STUDY ON THE DELIGNIFICATION OF FIBER ON THE TEMPERATURE AND NAOH ADDED CONCENTRATION BY ULTRASOUND ASSISTANCE

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LIST OF ABBREVIATION

EFB	Empty Fruit Bunch
FTIR	Fourier Transform Infra-Red
HPLC	High Performance Liquid Chromatography
OPEFB	Oil Palm Empty Fruit Bunch
SEM	Scanning Electron Microscopy
TGA	Thermogravimetric Analysis

KAJIAN TERHADAP DELIGNIFIKASI SERAT BERDASARKAN SUHU DAN PENAMBAHAN KEPEKATAN NAOH DIIRINGI ULTRASOUND

ABSTRAK

Minyak sawit adalah salah satu industri yang paling penting di Malaysia yang meninggalkan bahan lignoselulosik besar sebagai produk sisa. Produk ini mempunyai peratusan lignin yang tinggi yang boleh digunakan untuk membuat bahan-bahan yang berbeza dan komposit. Kajian ini bertujuan untuk mengkaji kaedah yang sesuai untuk menentukan jumlah lignin dalam serat dan untuk mengkaji kaedah ultrasound pada delignifikasi serat. Serat melalui langkah penyediaan bahan mentah dan diikuti oleh pencirian bahan menggunakan kaedah Klason-Lignin. Delignifikasi telah dijalankan dengan menggunakan kaedah ultrasound dan sampel telah diuji dengan Fourier Transformation Infrared Spectroscopy (FTIR), Analisis Kekuatan Tegangan dan Ujian Lignin untuk menganalisis keputusan yang diperolehi. Ia dijangka menghasilkan lebih daripada 15% daripada delignifikasi serat dari penyelidikan ini.

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ABSTRACT

Oil palm is one of the most important industries in Malaysia which leaves behind huge lignocellulosic material as waste product. This product has high percentage of lignin which can be used to make different materials and composites. This research aims to study on the suitable method to determine amount of lignin in fiber and to study on sonokinetic method on delignification of fiber. Fiber was subjected to raw material preparation and followed by material characterization using Klason-Lignin method. Delignification was carried out using ultrasound method and the samples were subjected to Fourier Transform Infrared Scpectroscopy (FTIR), Tensile Strength Analysis and Lignin Test for analysis of the results obtained. It is expected to produce more than 15% of delignification of fiber from the research.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Oil palm (***Elaeis guineensis*** Jacq.) is the most important yield in Malaysia that plays a significant role in the progress of economy for countries in West Africa and Southeast Asian (Shinoj, Visvanathan, Panigrahi and Kochubabu, 2011). Palm oil which has commercial value is mainly obtained through oil palm plant. Malaysia, falling under one of the Southeast Asian country is a huge producer of oil palm that produces more than 8 million tons of oil yearly. Huge lignocelluloses waste is left behind from oil palm industry like frond, trunk, and empty fruit bunch (EFB). These materials are normally thrown away or burned down and hardly made into useful product when fibers extracted from the residues can be raw material for many industries. According to Wirjosentono et al., 2004; Shinoj, Visvanathan, Panigrahi, & Kochubabu (2011), from all the parts of oil palm, EFB yields the highest percentage of fiber which is about 73%.

In the recent years, fiber has become a very important material that is largely being used for producing composite materials, string and filaments, and even to

make sheets of papers. Engineering materials are made of fibers. Fibers can be obtained naturally or made synthetically. Fibers that come naturally have lignin surrounding them. Lignin is a complex aromatic heteropolymer which prevents deterioration of polysaccharide in plants (Brown, 1985; Jung and Deetz, 1993; Grabber, 2005). As plant get older, lignin content increases thus reducing plant's deterioration through primary wall lignifications (Chesson, 1993; Jung and Deetz, 1993; Grabber, 2005).

Therefore, there are three notable challenges in delignification a fiber. The first is determining the content of lignin in a particular sample which is a great deal as it contains different components as well as covalently-linked carbohydrate, proteins and other compounds (Brinkmann, Blaschke and Polle, 2002). The second is delignification method effective and solutions to be used to remove lignin without altering the chemical and physical structure of fiber. The third is the process of determining quantity of lignin removed which is the analysis.

