

**CELLULOSE RECOVERY FROM SAWDUST: OPTIMIZATION OF  
CONCENTRATION, PRETREATMENT TIME AND TEMPERATURE BY  
USING RESPONSE SURFACE METHODOLOGY**

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## ABSTRACT

Cellulose is the most abundant organic material on earth that easily found nowadays in large amount which make it as a promising raw materials for the bio-energy production. The raw material that been used in this experiment is cellulose from meranti sawdust. The objective of this experiment is to obtain the maximum cellulose recovery from the sawdust by using the pretreatment methods and optimization using response surface methodology (RSM). The pretreatment process involved in this experiment were dilute sodium hydroxide NaOH, followed by peracetic acid pretreatment, PAA and dilute acid sulphuric H<sub>2</sub>SO<sub>4</sub> pretreatment, followed by extraction and analysis using UV- Vis Spectrophotometer at the wavelength 320 nm. The conditions before the optimization are at temperature 150°C, 2.0% (v/v) diluted H<sub>2</sub>SO<sub>4</sub> and 120 minutes of pretreatment time. After the optimization, the highest cellulose concentration (4.15922 mg/ml) can be achieved at 75°C, 82.96 minutes pretreatment time and at concentration 2.0% (v/v) diluted H<sub>2</sub>SO<sub>4</sub>.

## ABSTRAK

Selulosa adalah bahan organik yang paling banyak di bumi yang mudah ditemui sekarang dalam jumlah yang besar yang menjadikannya sebagai bahan mentah yang berpotensi untuk penghasilan bio-tenaga. Bahan mentah yang digunakan dalam kajian ini adalah selulosa daripada serbuk kayu meranti. Objektif kajian ini adalah untuk mendapatkan pemulihan selulosa pada kadar maksimum dengan menggunakan kaedah pra-rawatan dan pengoptimuman menggunakan kaedah permukaan respon (RSM). Proses pra-rawatan yang terlibat dalam kajian ini adalah dengan menggunakan natrium hidroksida (NaOH), diikuti dengan penggunaan asid perasetat, (PAA) dan menggunakan asid sulfurik ( $H_2SO_4$ ), diikuti oleh pengekstrakan dan analisis menggunakan Spektrofotometer Ultralembayung-Nampak (Uv-Vis) pada panjang gelombang 320 nm. Keadaan operasi sebelum pengoptimuman adalah pada suhu  $150^{\circ}C$ , kepekatan 2.0% (v/v)  $H_2SO_4$  dan 120 minit masa pra-rawatan. Setelah pengoptimuman, kepekatan selulosa tertinggi (4.15922 mg/ml) boleh dicapai pada  $75^{\circ}C$ , 82,96 minit masa pra-rawatan dan pada kepekatan 2.0% (v/v)  $H_2SO_4$ .

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**LIST OF ABBREVIATIONS**

g	-	Gram
C	-	Celcius
NaOH	-	Natrium Hydroxide
H <sub>2</sub> SO <sub>4</sub>	-	Sulphuric Acid
PAA	-	Peracetic Acid
UV-Vis	-	Ultraviolet-Visible
nm	-	Nanometer
v/v	-	Volume/volume
w/v	-	Weight/volume
w/w	-	Weight/weight
mL	-	Mililiter
3-D	-	3-dimension
RSM	-	Response Surface Methodology

## **CHAPTER 1**

### **INTRODUCTION**

#### **1.1 Background of Study**

The concerns about the environmental impact of fossil fuels are higher nowadays, leading researcher on developing alternatives energy resources from biomass that are less pollution to the environment. Biomass, which includes animal and human waste, wood products, grasses and agricultural residues such as wheat straw, corn stover and rice straw, is a renewable resource that stores energy from sunlight in its chemical bonds (McKendry, 2002). The biomass can be process into bio-fuels either chemically or biologically by breaking the chemicals bond and produce bio-fuels like bio-ethanol (Rebecca *et al.*, 2006).

The ethanol demands are higher nowadays. The lignocelluloses are one of raw material used for the production of ethanol due to its high composition in cellulose and hemicelluloses. Lignocelluloses, often found in wood industries are less expensive and easy to get in large amount. Forest biomass like sawdust is believed to be the most abundant sources of sugars.

Cellulose is a linear polymer of glucose that linked by b-1,4 glycosidic bonds (Fengel and Wegener, 1984). In plant, cellulose consists of crystalline (organized) structure, and parts with a, not well-organized, amorphous structure. The cellulose

strains are ‘bundled’ together and form so called cellulose fibrils or cellulose bundles that are independent and bound by hydrogen bonding (Laureano-Perez *et al.*, 2005).

