DILUTE SULFURIC ACID PRETREATMENT FOR CELLULOSE RECOVERY FROM SAWDUST

ROZIALFI BINTI ALWANER

UNIVERSITI MALAYSIA PAHANG

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Signature : ……

Name of Supervisor : Dr Mimi Sakinah Abd Munaim

Date : ……

DILUTE SULFURIC ACID PRETREATMENT FOR CELLULOSE RECOVERY FROM SAWDUST

ROZIALFI BINTI ALWANER

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
Universiti Malaysia Pahang

APRIL 2010

I declare that this thesis entitled “Dilute sulfuric acid pretreatment for cellulose recovery from sawdust” 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 :……

Name : Rozialfi binti Alwaner

Date : 30 April 2010

Special dedication of this grateful feeling to my:

Beloved father and mother:

Mr Alwaner bin Saimir and Mrs Elfianis binti Rayhanis

Loving brothers and sisters:

Hennialfi, Mohd. Fazly and Mohd. Alhafiz

Supportive friend:

Mohd. Nabil bin Abd Hamid

For their loves, supports and best wishes.

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ABSTRACT

Cellulose has much function in producing valuable product such as bio-ethanol that has the same function with the crude oil and produce using cheaper and abundant raw material (biomass). The biomass that has been used in this research is sawdust. In order to recover cellulose from sawdust, it is necessary to treat the sawdust using dilute sulfuric acid pretreatment as to remove the lignin and hemicelluloses that bonding the cellulose structure. Compared with the untreated sawdust, 3.4 g/l glucose was dissolved from the cellulose, whereas hemicelluloses which are xylose and arabinose in pre-treated sawdust decreased to 2.5 g/l and 6.8 g/l, respectively. The results of infrared spectra (IR) and scanning electron microscope (SEM) analysis also showed that the structure and the surface of the sawdust were changed through pretreatment and crystalline cellulose in sawdust pre-treated was disrupted. The maximum cellulose recovery of sawdust was achieved at a sulfuric acid concentration of 4 % and pretreatment time of 120 minutes.

ABSTRAK

Terdapat pelbagai kegunaan selulosa seperti bio-etanol, yang mempunyai fungsi yang sama dengan minyak mentah, dan boleh dihasilkan daripada bahan mentah yang murah seperti biojisim. Bahan mentah yang digunakan dalam kajian ini adalah sisa habuk kayu gergaji. Untuk mendapatkan selulosa daripada sisa habuk kayu gergaji, sisa habuk kayu gergaji mestilah di rawat menggunakan prarawatan asid sulfurik cair bagi menyingkirkan lignin dan hemiselulosa daripada ikatan struktur selulosa. Perbandingan antara sisa habuk kayu gergaji yang dirawat dengan yang tidak dirawat adalah 3.4 g/l telah larut daripada selulosa kepada glukosa manakala hemiselulosa iaitu xilosa dan arabinosa menurun sebanyak 2.5 g/l dan 6.8 g/l. Keputusan analisa daripada spektrum infra merah (FTIR) dan imbasan mikroskopi electron (SEM) menunjukkan perubahan struktur dan permukaan sisa habuk kayu gergaji selepas prarawatan dan selulosa kristalin dalam serbuk gergaji prarawatan terganggu. Penghasilan maksimum selulosa daripada habuk kayu gergaji pada kepekatan asid sulfurik cair 4% dan masa prarawatan 120 minit.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF NOMENCLATURES

$℃$	Celcius
HPLC	High performance liquid chromatography
H_2SO_4	Sulfuric acid
FTIR	Fourier transform infrared
SEM	Scanning electron microscopy
NaOH	Sodium hydroxide

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of study

Nowadays, ethanol has higher demand because it use as a vehicles fuel because of the environment problem in recent years. Lignocelluloses such as cellulose, hemicelluloses and lignin are usually use as a raw materials in the production of ethanol. Lignocelluloses biomass is believed to be less expensive and more plentiful than either starch or sucrose containing feedstock. If the materials such as forest residues like sawdust and wood bark, agricultural residues like corn stover or herbaceous grass like switch grass as well as municipal waste are used as feedstock, lignocelluloses based bio-fuels could replace about 30% petroleum currently consumed by the USA. Forest biomass such as sawdust and wood bark are believed to be one of the most abundant sources of sugars, although much research has been reported on herbaceous grass such as switch grass, agricultural residue such as corn stover and municipal waste (Hu *et al*., 2008).

Besides that, the polysaccharides which are cellulose and hemicelluloses present in the lignocelluloses biomass need to be hydrolyzed with acids or enzymes in order to produce fermentable sugars. In many processes in the enzymatic conversion of lignocelluloses biomass to ethanol and other chemical products, a pretreatment stage is required to break the lignin structure and to partially solubilize the polysaccharides (Camassola and Dillon, 2008). Cellulose is a linear polymer of glucose in plant and woody materials. It is contains with hemicelluloses, other structural polysaccharides and surrounded by a lignin seal. Lignin is a complex 3-

dimensional polyaromatic matrix that forms a seal around cellulose micro fibrils and exhibits limited covalent associated with hemicelluloses. This prevents enzymes and acids from accessing some regions of the cellulose polymers (Weil *et al*., 1994).

Pretreatment is an important tool for practical cellulose conversion processes. Pretreatment is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars. Pretreatment also has great potential for improvement of efficiency and lowering of cost through research and development (Mosier *et al*., 2004). Several pretreatment methods such as steam explosion, solvent extraction, and thermal pretreatment using acids or bases and also biological pretreatments have been widely investigated. Otherwise, many pretreatment processes require expensive equipment and large quantities of energy (Camassola and Dillon, 2008). Dilute acid pretreatment has been widely investigated. This is because it is effective and inexpensive among all the pretreatment methods. Beside it can improve cellulose conversion; it also can effectively solubilize hemicelluloses into monomeric sugars and soluble oligomers (Sun and Cheng, 2004).

1.2 Problem Statement

As we know, the waste of wood such as sawdust was abundant in Malaysia. This is because in Malaysia, many products from wood such as furniture, papers, and houses. The abundant of the sawdust can increase the pollution of the environment. Furthermore, cellulose cannot biodegradable by mammalian digestive enzymes because it has very long chain and complex structure. It will take a long time to biodegradable so it consider as a non biodegradable.

1.3 Research Objectives

The objective of this research is to study the recovery of the cellulose from the sawdust.

1.4 Scope of Study

In order to achieve the objective of the research study, several scope of study has been identified such as to study the effects of parameters which are H_2SO_4 concentration and residences time for dilute acid pretreatment process. Besides that, to investigate the cellulose, hemicelluloses and lignin composition in sawdust using Fourier Transform Infrared (FTIR) and Scanning Electron Microscopy (SEM) and to analyze the monomer sugars using High Performance Liquid Chromatography (HPLC).

1.5 Significant of the Study

In using of sawdust as a raw material can consider as a low cost because sawdust was abundant and inexpensive in Malaysia. Otherwise, the composition of the cellulose is plenty in sawdust. The food industry, medical industry and also chemical industry can get more profit because of the inexpensive of the sawdust. The reuse of the sawdust also can reduce the pollution of the environment. Besides that,

the production of cellulose has a potential in a future because from the cellulose, many valuable product can be produce such as a bio-ethanol which is the fuel that has same function with the crude oil like petrol but the bio-ethanol has a lower cost and lower price than it. Besides that, the production of sorbitol also one of the products from cellulose. Sorbitol has higher demand because it widely used in the food industry, not only as a sweetener but also as a humectants, texturizer, and softener. Its caloric value is similar to glucose, but it is less capable of causing hyperglycemia because it is converted to fructose in the liver. Other sorbitol applications include pharmaceutical, cosmetic, textile, and paper goods.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Lignocelluloses biomass is mainly composed of cellulose, hemicelluloses and lignin. In enzymatic hydrolysis, cellulose was hydrolyzed to its monomeric constituents and then fermented to ethanol or other products. Otherwise, the network between lignin-hemicelluloses were embedded cellulose fibers was slow the cellulose biodegradation by cellulolytic enzymes. Because of that, pretreatment process is important to remove the protecting shield of lignin-hemicelluloses, and make the cellulose that produce is suitable for enzymatic hydrolysis (Esteghlalian *et al*., 1996).

Otherwise, the lignocellulosic feedstock was very effective raw material because it can reduce the cost production of ethanol because it is less expensive and also available in large quantities (Silverstein *et al*., 2007).

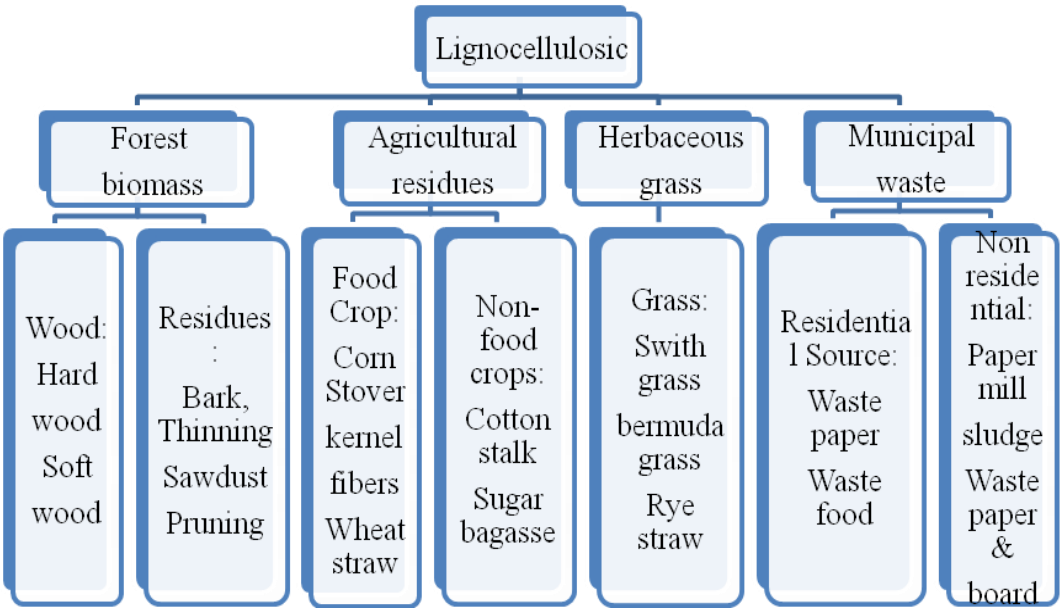

Figure 2.1 : Sources of Lignocellulosic.

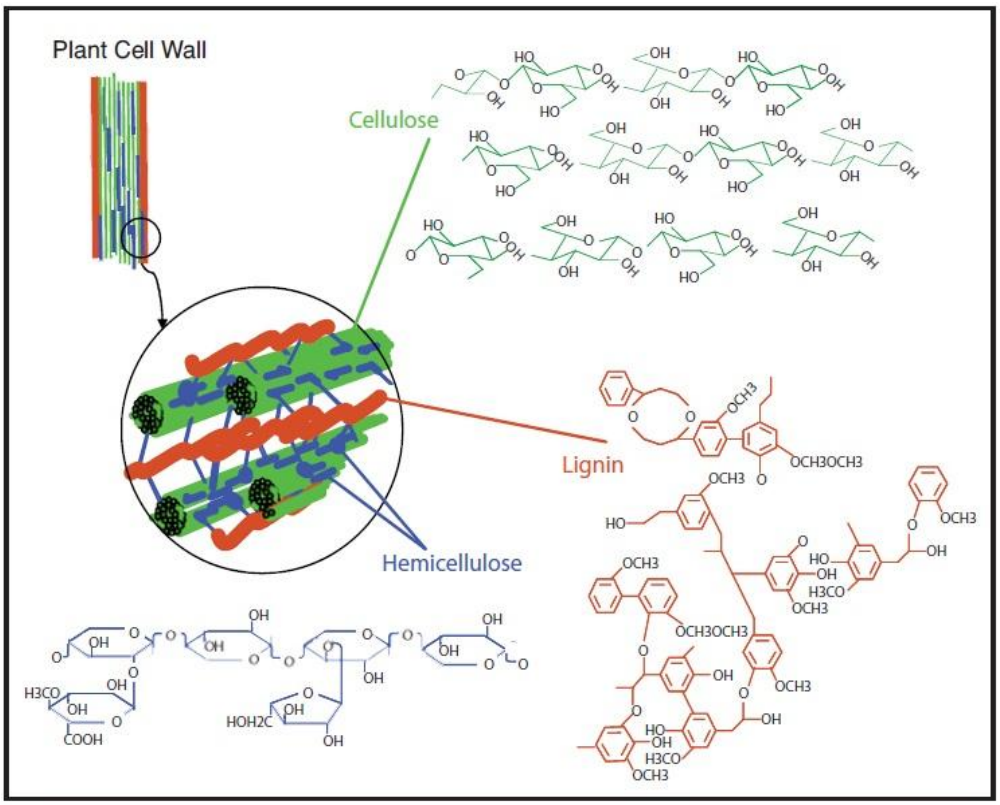

Figure 2.2 : Structure of cellulose, hemicelluloses, and lignin.