To date, there are many methods that have been introduced for determination of lignin. Many of the methods fall under two basic principles which are gravimetric method that isolates lignin and cell wall and spectrophotometric method which decomposes lignin into soluble degradable components (van Soest, 1963; Allen, 1989; Brinkmann, Blaschke and Polle, 2002). Due to long procedure of chemical preparation, both principles are time consuming. While there are no distinctive results over the years to determine the best method in delignification (Hatfield and Fukushima, 2005), spectrophotometric method can be a better method as it reduces the chemical alteration of lignin.

Different pulping methods have been introduced to delignify fiber. Common pulping method is through the use of chemicals such as Kraft pulping. However, this method makes chemical alteration towards fiber. Delignification through enzyme kinetics and sonokinetic are methods that gained attention lately. Apart from that, solvent used for pulping also affects the percentage of delignification. Suitable solvent for pulping are also being studied to give better delignification. Aqueous soda solution, sulfuric acid and acetic acid are the common solvents used. Among these three solvents, aqueous soda solution is found to be giving better percentage of lignin removal (Romdhane and Gourdon, 2002; Koberg et al., 2011; Garcia, Alriols, Llano-Ponte and Labidi, 2011).

Analysis of lignin removal can be made using various methods such as using Scanning Electron Microscopy (SEM) and High Performance Liquid Chromatography (HPLC). For this research purpose, Lignin Test and Tensile Strength Analysis analysis has to be carried out to evaluate the effectiveness of lignin removal. Selection of effective analysis to determine amount of lignin removed will result in better estimation of success of delignification.

Recent study shows that application of sonokinetic for delignification of fiber serves as a promising technology which is relatively easy to be implemented (Romdhane and Gourdon, 2002; Koberg et al., 2011; Garcia, Alriols, Llano-Ponte and Labidi, 2011).This method will be replacing the common pulping methods available. This paper will study on the application of sonokinetic on delignification

of fiber as well as the effective methods to determine amount of fiber and lignin removed.

1.2 Problem Statement

Delignification is an important process in obtaining fiber. However, the process can be very tedious and the success rate can be very discouraging. Fiber is enclosed with many complex parts of plant cell. This includes cell wall carbohydrates, proteins, fibers as well as lignin. The quantification of lignin may be interfered from all these compounds leading to over or underestimation. The exact quantification of lignin before proceeding with pulping process is important to determine the amount of lignin removed after pulping process is carried out. Therefore, determination and quantification of lignin are one of the major problems in delignification process.

Another notable problem in delignification is the determination of suitable solvent that will assist delignification process. Fiber is a complex chemical compound that is made of combination of different chemical compound. It is not easily degradable. A particular amount or part of fiber will be soluble in acid whereas other parts will be soluble in alkali. Employing multiple solvents for delignification will not be cost effective. Hence, selection of solvent for pulping process is vital to ensure all lignin content is removed from fiber strand cost effectively.

1.3 Research Objectives

i. To study on ultrasound method on delignification of fiber.

ii. To study on the Klason-Lignin method to determine the amount of lignin in fiber strand.

iii. To study on the tensile strength of fiber before and after delignification.

1.4 Scope of the Study

i. To study on the use of ultrasound on the delignification of fiber.

ii. Oil palm empty fruit bunch fiber is used as raw material.

iii. Determination of lignin content was made through Klason-Lignin method.

iv. Temperature and concentration of alkali solvent are parameters taken as manipulated variables.

v. The tensile strength analysis of fiber is carried out to study the effect of delignification.

1.5 Significance of the Study

Ultrasound method has an advantage compared to other pulping methods as it does not do damage towards fiber, either physically or chemically. This will help in ensuring the quality of fiber strand obtained. On the other hand, issues pertaining solubility of lignin in acids and alkali will not affect the amount of lignin removed as

chemicals are not used to separate lignin from fiber but only to break the cell wall of fiber.

To date, there are very few researches made on delignification using ultrasound. The existing research uses gravimetric method to determine the amount of lignin in fiber. Gravimetric method is proven to be least effective method for this purpose which renders the current researches inaccurate as the amount of lignin removed is expected not to yield proper result.

