EFFECT OF WHITE ROT FUNGI VARIATION ON THE BIOPULPING OF OIL PALM TRUNK

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EFFECT OF WHITE ROT FUNGI VARIATION ON THE BIOPULPING OF OIL PALM TRUNK

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
°C	Degree Celsius
BOD	Biological oxygen demand
BRF	Brown-rot fungi
C. subvermispora	*Ceriporiopsis subvermispora*
CAZymes	Carbohydrate active enzymes
CSL	Corn steep liquor
CTMP	Chemithermomechanical pulping
CMC	Carboxy methyl cellulose
CSF	Canadian Standard of freeness
DNS	3,5 Dinitro-salicylic acid
DP	Degree of polymerization
E. globules	*Eucalyptus globules*
EFB	Empty fruit bunch
FPPRI	Forestry and Forest Products Research Institute
FRIM	Forest Research Institute Malaysia
FSP	Fibre saturation point
g/m^2	Grammage
Lac	Laccase
LiP	Lignin peroxidase
mL	Millilitre
MnP	Manganese peroxidase

MOE	Modulus of elasticity
MOR	Modulus of rupture
OA	Oxalic acid
OPB	Oil palm biomass
OPF	Oil palm frond
OPT	Oil palm trunk
P. chrysosporium	*Phanerochaete chrysosporium*
P. sanguineus	*Pycnoporus sanguineus*
RMP	Refiner mechanical pulp
S. commune	*Schizophyllum commune*
SEM	Scanning electron microscopy
SSF	Solid state fermentation
T. versicolor	*Trametes versicolor*
TMP	Thermo-mechanical pulping
WRF	White rot fungi
Xyl	Xylanase
Α	Alpha
EGs	Endoglucanases
$NaClO_2$	Sodium chlorite