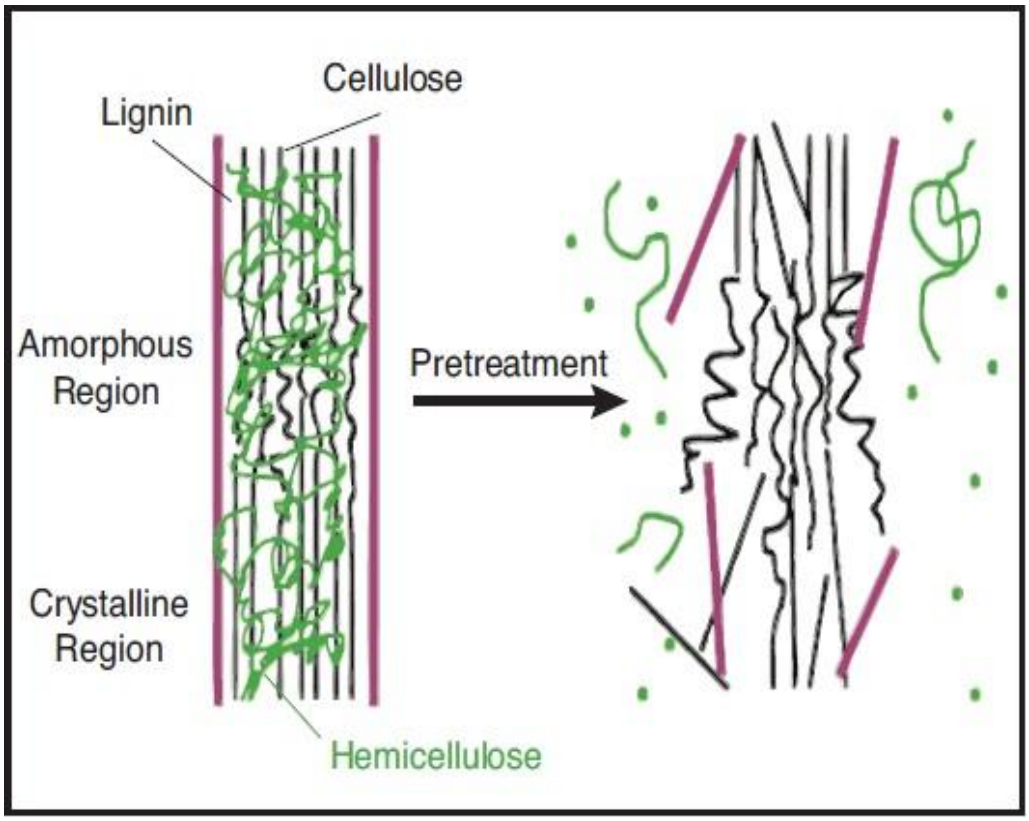

Figure 2.3 : Schematic of goals of pretreatment on lignocellulosic material.

Many ways of pretreatment process such as physical treatment like a high energy radiation, steam explosion and ball milling, chemical treatment with acid or basic catalysts, and biological treatments. Pretreatment can affect the structure of biomass by solubilizing or otherwise altering hemicelluloses, altering lignin structure, reducing cellulose crystallinity and increasing the available surface area and pore volume of the substrate. During pretreatment, hemicelluloses may be hydrolyzed to their monomeric constituents and lignin- hemicelluloses-cellulose interactions partially disrupted (Esteghlalian *et al*., 1996).

2.2 Raw material for recovery cellulose.

There are several feedstocks for recovery cellulose which is starch, corn stover, wheat straw, sugar bagasse that are among the agricultural residues. Table 2.1 show the percent dry weight composition of lignocelluloses in biomass feedstock.

Table 2.1 : Percent dry weight composition of lignocelluloses.

Feedstock	Glucan (cellulose)	Xylan (hemicellulose)	Lignin
Corn stover[a]	37.5	22.4	17.6
Corn fiber[b,c]	14.28	16.8	8.4
Pine wood[d]	46.4	8.8	29.4
Popular[d]	49.9	17.4	18.1
Wheat straw[d]	38.2	21.2	23.4
Switch grass[d]	31.0	20.4	17.6
Office paper[d]	68.6	12.4	11.3

Lignocellulosic complex is the most abundant biopolymer in the Earth. It is considered that lignocellulosic biomass comprises about 50% of world biomass and its annual production was estimated in 10–50 billion ton. Many lignocellulosic materials have been tested for bioethanol production as observed. In general, prospective lignocellulosic materials for fuel ethanol production can be divided into six main groups: crop residues such as cane bagasse, corn stover, wheat straw, rice straw, rice hulls, barley straw, sweet sorghum bagasse, olive stones and pulp, hardwood such as aspen and poplar, softwood such as pine and spruce, cellulose wastes such as newsprint, waste office paper and recycled paper sludge, herbaceous biomass such as alfalfa hay, switchgrass, reed canary grass, coastal Bermudagrass and thimothy grass.

Figure 2.4 : Sugarcane crop for feedstock of recovery cellulose.

Figure 2.5 : Corn stover as a raw material for recovery cellulose.

Figure 2.6 : Rice straw as a feedstock of recovery cellulose.

Figure 2.7 : Cellulosic waste use as a raw material for recovery cellulose.

Nowadays, the forest product industry was discarded large quantities of cellulosic waste products because they cannot be utilized as food for man in their present forms. Besides that, woody biomass such as sawdust was containing 70 to 80% carbohydrates. Furthermore, woody biomass has physically larger and structurally stronger and denser and also has higher lignin content (Zhu and Pan, 2009). Besides that, sawdust from hardwood has high lignin and low intracellular nutrient content than softwood sawdust. Because of that, only a small percentage of these carbohydrates can be utilized by the ruminant (Keith and Daniels, 1976).

Figure 2.8 : Sawdust from sawmill.

Figure 2.9 : Hardwood sawdust for cellulose recovery.

Sawdust is a waste by-product of the timber industry that is either used as cooking fuel or a packing material. It is composed of three important constituents such as cellulose, lignin, and hemicelluloses. Sawdust is not only abundant, but also it is actually an efficient adsorbent that is effective to many types of pollutants, such as dyes, oil, salt and heavy metals. Many agricultural by-products are little or no economic value, and some, such as sawdust, which are available in large quantities in lumber mills, are often present a disposal problem (Pekkuz, 2007).

2.3 Cellulose (Product)

In herbaceous and woody plants, cellulose exists as a linear polymer of glucose. Besides that, cellulose also associated with another polysaccharide, hemicelluloses and seal with lignin which is a complex three dimensional polychromatic compound that is resistant to enzyme and acid hydrolysis (Weil *et al*., 1998).

Cellulose exists of D-glucose subunits, linked by b-1, 4 glycosidic bonds. In plant consists two part of cellulose which is organized part that contain a crystalline structure and another part is not well organized that contain amorphous structure. Cellulose fibrils or cellulose bundles were the cellulose strains that 'bundled' together. These cellulose fibrils are mostly independent and weakly bound through hydrogen bonding (Hendriks and Zeeman, 2008).

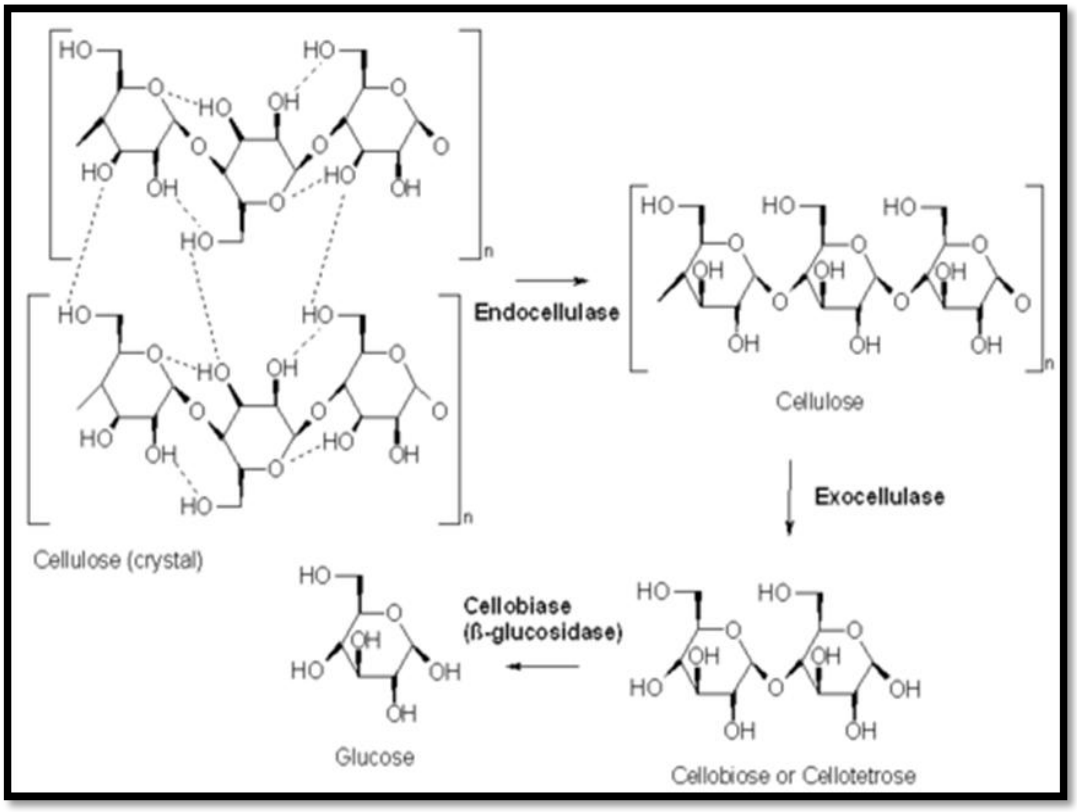

Figure 2.10 : Structure of Cellulose.

Cellulose, like starch, is a polymer of glucose. However, unlike starch, the specific structure of cellulose favors the ordering of the polymer chains into tightly packed, highly crystalline structures that is water insoluble and resistant to depolymerization (Mosier *et al*., 2005). Besides that, cellulose can be enzymatically hydrolyzed to its monomeric constituents (glucose units) and then fermented to ethanol or other products (Esteghlalian *et al*., 1997).

2.4 Pretreatment Process

In general, pretreatment can be classified into biological pretreatment, physical pretreatment and also chemical pretreatment according to the different force or energy consumed in the pretreatment process. Some pretreatment combines any two or all of these pretreatment and can be produce subcategories. Biological pretreatment has not attached much attention, probably because of kinetic and economic considerations; although there have been various researches showing biological pretreatment can be an effective way to recover sugars from different species of biomass.

Physical and chemical pretreatments have been the subject of intensive research. Steam and water are usually excluded from being considered as chemical agent for pretreatment, since no extra chemical are added to the biomass. Physical pretreatment include comminution, in which the particle sizes of the biomass are reduced with mechanical forces, steam explosion, and hydrothermalysis.

Acids or bases promote hydrolysis and improve sugar recovery yield from cellulose by removing hemicelluloses or lignin during pretreatment. Sulfuric acid and sodium hydroxide are the most commonly used acid and base, respectively. Another approach of for pretreatment is to use liquid formulations capable for acting as solvent for cellulose. Work with cellulose solvent systems has shown the enzymatic hydrolysis could be greatly improved, but the work mainly has been restricted to agricultural residues and herbaceous grass.