Therefore, it is expected that by employing the Klason-lignin method to determine lignin content and alkali solvent to break cell wall of fibers, more amount of lignin removal from ultrasound method on delignification can be obtained. This research has commercial value in the industries using fiber as raw material. If this study is able to produce about 18% to 25% of delignification, then this will be the very first method to obtain fiber through better delignification process. This is a novel research and it has wide potential for patenting the research for further publication and commercialization.

CHAPTER 2

LITERATURE REVIEW

2.1 Lignin: Structure And Composition

Lignin is a complex structure that is particularly known for its contribution towards the strength of plants. It is derived from free radical reaction of an alcohol component with other cell wall constituents. It is an integral component of secondary cell wall with common molecular structure of $C_9H_{10}O_2$, $C_{10}H_{12}O_3$, or $C_{11}H_{14}O_4$. Lignin has cross-links between macromolecules which have molecular weight that normally exceeds ten thousand.

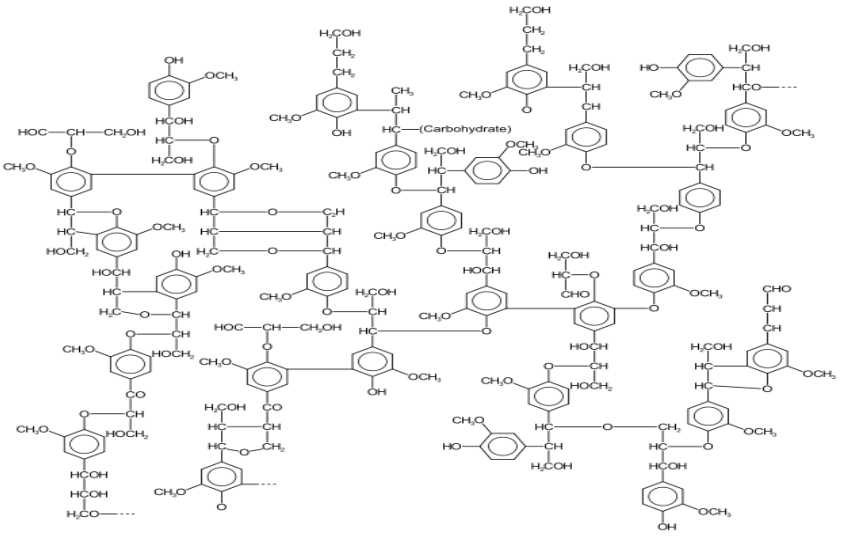

Figure 2.1 Structure of Lignin

Lignin has high commercial value and it has wide scope of function when it comes to biological and ecological function. It plays vital role in improving water conduction in plants through xylem, controlling the attacks of pathogens and also strengthening fibers (Grabber, 2005). Lignin also plays an important role in sustaining the carbon cycle, isolating carbon in atmosphere into plants. As it slowly decomposes, it becomes humus which helps to maintain water retention in plants when ecological changes occur. Lignin has commercial values as it acts as dispersant in cement application, additives in oil field applications and raw material for several chemicals.

Lignin plays a vital role in plant growth and develop-ment by improving water conduction through xy-lem tracheary elements, enhancing the strength of fi-brous tissues, and limiting the spread of pathogens in plant tissues (Iiyama et al., 1994). Lignin restricts the degradation of structural polysaccharides by hydrolytic enzymes, thereby limiting the bioconversion of forages and fibrous crops into animal products or into liquid fuels and other industrial products (Brown, 1985; Jung and Deetz, 1993). Lignified dietary fiber also plays an important role in maintaining gastrointestinal function and health in humans (Ferguson et al., 2001).

The enzymatic degradability of cell walls in leaves and particularly stems of plants declines during maturation because of accumulation and progressive lignification of primary and secondary cell walls of vascular and sclerenchyma tissues. As plants reach physiological ma turity, the degradability of stems, and to a lesser degree leaves, is further depressed by lignification of primary-walled parenchyma and epidermal tissues (Wilson and Hatfield, 1997). These reductions in

degradability are partly related to the increased lignin content of cell walls; however, variations in three-dimensional struc-ture and composition of lignin and its hydrophobicity, encrustation, and cross-linking to other matrix compo-nents also have been implicated (Chesson, 1993; Jung and Deetz, 1993).

