellulose and lignin. It is form in linear homopolymer anhydro D-glucose unit linked together by β-1,4-polyacetal of cellobiose (4-O-β-D-glucopyranosyl-D-glucose) (Figure 2.4). Highly ordered crystalline regions are formed in the formation of hydrogen bonding between cellulose molecules. The crystalline molecules are formed approximately 50 to 90% of the total cellulose (Foyle *et al*., 2004). It is commonly considered as a polymer of glucose because cellobiose consists of two molecules of glucose. The chemical formula of cellulose is $(C_6H_{10}O_5)n$ and the structure of one chain of the polymer is presented in O.

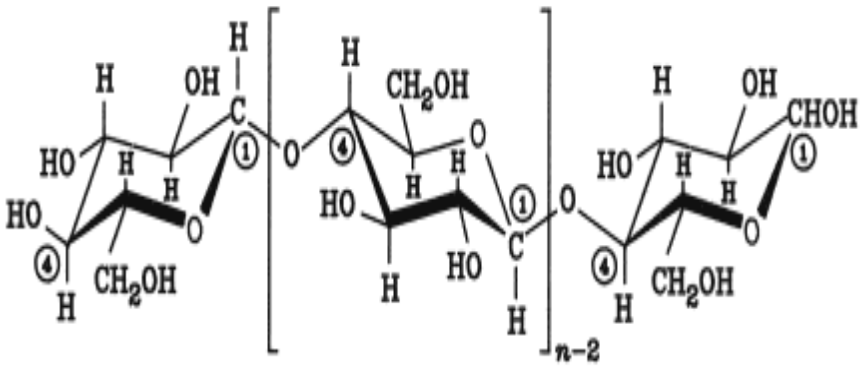

Figure 2.4: Cellulose structure in lignocellulose biomass

2.2.3 Hemicellulose

Hemicellulose is a complex and consists of highly branches polysaccharide. Commonly, the hemicelluloses are located in the secondary cell walls structure together with cellulose and lignin. It is chemically heterogeneous polymer, low degree of polymerization, amorphous and consisting several differences sugars and sugar derivatives. The basic monomeric residues that present are xylose, arabinose, mannose, glucose, galactose, glucoronic acid and methyl ester.

It is mainly composed of xylans that consist of xylose and xylo-ligosaccharides which have highly potential in differences area in chemical food and pharmaceutical industry. Xylan that formed in hemicellulose has a backbone of 1,4-β-linked xylose residues (Figure 2.5). Usually, 75% of the monomers for hemicellulose are pentoses and D-xylose is roughly consist 75% of these sugar. However, hemicellulose has weaker bonding compared to cellulose. It is easily to broken using suitable kinds of pretreatments, such as dilute acid hydrolysis (Saha *et al*., 2005).

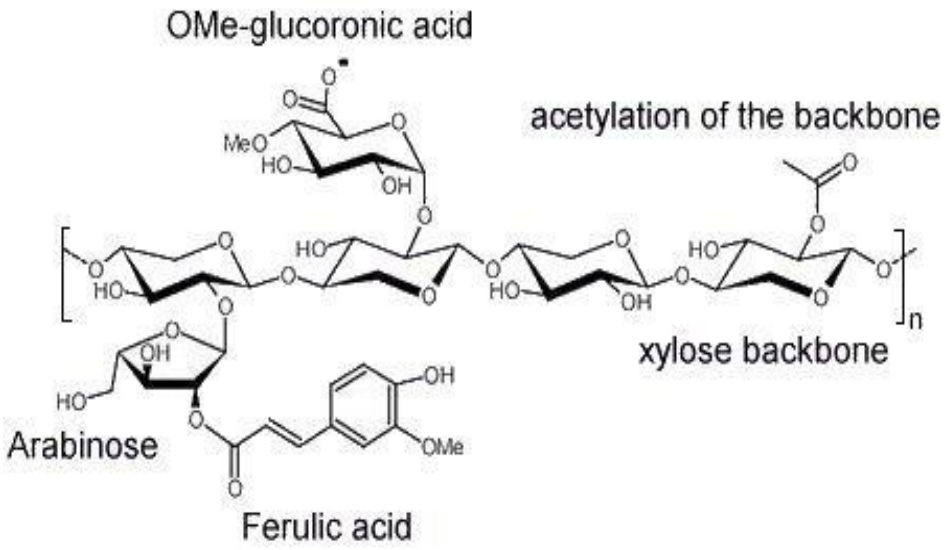

Figure 2.5: Hemicellulose structure in lignocellulose biomass.

2.3 RAW MATERIAL

Wood is a hard, fibrous tissue that found in biological origin in many tree. It has a very complex structure and produced as a secondary xylem in the woody plants. There are consisting two groups of wood species which are hardwoods and softwoods. Generally, softwood species contain more lignin and less hemicellulose than hardwoods. The lignin content of hardwoods is around 18%-25%, while amount of lignin in softwoods is around 25%-35% of the wood dry solids.

2.3.1 Softwood Species

2.3.1.1 Rice Straw

Rice Straw is a byproduct waste from agricultural field in all rice producing countries (Figure 2.6). It is one of the abundant lignocellulosic waste materials in the world. The annual output of rice straw in the global production is estimated about 600-900 million tons respectively. On the other hand, every kilogram of grain harvested will approximately produce 1 to 1.5 kg of the straw. Usually, the major practice for removing Rice Straw is by open burning in the field. However, it is increases the air pollution and consequently affects the public health (Karimi *et al*., 2006).

Rice Straw is one kind of good raw materials to produce D-xylose. It is consist of three main fractions of the rice straw structure which are cellulose (32–47%), hemicellulose (19–27%) and lignin (5–24%) (Karimi *et al*., 2006). The pentose sugar are dominant in hemicelluloses, a heteropolymer composed mainly of xylose in which xylose is the most important sugar followed by arabinose and hexoses (Zhang *et al*., 2010). The main product of D-xylose is obtained when xylan is hydrolyzed by chemically or enzymatically (Saha *et al*., 2005; Yu *et al*., 2008).

Figure 2.6: Rice Straw lignocellulosic material

2.3.1.2 Oil Palm Empty Fruit Brunch (OPEFB)

Oil palm empty fruit bunch fiber is a lignocellulosic waste residue from palm oil mills (Figure 2.7). Malaysia is one of the main oil palm producers and exporter in the world where approximately accounting for 47% of global palm oil production and 89% of exports (Najafpour *et al*., 2007; Saleh *et al*., 2011). Fifteen million tons of oil palm empty fruit brunch (OPEFB) biomass waste is generated annually throughout Malaysia by palm oil mills (Rahman *et al*., 2005). Where, 27% of total weight of the fresh fruit bunch would be the crude oil whereas the other portion left was the solid wastes; 23 % Empty Fruit Brunch, 14-15% fibre,6-7 % kernel and 6-7 % shell. Commonly, the OPEFB residues are composed by open burning in the field, which causes major air pollution to the environment (Najafpour *et al*., 2007).

The OPEFB biomass contains three major polymeric components which are cellulose, hemicellulose and lignin (Wyman *et al*., 1994). However, it is consists of high amount of cellulose (50 % cellulose) in compare to other sources of biomass such as sugar cane bagasse, rice straw, sorghum straw and corn cobs (Najafpour *et al*., 2007). It

Figure 2.7: Oil Palm Empty Fruit Branch (OPEFB) lignocellulosic material

2.3.1.3 Sugarcane Bagasse

Chemical composition of sugarcane bagasse shown that lignocellulosic biomass is mainly formed by cellulose, hemicellulose and lignin (Figure 2.8). Bagasse fibre has been extended on investigating acid catalysed hydrolysis to cleave the intrachain linkages in hemicellulose and cellulose chains contained in bagasse to produce commercial quantities of xylose, glucose and other sugars (Lavarack *et al*., 2001).

The hemicellulosic fraction is 26%, with preponderance of xylose (92%) as monosaccharide which suggests the availability of only slightly branched xylan in the hemicellulosic fractions. Bagasse also contains high amount of lignin (20%) comparable to that in wheat straw. Lignin strengthens the bagasse structure and influences its suspension texture in water. However, lignin consist of hydrophobic character considerably reduces the bagasse moisturizing facility (Boussarsar *et al*., 2009).

Figure 2.9: Sago bark lignocellulosic material

2.3.1.5 Summary of Softwood Species

Table 2.3 shows the summary of softwood species raw material that used in the xylose production from the previous studies.

Table 2.3: Summary of softwood species raw material that used in xylose production

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
Modeling and optimization of the diluted sulfuric acid pretreatment of corn stover, poplar and switchgrass	corn stover, and switchgrass	Xylose	Estenghlalian *et al*., 1996
Effect of autohydrolysis and enzymatic treatment on oil palm (Elaeis guineensis Jacq.) frond fibres for xylose and xylooligosaccharides production	Oil palm fronds (OPF)	Xylose and xylooligosaccharides	Hanim *et al*., 2011
A study on consecutive preparation of D-xylose and pure superfine silica from rice husk	Rice Husk	D-xylose and pure superfine silica	Zhang *et al*., 2010
Optimization of sugarcane bagasse conversion by hydrothermal treatment for recovery of xylose	Sugarcane bagasse	Xylose	Boussarsar *et al*., 2009
The acid hydrolysis of sugarcane bagasse hemicelluloses to produce xylose, arabinose, glucose, and other products	Sugarcane bagasse	xylose, arabinose, glucose, and other products	Lavarack *et al*., 2002

Optimization of xylose production from sago trunk cortex by acid hydrolysis	Sago barks	Xylose	Nurul Lina *et al*., 2011
A simple method for D-xylose extraction from Jute stick and rice husk	Jute stick and rice husk	D-xylose	Zakaria *et al*., 2001
Conversion of rice straw to sugars by diluted-acid hydrolysis	Rice straw	Xylose	Karimi *et al.*, 2006
Diluted-acid hydrolysis for optimization of xylose recovery from rice straw in a semi-pilot reactor	Rice straw	Xylose	Roberto *et al.*, 2002

2.3.2 Hardwood Species

2.3.2.1 Eucalyptus Wood

Eucalyptus globulus wood (Figure 2.10) is a cheap and widespread resource having xylan as the main constituent of its hemicellulosic fraction (Parajo *et al*., 1996). It's were treated with water under selected operational conditions which is autohydrolysis reaction to obtain a liquid phase containing hemicelluloses-decompasition products (mainly acetylated xylooligosaccharide, xylose and acetic acid) (Garrote *et al*., 2001). Hydrolysate from Eucalyptus wood cantain about 71% w/w sugar, 2% w/w monomeric arabinose, 8% w/w monomeric xylose and 61% w/w are oligosaccharides mainly composed of xylose (68 mol%) and Uronic acid (17 mol%) (Kabel *et al*., 2002).

Figure 2.10: Eucalyptus globulus sawdust lignocellulosic material

2.3.2.2 Red *Meranti* Wood

Red *Meranti* wood (RMW) is a most common and popular species of hardwood in Malaysia. Most of sawmill has processed this species of wood since it's a lot of growth in peninsular and east of Malaysia. Because of that, there are abundant amount of sawdust waste produced from the plant. It is contains of three main component of biopolymer which are cellulose, hemicellulose and lignin. It is estimated that RMW biomass consist of 29% of xylan which is a sugar polymer that made up from pentose sugar xylose. However, there are still limited uses of hardwood species in Malaysia. Usually, it is widely used as animal feed, fuel in manufacturing plants and local utilities (Rafiqul *et al.*, 2011).

2.3.2.3 Summary of Hardwood Species

Table 2.3 shows the summary of softwood species raw material that used in the xylose production from the previous studies.

Table 2.4: 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 globulus wood by autohydrolysis-posthydrolysis process: posthydrolysis kinetics	Eucalyptus globulus 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

2.3.3 Summary of Raw Material

Most of the prior research on the production of xylose, there are limited used hardwood as a source of raw material. Softwood species of lignocellulosic material was commonly chosen than hardwood. This is due to softwood species has a much weaker structure compared to the hardwood species. Although hardwood species consisting strong structure, but higher amount of hemicellulose, which consist of xylose. Hence, for the selection of the raw material used, hardwood species is chose due its availability. In Malaysia, hardwood species of sawdust can be easily found since there are many sawmills and wood industries operating in Peninsular Malaysia and East Malaysia (Miskam *et al*., 2004).

2.4 PARTICLE SIZE OF SAWDUST

Sawdust in industrial environment is an undesirable byproduct considered as a pollutant. Hence, it is needs proper management. In the saw mill industries, the sawdust was produce when the size of lignocellulosic material was reduced to a very small fine particle powered during the grinding mill process. Dust particle occur over a range of particle size. From occupational health view point, dust are categorized as respirable (< 10 μm), inhalable (median diameter 10 μm), and total dust covering both. When the dust particle size are small and depending on the particle density, they become airborne and pose more serious issues than larger particles that easily settle out (Igathinathane *et al*., 2009). In the acid hydrolysis process to xylose production, smaller particle size of sawdust will increase the surface area and tendency reduces the diffusion problem related to the reactant involved.

2.5 XYLOSE

2.5.1 Xylose Production

Xylose is a pentose sugar and commonly hydrolyzed from xylan like wood, rice husk, corn stalk, wheat straw and flax straw (Sjoman *et al*., 2008). It is appears to be a major component of hemicellulose in biomass and agricultural waste residue (David *et al*., 2003). It can be used as a substrate for production of wide variety of compound 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). Nowadays, there are three types of industry that becoming interest to used xylose as a part of their production which are liquid fuel production, food industry, odontological and pharmaceutical industry.

2.5.2 Structure of Xylose

Figure 2.11 shows the xylose structure.