Pretreatment in biomass is an essential to break then lignin and obtain high yield of cellulose and to increase its enzymatic digestibility. The final purpose of pretreatment is to obtain high sugar yields from the overall process at low cost, increase the enzymatic digestibility, remove lignin and hemicelluloses, disrupt the cellulose crystallinity and make cellulose more accessible to the cellulase (Mosier *et al.*, 2005). Many pretreatment approaches have been investigated on a wide variety of feedstocks types and there are several recent review articles which provide a general overview of the field (Hendriks and Zeeman, 2009).

Alkali pretreatment will increase the cellulose digestibility and lignin solubility (Alvira *et al.*, 2010). The acid pretreatment will increase the hemicelluloses solubility; solubilize the hemicelluloses into monomeric sugars and oligomers and make the cellulose to be more accessible to enzymes (Sun and Cheng, 2004). Diluted acid pretreatment is a favourable method for industrial applications due to its effectiveness and inexpensive among all the pretreatment methods.

The optimization in RSM has demonstrated the use of a central composite design by determining the optimum conditions that lead to the high yield of cellulose concentration. Thus, smaller and less time consuming experimental designs could generally optimize the pretreatment process.

## **1.2 Problem Statement**

The wood waste from the industries were abundant in our country. Without proper handling, the unused waste from the wood can increase the pollution to the environment. Large amount of wood waste are discard because it cannot be used as food. Cellulose consists of complex microstructure system composed of lignin and hemicelluloses that cannot biodegradable by mammalian digestive enzymes.

## **1.3 Research Objectives**

The objective of this research is to obtain the maximum condition (concentration, temperature and pretreatment time) for the cellulose recovery by using Response Surface Methodology (RSM).

## **1.4 Scope of Study**

In order to achieve the objective of this research study, several scope of study has been identified such as effect of concentration  $H_2SO_4$ , temperature used to obtain maximum cellulose hydrolysis and pretreatment time. The pretreatment methods used in this research also include sodium hydroxide (NaOH) and peracetic acid pretreatment (PAA). To analysis the concentration of cellulose, the UV-Vis Spectrophotometer is used. The optimization is done by using the Surface Response Methodology (RSM) by selecting the low and high range for each parameter.

## **1.5 Significant of the Study**

Sawdust is low in cost and easy to get. Besides, it is an abundant material in Malaysia. Sawdust also contains high cellulose composition. The used of sawdust in research can reduce the pollution. As addition, production of cellulose has a potential due to the fact that many product like fuel; bio-ethanol can be produced from it. This fuel is low in cost. Sorbitol is other product from cellulose that is highly demand in the food industry, pharmaceutical, cosmetic and paper good. The important of this research is to reduce the environmental pollution by treating the sawdust and optimizing the cellulose concentration by using Response Surface Methodology (RSM).