Because of the anatomical, morphological, and devel-opmental complexity of cell wall and lignin formation in various plant tissues, studies with normal, mutant, or transgenic germplasm often do not show consistent relationships between lignin-matrix characteristics and cell wall degradability at the whole plant, plant part, or even tissue level (Grabber et al., 1992; Jung and Vogel, 1992; Jung et al., 2000). Even when plant selection or enzyme downregulation is targeted at specific lignin properties or lignin-matrix interactions, compensatory or associative changes in other cell wall characteristics often occur, making it difficult to identify underlying mechanisms controlling cell wall degradability. Plants may, for example, respond to lower lignification by in-creasing the amount of cross-linking (Chabannes et al., 2001), perhaps yielding no net change in digestibility. A common but faulty assumption is that correlations between a particular lignin trait and degradability dem-onstrate a cause and effect relationship. Because of the complexity of cell wall development, associations be-tween one lignin characteristic (e.g., lignin composition) and cell wall degradability can be masked, confounded, or merely correlated to concurrent changes in other lignin properties (e.g., lignin cross-linking) that influ-ence cell wall degradability. Because of these factors, the underlying mechanisms by which lignin restricts de-gradability of plant cell walls are poorly understood.

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Because of the developmental and morphological complexity of plants, a variety of simpler model systems have been developed in an attempt to isolate and iden-tify potential interactions between lignin and matrix components and, in some cases, to assess how these interactions influence cell wall hydrolysis by enzymes or rumen microflora. One approach involves adding, removing, or modifying lignins or other matrix compo-nents in isolated cell walls or cellulose by chemical or enzymatic treatments to assess their role in controlling cell wall degradability. The simplest studies (Jung and Ralph, 1990; Sewalt et al., 1996a) involved adding pow-dered wood lignin or an artificial lignin (polyeugenol) to cellulose before hydrolysis by rumen microflora. More intimate associations between components were ob-tained by forming lignocellulosic hydrogels or by nonspe-cific coupling of polyeugenol to cellulose. These studies yielded contradictory results with cellulose hydrolysis being inhibited by added wood lignins but not by added polyeugenol. In contrast, bound polyeugenol severely restricted cellulose hydrolysis whereas intimate associa-tion of lignin with cellulose in hydrogels actually en-hanced cellulose hydrolysis. Various chemical or biolog-ical treatments have been used to remove or modify lignins and to cleave lignin-matrix cross-links in cell walls (Fritz et al., 1991; Jung et al., 1992; Morrison, 1991), but the specificity of these treatments was poor (Fry, 1986), making it difficult to attribute changes in degradability to specific changes in cell wall properties. In contrast, enzymes like cinnamoyl esterases can attack specific linkages found in cell walls (Garcia-Conesa et al., 1999a), but their efficacy *within* lignified cell walls remains unproven. Alternatively, the activity of many enzymes involved in cell wall biosynthesis can be manip-ulated in plants by inhibitors and elicitors (Grand et al., 1985; Reddy et al., 1999). Unfortunately, the utility of elicitors or inhibitors in cell wall degradability studies would be limited because they often lead to multiple

changes in cell wall characteristics.