Little has been reported about the use of cellulose solvents in pretreating forest biomass such as wood, bark, or mixtures of such residue. A broad range of chemical pretreatment, such as concentrated mineral acids which is sulfuric acid and hydrochloric acid, ammonia based solvent, aprotic solvents, as well as wet oxidation also reduce cellulose crystalline, disrupt the association of lignin with cellulose, and dissolve cellulose. However, the economics of these methods do not permit any practical application when compared to the value of glucose. Lime pretreatment and ammonium pretreatment have seemed to be the most attractive alkaline pretreatment, while most attention in acid pretreatment has been concentrated on the use of sulfur

dioxide and sulfuric acid. These two acid pretreatment have also been combined to the steam pretreatment (Hu *et al.*, 2008).

Pretreatment is required to disrupt the structure of lignocellulosic materials during cellulosic ethanol production, because the extensive interactions among cellulose, hemicellulose and lignin, and the barrier nature of lignin minimize enzyme access to the carbohydrates and result in poor yields of fermentable sugars. The major effect of alkaline pretreatments is the delignification of lignocellulosic biomass, thus enhancing the reactivity of the remaining carbohydrates.

Pretreatment can affect the structure of biomass by solubilizing or otherwise altering hemicelluloses, altering lignin structure, reducing cellulose crystallinity and increasing the available surface area and pore volume of the substrate. During pretreatment, hemicelluloses may be hydrolyzed to their monomeric constituents and lignin-hemicelluloses-cellulose interactions partially disrupted (Esteghlalian *et al*., 1997).

Table 2.2 : Pretreatment Method for Lignocellulose.

Pretreatment	Energy		Effect
	Source	Means	
Biological Pretreatment	Microbe	Fungi	Reduce DP of cellulose and hemicellulose
		Actinomycetes	Remove Lignin
Physical Pretreatment	Comminution	Ball Milling	Decrease particle size, cellulose crystallinity & DP
		Colloid Milling	
		Hammer Milling	
		Compression Milling	
	Irradiation	Electron Beam	Increase surface area and pore sizes
		Gamma-ray	Soften and partially deploymerize lignin
		Microwave	
	Hydrothermolysis	Liquid Hot Water	
	Steam Explosion	High Pressure Steam	Partialy hydrolyze hemicellulose
	Other Mechanical Energy	Expansion	
		Extrusion	
Chemical Pretreatment	Acid	carbonic acid	Decrease Crystallinity of Cellulose and its DP Partial or complete hydrolysis of hemicellulose Delignification
		hydrocholric acid	
		hydrofluoric acid	
		nitric acid	
		peracetic acid	
		phosphoric acid	
		sulfur dioxide	
		sulfuric acid	
	Alkaline	lime	
		sodium hydroxide	
		sodium carbonate	
		ammonia	
		ammonium sulfite	
	Gas	Chlorine dioxide	
		Nitrogen Dioxide	
	Oxidant	Hydrogen Peroxide	
		Ozone	
		Wet Oxidation	
	Cellulose Solvent	Cadoxen	
		CMCS	
		DMSO	
	Extraction of Lignin	Hydrozine	
		Ethanol-Water	
		Benzene-Water	
		Ethylen Glycol	
		Butanol-Water	
		Swelling Agent	

Table 2.3 : Effect of pretreatment on the chemical composition and chemical or physical structure of lignocelluloses biomass.

Pretreament Method	Accessible surface area increases	Cellulose Decrystalization	Hemicellulose Removal	Lignin Removal	Lignin Structure Alteration
Uncatalyzed steam explosion	●		●		○
Liquid hot water	●	ND	●		○
pH controlled hot water	●	ND	●		ND
Flow-through liquid hot water	●	ND	●	○	○
Dilute acid	●		●		●
Flow-through acid	●		●	○	●
Lime	●	ND	○	●	●
Ammonia freeze explosion(AFEX)	●	●	○	●	●
Ammonia recycled percolation(ARP)	●	●	○	●	●

●: Major Effect
○: Minor Effect
ND: Not Determined

Biological pretreatment has low energy requirements and mild environmental conditions. However, most of these processes are too slow limiting its application at industrial level. Many white-rot fungi degrade the lignin and, for this reason, they have been utilized for ligninases production and lignocellulose degradation. Reports the main microorganisms producing lignin-degrading enzymes and indicates the fermentation processes for producing them by both submerged culture and solid-state fermentation. In fact, the fungus *Phanerochaete chrysosporium* has been proposed in the patent of for degrading the lignin in a biomass-to-ethanol process scheme involving the separate fermentation of pentoses and hexoses and highlight the viability of producing cellulases and hemicellulases by solid-state fermentation. According to preliminary evaluations of the NREL, the cost of cellulases produced *in situ* by submerged culture is US$0.38/100,000 FPU (Filter Paper Units, a way for measuring cellulase activity). Thus, cellulase costs comprise 20% of ethanol production costs assuming them in US$1.5/gallon. On the other hand, commercial cellulase cost (US$16/100,000 FPU) is prohibitive for this process. In contrast, these authors indicate that the cost of producing cellulases by solid-state fermentation of corn stover could reach US$0.15/100,000 FPU that would correspond to US$0.118/gal EtOH, i.e. near 8% of total costs.

One of the main problems during the pretreatment and hydrolysis of biomass is the variability in the content of lignin and hemicellulose. This variability depends on factors as the type of plant from which the biomassis obtained, crop age, method of harvesting, etc. This makes that no one of the pretreatment methods could be applied in a generic way for many different feedstocks. The future trends for improving the pretreatment of lignocellulosic feedstocks also include the production of genetically modified plant materials with higher carbohydrate content or modified plant structure to facilitate pretreatment in milder conditions or using hemicellulases. It is estimated that the use of these new materials along with improved conversion technologies, could reduce the ethanol cost from lignocellulosic biomass in US$0.11/L in the next ten years (Sa´ nchez and Cardona, 2008)

Several studies have shown the potential of sodium hydroxide pretreatment on a variety of lignocellulosic materials. Furthermore, sodium hydroxide can enhances lignocelluloses digestibility by increasing internal surface area, decreasing the degree of polymerization and the crystallinity of cellulose, and separating structural linkages between lignin and carbohydrates effectively. Besides that, the digestibility of sodium hydroxide treated hardwood increased with the decrease of lignin content (Wang *et al.*, 2010).

Otherwise, the porosity of the lignocellulosic materials increases with the removal of the crosslinks which is lignin (Sun and Cheng, 2002). The major effect of alkaline pretreatments is the delignification of lignocellulosic biomass, thus enhancing the reactivity of the remaining carbohydrates (Wang *et al.*, 2010).

Besides that, based on the prominently researched and promosing technology, dilute acid pretreatment was chosen as the method for pretreatment. The function of acid in this pretreatment is to break down hemicellulose and opens the remaining structure for subsequent enzymatic hydrolysis. Furthermore, reaction conditions which favor the production of xylose monomer while minimizing degradation to furfural is preferred so as they do not inhibit subsequent enzymatic hydrolysis. Otherwise, the previous study shows that the optimal pretreatment conditions for these two processes are not necessarily the same (Jensen *et al*., 2010).

Pre-treatment of biomass with dilute sulfuric acid at high temperatures can effectively dissolve the hemicelluloses and increase the enzymatic digestibility of celluloses. The advantages of the dilute sulfuric acid pretreatment were high reaction rates, low acid consumption, and low cost of sulfuric acid. A dilute-acid pretreatment plant will not require an acid-recovery system, which seems to be essential for a pre-treatment plant using concentrated acid (Esteghlalian *et al*., 1996).

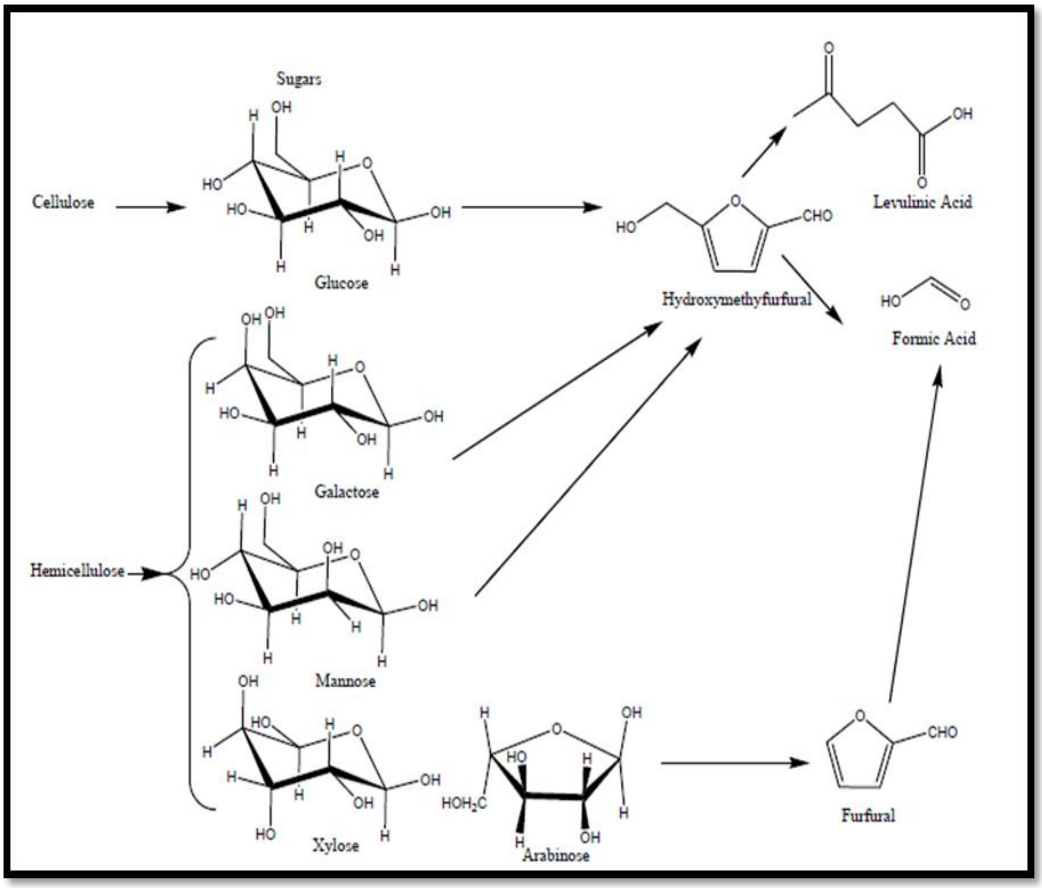

Figure 2.11 : Reaction occurring to carbohydrates during hydrolysis of lignocellulosic material.

The application of dilute acid pretreatment to woody biomass can achieve some level of success that can provide satisfactory cellulose conversion with certain hardwood species (Zhu and Pan, 2009). The dilute sulfuric acid pretreatment can effectively solubilize hemicelluloses into monomeric sugars which is arabinose, galactose, glucose, mannose, and xylose and soluble oligomers, thus improving cellulose conversion. Compared to other pretreatment methods, it is especially useful for the conversion of xylan in hemicelluloses to xylose that can be further fermented to ethanol by many microorganisms (Sun and Cheng, 2005).

Otherwise, dilute sulfuric acid pretreatment is effective due to be relatively inexpensive and to produce high hemicelluloses recoveries and cellulose digestibilities (Cara *et al*., 2008). Besides that, dilute acid pretreatment with sulfuric acid has been extensively researched because it is inexpensive and effective, although other acid such as nitric acid, hydrochloric acid and phosphoric acid has also been tested (Hu *et al*., 2008).

2.5 High Performance Liquid Chromatography (HPLC) Analysis

High-performance liquid chromatography (HPLC) is a form of column chromatography used frequently in biochemistry and analytical chemistry to separate, identify, and quantify compounds. HPLC utilizes a column that holds chromatographic packing material which is stationary phase, a pump that moves the mobile phases through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phases, the molecules being analyzed, and the solvents used (Xiang, 2006).

Besides that, high performance liquid chromatography (HPLC) analysis can use to analyze sugars of the hydrolysate. The advantage of this analysis is relatively fast, it is less sensitive for low sugar concentrations especially when the refractive index detector is used. Column regeneration is challenging and sample preparation is time-consuming. Furthermore, the resolution of sugars such as fructose and mannose are poor (Agblevor *et al*., 2004).