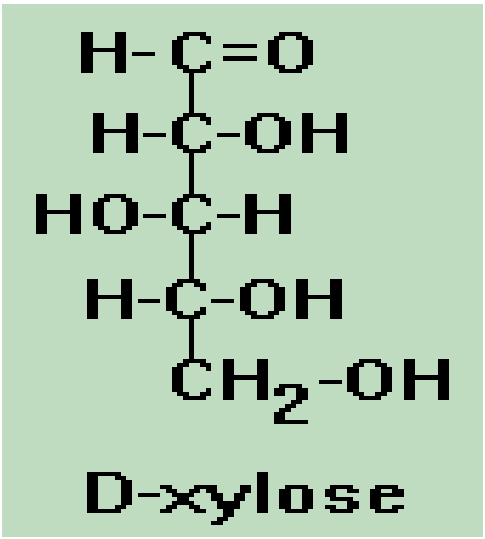

Figure 2.11: Structure of xylose

2.5.3 Applications of Xylose

2.5.3.1 Xylitol Production

Xylose is a good resource for xylitol production and function as intermediate product in xylitol production. In order to produce pure fermentable sugar (five carbon polyols), the xylose from complicated structure of lignocellulosic biomass is necessary to pretreat by chemical or biological hydrolysis method. Then, metabolic intermediate fermentation process of xylose is occurred by yeasts which can formed sugar alcohol (xylitol) (Chen *et al*., 2010). Xylitol is a hydrogenated carbohydrate and has been used as sugar replacers in food additives and sweetening agent, especially for non-insulin dependent diabetics. Figure 2.15 shows the xylitol production process from lignocellulosic material.

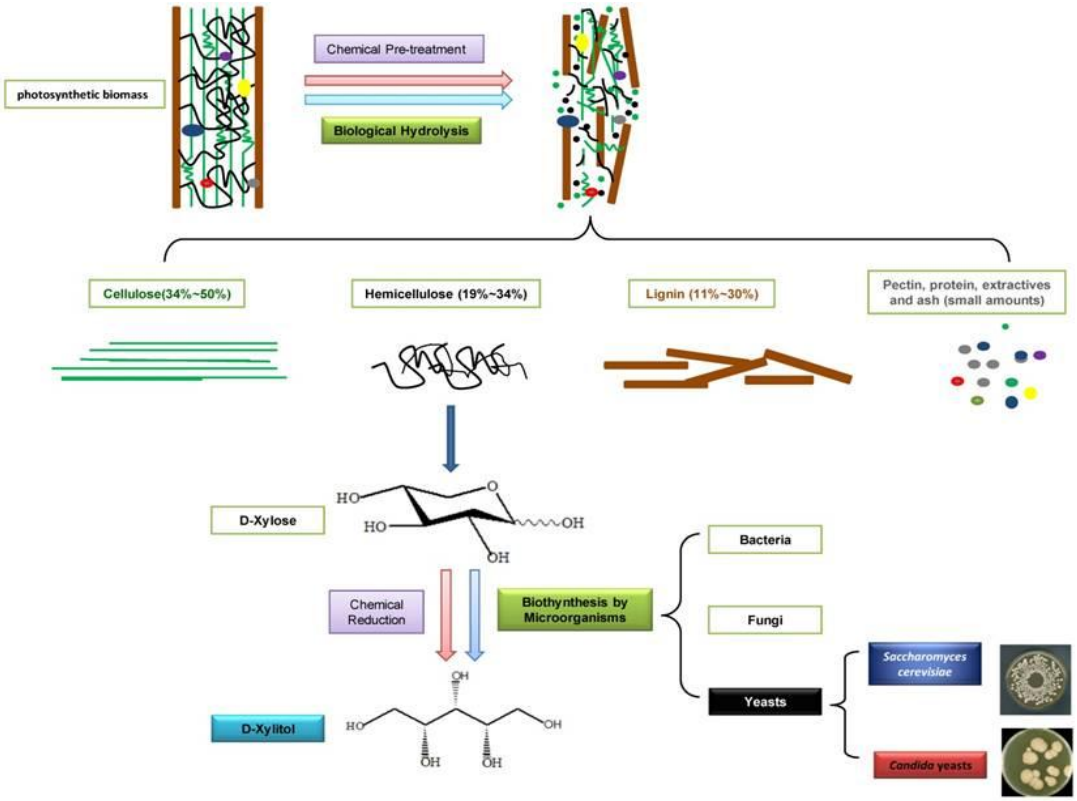

Figure 2.12: Xylitol production process

Furthermore, xylitol is a sugar alcohol which extensively as an alternative to sucrose as sweetener in the food industries where the sweetness is equal to sucrose but it does not cause dental carries (Roberto *et al*., 2002). It is alternative sweetener for diabetics' diets where it has low glycemic index and 40% fewer calories than sugar. It will produce insulin independent metabolism in the body and take up slowly from the intestine so that the blood glucose in the body will change.

Other than that, xylitol is also used in the odontological industries where it is function as anticariogenicity, tooth rehardening and remineralization properties. Since xylitol has non-cariogenic five carbon structures, it can reduce plaque growth on the dental surface and stopping the production of tooth-decay causing by creating acid in mouth. Hence, in these industries, xylitol is commonly formulated in the tooth paste where it is daily applied to protect the mouth by brushing and flossing for adults and children.

Finally, xylitol is used in pharmaceutical industries where it's capability of preventing otitis and its possibility of being used as a sweetener in syrups, tonics and vitamin formulations. Other than that, it is used as the tablet coating in tablet manufacturing process like in supplement tablet production (Roberto *et al*., 2002).

2.5.3.2 Liquid Fuel

Biomass can be used to meet a variety of energy needs, including generating electricity, fueling vehicles and providing process heat for industrial facilities (Canettieri *et al*., 2006). Since few decades ago, the demand of fuel production from recycling biomass has increased drastically for two main reasons. Firstly, the oil crisis problem in the world and the second is to control the greenhouse effect caused by emission of carbon dioxide (CO_2). In the fuel production plans, there are still produces CO_2 from the biomass combustion process but there are results in zero net emissions of greenhouse gasses (Miskam *et al.*, 2004)

However, hemicellulose sugar from the conversion of lignocellulosic biomass can be utilized to produce fuel ethanol. Hemicellulose that contain in lignocellulosic biomass has generated a mixture of sugar by acid hydrolysis or enzymatic pretreatment. Then, xylose can be converted to xylulose using the enzyme xylose isomerase and yeast in the fermentation process of xylulose to ethanol. Commonly, there are three types of yeast that have the capability to use in fermentation process. The yeasts are *Pachysolen tannophilus*, *Pichia stipitis*, and *Candida shehate* (Badal; 2003).

2.6 ACID HYDROLYSIS OF LIGNOCELLULOSIC BIOMASS

Acid hydrolysis is an effective and inexpensive method that widely used for biomass conversion in the production of xylose (Figure 2.13). Xylose is produced as main sugar components from hemicellulose while other by product component also generated in low amount during acid hydrolysis (Dominguez *et al.,* 1997). Usually, a single stage hydrolysis with diluted acid (less than 5% acid concentration) used rather than concentrated acid solution. In the under control treatment conditions, acid act catalyst in acid hydrolysis of lignocellulosic biomass that mainly produce xylose from hemicellulose with the cellulose and lignin component remain unaltered.

Furthermore, under the controlled conditions, the diluted acid hydrolysis of lignocellulosic material mainly produces xylose from hemicellulose in the liquid phase. While, the cellulose and lignin are unaltered structure that remained as solid phase. Hemicellulose is easily to degraded during acid hydrolysis due to it's amorphous, brunched structure compared to cellulose which need severe treatment conditions due to its crystalline nature (Parajo *et al.*, 1998)

Other than that, acid concentration is the most important parameter that affecting the sugar yields. Higher temperature is needed to achieve maximum yield of xylose production from lignocellulosic material conversion within in a short time. However, implementations of high temperature will causes the contamination occurs with the presence of soluble derivatives, furfural, hydroxy-methyl furfural and sugar alcohol respectively.

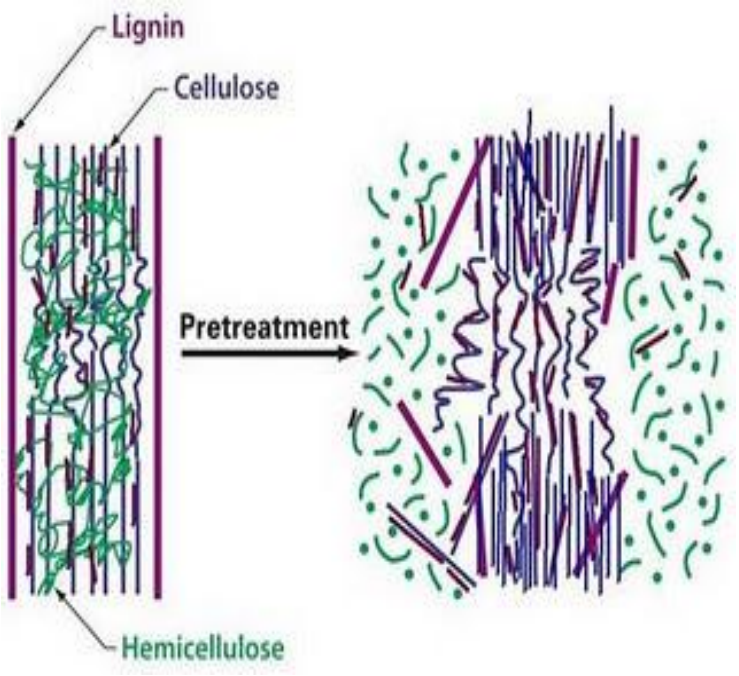

Figure 2.13: Lignocellulosic material structure during acid hydrolysis process

Table 2.5: Summary of pretreatment method of lignocellulosic biomass

Title	Raw Material	Method	Product	References
Modeling and optimization of the diluted sulfuric acid pretreatment of corn stover, poplar and switchgrass	Poplar	Acid hydrolysis (Sulfuric acid)	Xylose	Estenghlalian *et al.*, 1996
Production of xylitol from concentrated wood hydrolysates by *Bebaryomyces hansenii* : Effect of the initial cell concentration	Eucalyptus globulus wood chips	Acid hydrolysis (Sulfuric acid) and fermentation	Xylitol	Parajo *et al*., 1996
Generation of xylose solutions from *Eucalyptus globulus* wood by autohydrolysis-posthydrolysis processes: posthydrolysis kinetics	Eucalyptus globulus wood	Autohydrolysis and posthydrolysis	Xylose	Garrote *et al*., 2001
Response surface optimization of oxalic acid pretreatment of yellow poplar (*Liriodendron tulipifera*) for production of glucose and xylose monosaccarides	Yellow poplar stems	Acid hydrolysis (Oxalic acid)	Glucose and xylose	Kim *et al*., 2011

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 of xylose production from sago trunk cortex by acid hydrolysis	Sago trunk cortex	Acid hydrolysis (Sulfuric acid)	Xylose	Nurul Lina *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

2.7 ANALYTICAL METHOD FOR XYLOSE PRODUCTION

2.7.1 High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) is a separation method that used chromatographic technique to separate a mixture of compounds to identify, quantify and purify the individual components of mixture. It is high versatility compared to other chromatographic system and ability to separate a variety of chemical mixture. It is typically utilizes differences types of stationary phase where form in the solid or liquid phase. The component is dissolved with the solvent and it is relatively differences in the travel through the column due to the pressure exist through the mobile phase. Because of that, the separation of compound is occurred.

An amount of resolution is depending on the extended of interaction between the solute component and stationary phase. The compounds of the mixture travel with different rates due to their relative affinities with the solvent and stationary phase. Compounds with higher affinity towards stationary phase of the column travels slowly and vice-versa. The separation is more effective due to greater surface area achieved due to very small particle size of stationary phase in comparison to that used in column chromatography. Table 2.6 shows the summary of the HPLC method from the previous studies.

Table 2.6: Summary of HPLC method

Title	Column	Mobile Phase	Detect	Flowrate	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
Responnse surface optimization of oxalic acid pretreatment of yellow poplar (Liriodendron tulipifera) for production of glucose and xylose monosaccarides	BioRad Aminex HPX-87H	5mM H_2SO_4	RI	0.3 mL/min	55 ^{o}C	Kim *et al.*, 2011
Design of the process parameters for the production of xylose from wood sawdust	Razex RHM Monosaccharides column H^+	Ultra-pure water	RI	0.6 ml/min	80^{o}C	Parajo *et al.*, 1996

2.7.2 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is a tool that uses for qualitative analysis in the identifying types of chemical bonds (functional groups). It is useful for identifying chemicals either organic or inorganic component. Usually, it is applied for the analysis of solids, liquids and gasses. Molecular bond is vibrating at various frequencies depending on the elements and the types of bonds. For any given bond, there are several specific frequencies where it can be vibrated. The wavelength of the light absorbed is characteristics of the chemical bond in the spectrum. Figure 2.14 shows the principle of the FTIR.