Plant tissue culture also can be used to model cell wall formation and lignification, the most successful system being the differentiation of up to 60% of *Zinnia elegans* L. mesophyll cells into tracheary elements (Fukuda, 1992). This system has been extremely valuable for cell biology and gene expression studies of vascular differen-tiation, secondary wall formation, and lignification (Mil-ioni et al., 2001). Unfortunately, each *Zinnia* culture produces only a few milligrams of cell walls, limiting its application in cell wall degradability studies. Gram quantities of cell walls can be produced with other tissue culture systems, but the degree of differentiation and lignification is typically much lower, except perhaps for cell cultures with high endogenous levels of cytokinin (Blee et al., 2001). In all cases, differentiating cultures produce a mixture of primary- and secondary-walled cell types with nonlignified cells providing phytohormones, monolignols, or other factors needed by differentiat-ing cells for secondary wall formation and lignification (Hosokawa et al., 2001). As a result, characterization of specific cell types in differentiated culture systems would require the same type of fractionation procedures as used to isolate tissues from intact plants (Grabber and Jung, 1991; Hatfield et al., 1999). To a greater degree than with plants, cell walls from cultured cells can be manipulated to varying degrees by chemical, enzymatic, biological, elicitor, or enzyme inhibitor treatments (Kal-iamoorthy and Krishnamurthy, 1998; Kauss et al., 1993; Taylor and Haigler, 1993) or by altered gene expression (Stasolla et al., 2003), but specific alteration of individual lignin characteristics without compensatory changes in other lignin-matrix interactions would be difficult to obtain. In addition, interactions between matrix compo-nents and lignin in cell

culture systems, as with plants, are difficult to quantify because the deposition of matrix components and lignin are often overlapping processes during cell wall formation. This difficulty is further com-pounded by our inability to fully isolate or characterize, by solvolytic or spectroscopic methods, lignin and lignin-matrix structures in cell walls. The prospects for lignin and cell wall characterization have, however, improved considerably with the recent discovery of solvent sys-tems capable of fully dissolving ball-milled cell walls for analysis by NMR and other analytical methods (Lu and Ralph, 2003).

Another approach, often enabling specific alteration of individual lignin characteristics or lignin-matrix inter-actions, is the in vitro oxidative polymerization of lignin precursors (i.e., monolignols) into DHPs, either alone or in the presence of natural or artificially synthesized cell wall components (Higuchi et al., 1971; McDougall et al., 1996; Ohnishi et al., 1992; Ralph et al., 1992; Terashima et al., 1995; Touzel et al., 2003). In this system, a wide variety of monoclonal types, matrix components, and polymerization conditions may be used, pro-viding the investigator with a much greater control over the composition and structure of lignin and lignin-matrix interactions formed than is possible with living plant systems. Characterization of matrix components and monolignols before and after DHP formation permits the characterization of lignin-matrix interactions to a degree that is not possible in natural plant systems where matrix components and lignin are often deposited concurrently into composite structures that are difficult to analyze by spectroscopic or solvolytic methods. Although this in vitro system has been extremely valuable for modeling many aspects of cell wall lignification, artificial DHP lignins do not fully mimic the structure of natural plant lignins (Terashima et

al., 1996), and they do not adequately model the three-dimensional matrix of cell walls.

The limitations of the tissue culture and DHP model systems are largely overcome by using in situ proxy-das Es to polymerize monolignols added to isolate cell walls. In the late 1970s, Whitmore (1978) was the first to form such DHP-cell wall complexes to study lignin-protein and lignin-carbohydrate interactions in cell walls isolated from callus of *Pinus elliottii* Engelm. Since the early 1990s, our objective has been to develop, evaluate, and utilize DHP-CW complexes as a model for studying lignin formation and lignin-matrix interactions in plants and for assessing how lignins restrict the enzymatic degradation of cell walls.

Lignin is complexly linked with fiber. According to Grabber (2005), interaction between plant components and lignin is difficult to analyze as lignin and other components often overlaps during its formation, making the structural study harder. The challenge in delignification process is to determine or quantifying the amount of lignin there is in a particular sample and separating lignin without making alteration to other components of plant. A definitive molecular structure cannot be drawn for lignin and lignin concentration estimates are empirical which results in varying lignin concentration estimates for different empirical method (Jung, Varel, Weiber, & Ralph, 1999). Grabber (2005) found that even when delignification is targeted at specific lignin part of plant eventually this process changes other cell wall structure. Due to this complexity also, the degradability of lignin cannot be determined by one characteristic of lignin alone when many factors like composition and cross-linking are determinant factor in delignification. Therefore, lignin cannot

be efficiently separated by solvolytic or spectroscopic method (Grabber, 2005). On the other hand, due to structural similarities between other components of plant and lignin, analysis of delignification is made tedious as some other component fractions that may be isolated together with lignin could be mistakenly taken into account as lignin. These are the reason why the mechanism by which lignin restricts fiber delignification is difficult to be studied.