Figure 2.12 : High Performance Liquid Chromatography (HPLC) that using SUPELCOSIL LC – NH_2 as a column.

Furthermore, HPLC separations of carbohydrates depend on differences in conformation, configuration, and column type. For silica-based SUPELCOSIL LC-NH_2 columns, it uses to separate monosaccharides, disaccharides, and some trisaccharides. By using this column for separation, sugar retention decreases when ratio between water and acetonitrile in the mobile phase is increased. Sugars generally will be eluted in order of increasing molecular weight.

There are severel types of High Performance Liquid Chromatography (HPLC). One of it is Normal phase HPLC (NP-HPLC) or also known as adsorption chromatography. The concept of this type of HPLC is separates analytes based on adsorption to a stationary surface chemistry and by polarity. Otherwise, the Normal phase HPLC was one of the first kinds of HPLC that chemists developed. In this NP-HPLC, it uses a polar stationary phase and a non-polar for non-aqueous mobile phase.

This NP-HPLC works effectively for separating analytes readily soluble in non-polar solvents. The analyte associates with and is retained by the polar stationary phase. For the theory of NP-HPLC, elution time will increase when the interaction between the polar analyte and the polar stationary phase increase. This polarity is increased when the adsorption strengths increase (Snyder and Dolan, 2006).

2.6 Fourier Transform Infrared (FTIR) Analysis

FTIR is most useful for identifying chemicals that are either organic or inorganic. It can be utilized to measure some components of an unknown mixture. Besides that, FTIR can be applied to the analysis of solids, liquids, and gasses.

The term Fourier Transform Infrared Spectroscopy (FTIR) refers to a fairly recent development in the manner in which the data is collected and converted from an interference pattern to a spectrum. Today's FTIR instruments are computerized which makes them faster and more sensitive than the older dispersive instruments.

FTIR can be used to identify chemicals from spills, paints, polymers, coatings, drugs, and contaminants. FTIR is perhaps the most powerful tool for identifying types of chemical bonds which is functional groups (Smith, 1996).

Figure 2.13 : Fourier Transform Infrared Spectroscopy (FTIR).

Besides that, FTIR is a useful technique for studying wood decay chemistry. This is because by using FTIR, the sample that required running the analysis is in very small quantities then using conventional gravimetric techniques where several grams are required. Otherwise, FTIR has previously been used to characterize the chemistry of wood and determine lignin content in pulp, paper and wood. It has also been used for analyzing chemical changes that occur in wood during weathering, decay and chemical treatments (Pandey and Pitman, 2003).

2.7 Scanning Electron Microscopy (SEM) Analysis

Scanning electron microscopy (SEM) is an ideal technique for examining plant surfaces at high resolution. Plant tissues must be preserved by dehydration for observation in an electron microscope because the coating system and the microscopes operate under high vacuum and most specimens cannot withstand water removal by the vacuum system without distortion.

In order to examine the native structure of the sample, some microscopes are designed to image frozen hydrated samples and more recently environmental SEM microscopes have been developed which can image the sample in their native-hydrated state. These microscopes are specialized equipments and may not be available in many labs. Hence, sample preparation by dehydration is still an important consideration for observation in conventional microscopes.

Figure 2.14 : Scanning Electron Microscopy (SEM).

For samples that necessitate dehydration, many techniques other than just air-drying have been developed to remove water from the sample, all aiming at minimal distortion of the cell and maximal preservation of the original form and structure. These techniques include freeze-drying, critical point drying, and various types of chemical fixation treatments prior to dehydration of samples. However, acceptable

methods offer less than ideal preservation for some plant species and may be inconsistent.

The inconsistency is largely due to diversity in tissue types, form, structure and composition of plants. Inconsistencies also arise from variation in individual skills and equipment used across different labs. Hence, new or modified techniques are continually being tested and developed for the preparation of specific plant tissues for visualization under electron microscopes (Pathan *et al*., 2008).

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Raw Material (Sawdust)

For pretreatment process, sawdust such as hardwood sawdust as a raw material was taken from the saw mill factory at Gambang. For the preparation of sawdust before pretreatment, sawdust was dried in the oven at 60˚C about 24 hours. After that, dry sawdust was sieved using shack sieve that have size 6 mm to provide fine size class. Then, it will store in seal bags at room temperature until further process (Guo *et al.*, 2007).

Figure 3.1 : Sawdust that taken from the sawmill factory, Gambang, Pahang.

Figure 3.2 : Sawdust was sieve using shack sieve.

Figure 3.3 : Sawdust that store in seal bags.

3.2 Sodium Hydroxide (NaOH) Pretreatment

For the preparation of the 0.1M sodium hydroxide (NaOH) pretreatment, firstly, 10 g of the sodium hydroxide (solid) was weight using analytical balance. After that, 10 g of the NaOH was diluted with some of the distilled water in the beaker. Then, the NaOH solution was added in the volumetric flask 1000 mL and then top up with the distilled water until reach the line at the volumetric flask. The sodium hydroxide solution was stored in the chemical cabinet until it used for the pretreatment.

For the sodium hydroxide pretreatment, firstly, 50 g of sawdust was weight using analytical balance. Then, the sawdust was mixed and stirer with 500 mL NaOH solution in the Erlenmeyer flask. After that, the pretreatment was done in the autoclave about 30 minutes at 121˚C (Wang *et al*., 2008). After the autoclave was done, the sample was cool down at room temperature in the fume hood. When the sample was cool, the filtration step was done. The sample was filtrate using filter to separate the solid and liquid. For the solid residue, it was dried using oven at 60˚C in 24 hours and then the dry sawdust was stored in the seal bag at the room temperature until it use for dilute sulfuric acid pretreatment.

Figure 3.4 : Preparation of sample before NaOH Pretreatment.

Figure 3.5 : Pretreatment Process in autoclave.

Figure 3.6 : Sample was cool down after autoclave.

Figure 3.7 : Solid residue after NaOH pretreatment was dried using oven.

3.3 Dilute Sulfuric Acid (H_2SO_4) Pretreatment

For the dilute sulfuric acid (H_2SO_4) pretreatment, 0.01M dilute H_2SO_4, 0.02M dilute H_2SO_4, 0.03M dilute H_2SO_4 and 0.04M dilute H_2SO_4 solution was prepared. For the 0.01M dilute H_2SO_4 preparation, firstly, 10.2 mL of H_2SO_4 was measured using measuring cylinder. Then, 10.2 mL of the sulfuric acid was mixed with 989.8 mL distilled water in the 1000 mL volumetric flask. After that, the 0.01M dilute sulfuric acid was stored in the chemical cabinet at the room temperature until it used for the pretreatment. The step was repeated for the 0.02M dilute H_2SO_4, 0.03M dilute H_2SO_4 and 0.04M dilute H_2SO_4 solution.

For the sulfuric acid pretreatment, firstly, 10 g of sawdust was weight using analytical balance. Then, 10 g of sawdust was mixed with the 100 mL 0.01M dilute sulfuric acid solution in the Schott Bottle. After that, the sample was heat up at 121 ˚C in the oven for the pretreatment. The sample was holding up in different times which are 30 minutes, 60 minutes, 90 minutes and 120 minutes in the oven. For 30 minutes experiment, after 30 minutes, the sample was cool down at the room temperature in the fume hood. After the temperature of the sample drop, samples were then quickly separated into solid and liquid fractions by filtration. For the solid residue, it was dried in the oven at 60˚C about 24 hours. After 24 hours, the dry solid was weight using analytical balance and record the mass. The solid sawdust was stored in the seal bag at the room temperature until it used for the Fourier transform infrared (FTIR) analysis on both the original sawdust before pre-treatment and the maximally pretreated solid residue after dilute acid hydrolysis and scanning electron microscopy (SEM) analysis. For the filtrate, it was stored in the vial in refrigerator 4˚C until it use for the high performance liquid chromatography (HPLC) analysis to determine the concentration of glucose, xylose, arabinose and galactose (Guo *et al.*, 2007).

Figure 3.8 : Preparation of sawdust for dilute sulfuric acid pretreatment.

Figure 3.9 : Dilute sulfuric acid pretreatment was done in the oven.

Figure 3.10 : Sample was cool down in fume hood.

Figure 3.11 : Sawdust after pretreatment process before dried in the oven.

3.4 High Performance Liquid Chromatography (HPLC) Analysis

For the sample preparation before run the HPLC, firstly, all samples were filtered through a 0.45 μm filter. The quantitative analysis for glucose, xylose, arabinose, galactose were performed at ambient temperature using an HPLC system (Agilent 1200 series, Agilent Technologies) equipped with a refractive index detector. The separation involved a SUPERCOSIL LC-NH_2 column with a length of 25 cm, an inner diameter 4.6 mm and the size of particle is 5μm particles. The mobile phase that uses is acetonitrile and water that have ratio 75:25. The flow rate that use is 1 mL/min (Guo *et al.*, 2007).

Figure 3.12 : Preparation of HPLC mobile phase.

3.5 Fourier Transform Infrared (FTIR) Analysis

For Fourier Transform Infrared (FTIR) analysis, untreated sawdust and treated sawdust at 1% dilute sulfuric acid in 120 minutes was prepared. The sample was drying in the oven at 60˚C about 24 hours. After the sample was dried, the sample was used to analysis using FTIR Nicolet Avatar 370 DTGS.

Figure 3.13 : Fourier Transform Infrared (FTIR).

3.6 Scanning Electron Microscopy (SEM) Analysis

For the SEM analysis, the untreated and treated sawdust before and after pretreatment using 1% dilute sulfuric acid at 120 minutes were dried in the oven at 60˚C about 24 hours. After that, the sawdust was used to analysis using scanning electron microscope (SEM) EDX Spectrometer EVO 50 using 5kV accelerating voltage. The magnification of the SEM is 1000 x.

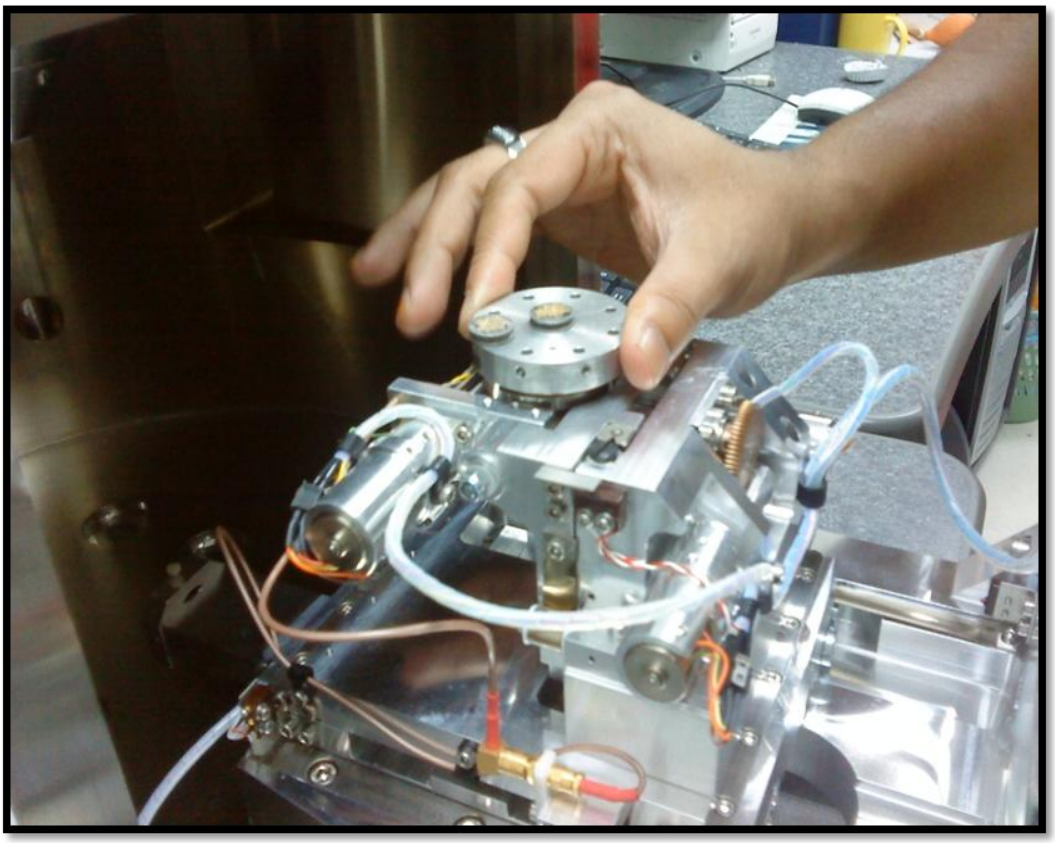

Figure 3.14 : Preparation for SEM Analysis.