## **CHAPTER 2**

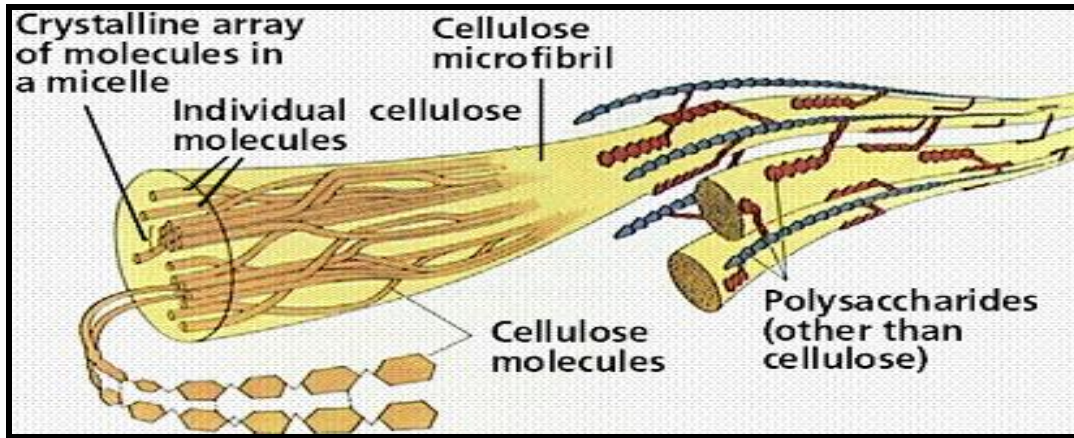
### **LITERATURE REVIEW**

#### **2.1 Introduction**

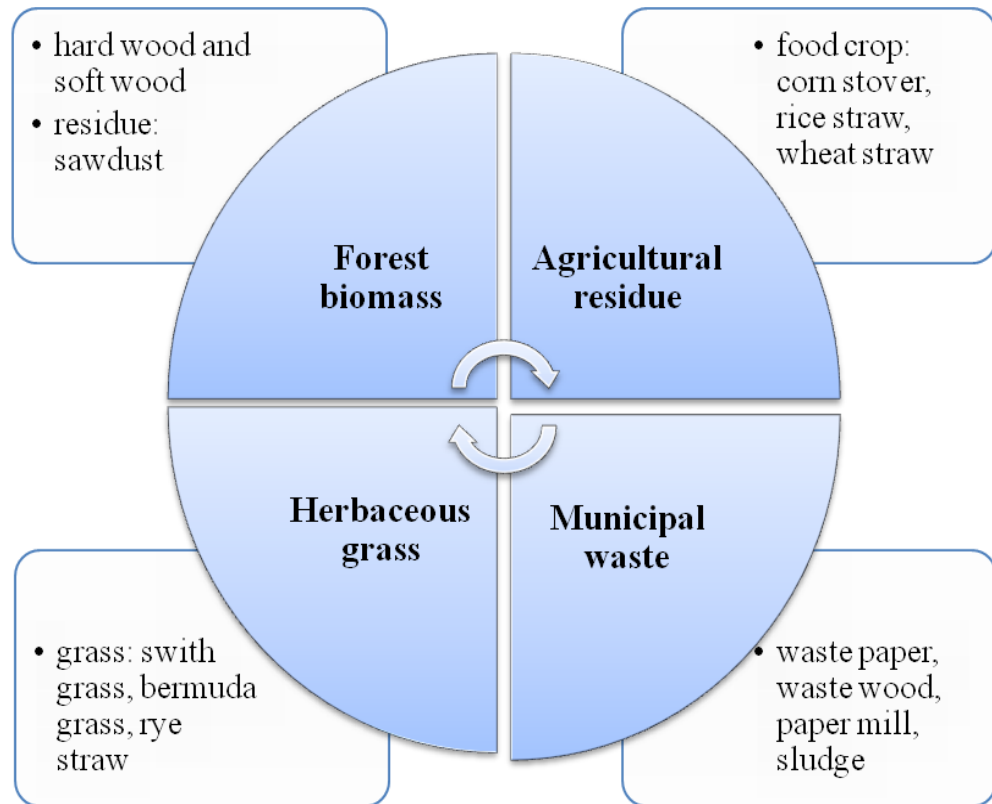
Lignocelluloses biomass composed of cellulose, hemicelluloses and lignin. It is an effective raw material as it is easy to find in large amount and less expensive (Silverstein *et. al.*, 2007). Lignocelluloses waste can be found from forest biomass, agricultural residue, herbaceous grass and municipal waste.

The lignocellulosic materials were chosen as the sources for the bio-fuel productions which can be divided into: crop residue (corn stover, bagasse, wheat straw, rice straw), wood residue (hard wood and soft wood), municipal waste paper (office paper, sludge) and herbaceous grass (bermuda grass, rye straw). Figure 2.1 shows the structure of lignocellulosic. Figure 2.2 to Figure 2.6 show the sources of cellulose recovery.





**Figure 2.1:** Structure of cellulose, hemicelluloses and lignin



**Figure 2.2:** Sources of lignocellulosic



**Figure 2.3:** Corn stover as the raw material for cellulose recovery



**Figure 2.4:** Sugarcane bagasse as the raw material for the cellulose recovery



**Figure 2.5:** Paper waste as the feedstock for cellulose recovery



**Figure 2.6:** Rice straw as the feedstock for the cellulose recovery

## 2.2 Sawdust

In the wood industries, as example furniture industries, there is plenty of sawdust formed as waste. Meranti tree is widely used for furniture making in Malaysia (Ahmad *et. al.*, 2009). Meranti sawdust is also the most common wood found in the furniture industries in Thailand (Jutarat and Kanita , 2003). Large quantity of forest industries product are being discard because cannot be used for the food (Keith and Daniel, 1976). Holland and Yocum (1970) have estimated that the total amount of unused wood residues, not including from paper industry in the United States exceeds one billion cubic feet annually.

The sawdust is generally looks not useful and always dumped at the land field without people realizing the value of it. These unused materials are usually applied as a fuel source or for the production of ply woods (Jutarat and Kanita , 2003). Due to the high availability and low cost of the sawdust, it can be more versatile and valuable by compounding the sawdust with plastics in order to improve plastic properties, for example, high strength and easy processing that are better than wood or plastics.