2.2 Ultrasound

Pre-treatment process is one of the essential steps in delignification. Pre-treatment is process of disrupting carbohydrate-lignin shield that hinders enzyme or chemicals to reach cellulose (R.Yunus et al., 2010). Many types of pre-treatment methods have been developed throughout the years but better methods are still in search to cut pre-treatment cost. Ultrasonic is a new method of pre-treatment compared to the existing chemical, physical, physiochemical and biological pre-treatment. When material is treated by ultrasonic, the molecules will reduce in size due to erosion (Jun et al., 2009; R.Yunus et al., 2010). This method has the ability of cracking cell walls and cause fibrillation by giving more exposure (Tang and Liang, 2000; R.Yunus et al., 2010). According to Kadimaliev et al., 2003; R.Yunus et al., 2010, this method could be used for intense bioconversion like bioethanol. Therefore, this method is expected to give reasonably good result of delignification of fiber of oil palm empty fruit bunch (OPEFB).

Pre-treatment of lignocellulose biomass depends on pre-treat-ment processing conditions and it must be tailored to the specific chemical and structural composition of the various, and variable, sources of lignocellulosic biomass. Ultrasonic pre-treatment is rel-atively new in biomass pre-treatment technology. The effect of ultrasound on lignocellulosic biomass has been employed in order to improve the extractability of hemicelluloses, cellulose, lignin or to get clean cellulosic fibre from used paper but only a few at-tempts to improve the susceptibility of lignocellulosic materials to biodegradation by using ultrasound power have been described (Asakura et al., 2008). The ultrasonically assisted pre-treatment does not hydrolyse the biomass to soluble sugars. The function is to generate a pre-treated substrate that is more easily hydrolysed via increasing the accessible surface area and affecting the crystal-linity (Toma et al., 2007). The efficiency of acid hydrolysis has been evaluated to determine the optimal conditions of ultrasonic expo-sure for the pre-treatment of lignocellulosic biomass.

When materials in a liquid suspension are treated by ultrasonic energy, the particles are subjected to either surface erosion via cavitational collapse in the surrounding liquid, or size reduction due to fission through interparticle collision or the collapse of cav-itation bubbles formed on the surface (Jun et al., 2009). Ultrasonic energy could crack the cell walls, dislocate the secondary wall of the middle layer, and cause exposure and fibrillation (Tang and Liang, 2000). The propagation of ultrasound waves through liquids at frequencies ranging from 20 kHz to a few megahertz results in cavitation, which causes various physical and chemical effects (Asakura et al., 2008). Some studies suggested that pre-treatment of lignocellulose substrates with ultrasonic energy could be recom-mended for the

intensification of bioconversion both in nature and under production conditions (Kadimaliev et al., 2003).

The effect of ultrasonication on the OPEFB fibre prior to acid hydrolysis was evaluated. The main objective of the study was to determine if ultrasonic energy could function as a pre-treatment methodology for bioethanol production. In doing so, ultrasonic en-ergy would be evaluated for its ability to open up the hemicellu-loses structure as well as decrystallize cellulose such that the chemicals or enzymes can penetrate easily. The impact of ultra-sonic energy treatment on the efficiency of OPEFB hydrolysis at low temperature was measured by the level of sugars released. The samples were then analysed using a scanning electron micro-scope (SEM) to describe the morphological changes of OPEFB fibre under different acid hydrolysis conditions. The optimum ultrason-ication condition was employed to study the effect of acid hydro-lysis on the production of xylose at different temperatures using a batch reactor.

The application of ultrasounds should be considered as an auxiliary source of energy (like agitation or heating) in solid-liquid extraction processes. The formation of microbubbles during sonication treatments (cavitation phenomenon, which involves the increase of temperature and pressure at the solid – solvent interface) improves diffusivity or mass transfer processes. Therefore, the mechanical energy associated to the ultrasound application (vibration and stirring) promotes the decrease of solid particles size. When vegetal materials are ultrasound-treated the cellular wall is affected by this energy improving the extractability of its components into the solvent media (Ebringerová and Hromádková, 2002; Liu et al., 2007).