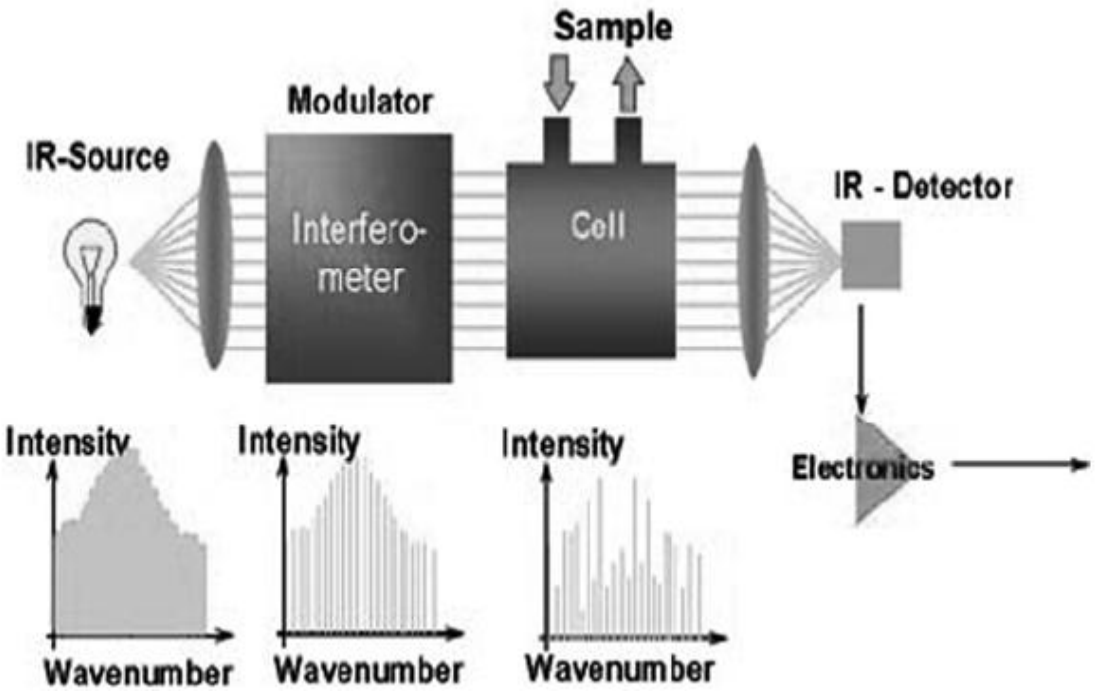

Figure 2.14: Principle of Fourier Transform Infrared Spectroscopy (FTIR)

2.7.3 Scanning Electron Microscope (SEM)

Scanning Electron Microscope (SEM) is a microscope that uses an electrons rather than light to form an image. It is allows more of a specimen to be focus at one time and has much higher resolution, so closely spaced specimens can be magnified at much higher levels. Then, a beam of electrons is produced at the top of the microscope by an electron gun. The electron beam follows a vertical path through the microscope, which is held within a vacuum. The beam travels through electromagnetic fields and lenses, which focus the beam down toward the sample. Once the beam hits the sample, electrons and X-rays are ejected from the sample. Detectors collect these X-rays, backscattered electrons, and secondary electrons and convert them into a signal that is sent to a screen similar to a television screen and produce the final image. Figure 2.15 shows the principle of the Scanning Electron Microscope (SEM).

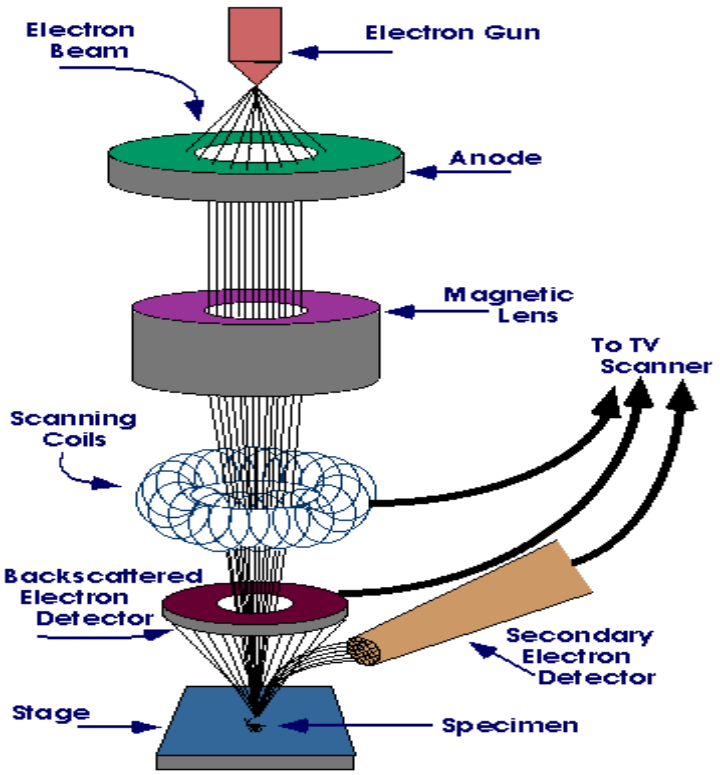

Figure 2.15: Principle of Scanning Electron Microscope (SEM)

CHAPTER 3

RESEARCH METHODOLOGY

3.1 PRETREATMENT OF RAW MATERIAL

Raw materials used were hardwood species of sawdust (*Meranti*, *Keruing* and *Resak*). It was collected from saw mill industry at Gambang and Kuantan, Pahang. The samples were sundried for a day. After that, the samples were sieved into five differences particle size of sawdust (800, 615, 400, 315 and 200 µm) by using vibrator sieve shaker (Figure 3.1). Then, it was stored in schott bottle and autoclave it to sterile the samples (Figure 3.2). The samples were dried in the oven at 60^{o}C for 48 hours to remove moisture content in the sawdust (Figure 3.3). Finally, the samples were stored at room temperature for the further process (Relova et al., 2009).

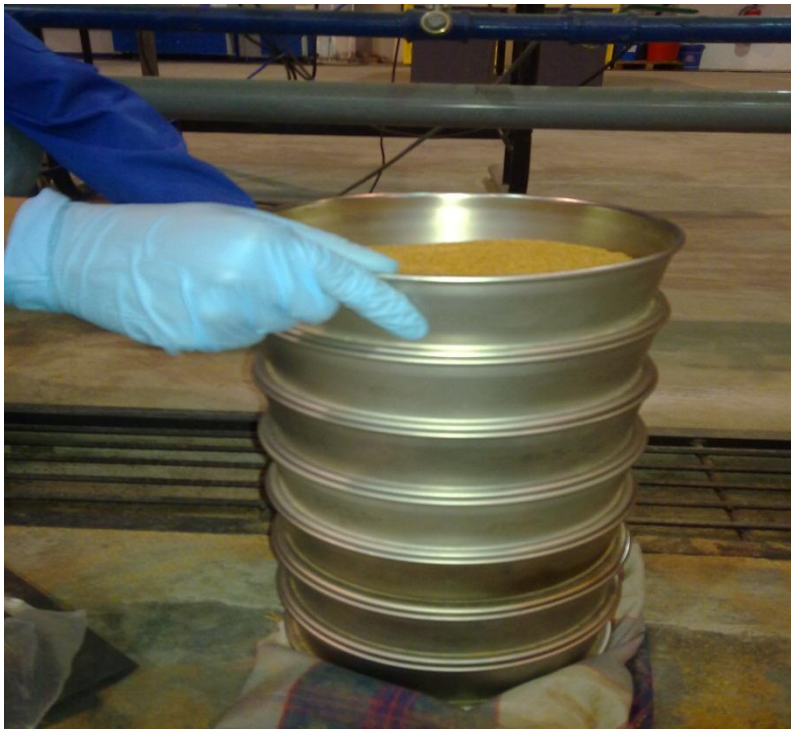

Figure 3.1: Sieving Process of Sawdust

Figure 3.2: Drying Process

Figure 3.3: Stored the raw material

3.2 ACID HYDROLYSIS

Oven dried hardwood sawdust was mixed with 3.24% w/w concentration of Sulfuric Acid in 100 ml Erlenmeyer flasks (Lavarack et al., 2002). The flasks were loaded with samples to a solid liquid ratio of 1:8 (Figure 3.4). The slurries were allowed to stir using magnetic stirrer for 15 minutes at room temperature until completely mixed. Then, the hydrolysis process (Figure 3.5) was carried out in an autoclave (Hiclave HVE-50, Hirayama, Japan) at constant temperature of 121^{o}C for 40 minutes (Mohamed et al., 2011).

After completing the acid hydrolysis process, the flasks were cooled at room temperature for desired length of time. Then, the content of the flasks were filtered using a filter paper (Whatman No. 1) (Figure 3.6). The hemicellulosic hydrolysate was obtained at the filtrate at pH 1.25 (Figure 3.6) and the cakes were contained cellulose and lignin (Musatto *et al*., 2005). Then, the filter cake remained in the filter paper was oven dry at 60^{o}C for 3 hours.

Figure 3.4: Sawdust measurement for acid hydrolysis process

Figure 3.5: Acid Hydrolysis process

Figure 3.6: Filtration process

Figure 3.7: pH Measurement after Acid Hydrolysis

3.3 NEUTRALIZATION

From the filtration process, the filtrate solution that obtains in acidic solution was neutralized with calcium Hydroxide (Figure 3.8). The calcium hydroxide was added a little bit and stirred the solution by using magnetic stirrer for 15 minutes. After that, the stirred solution was checked the pH reading until it reach an average pH 6.5. If the solution did not reach the neutral solution, calcium hydroxides were added more. The neutral sugar solution will be separated by filtration to obtain the clear solution. Then, the concentrated sugar solutions were stored at 4^{o}C for analysis process. (Bludworth *et al*., 1993)

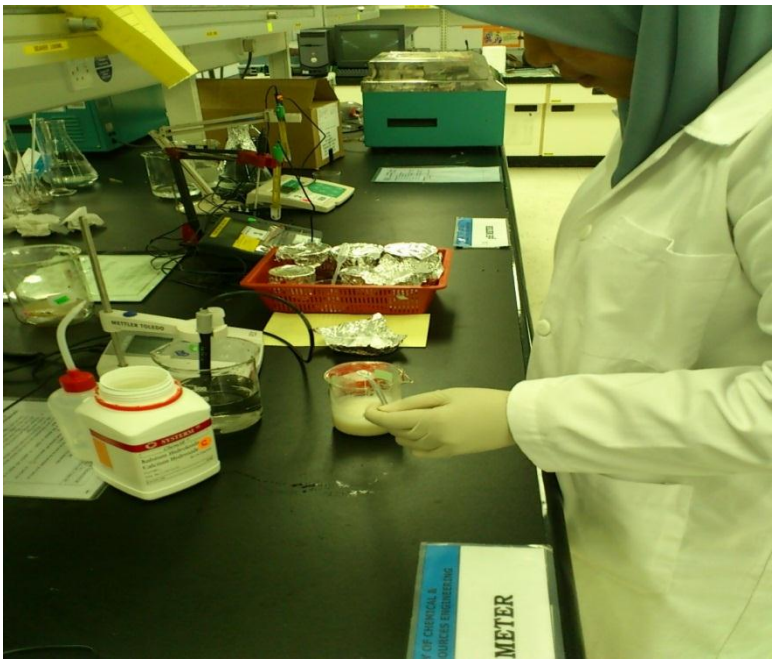

Figure 3.8: Neutralization process by using calcium hydroxide

3.4 ANALYSIS OF SAMPLES

3.4.1 High Performance Liquid Chromatography (HPLC)

After neutralization procedure, the filtrate solutions were analyzed by using high performance liquid chromatography (HPLC) to determine the concentration of xylose, glucose, and arabinose (Figure 3.9). The concentration of the component was detect with Refrative Index (RI) detector using SUPERCOGEL Ca, 30cm x 7.8 mm column aluted at 80^{o}C using water as mobile phase, 1.5 ml/min flowrate and 10µL as an injection volumn.

Before the HPLC analysis run, the column was washing first by purging process for 3 hours. The samples were filter by using 0.2µm membrane filer and transfer in HPLC vial. The standards solutions that consist of xylose, glucose and arabinose were diluted in five difference concentration. The standard solutions also were filter and transfer in HPLC vial. The retention time needed for this analysis was 20 minutes for each sample (Nurul Lina *et al.*, 2011).

Figure 3.9: High Performance Liquid Chromatography (HPLC)

3.4.2 Scanning Electron Microscope (SEM)

The surface morphology of the treated and untreated sawdust were examined the structure by a scanning electron microscope (EVO 50) (Figure 3.10). The samples were sputter with platinum and observed under SEM. The micrographs were taken at magnification 300, 500, 1000 and 1500 (Abdul Muna'im *et al.,* 2011).

Figure 3.10: Scanning Electron Microscope (SEM)

3.4.3 Fourier Transform Infrared Spectroscopy (FTIR)

The infrared spectra of the raw sawdust and treated sawdust specimen were recorded with OMNIC ESP software (Thermo Nicolet Instrument Corporation, Madison, WI) (Figure 3.11). Dried raw sawdust and treated sawdust were prepared before analyzed with FTIR. FTIR was used to determine functional groups associated in the surface of untreated and treated sawdust (Abdul Muna'im *et al.*, 2011).

Figure 3.11: Fourier Transform Infrared Spectroscopy (FTIR)

3.4.4 Lignin Content Determination

An amount of 0.1 g dried sawdust was added into a mixture of 20 mL of 0.02 M potassium permanganate ($KMnO_4$) with 5 mL of 2.0 M sulfuric acid (H_2SO_4) and mixed well for three minutes. Solid sample was separated from the solution through filtration using filter paper (Whatman no 1). The filtrate was measured using UV-Spectrophotometer (U1800, Hitachi) at 546 nm of wavelength. Value of kappa number was determined using equation 1.

$$K = \frac{a}{w}\left(\frac{Ao-Ae}{Ao}\right) \quad (1)$$

Where,

K = Kappa Number
a = the volume of KMnO4 used in the solution
w = Weight of moisture-free sample used
Ao = Spectral intensities at time t = 0 (before sample is being added)
Ae = Spectral intensities at the end of the reaction.

Accordingly, lignin content in the sample was calculated from the values of Kappa Number, K using Eq. (2), where the percent of lignin degradation is illustrated in Eq. (3)

Lignin Content (wt%) = 0.15 K (2)

$$Lignin\ degradation = \frac{Lignin\ wt\%\ (Untreated) - Lignin\ wt\%\ (Treated)}{Lignin\ wt\%\ (Untreated)} \quad (3)$$

3.5 SUMMARY OF METHODOLOGY

Figure 3.12 shows the overall process of xylose production from difference species and particle size of sawdust.