Sawdust contains 70 to 80% of total carbohydrates that consists of lignocellulosic (Keith and Daniel, 1976). Lignocellulosic material consists of mainly three different types of polymers, which are cellulose, hemicellulose and lignin, which are associated which each other (Fengel and Wegener, 1984). Lignocellulosic materials contain cellulose and hemicellulose that are bound together by lignin, meanwhile cellulose and hemicellulose are both polymers (Petterson *et. al.*, 2007).



**Figure 2.7:** Sawdust as the cellulose recovery

### **2.3 Lignin**

Lignin is a composition in the lignocellulosic material. Lignin is, after cellulose and hemicellulose, one of the most abundant polymers in nature and is present in the cellular wall. It is an amorphous heteropolymer consisting of three different phenylpropane units (p-coumaryl, coniferyl and sinapyl alcohol that are held together by different kind of linkages and the main purpose of lignin is to give the plant structural support, impermeability, and resistance against microbial attack and oxidative stress (Hendriks and Zeeman, 2008). The amorphous heteropolymer is also non-water soluble and optically inactive; all this makes the degradation of lignin very tough (Fengel and Wegener, 1984).

Lignin, just like hemicellulose, normally starts to dissolve into water around 180°C under neutral conditions (Bobleter, 1994) The solubility of the lignin in acid, neutral or alkaline environments depends however on the precursor (p-coumaryl, coniferyl, sinapyl alcohol or combinations of them of the lignin (Grabber 2005).

## 2.4 Hemicellulose

Hemicellulose is a complex carbohydrate structure that consists pentoses (example; xylose and arabinose), hexoses (example; mannose, glucose and galactose), and sugar acids. Xylan is found mostly in hardwood and glucomannan the most common hemicelluloses present in softwood and forest industry products (Fengel and Wegener, 1984; Saha, 2003).

Hemicellulose is easy to hydrolyze compared to cellulose due to its lower molecular weight and it branches with short lateral chains that consist of different sugars, making it easy to react during pretreatment (Fengel and Wegener, 1984). Hemicellulose also known as a connection between the lignin and the cellulose fibers and gives the whole cellulose–hemicellulose–lignin network more rigidity (Laureano-Perez *et. al.*, 2005). The solubility of the different hemicellulose compounds is in descending order: mannose, xylose, glucose, arabinose, and galactose (Hendriks and Zeeman, 2008). Hendriks and Zeeman (2008) also found that the solubility of hemicelluloses will increase with temperature, when the temperature is high, the hemicelluloses is more soluble.

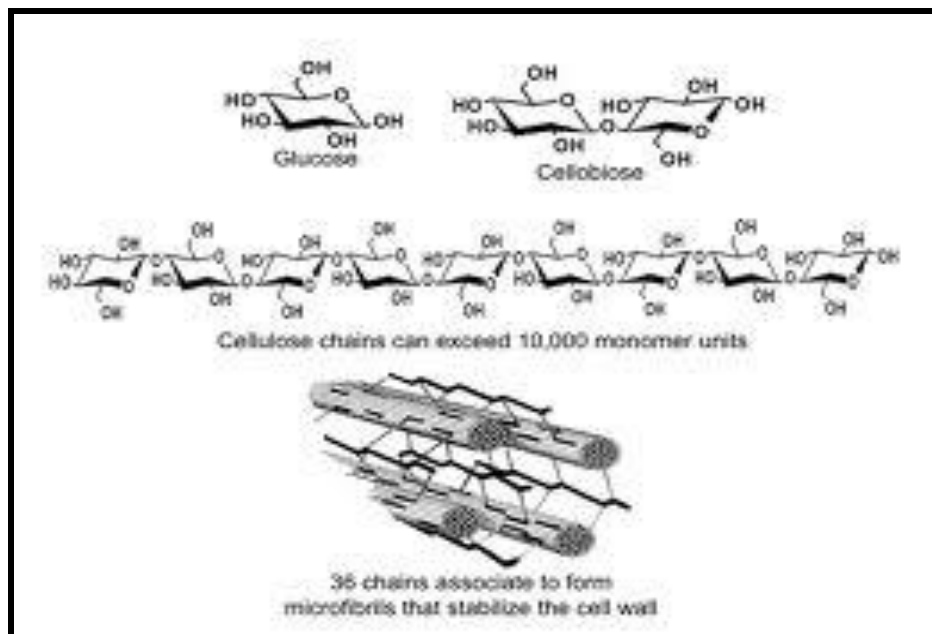
Due to the unknown boiling point of the material in hemicelluloses, the solubility's of higher molecular polymers could not be predicted, (Gray *et. al.*, 2003). Bobleter (1994) stated that the solubilization of hemicellulose compounds into the water starts around 180°C under neutral conditions. But Garote *et. al.* (1999) found that some part of hemicelluloses already soluble at 150°C. The solubilization of lignocelluloses

components also depend on the moisture content and pH of the wood tested, not only depends on temperature (Fengel and Wegener, 1984).