KESAN PELBAGAIAN KULAT PEREPUT PUTIH TERHADAP BIO PEMULPAAN BATANG KELAPA SAWIT

ABSTRAK

Biojisim lignoselulosik adalah suatu sumber semulajadi yang penting dan berterusan bagi pembuatan kertas. Kayu merupakan sumber utama yang mengandungi gentian selulosa yang fleksibel, pra rawatan menghasilkan selulosa yang lebih cenderung untuk hidrolisis berenzim melalui pengubahsuaian penghalang lignin. Pemulpaan biologi merupakan rawatan mendegradasi lignin secara pengoksidaan bahan-bahan lignoselulosik oleh kulat pereput putih untuk proses pemulpaan. Mikroorganisma ini merembes enzim yang mendegradasi lignin dengan kehilangan polisakarida yang minimum. Walaupun sistem pra rawatan ini tidak berkembang dengan baik, tetapi ia membuka peluang kepada pra rawatan yang lain kerana ia tidak membawa keburukan kepada persekitaran dengan penggunaan bahan kimia yang berbahaya. Kajian terhadap pra rawatan biologi menunjukkan kehilangan berat lignin dan kualiti kertas meningkat. Walaubagaimanapun, hanya beberapa kulat pereput putih yang telah dicirikan dikaji. Dalam kajian ini penyiasatan terhadap potensi bagi empat jenis kulat reput putih iaitu *Phanerochaete chrysosporium* Burds, *Trametes versicolor, Pycnoporus sanguineus* (KUM 70097) dan *Schizophyllum commune* (KUM 793066) telah dijalankan. Ia dipilih berdasarkan kebolehan yang terkenal bagi pengurai pokok. Kajian ini lebih tertumpu terhadap penggunaan batang kelapa sawit sebagai substrat lignoselulosik dengan menggunakan fermentasi dalam keadaan pepejal bagi pra rawatan selama 28 hari. Kesan terhadap parameter pertumbuhan seperti tempoh inkubasi, tahap kelembapan, medium, kepekatan, pH dan suhu juga dioptimumkan. Kesan terhadap pra rawatan biologi untuk proses pembuatan kertas telah ditentukan. Setelah empat minggu inkubasi pada suhu 28°C dengan kandungan lembapan awal 60% dan pengudaraan yang malar, serpihan batang kelapa sawit telah dihkasilkan. Piawaian protokol (Piawaian Metadologi TAPPI), selalunya digunakan oleh industri kertas dan pulpa ntuk menentukan peratusan selulosa, lignin Klason serta ekstraktif dalam serpihan batang kelapa sawit. Kulat-kulat ini menghasilkan enzim ligninolitik, kebanyakannya peroksidase mangan (MnP), peroksidase lignin (LiP), dan lakase (Lac) dengan kombinasi yang berbeza. Selain itu, didapati bahawa enzim pencerna polisakarida juga dihasilkan oleh kulat dalam kuantiti yang kecil. Keputusan yang diperolehi selari dengan penemuan yang lain di mana kulat reput putih *Pycnoporus sanguineus, Phanerochaete chrysosporium* dan *Trametes versicolor* menunjukkan corak kebolehannyan lignin yang serentak ketika membiak pada biojisim kelapa sawit. Pra rawatan dengan kulat pereput putih menyebabkan ligninolisis dan selulosa dengan kebolehcernaan yang lebih baik diperolehi dengan kehilangan lignin yang tinggi. Seterusnya penggunaan tenaga penapisan menunjukkan pengurangan yang ketara dan berubah dari 8 hingga 23%. Apabila serpih batang kelapa sawit dipulpa secara mekanik, nombor Kappa, hasil pulpa, dan hasil pulpa yang ditapis berkurang dengan ketara dan kekuatan kertas meningkat pada suatu tahap tertentu dengan masa pendedahan. Ciri-ciri kertas uji kaji juga meningkat secara ketara dengan rawatan kulat. Kehilangan berat, lignin, selulosa dan holoselulosa dikira dan takat penguraian dinding sel dalam kombinasi dengan modifikasi lignin dapat dilihat daripada mikroskop pengimbasan elektron (SEM). Takat bio nyah lignin jelas menunjukkan kebaikan pra rawatan kulat pada proses bio pemulpaan menambah penghasilan enzim, menyebabkan degradasi lignin, dan meningkatkan kebolehcapaian selulosa untuk hidrolisis enzim. Kajian ini membuktikan bahawa biojisin kelapa sawit ialah substrat yang sesuai bagi bio pemulpaan dan berpotensi untuk digunakan dalam proses bioteknologi secara besar-besaran. Berdasarkan keempat-empat fungi dan peranan keseluruhannya dalam sifat pemulpaan dan pembuatan kertas, *Trametes versicolor* boleh dipilih sebagai yang terbaik berbanding dengan yang lain. Kajian ini akan memberi pemahaman untuk mengetahui sebab munasabah dari segi ekonomi bagi mengkomersialkan bio pemulpaan dalam aplikasi perindustrian.

Kata kunci: Bio pemulpaan, Deligninfikasi, Kulat Reput Putih, Lignin, Selulosa, Tenaga Penapisan.