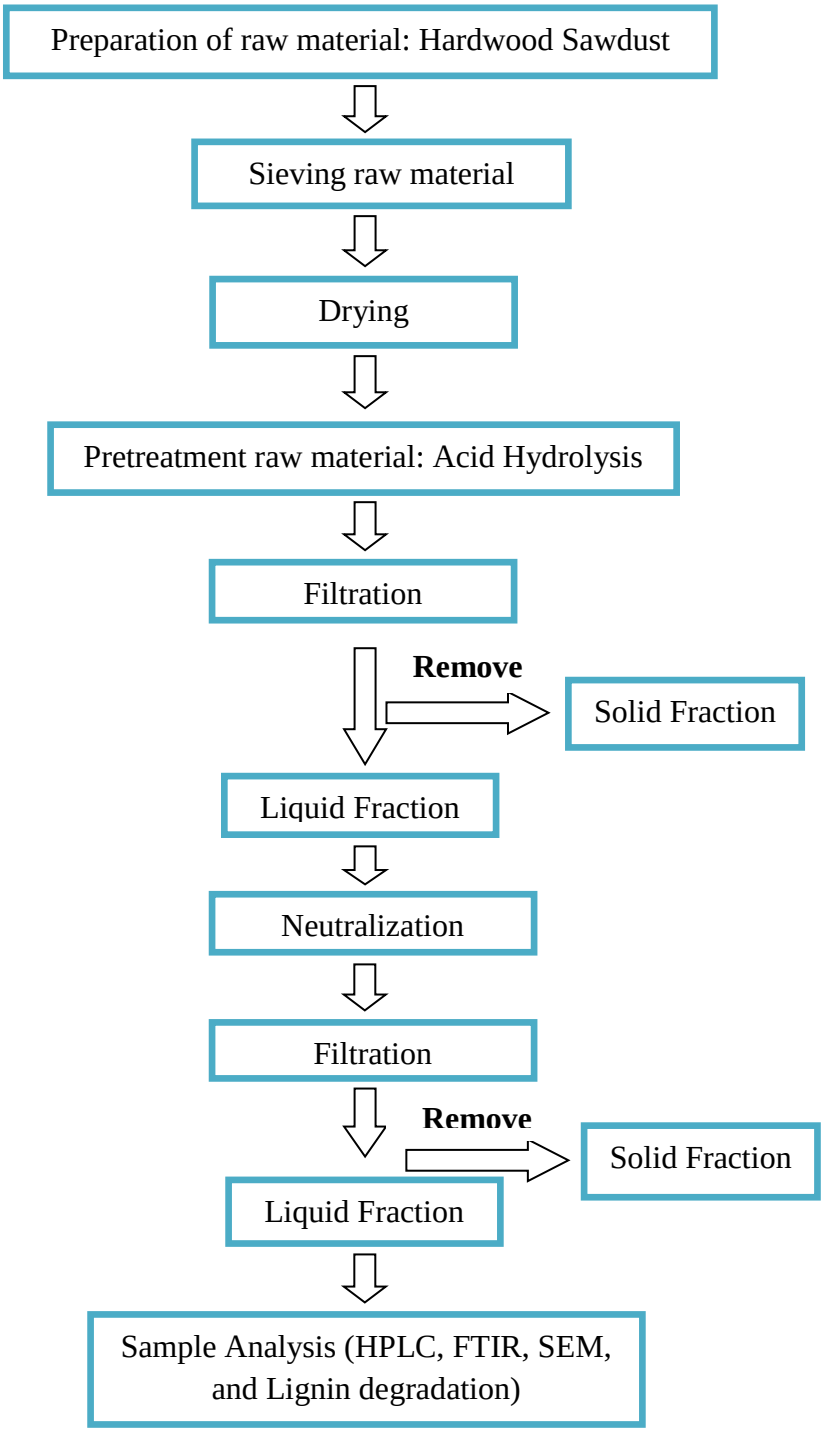

Figure 3.12: A process for xylose production

CHAPTER 4

RESULT AND DISCUSSION

4.1 INTRODUCTION

In this chapter, the results obtained from the experiment were discussed. The experiment was performed to study the effect of sawdust species and particle size on xylose production. In order to achieve the research objectives, acid hydrolysis process was come out to degrade the amount of hemicellulose and xylose from the structure of sawdust. This was due to the lower accessibility and solubility of crystalline cellulose compared to the open branches structure in hemicellulose (Parajo *et al.*, 1998). Hence, the qualitative analysis (FTIR and SEM) and quantitative analysis (HPLC and Kappa Number) was done to find out the best type of species and particle size of sawdust species and particle size.

4.2 COMPOSITION OF HARDWOOD SPECIES OF SAWDUST

Differences level of particle size ranging from 200 to 800 µm from three types of species (*Meranti*, *Keruing* and *Resak*) were selected to determine the maximize amount of xylose concentration and minimize amount of by-product (Glucose, Arabinose, Mannose and Galactose) production.

Table 4.1: Composition of Hardwood species of sawdust on dry basis

Main fraction	Percent (%)
Cellulose	41.06
Hemicellulose	30.64
Lignin	25.22
Others	3.08

Table 4.2: Sugar compound detected by HPLC in hardwood species of sawdust hydrolysate

Species / Particle size	Sugar Recoveries		
	Xylose (g/l)	Glucose (g/l)	Arabinose (g/l)
Keruing 315µm	30.17	9.33	ND
Keruing 400µm	21.17	14.42	ND
Meranti 200µm	17.66	16.16	ND
Meranti 630µm	14.70	14.63	ND
Resak 200µm	22.52	16.16	ND
Resak 630µm	19.62	15.22	ND

Notes: ND = Not Detected

Table 4.1 presents the results from HPLC analysis of sugar content of the three types of sawdust from hardwood species (*Meranti*, *Keruing* and *Resak*) which produce higher and lower xylose production for each species. The analysis of sugar content revealed that the hardwood species of sawdust contained primarily xylose and glucose. However, Arabinose, Mannose and Galactose were not detected in this substrate.

From the composition on dry basis, 30.64% of hemicellulose was portion in the Hardwood species of sawdust. Xylose yielded for each species and particle sizes were difference. From the results, xylose yielded for *Keruing* 315µm, *Keruing* 400µm, *Meranti* 200µm, *Meranti* 630µm, *Resak* 200µm and *Resak* 630µm were 76.38%, 59.48%, 52.22%, 50.12%, 54.03% and 56.31% respectively from the total sugar production for each species.

According to Rahman et al. (2006) was successfully obtaining the xylose yield of 90.35% from 24.01% of hemicellulose from oil palm empty fruit bunch. In addition, Xylose yield of 88% was obtained in the rice straw that contained 25% of hemicellulose (Roberto *et al.*, 2003). Then, 91.4% of xylose was yield in the sago trunk cortex that contained 21.09% of hemicellulose portion (Nurul Lina *et al.*, 2011). From the results obtained, it is clearly understood that differences material will produce differences composition of hemicellulose and its sugar compounds. Hardwood species of sawdust will aid to select as a substrate for hydrolysis to simple sugars mainly xylose and glucose.

4.3 EFFECT OF PARTICLE SIZE OF SAWDUST IN XYLOSE PRODUCTION

In this research, the particle size of sawdust effect was studies during the acid hydrolysis process in the xylose production. According to the mass transfer theory, smaller particle size of sawdust will increase the surface area and tendency reduces the diffusion problem related to the reactant involved. However, from the result, the xylose production of the hardwood species of sawdust was not dependent on the particle size of sawdust. This is due to other factor that tendency effected the xylose production. According to the Gong et al. (2006) the structure of wood itself can influence the sugar monomer production during acid hydrolysis process. Figure 4.1, Figure 4.2 and Figure 4.3 shows the effect of particle size on the formation of xylose at constant LSR (8g/g), H_2SO_4 concentration (3.24% w/w), residence time (40 minutes) and temperature (121°C) for three types of species.

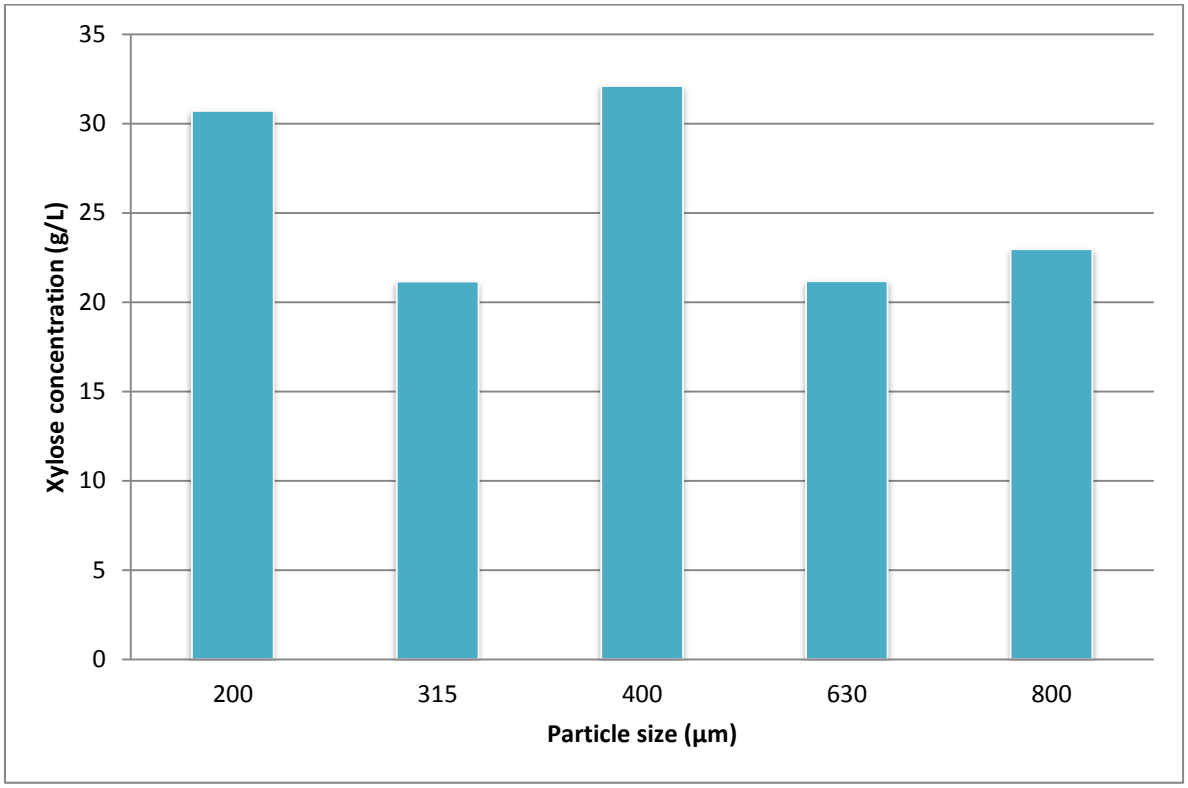

Figure 4.1: Xylose concentration for *Keruing* species

From the results in the *Keruing* species, higher xylose production was obtained at 400µm particle size of sawdust while lower xylose production produced at 315µm.

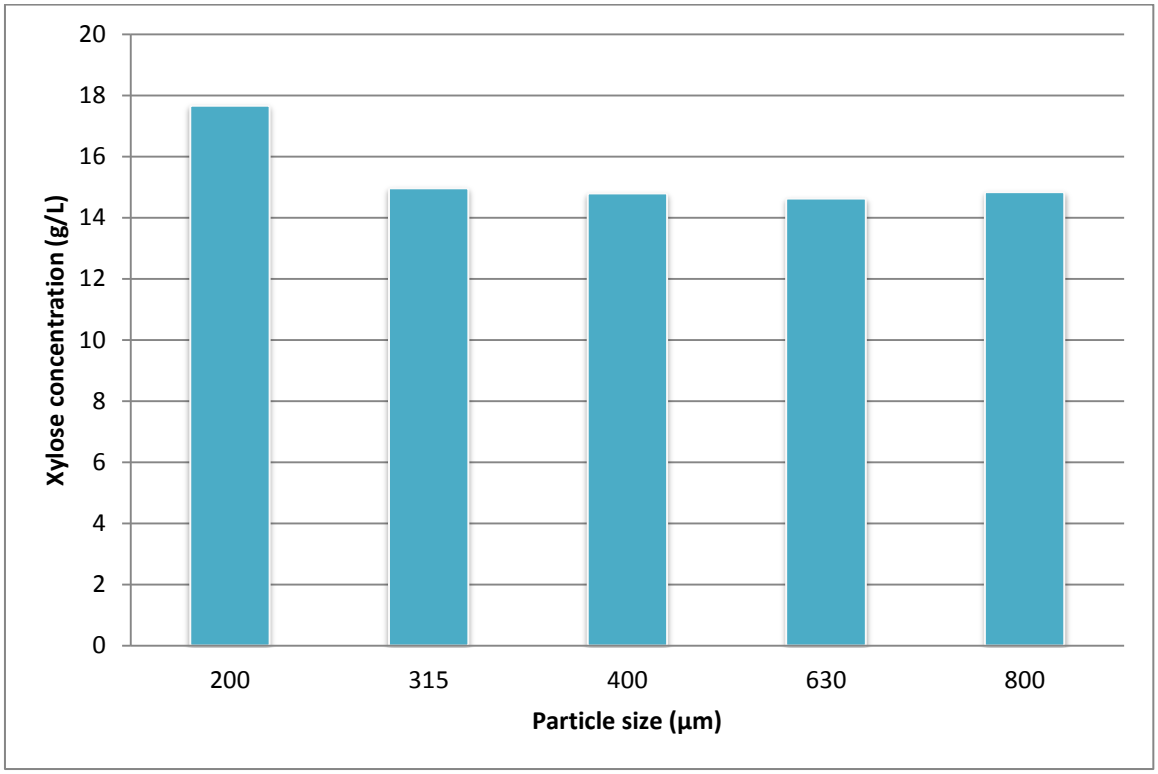

Figure 4.2: Xylose concentration for *Meranti* species

From the results in the *Meranti* species, higher xylose production was obtained at smaller particle size of sawdust (200µm) while lower xylose production produced at 630µm.