3.7 Summary for Dilute Sulfuric Acid Pretreatment method

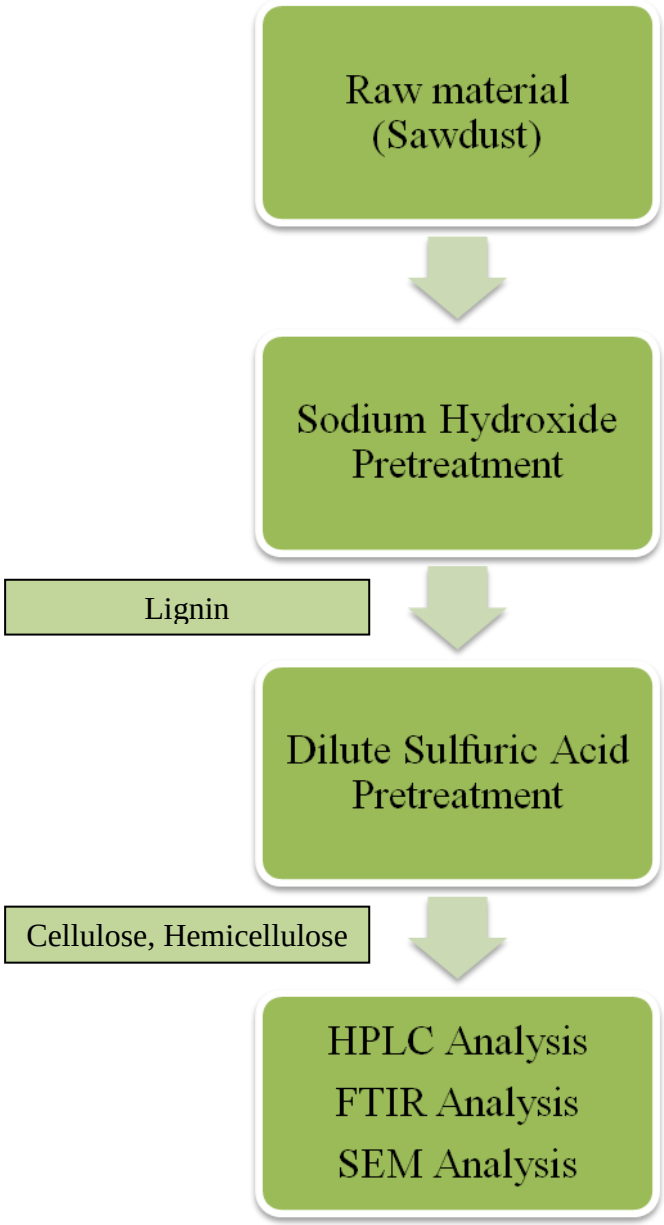

Figure 3.15 : The process flow for recovery cellulose.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 High Performance Liquid Chromatography (HPLC)

In this experiment, High Performance Liquid Chromatography (HPLC) was used as quantitative analysis to measure the concentration of monomer sugars which is xylose, arabinose, and glucose that hydrolyzed from cellulose and hemicelluloses during the pretreatment. From the pretreatment of sawdust, some of cellulose was converted to glucose and hemicelluloses were hydrolyzed to xylose and arabinose. The figures below were shown the standard concentration of xylose, arabinose and glucose.

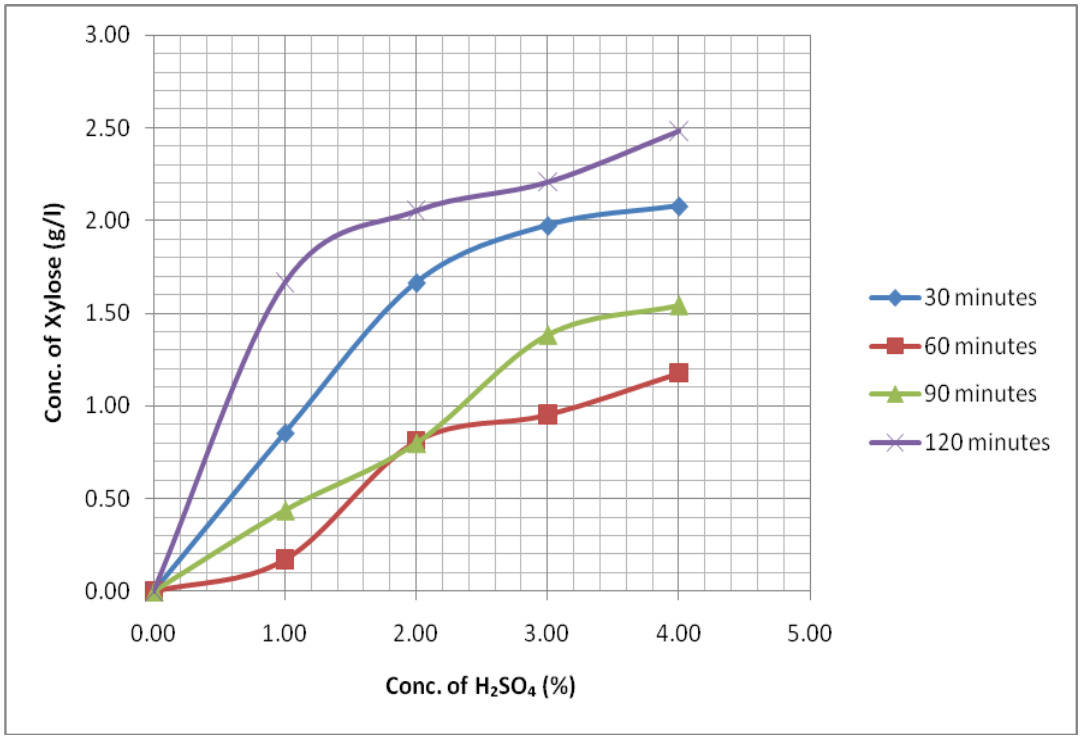

Figure 4.1. : Graph for effect of xylose concentration towards acid concentration.

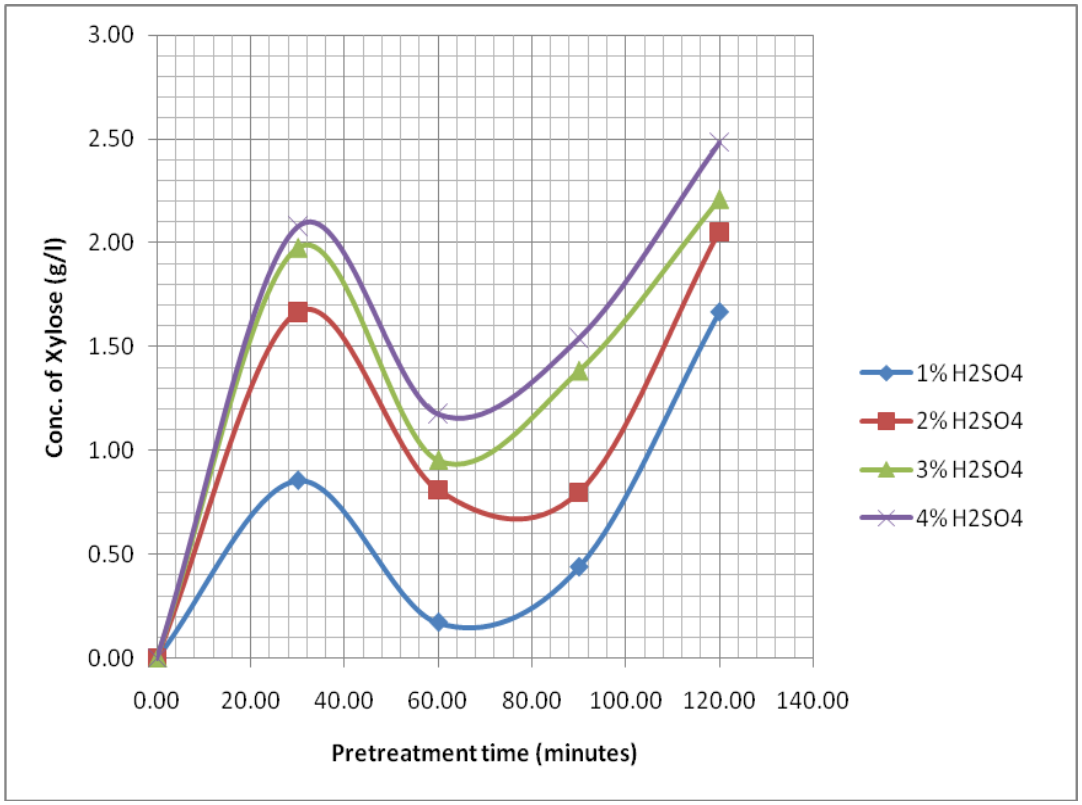

Figure 4.2 : Graph for effect of xylose towards pretreatment time.

From the figure 4.1 and figure 4.2 above, it show that concentration of xylose was not significantly influence by the pretreatment time which is 30 minutes, 60 minutes, 90 minutes and 120 minutes but it was significantly influence by the concentration dilute sulfuric acid which is 1%, 2%, 3% and 4. From that, it shows when the concentration of dilute sulfuric acid was increased, the concentration of xylose release also increased. But for pretreatment time, it will not influence the concentration of xylose release. This is show that the amount of xylose in the hemicelluloses was small so the change of xylan to xylose was not much. From the graph, the maximum concentration of xylose release is 2.5 g/l at 4% dilute sulfuric acid at 120 minutes.

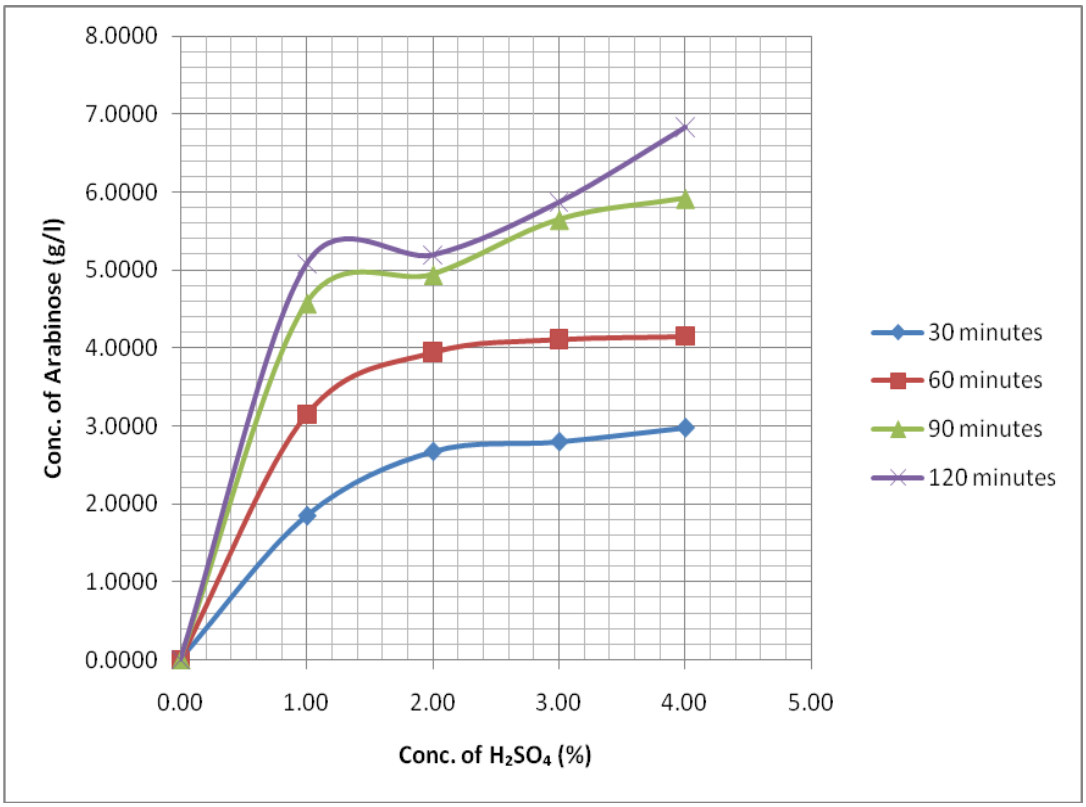

Figure 4.3 : Graph for effect of arabinose concentration towards acid concentration.