The xylan of hemicellulose can be extracted quite well in an acid or alkaline environment, while glucomannan can hardly be extracted in an acid environment and needs a stronger alkaline environment than xylan to be extracted (Balaban and Ucar, 1999; Fengel and Wegener, 1984; Lawther *et. al.*, 1996). Xylan appears to be the part that can be extracted the most easily. Of cellulose, hemicellulose and lignin the hemicelluloses are the most thermal-chemically sensitive (Levan *et. al.*, 1990; Winandy, 1995). During thermal-chemical pretreatment firstly the side groups of hemicellulose react, followed by the hemicellulose backbone (Sweet and Winandy, 1999).

## 2.5 Cellulose

Cellulose,  $((C_6H_{10}O_5)_n)$ , the major component in lignocellulosic materials consists of 35 to 50% of carbohydrates, ( $1000 < DP < 15,000$ ) composed of  $\beta$  (1,4)-linked D-glucose units, which has been recognized as a potential renewable source for biofuels and bio-based chemicals production (O'Sullivan, 1997). Cellulose exists of D-glucose subunits, linked by  $\beta$ -1,4 glycosidic bonds (Fengel and Wegener, 1984). Cellulose is insoluble in water and most organic solvents due to its hydrogen bonding between its fibrils (Kim *et. al.*, 2010). These cellulose fibrils are mostly independent and weakly bound through hydrogen bonding (Laureano-Perez *et. al.*, 2005). Cellulose favors the ordering of the polymer chains into tightly packed, highly crystalline structures that are water insoluble and resistant to depolymerization (Mosier *et. al.*, 2005).



**Figure 2.8:** Structure of cellulose

The high order structure and crystallinity of cellulose makes it recalcitrant to hydrolysis (Kuo and Lee, 2008). Murakami *et. al.*, (2007) also found that the most suitable method to process cellulose is by hydrolyzing it into solution. Kim *et. al.*, (2010) suggested that the cellulose is insoluble in water or organic solvents due to its extensive network of inter- and intra-molecular hydrogen bonding between its fibrils.

A pretreatment process is required to make cellulose more susceptible for subsequent process. The economically pretreatment is important in the cellulose processing (Mosier *et. al.*, 2005; Hamelinck *et. al.*, 2005). Many numerous methods have been applied for cellulose pretreatment prior to hydrolysis (Mosier *et. al.*, 2005; Hamelinck *et. al.*, 2005). Although several processes including alkali and acid, oxidation and other chemical treatments have been reported to be a capable method in reducing the crystallinity of cellulose, they are high energy-demanding and often require severe conditions (Taherzadeh and Karimi, 2008).

## 2.6 Pretreatment



McMillan (1994) stated that the effect and the usage of pretreatment of lignocellulosic materials have been recognized for a long time. The purpose of the pretreatment is to remove lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the material (Sun and Cheng, 2001). The purpose of pretreatment is also to obtain high sugar yields from at low cost, to increase the enzymatic digestibility is to remove lignin and hemicelluloses, disrupt the cellulose crystallinity, and increase the porosity of the materials to make cellulose more accessible to the cellulases (Zhao *et. al.*, 2009). The porosity (accessible surface area) of the waste materials, cellulose fiber crystallinity, and lignin and hemicellulose will determine the pretreatment used; which hydrolysis is the selected pretreatment in many raw materials (McMillan, 1994).

Pretreatment process is require in order making cellulose more susceptible for subsequent process, with the considerations such as effective and economical type of pretreatment are important for the cellulose processing for the further hydrolysis.(Mosier *et. al.*, 2005; Hamelinck *et. al.*, 2005). Pretreatment is an essential needed in order to make biomass is accessible by breaking the lignin seal, removing hemicellulose, or disrupting the crystalline structure of cellulose (Fan *et. al.*, 1981).

Pretreatment must improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis, avoid the degradation or loss of carbohydrate and to avoid the formation of byproducts inhibitory to the subsequent hydrolysis (Sun and Cheng, 2001).