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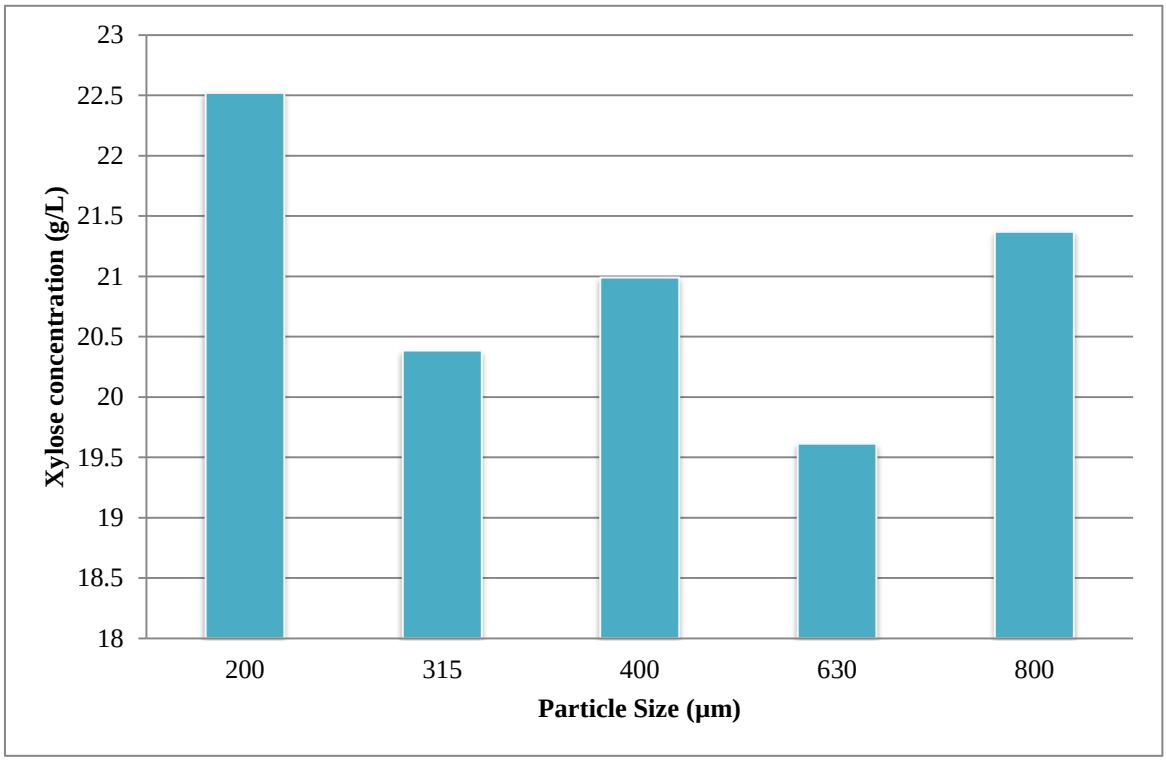

Figure 4.3: Xylose concentration for *Resak* species

From the results in the *Resak* species, higher xylose production was obtained at smaller particle size of sawdust (200µm) while lower xylose production produced at 630µm.

From the results obtained in Figure 4.4, it shows that, every species of sawdust was produce difference amount of xylose. This is because differences species of sawdust have differences amount of its composition such as lignin, hemicellulose and cellulose. Xylose is a sugar derivative that located in hemicellulose. The amount of the xylose production is depends on the amount of hemicellulose that contain in the lignocellulosic biomass itself.

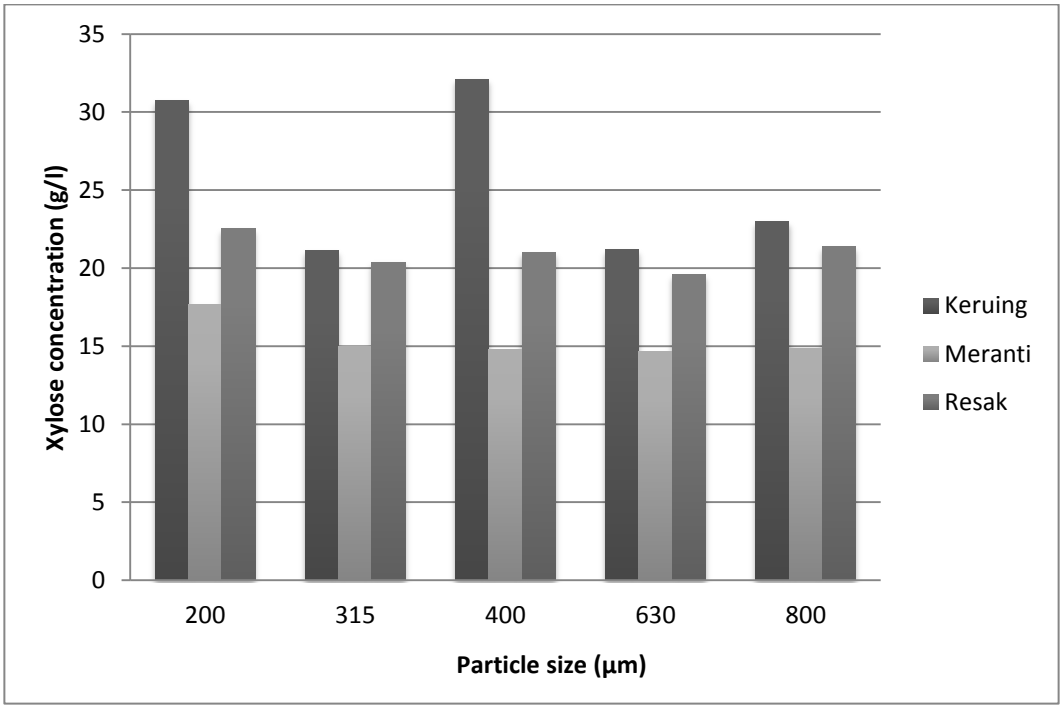

Figure 4.4: Effect of particle size on the formation of xylose at three differences particle size.

Other than that, the particle size was not the main factor that affected in xylose production. According to Gong *et al*., (2006) the strength of the component of lignin, cellulose and hemicellulose in the wood are differences in every species. Hence, every species of sawdust has its own structure characteristics. Because of that, it is influence the xylose production during acid hydrolysis process. The result shows that, the higher production of xylose is not come out from smaller particle size of sawdust. Although the

smaller particle size of sawdust has higher surface area of the particle but it not mainly affected the xylose production in the acid hydrolysis process.

From the results, highest xylose concentration was not obtained at smaller particle size but it is produced at 400µm particle size from *Keruing* species of sawdust. The higher xylose concentration value is 32.12 g/l. While, the lowest xylose concentration obtained at 630µm particle size from *Meranti* species of sawdust. The lower xylose concentration value is 14.63 g/l.

4.4 EFFECT OF PARTICLE SIZE STRUCTURE OF SAWDUST AFTER HYDROLYSIS PROCESS.

The solid material of sawdust recovered after acid hydrolysis process mainly consists of lignin, cellulose and untreated hemicellulose while hemicellulose hydrolysate was produced in the liquid form. Scanning electron microscope (SEM) was done to see differences structure of untreated and treated sawdust with diluted sulfuric acid (3.24% w/w). The morphology results for all species structure were shown in the Figure 4.5, Figure 4.6 and Figure 4.7.

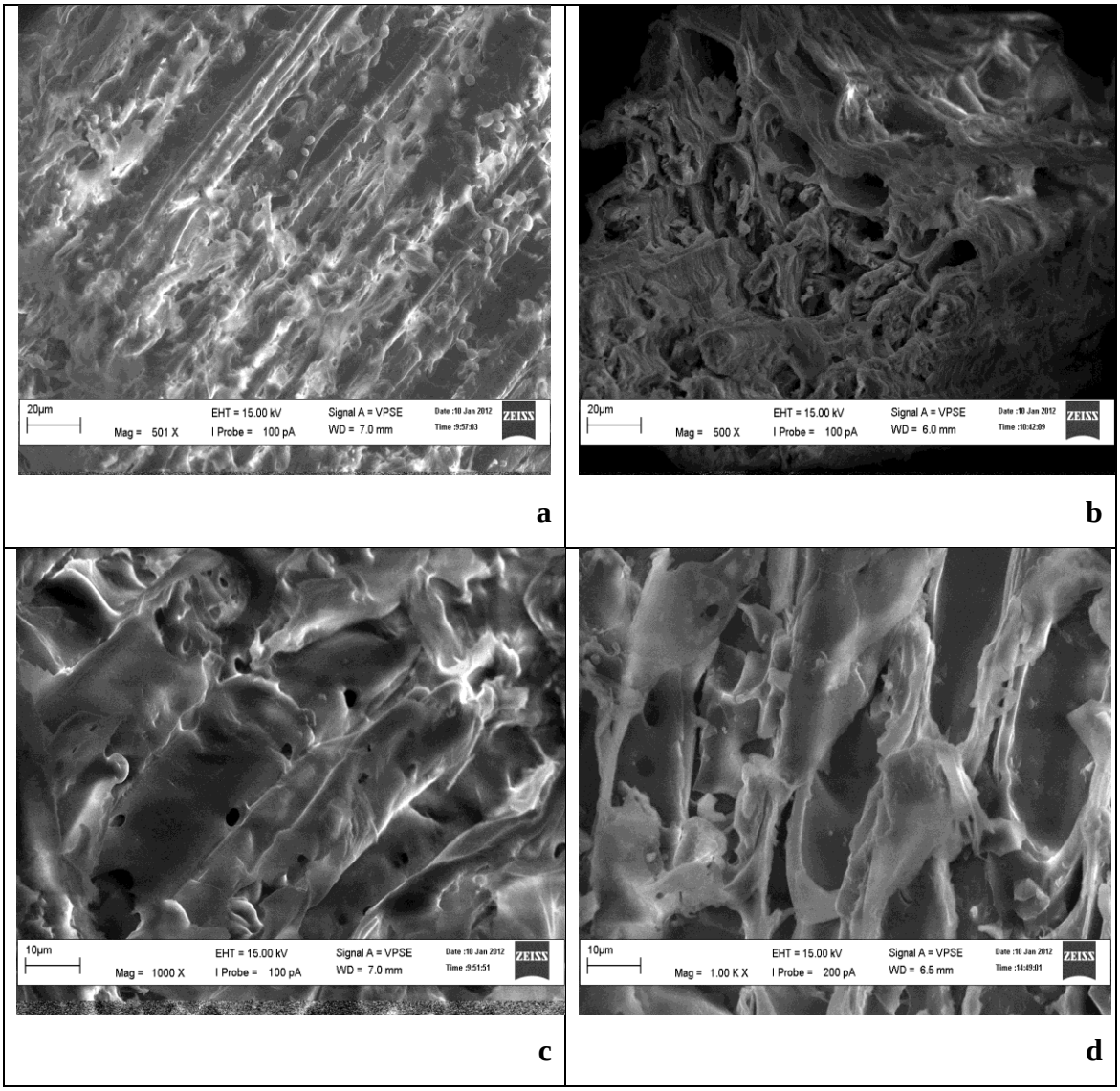

Figure 4.5: a) SEM micrograph of untreated *Keruing* species of sawdust at 315µm. b) SEM micrograph of treated *Keruing* species of sawdust at 315µm. c) SEM micrograph of untreated *Keruing* species of sawdust at 400µm. d) SEM micrograph of treated *Keruing* species of sawdust at 400µm

From the results, xylose production was higher at 400µm particle size which is 32.12 g/l. However, the xylose production was lower at 315µm particle size which is 21.15 g/l. It has been observed that surface morphology of untreated and treated *Keruing* species of sawdust composites is totally difference. The untreated sawdust was in the roughness surface like many bundles of chopsticks and flacks.

Then, hollow was obtained in the treated sawdust. The hollow was obtained after the treatment due to the degradation of hemicellulose from the acid hydrolysis process using diluted sulfuric acid. When the xylose was come out from the sawdust structure, the hollow was present in the structure. The amount of hollow that presents is dependent on the amount of xylose production. From the observation, the hollow that produces from the treated *keruing* species of sawdust at 400µm was like to disrupt due to the species was produced higher amount of xylose.