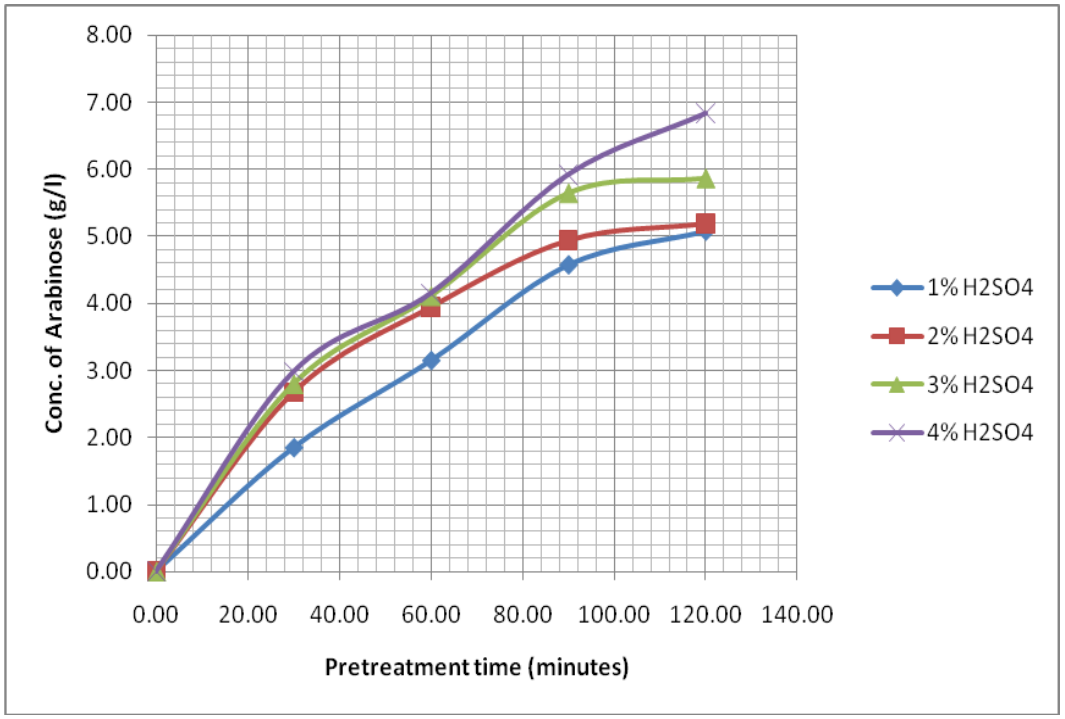

Figure 4.4 : Graph for effect of arabinose concentration towards pretreatment time.

From the figure 4.3 and figure 4.4 above, it shows that concentration of arabinose release is significantly influence by concentration of dilute sulfuric acid which is 1%, 2%, 3% and 4% that used and also pretreatment time which is 30 minutes, 60 minutes, 90 minutes and 120 minutes. From that, it shows when the dilute sulfuric acid concentration and pretreatment time increased, the concentration of arabinose release also increased. This is because the concentration of arabinose in hemicelluloses that contain in sawdust was higher. The maximum concentration of arabinose release was 6.8 g/l at 4% dilute sulfuric acid and 120 minutes.

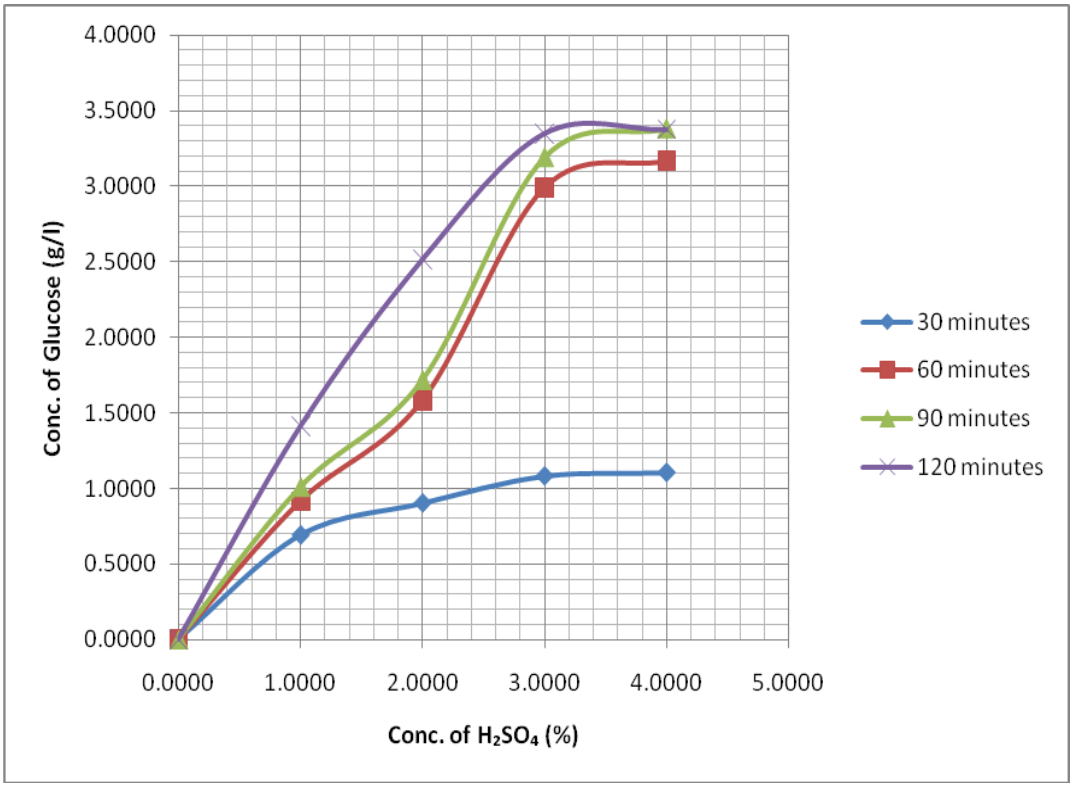

Figure 4.5 : Graph for effect of glucose concentration towards acid concentration.

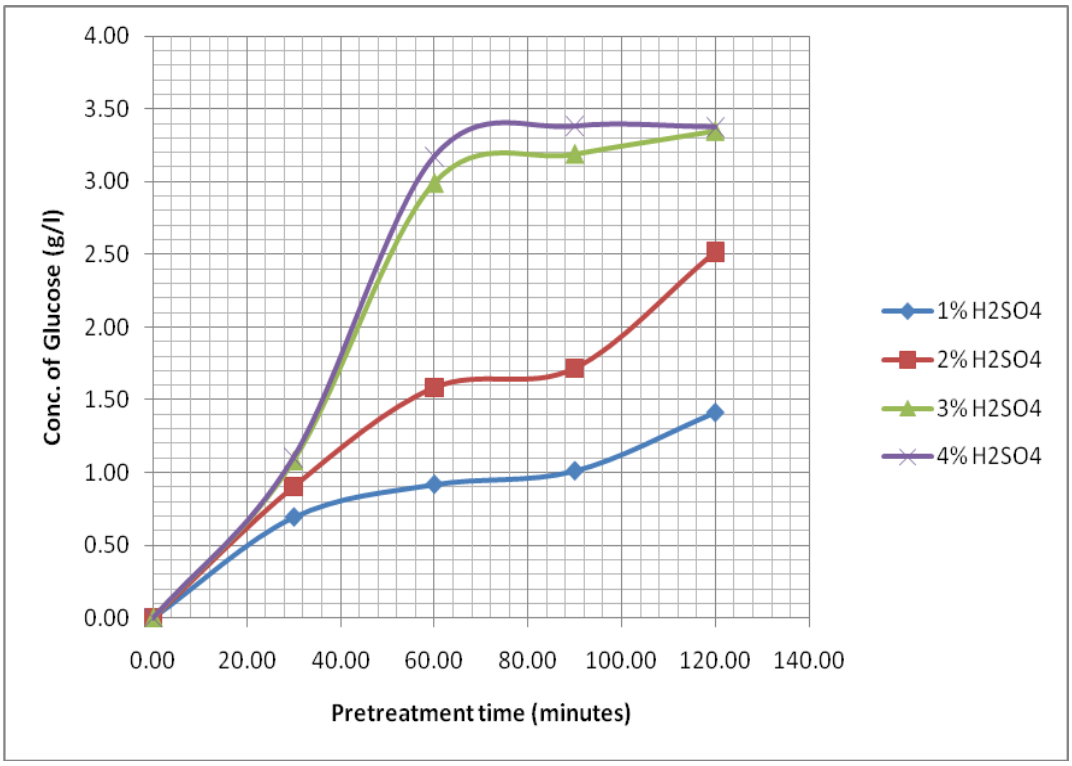

Figure 4.6 : Graph for effect of glucose concentration towards pretreatment time.

From the figure 4.5 and figure 4.6 above, it shows that the concentration of glucose release was significantly influence by the sulfuric acid concentration which is 1%, 2%, 3%, and 4% and also pretreatment time which is 30 minutes, 60 minutes, 90 minutes and 120 minutes. From that, the concentration of glucose will increased when concentration of dilute sulfuric acid increased which are 1%, 2%, 3%, and 4% and pretreatment time which are 30 minutes, 60 minutes, 90 minutes and 120 minutes increased. From the graph, at 4% dilute sulfuric acid, at pretreatment time 90 minutes and 120 minutes, the concentration of glucose release was same which is 3.4 g/l. It shows 4% dilute sulfuric acid was a maximum concentration to hydrolyzed glucose from cellulose. The maximum concentration of glucose release was 3.4 g/l at 4% dilute sulfuric acid and 90 minutes and 120 minutes.

4.2 Scanning Electron Microscopy (SEM)

In this experiment, pretreatment not only affect the chemical composition of the sawdust but also affected the physical appearance of the sawdust at microscopic level. Scanning Electron Microscopy (SEM) was used to analyze the different of the physical appearance of sawdust before pretreatment and after pretreatment was done. The figures below show the result for the Scanning Electron Microscopy (SEM) analyzed.