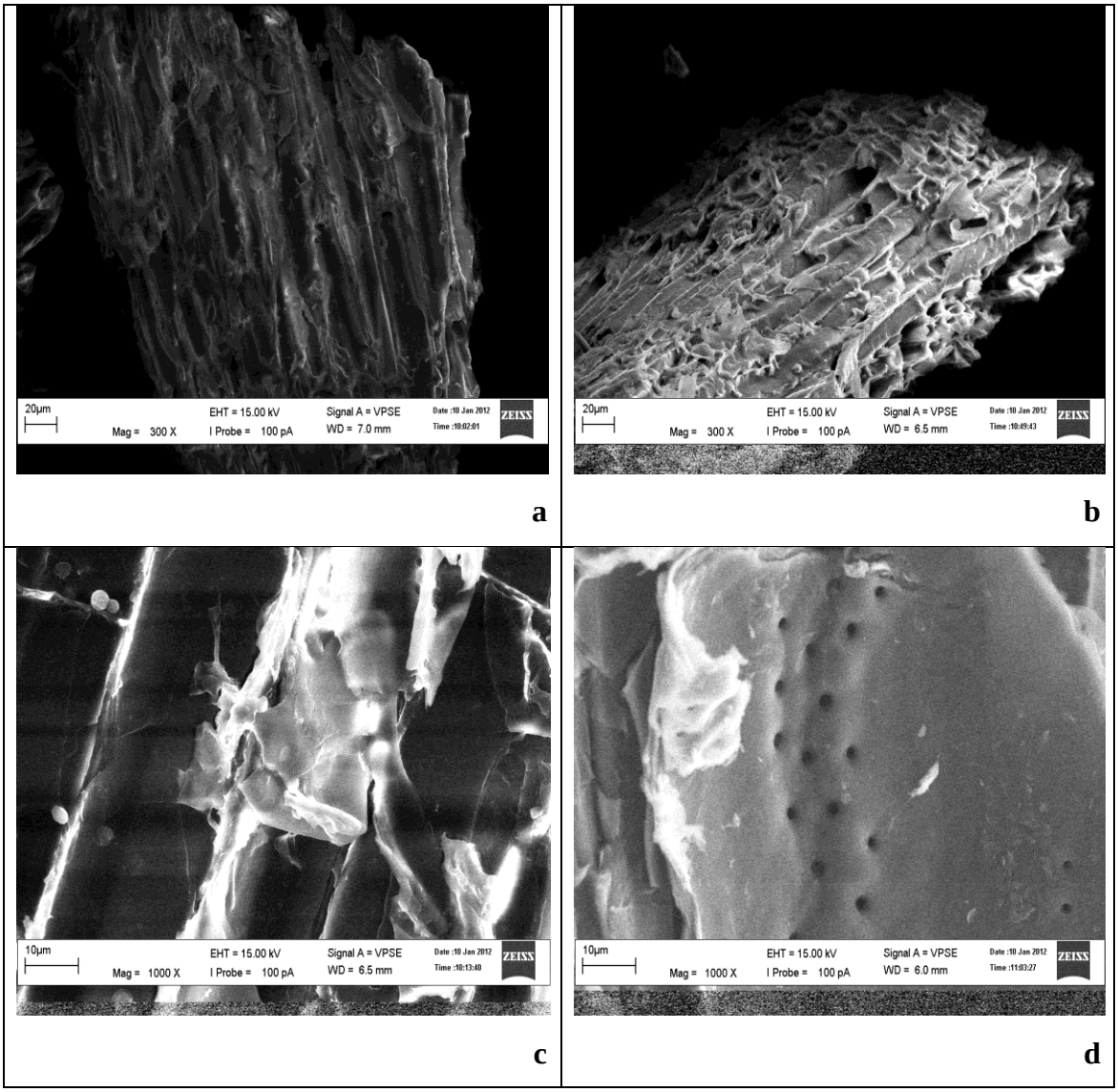

Figure 4.6: a) SEM micrograph of untreated *Meranti* sawdust at 200µm. b) SEM micrograph of treated *Meranti* sawdust at 200µm c) SEM micrograph of untreated *Meranti* sawdust at 630µm. d) SEM micrograph of treated *Meranti* sawdust at 630µm

From the results, xylose production was higher at 200µm particle size which is 17.66 g/l. However, the xylose production was lower at 630µm particle size which is 14.63 g/l. It has been observed that surface morphology of untreated and treated *Meranti* species of sawdust composites is totally difference. The untreated sawdust was in the roughness surface like many bundles of chopsticks and flacks.

Then, hollow was obtained in the treated sawdust. The hollow was obtained after the treatment due to the degradation of hemicellulose from the acid hydrolysis process using diluted sulfuric acid. When the xylose was come out from the sawdust structure, the hollow was present in the structure. The amount of hollow that presents is dependent on the amount of xylose production. Then, from the observation, in the treated 200 µm particle size of *Meranti* species, many hollows were produce at the roughness surface of sawdust.

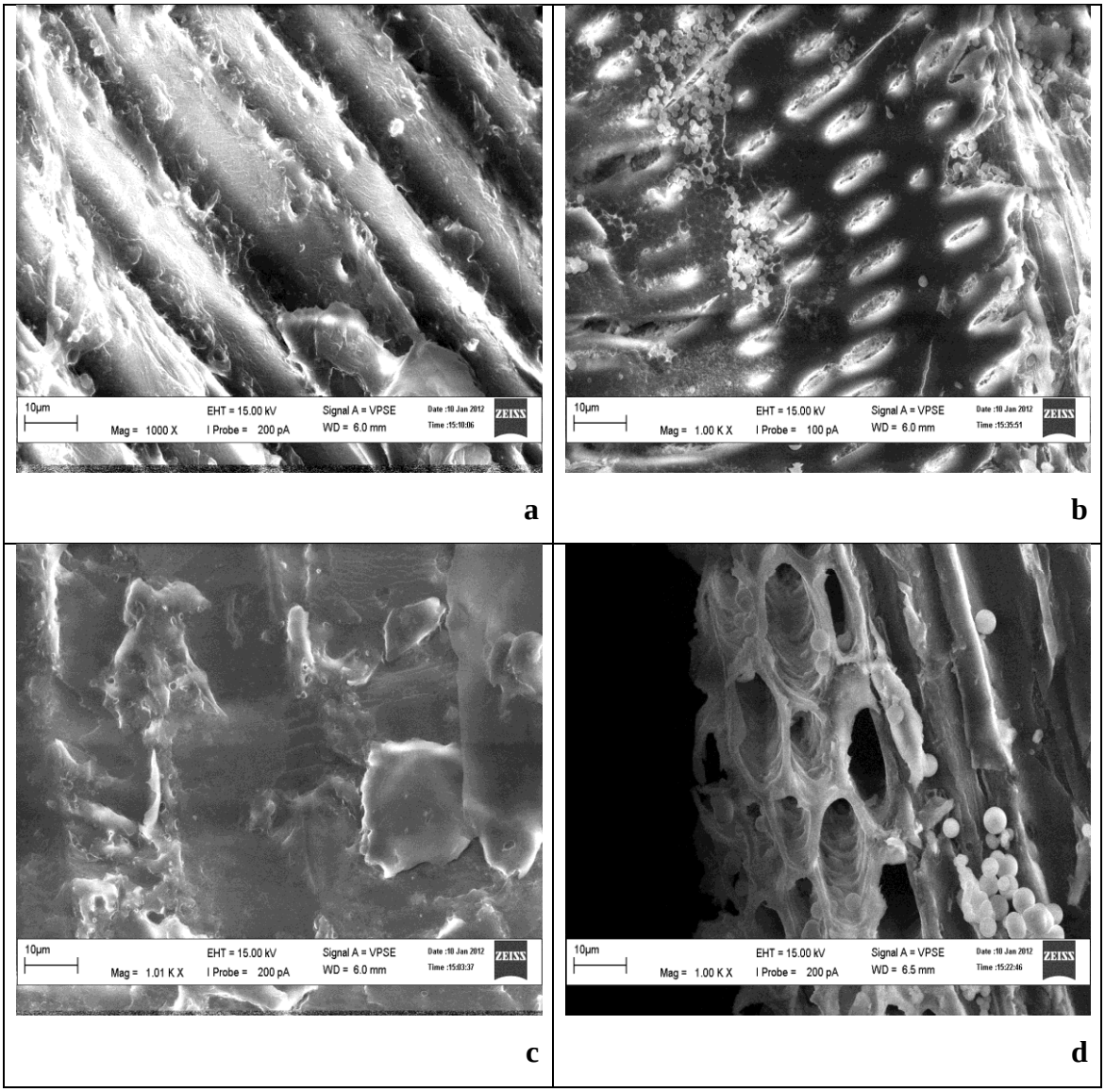

Figure 4.7: a) SEM micrograph of untreated *Resak* sawdust at 200µm. b) SEM micrograph of treated *Resak* sawdust at 200µm c) SEM micrograph of untreated *Resak* sawdust at 630µm. d) SEM micrograph of treated *Resak* sawdust at 630µm

From the results, xylose production was higher at 200µm particle size which is 22.52 g/l. However, the xylose production was lower at 630µm particle size which is 19.62 g/l. It has been observed that surface morphology of untreated and treated *Resak* species of sawdust composites is totally difference. The untreated sawdust was in the roughness surface like many bundles of chopsticks and flacks.

Then, hollow was obtained in the treated sawdust. The hollow was obtained after the treatment due to the degradation of hemicellulose from the acid hydrolysis process using diluted sulfuric acid. When the xylose was come out from the sawdust structure, the hollow was present in the structure. The amount of hollow that presents is dependent on the amount of xylose production. From the observation, in the treated 200 µm particle size of *Resak* species, many hollows were produce at the flacks' structure of sawdust.

4.5 LIGNIN DEGRADATION DETERMINATION

In the acid hydrolysis, lignin and cellulose was remained in the treated sawdust structure. However, to ensure that lignin is not degraded during the hydrolysis, direct spectroscopic Kappa number was employed to determine the lignin content in the sawdust samples (Table 4.3 and Table 4.4). Kappa number is an indication of the residual lignin content of wood by standardized analysis. It is used to monitor the lignin degradation in the hemicellulose extraction phase of hydrolysis process (Table 4.5).

According to one studies from Chai and Zhu (1998), the amount of kappa number that calculated from the samples is generally proportional to the lignin content. Lower sample kappa number represents lower contents of aliphatic hydroxyl groups and b-O-4 structures and contains higher phenolic hydroxyl groups and carboxylic acid groups. The untreated of sawdust was used in this experiment to employed as a control of the study for the basis of sawdust lignin content. From the results, it shows that, lignin degradation was present in small amount (less than 1). This is proved that, lignin

are not degraded into the hydrolysate phase during the acid hydrolysis process by using diluted sulfuric acid. It is remain the solid structure of treated sawdust.

Table 4.3: Lignin content for untreated sawdust

Species / Particle size (µm)	Kappa Number, K	Lignin content (wt%)
Keruing 315 µm	11.943	1.791
Keruing 400 µm	8.339	1.250
Meranti 200 µm	25.549	3.832
Meranti 630 µm	30.586	4.588
Resak 200 µm	18.643	2.796
Resak 630 µm	13.492	2.023

Table 4.4: Lignin content for treated sawdust

Species / Particle size (µm)	Kappa Number, K	Lignin content (wt%)
Keruing 315 µm	8.364	1.254
Keruing 400 µm	2.233	0.335
Meranti 200 µm	19.874	2.981
Meranti 630 µm	24.341	3.651
Resak 200 µm	13.037	1.955
Resak 630 µm	10.096	1.514

Table 4.5: Lignin degradation of sawdust

Species / Particle size (µm)	Lignin Degradation
Keruing 315 µm	0.299
Keruing 400 µm	0.732
Meranti 200 µm	0.222
Meranti 630 µm	0.204
Resak 200 µm	0.301
Resak 630 µm	0.251

4.6 Fourier Transform Infrared Spectroscopy (FTIR)

The formation of functional group in the hardwood species of untreated and treated sawdust was conducted using FTIR spectroscopic analysis. The FTIR spectrums of the untreated and treated wood sawdust from three species were come out with the same trend of spectrum. This is due to the all the samples are come from same functional group. The spectrum of untreated and treated wood sawdust as shown in Figure 4.8, Figure 4.9, Figure 4.10, Figure 4.11, Figure 4.12 and Figure 4.13.

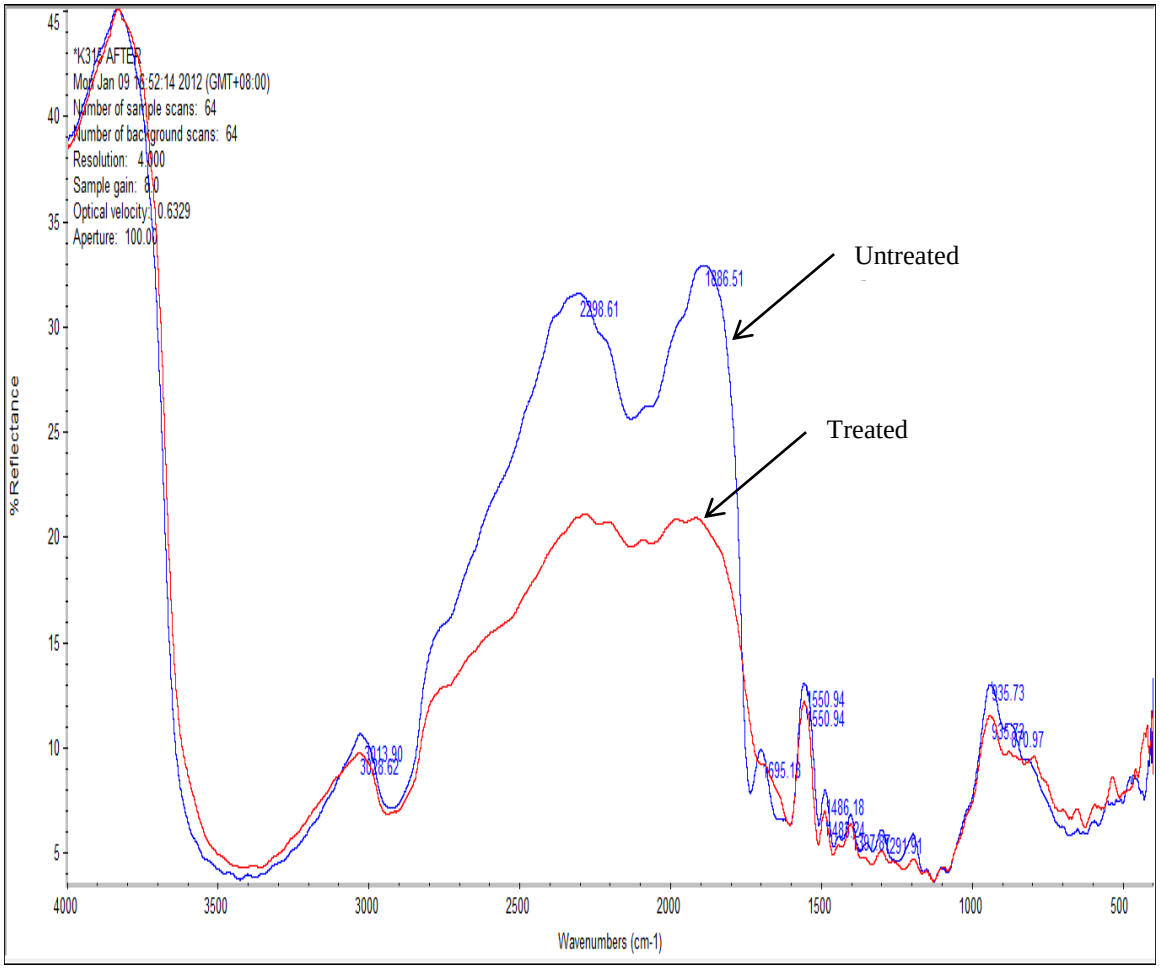

Figure 4.8: FTIR spectra of untreated and treated *Keruing* sawdust at 315µm

From the results of *Keruing* species at 315 µm, the FTIR spectrum of untreated wood sawdust shows the absorption bands in the region of 3800 cm^{-1}, 3013 cm^{-1} and 1995 cm^{-1} due to O-H stretching vibration, C-H stretching vibration, and C=O stretching vibration, respectively. These absorption bands are due to hydroxyl group in cellulose, carbonyl group of acetyl ester in hemicellulose, and carbonyl al-dehyde in lignin. However, there are consisting of impurities compound from thiol groups at the region of 2398 cm^{-1}.

Then, the absorption band at treated sawdust show at O-H which shifted towards 3775 cm^{-1} and at C-H the absorbance shifted towards into 3008 cm^{-1} respectively. From the observation, there are higher absorption bond produce at O-H groups due to the moister contained in the sawdust. It can be seen that, the carbonyl peak C=O at 1195 cm^{-1} was slightly shifted towards 1900 cm^{-1} in the spectra of treated sawdust because the ester carbonyl bonds in the hemicellulose was break due to the chemical treatment which is diluted acid hydrolysis process (Abdul Muna'im *et al.*, 2011). All the difference happens due to the chemical treatment using diluted sulfuric acid onto the sawdust.

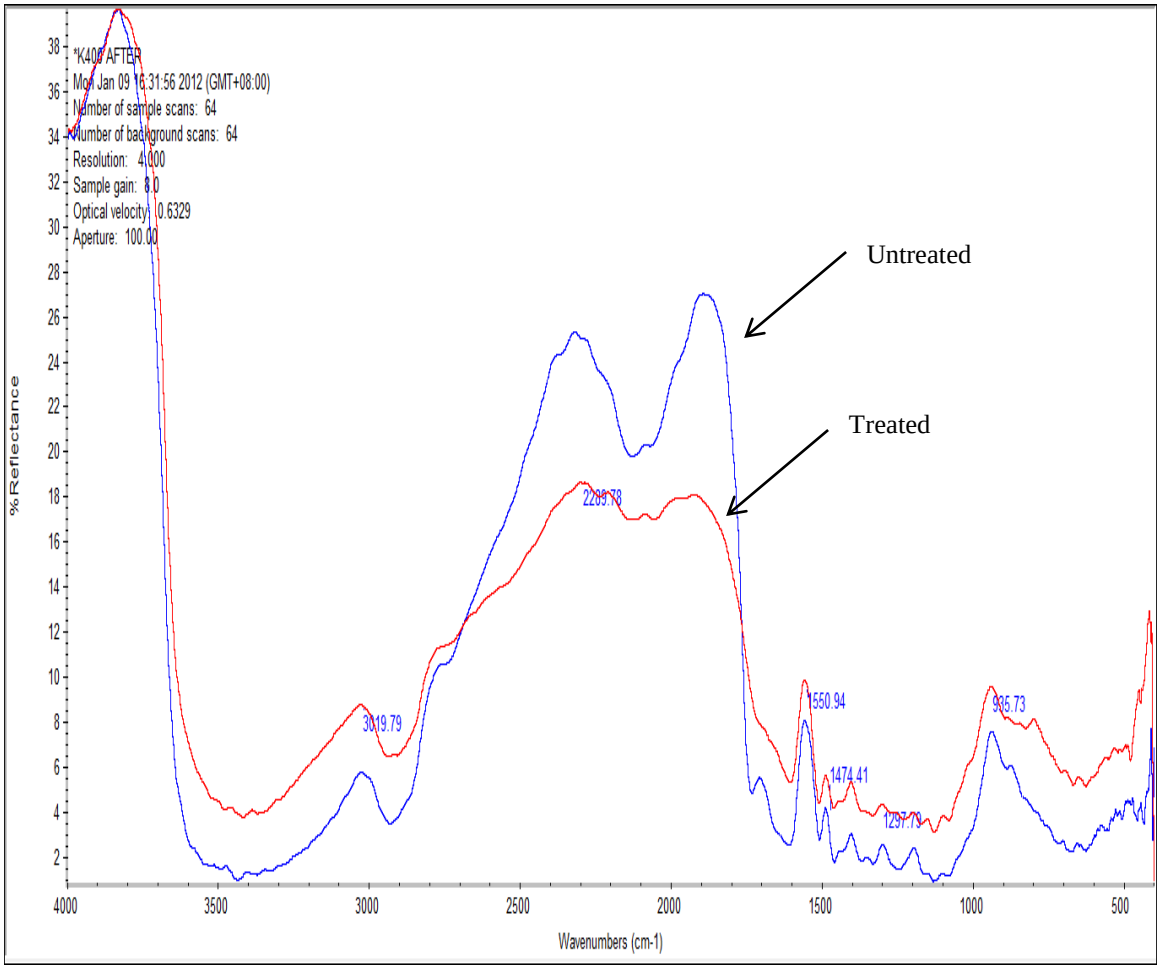

Figure 4.9: FTIR spectra of untreated and treated *Keruing* sawdust at 400µm

From the results of *Keruing* species at 400 µm, the FTIR spectrum of untreated wood sawdust shows the absorption bands in the region of 3820 cm^{-1}, 3019 cm^{-1} and 1711 cm^{-1} due to O-H stretching vibration, C-H stretching vibration, and C=O stretching vibration, respectively. These absorption bands are due to hydroxyl group in cellulose, carbonyl group of acetyl ester in hemicellulose, and carbonyl al-dehyde in lignin. However, there are consisting of impurities compound from thiol groups at the region of 2350 cm^{-1}.

Then, the absorption band at treated sawdust show at O-H remains unchanged at 3820 cm^{-1} and at C-H the absorbance shifted towards into 3015 cm^{-1} respectively. From the observation, there are higher absorption bond produce at O-H groups due to the moister contained in the sawdust. It can be seen that, the carbonyl peak C=O at 1711 cm^{-1} was slightly shifted towards 1700 cm^{-1} in the spectra of treated sawdust because the ester carbonyl bonds in the hemicellulose was break due to the chemical treatment which is diluted acid hydrolysis process (Abdul Muna'im *et al.,* 2011). All the difference happens due to the chemical treatment using diluted sulfuric acid onto the sawdust.

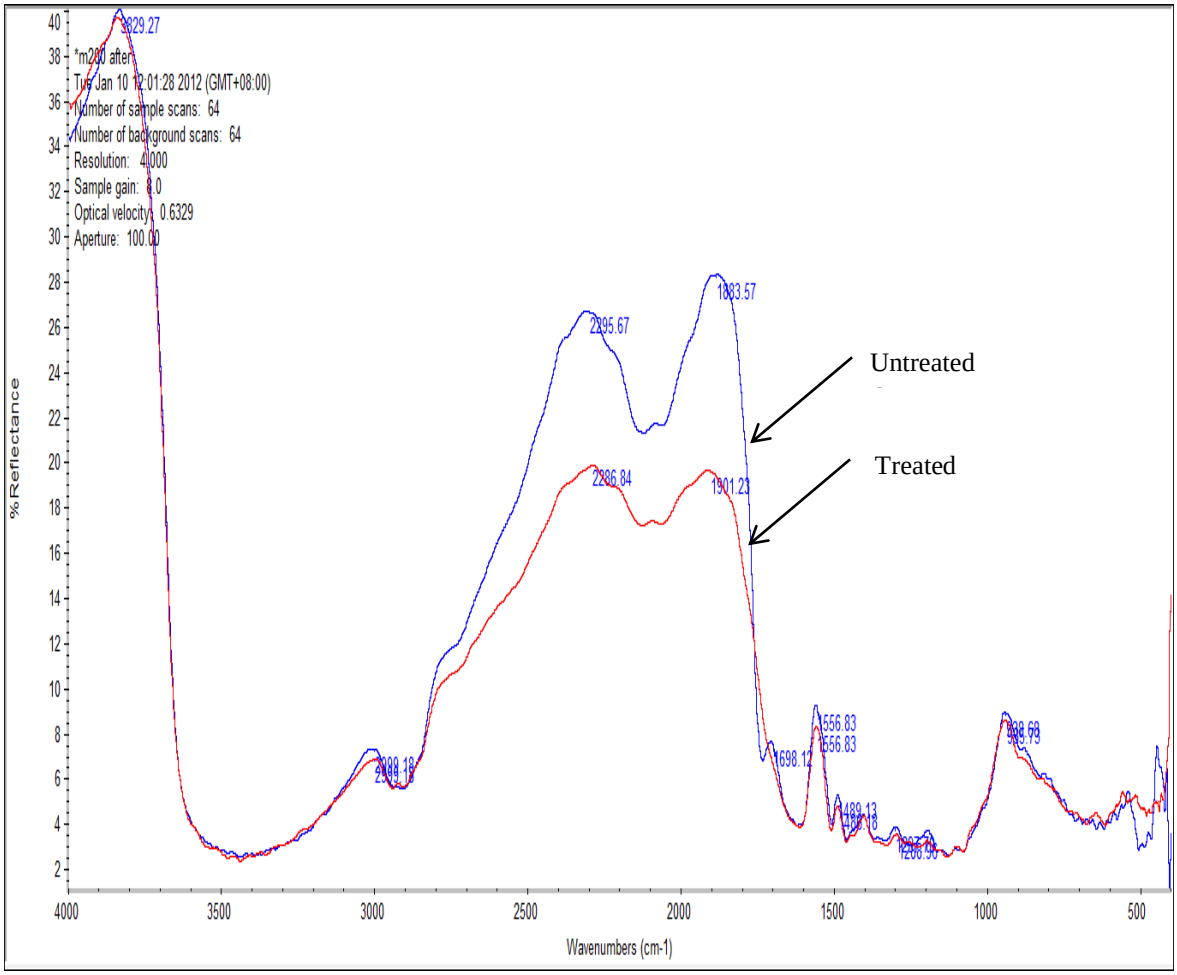

Figure 4.10: FTIR spectra of untreated and treated *Meranti* sawdust at 200µm

From the results of *Meranti* species at 200 µm, the FTIR spectrum of untreated wood sawdust shows the absorption bands in the region of 3829 cm^{-1}, 2999 cm^{-1} and 1556 cm^{-1} due to O-H stretching vibration, C-H stretching vibration, and C=O stretching vibration, respectively. These absorption bands are due to hydroxyl group in cellulose, carbonyl group of acetyl ester in hemicellulose, and carbonyl al-dehyde in lignin. However, there are consisting of impurities compound from thiol groups at the region of 2395 cm^{-1}.

Then, the absorption band at treated sawdust show at O-H shift to 3835 cm^{-1} and at C-H the absorbance shifted towards into 2955 cm^{-1} respectively. From the observation, there are higher absorption bond produce at O-H groups due to the moister contained in the sawdust. It can be seen that, the carbonyl peak C=O at 1556 cm^{-1} was slightly shifted towards 1554 cm^{-1} in the spectra of treated sawdust because the ester carbonyl bonds in the hemicellulose was break due to the chemical treatment which is diluted acid hydrolysis process (Abdul Muna'im *et al.*, 2011). All the difference happens due to the chemical treatment using diluted sulfuric acid onto the sawdust.

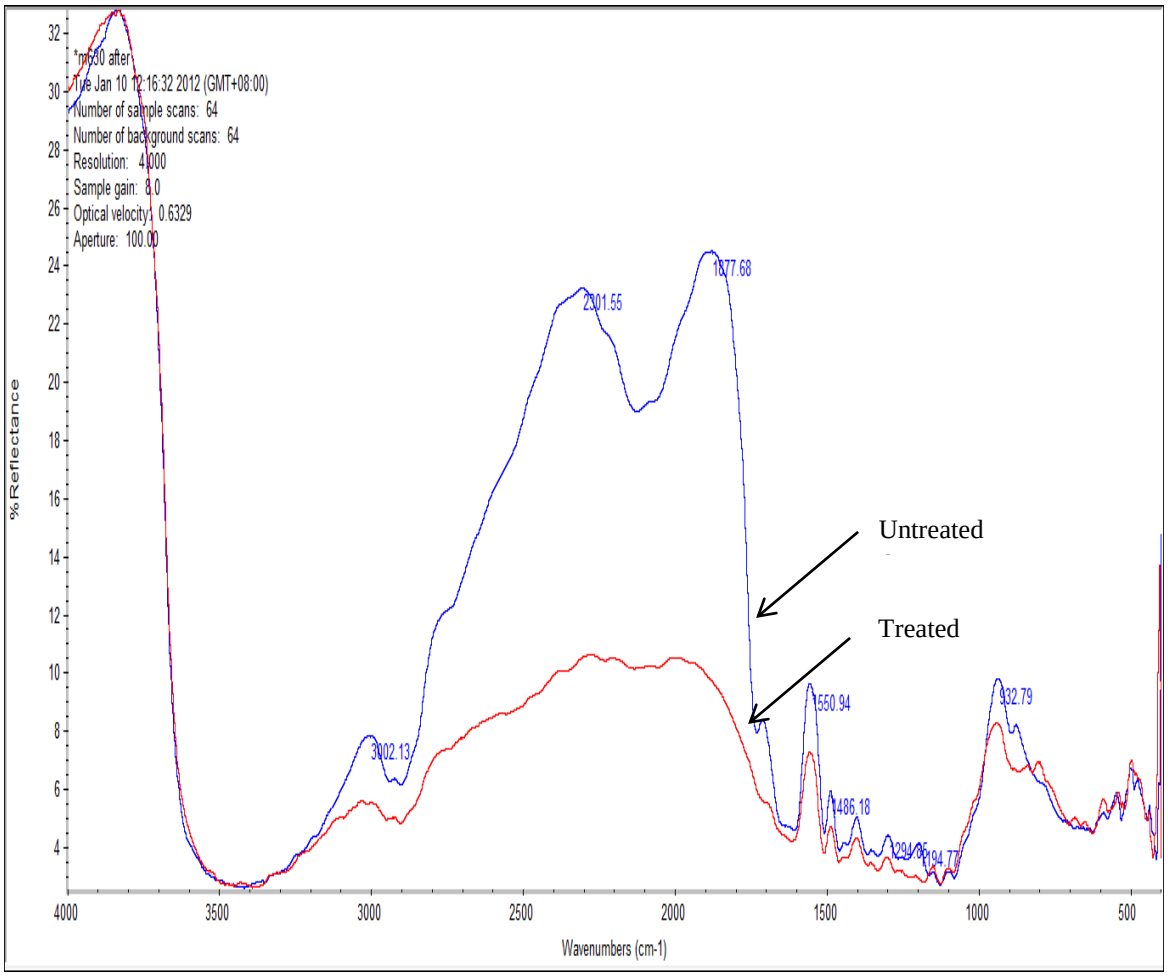

Figure 4.11: FTIR spectra of untreated and treated *Meranti* sawdust at 630µm

From the results of *Meranti* species at 630 µm, the FTIR spectrum of untreated wood sawdust shows the absorption bands in the region of 3800 cm^{-1}, 3012 cm^{-1} and 1550 cm^{-1} due to O-H stretching vibration, C-H stretching vibration, and C=O stretching vibration, respectively. These absorption bands are due to hydroxyl group in cellulose, carbonyl group of acetyl ester in hemicellulose, and carbonyl al-dehyde in lignin. However, there are consisting of impurities compound from thiol groups at the region of 2301 cm^{-1}.