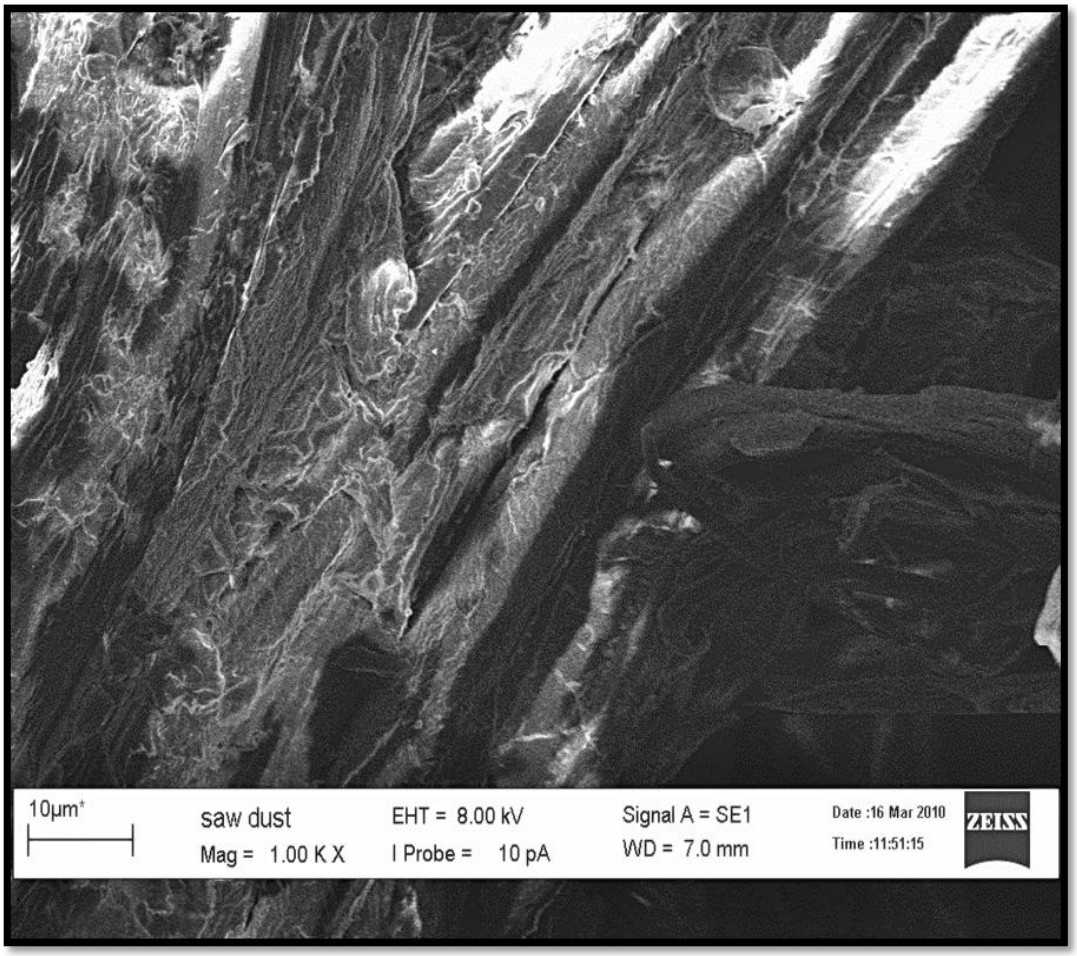

Figure 4.7 : Untreated sawdust at 1000x mignification.

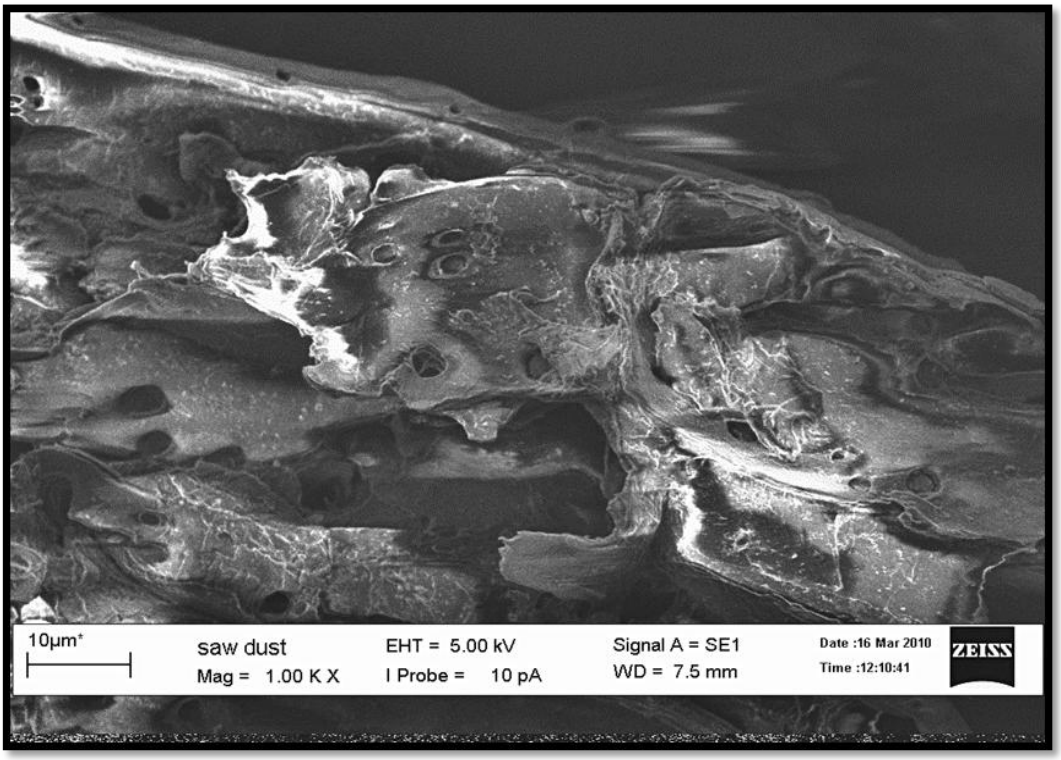

Figure 4.8 : Treated sawdust at 1000x mignification at 1% dilute H_2SO_4 solution, 90 minutes, 121˚C.

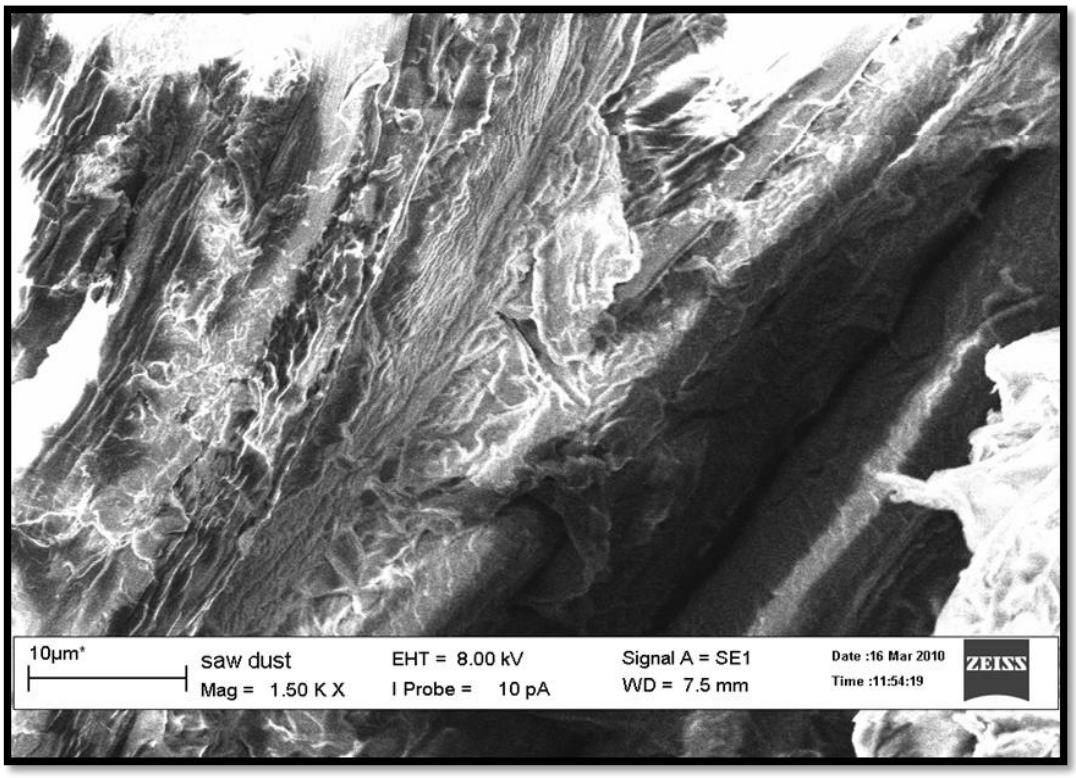

Figure 4.9 : Untreated sawdust at 1500x magnification.

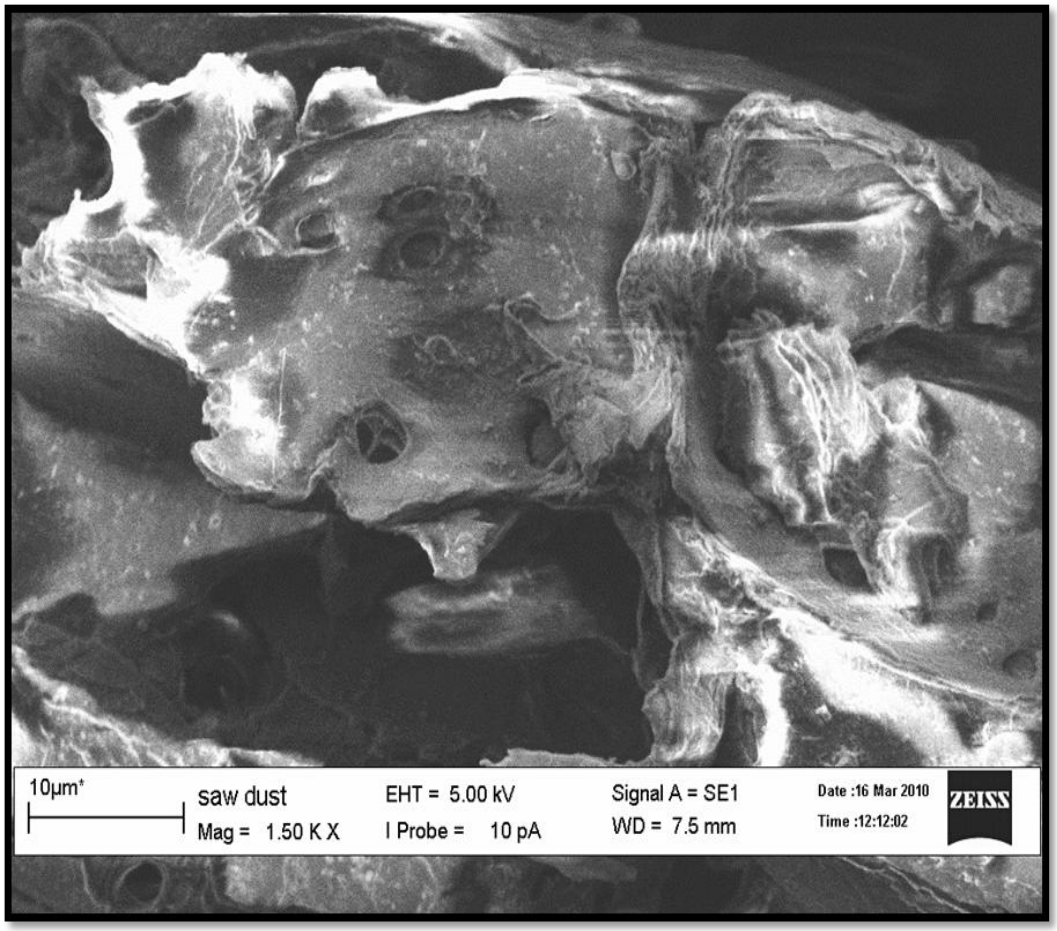

Figure 4.10 : Treated sawdust at 1500x magnification at 1% dilute H_2SO_4 solution, 90 minutes, 121˚C.

Figure 4.4 and figure 4.6 shows the SEM analysis at 1000x and 1500x magnification for untreated sawdust. From the pictures above, it shows that the surface the untreated sawdust has three layers which is cellulose, hemicelluloses and lignin.

Figure 4.5 and figure 4.7 shows the SEM analysis at 1000x and 1500x magnification for treated sawdust. From the pictures above, it shows that the surface of the sawdust after treat using 1% dilute H_2SO_4 solution in 90 minutes at temperature 121˚C. After the pretreatment, the smooth and contiguous surface of the sawdust was perforated. These pores may increase the enzyme-accessible surface area which increases the enzyme digestibility of the sawdust. Furthermore, the layer of sawdust after pretreatment was changed. The layer for cellulose was decreased because some of the cellulose was hydrolyzed to glucose. For the layer of hemicelluloses, the hemicelluloses were hydrolyzed to monomer sugars which are

arabinose and xylose during the dilute sulfuric acid pretreatment. For lignin, the layer was changed because the removal of lignin during the sodium hydroxide pretreatment.

4.3 Fourier Transform Infrared (FTIR)

Fourier Transform Infrared (FTIR) analysis was used to investigate the influence of dilute sulfuric acid pretreatment on structure of the sawdust. In this analysis, the changes of the structure of sawdust from the wave numbers that appears. The figures above shows the result for the FTIR analysis on untreated sawdust and treated sawdust at 1% dilute H_2SO_4 solution, 90 minutes, 121˚C.

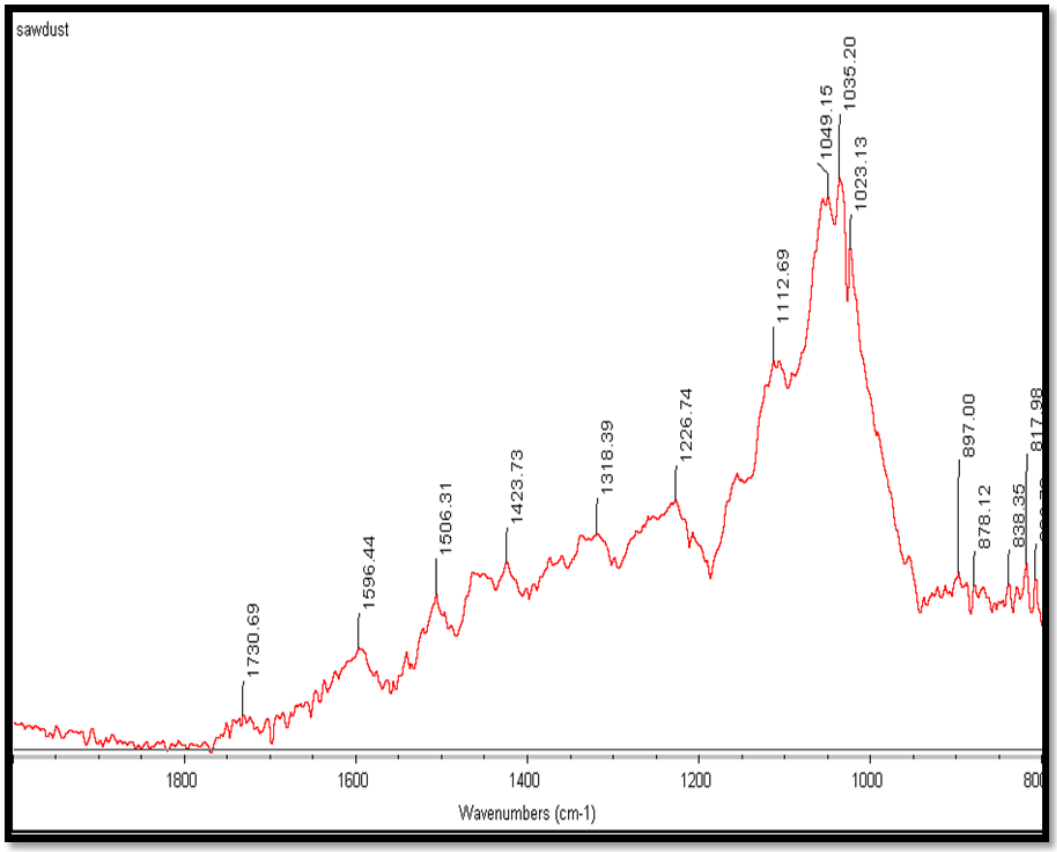

Figure 4.11: FTIR analysis result for untreated sawdust.

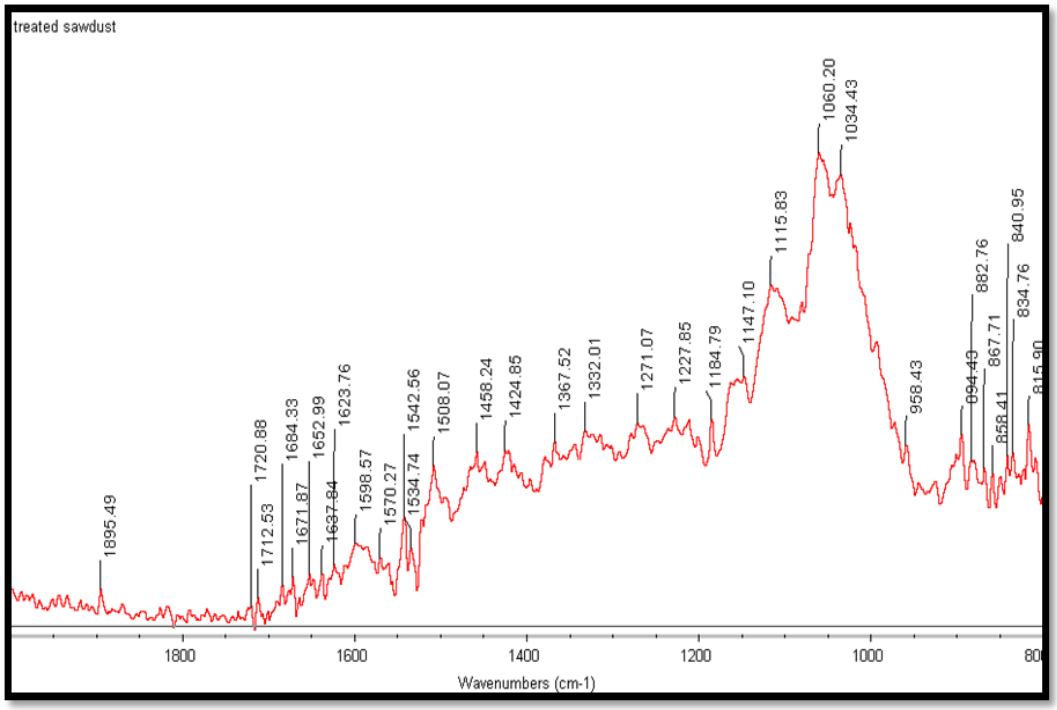

Figure 4.12 : FTIR analysis result for treated sawdust at 1% dilute H_2SO_4 solution, 90 minutes, 121˚C.

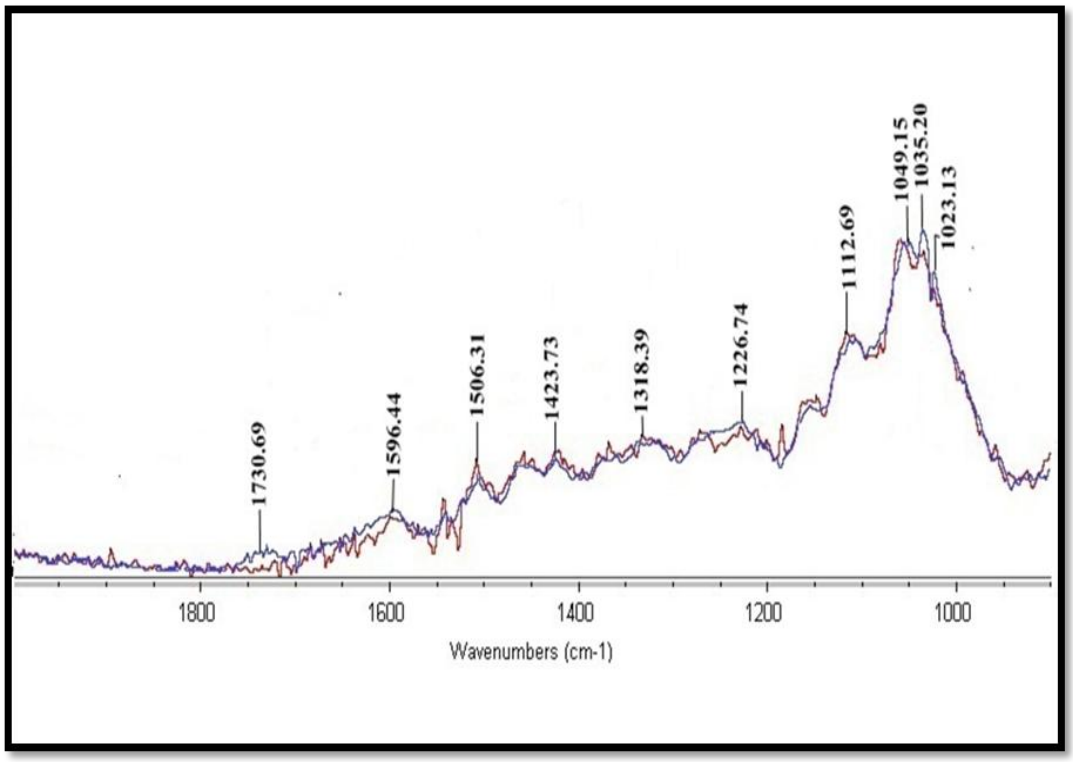

Figure 4.13 : The difference wave numbers for untreated sawdust (blue line) and treated sawdust (red line).

The figure 4.13 above, it shows the difference of wave numbers for untreated and treated sawdust. From the absorption peak at range 400 cm^{-1} to 1500 cm^{-1}, it was a fingerprint region. All the functional group at that range has single bond. Besides that, cellulose and hemicelluloses also in this range which is the wave number is 1030 cm^{-1} and 1240 cm^{-1}. Furthermore, the functional group that indicated the cellulose and hemicelluloses is C-OH. For lignin, it presents at range 1500 cm^{-1} to 2000 cm^{-1}. The wave number of lignin is 1590 cm^{-1}. The functional group in this range has double bond. The functional group that indicated the lignin is C=O. The wave number of lignin is 1590 cm^{-1}.

At the highest peak which is at absorbance range 1000 cm^{-1} to 1100 cm^{-1}. In that range the functional group of cellulose was appeared. From the figure 4.13, it shows that the absorbance peak was not much change. This is because the cellulose was not much effect during the pretreatment. That means a few of cellulose will be hydrolyzed to glucose. The cellulose will be fully hydrolyzed to glucose in further study which is enzymatic hydrolysis.

At the wave number in range 1200 cm-1 to 1300 cm-1, the hemicelluloses wave functional group was appeared. The wave number of hemicelluloses is 1240 cm^{-1}. From the figure 4.13, it shows that the absorbance peak was decrease after pretreatment. It means that hemicelluloses were hydrolyzed to monomer sugars such as xylose and arabinose after pretreatment using dilute sulfuric acid.

At the wave number in range 1500 cm^{-1} to 2000 cm^{-1}, the functional group of lignin was appeared. The wave number of lignin is 1590 cm^{-1}. From the figure 4.13, it shows that the absorbance peak also decrease after pretreatment using sodium hydroxide. It means that lignin was removed after the pretreatment.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

As a conclusion, the dilute sulfuric acid pretreatment is suitable to recovery cellulose from the sawdust. From the result it shows that the maximum concentration of xylose release is 2.5 g/l at 4% dilute sulfuric acid at 120 minutes. For arabinose, the maximum concentration release was 6.8 g/l at 4% dilute sulfuric acid and 120 minutes and for glucose, the maximum concentration release was 34 g/l at 4% dilute sulfuric acid and 90 minutes and 120 minutes. The conclusion for this result is hemicelluloses were hydrolyzed to xylose and arabinose using dilute sulfuric acid pretreatment and cellulose was hydrolyzed to glucose during the pretreatment. The maximum recovery cellulose is at 4% dilute sulfuric acid (H_2SO_4) at 120 minutes.

In this research, the study was related to the three elements which are environment, economics and engineering. For environment element, this research use sawdust as a raw material. Nowadays, sawdust was biomass that abundant nowadays and can cause pollution when the amount of sawdust was exceed. When the sawdust was use to produce the valuable product, it can decrease the pollution to the environment and it also related to the second element which is economics. When using sawdust as a raw material, the cost production also can reduce because the cost for raw material was cheaper. Besides that, the dilute sulfuric acid pretreatment was an effective and economic process. For third element which is engineering, this product which is cellulose can product the valuable product that can give benefit for community such as sorbitol, a low calorie sugars, that can give benefit to diabetes patient.

For recommendation, firstly is to further the study in producing glucose using enzymatic hydrolysis. This is because commercial glucose is produce from the starch that contain in cassava, potatoes and also rice. Use cassava, potatoes and also rice as raw materials is not effective because it’s also use as a food to the human. But if use biomass as a raw material it is very effective because biomass nowadays was abundant and also can reduce the pollution.

Secondly is further study with another pretreatment such as biological pretreatment, hot water pretreatment and ammonia pretreatment to compare the effectiveness of the pretreatment to recovery cellulose.

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APPENDIX A

Figure 1 : Sawdust that used for pretreatment.

Figure 2 : Sawdust after dilute sulfuric acid pretreatment.

APPENDIX B

TABLE OF DATA FOR HPLC ANALYSIS

(Refer to the File PDF)