Then, the absorption band at treated sawdust show at O-H remained unchanged at 3800cm^{-1} and at C-H the absorbance shifted towards into 3000 cm^{-1} respectively. From the observation, there are higher absorption bond produce at O-H groups due to the moister contained in the sawdust. It can be seen that, the carbonyl peak C=O at 1550 cm^{-1} was slightly shifted towards 1548 cm^{-1} in the spectra of treated sawdust because the ester carbonyl bonds in the hemicellulose was break due to the chemical treatment which is diluted acid hydrolysis process (Abdul Muna'im *et al.,* 2011). All the difference happens due to the chemical treatment using diluted sulfuric acid onto the sawdust.

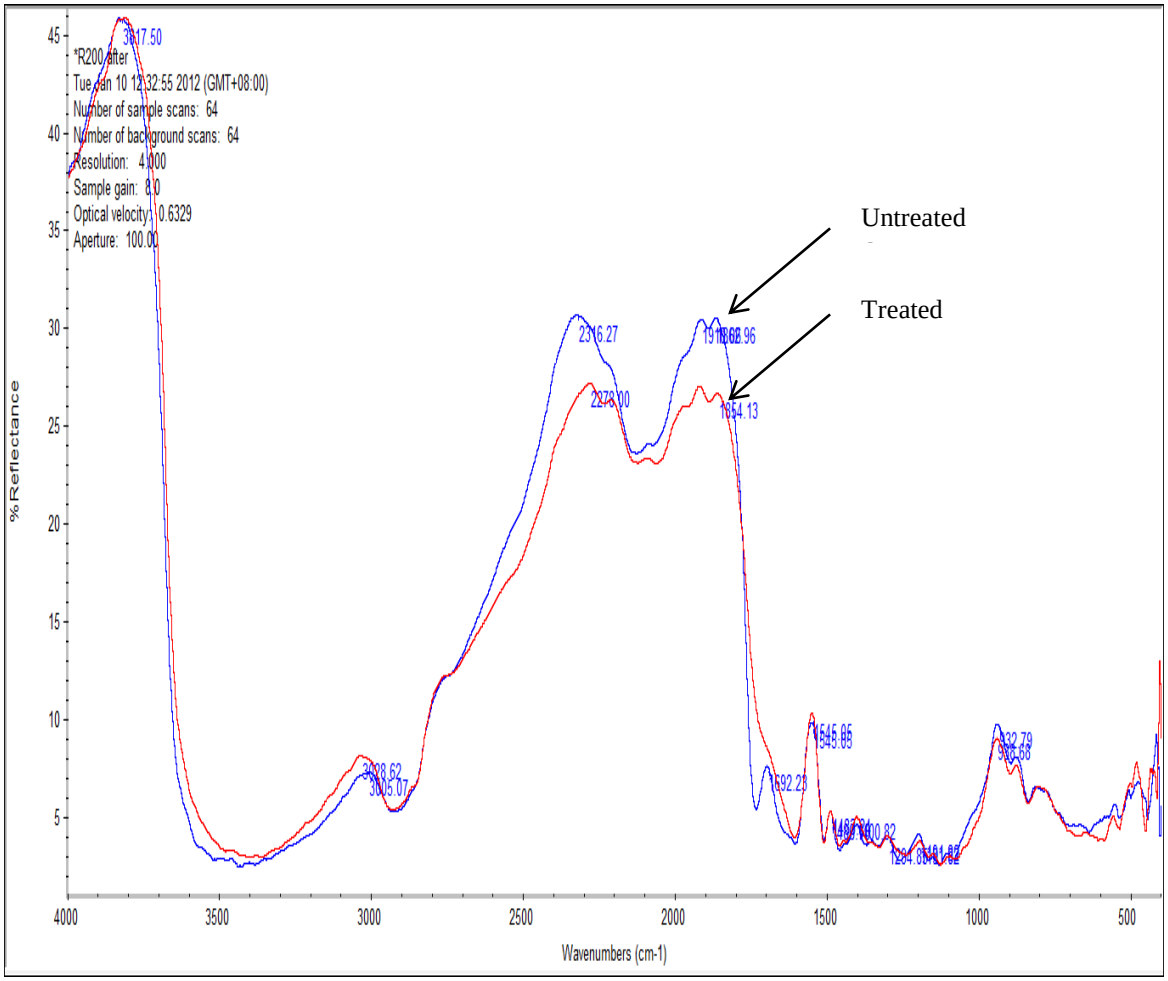

Figure 4.12: FTIR spectra of untreated and treated *Resak* sawdust at 200µm

From the results of *Resak* species at 200 µm, the FTIR spectrum of untreated wood sawdust shows the absorption bands in the region of 3817 cm^{-1}, 3098 cm^{-1} and 1545 cm^{-1} due to O-H stretching vibration, C-H stretching vibration, and C=O stretching vibration, respectively. These absorption bands are due to hydroxyl group in cellulose, carbonyl group of acetyl ester in hemicellulose, and carbonyl al-dehyde in lignin. However, there are consisting of impurities compound from thiol groups at the region of 2316 cm^{-1}.

Then, the absorption band at treated sawdust show at O-H remained unchanged at 3817cm^{-1} and at C-H the absorbance shifted towards into 3005 cm^{-1} respectively. From the observation, there are higher absorption bond produce at O-H groups due to the moister contained in the sawdust. It can be seen that, the carbonyl peak C=O at 1545 cm^{-1} was slightly shifted towards 1543 cm^{-1} in the spectra of treated sawdust because the ester carbonyl bonds in the hemicellulose was break due to the chemical treatment which is diluted acid hydrolysis process (Abdul Muna'im *et al.*, 2011). All the difference happens due to the chemical treatment using diluted sulfuric acid onto the sawdust.

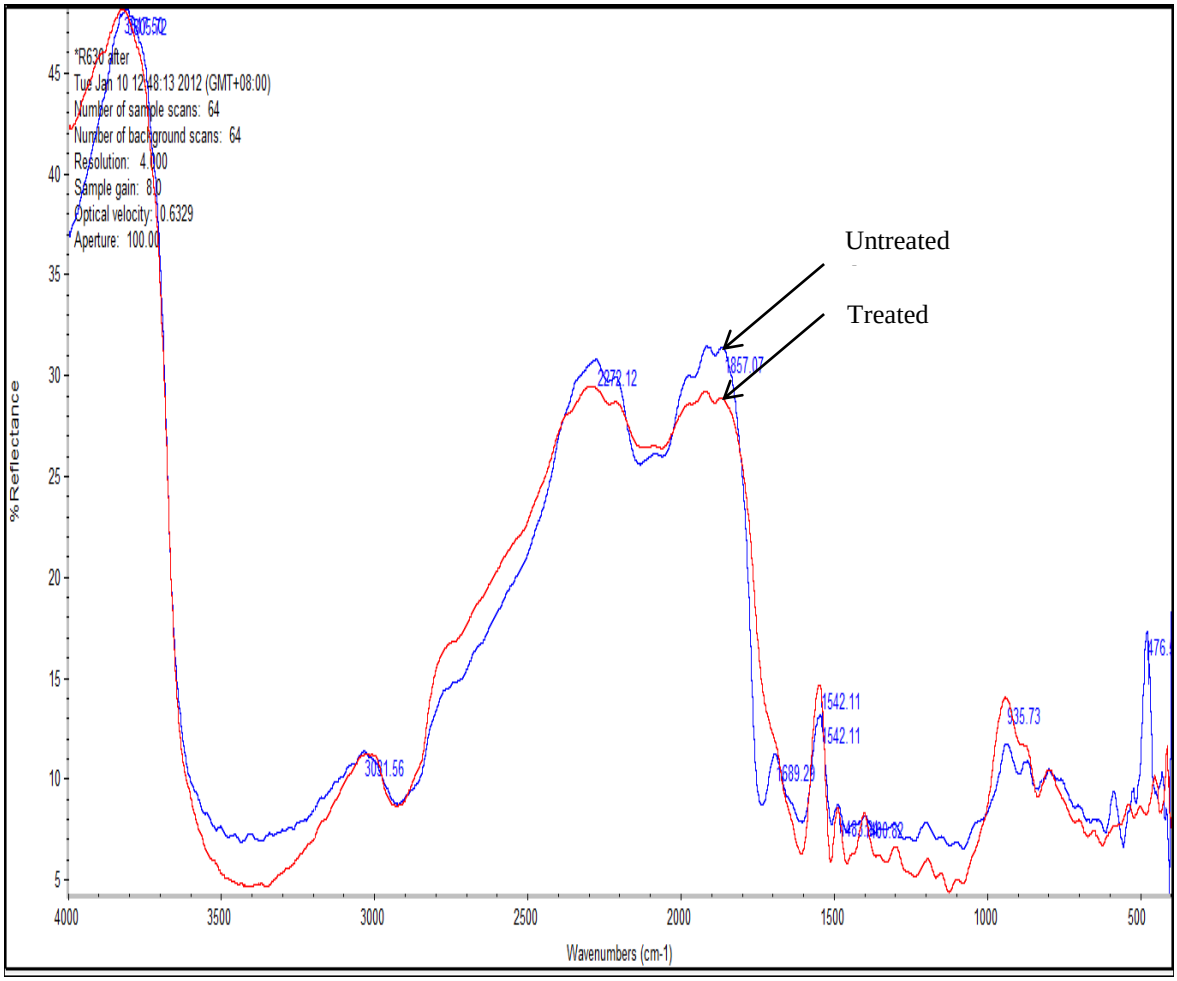

Figure 4.13: FTIR spectra of untreated and treated *Resak* sawdust at 630µm

From the results of *Resak* species at 6300 µm, the FTIR spectrum of untreated wood sawdust shows the absorption bands in the region of 3805 cm^{-1}, 3081 cm^{-1} and 1542 cm^{-1} due to O-H stretching vibration, C-H stretching vibration, and C=O stretching vibration, respectively. These absorption bands are due to hydroxyl group in cellulose, carbonyl group of acetyl ester in hemicellulose, and carbonyl al-dehyde in lignin. However, there are consisting of impurities compound from thiol groups at the region of 2272 cm^{-1}.

Then, the absorption band at treated sawdust show at O-H shift to 3800 cm^{-1} and at C-H the absorbance shifted towards into 3869 cm^{-1} respectively. From the observation, there are higher absorption bond produce at O-H groups due to the moister contained in the sawdust. It can be seen that, the carbonyl peak C=O at 1542 cm^{-1} was remained unchanged of treated sawdust because the ester carbonyl bonds in the hemicellulose was not completely break in the chemical treatment (Abdul Muna'im *et al.,* 2011). All the difference happens due to the chemical treatment using diluted sulfuric acid onto the sawdust.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Lignocellulosic material of hardwood species contains higher amount of hemicellulose, which is promising source of xylose. Hydrolysis of sawdust was carried out with dilute sulfuric acid (3.24%) at the temperature 121°C for 40 minutes to obtain a high concentration of xylose with low byproduct. However, the yield of xylose that obtained not depended on particle size but it is depend on structure itself. From the experiment, the higher xylose yield was 32.12 g/l from *Keruing* species at 400µm particle size. Then, the structure of sawdust was changes after the hydrolysis. A lot of hole was present in the structure of sawdust due to degradation of hemicellulose from the lignocellulosic material. Large amount of hemicellulose was degraded during acid hydrolysis while lignin is degraded in a small amount(less than 1%).

5.2 RECOMMENDATION

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

i. Studies on the structure of hardwood species should be done before continue to the acid hydrolysis process.
ii. Purification method for unwanted part should be done in order to get the pure xylose.
iii. Studies on the hazardous analysis of hardwood species of sawdust should be considered in order to prevent unwanted hazardous materials existing in the sugar production.

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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.

B) 0.02 M Sodium Precipitate ($KMnO_4$)

$$n = \frac{MV}{1000}$$

$$\frac{m}{Mw} = \frac{MV}{1000}$$

Where,

n = mol

m = mass of $KMnO_4$ needed

M = molarity @ concentration of $KMnO_4$ = 0.02M

V = volume of solvent (H_20), mL = 1000 mL

MW = molecular weight of NaOH, g/mol = 158.034 g/mol

Thus, 0.02M $KMnO_4$ was prepared as follow:

$$m = \frac{0.02 \text{ x } 1000}{1000} \; x \; 158.034$$

m = 3.16 g = **3.16 g of $KMnO_4$ (solid)** needed to be diluting with 1000 L distilled water to obtained 0.02M $KMnO_4$

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