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ABSTRACT

Lignocelluloses biomass is an important and sustainable natural resource for paper making. Wood the predominant source consists of flexible cellulose fibres, its pretreatment makes cellulose more accessible to enzymatic hydrolysis by modification of the lignin. Biological pulping is the treatment of lignocellulosic materials with oxidative lignin-degrading white rot fungi prior to pulping process. These microorganisms secrete enzymes that degrade lignin with minimum loss of polysaccharides. Though this pretreatment system is not well developed; but is advantageous over others because it does not burden the environment with harmful chemicals. Studies on biological pre-treatment have shown lignin loss, weight loss and improvement in paper quality. In the present study evaluation of the potential of four different white rot fungi namely *Phanerochaete chrysosporium* Burds, *Trametes versicolor, Pycnoporus sanguineus* (KUM70097) and *Schizophyllum commune* (KUM 793066) was conducted. They were chosen as they are well known inhabitant of tropical decomposing trees. The interest was to cultivate them on oil palm trunk as lignocellulosic substrate using solid state fermentation and determines their effect on paper making. After 28 days of pretreatment at 28±2°C with initial moisture content of 60% and constant aeration, the chips were harvested. Standard TAPPI methods, commonly used by the paper and pulp industry, were employed to determine the percentage cellulose, Klason lignin and extractives in the chips. These fungi produce ligninolytic enzymes, predominantly manganese peroxidase (MnP), lignin peroxidase (LiP), and laccase (Lac) in different combinations. The results supported the findings of others that white rots *Phanerochaete chrysosporium, Trametes versicolor, Pycnoporus sanguineus* and *Schizophyllum commune* can grow upon lignocellulosic substrates and produce considerable refining energy savings. The weight, lignin, cellulose and holocellulose loss was calculated and the extent of cell wall breakdown in combination with lignin modification was depicted from Scanning electron microscopy(SEM).The extent of bio delignification clearly shows the advantage of fungal pre-treatment in biopulping enhances production of enzymes, causing partial degradation of lignin and improving accessibility of cellulose. Additionally this study puts in evidence that oil palm biomass is a suitable substrate for biopulping and has potential for use in large-scale biotechnological processes. Considering all the four fungi and their performance overall in pulping and papermaking properties, *Trametes versicolor* can be considered to be the best amongst them. This fungi shows peak activities of lignocellulytic enzymes and in mechanical refining the chips pre-treated with *T.versicolor* consumed the least energy. After refining when pulp properties were studied it depicted that most of the mechanical properties were superior except for brightness. The Scanning electron microscopic examination revealed the development of cracks and collapse of cell walls. Thus due to afore mentioned qualities *T.versicolor* is found to be ideal for biopulping.

Keywords: Biopulping, White-rot fungi, Lignin, Cellulose, Pulp properties, Refining energy.

Chapter 1

INTRODUCTION

1.0 Research Background

Lignocellulose is the most significant structural component of vegetal biomass and represents a chief resource of renewable organic substance (Howard et al., 2003). Lignocellulose biomass is available in huge quantity and has been focused broadly for its use as substitute resource for pulp and paper, alcoholic fuel, chemicals and protein meant for food and feed using microbes for bioconversion processes. The cell walls of plants are unique in constitution of cellulose, hemicellulose and lignin (Martinez et al., 2009). In addition relatively low amounts of sugars, proteins and minerals also make up lignocellulose (Wyman et al., 2009).

The lignocellulose can be considered as a vast biological refinery that aims to generate renewable fuels, chemicals and new polymers from all of its components (Mahajan, 2011). The lignolytic and cellulolytic enzymes find multipurpose uses in various biotechnological applications, like in chemicals, textiles, food, pulp and paper industries (Bhat, 2000; Kuhad et al., 2011).

Traditionally pulp and paper is a highly investment demanding industry, that has been occasionally affected by over capacity (Kenealy and Jeffries, 2003). It is the large consumer of primary energy in the industrial sector and contributes significantly towards creating air and water pollution (Dudley et al., 1996). Paper is a major product of the forestry industry and has wide uses in our society. Currently wood is the most vital source for fibres. Manufacturing of pulp begins with raw material preparation, which includes debarking, chipping, beating and depithing the cellulosic pulp, using mechanical and chemical methods. At various stages of pulping process, chemicals are used to give the paper its specific properties (Hubbe et al., 2007).

Currently the paper consumption is rising throughout thus the world pulp production has set three imperative goals; they are to lower contamination, complete use of natural resources and to use more cost effective technologies (Gonzalez et al., 2002). Being one of the high demanding sectors globally, the pulp and paper production has been related with environmental problems (Sridach, 2010b). Nowadays, there is an ever-growing concern to maximize use of non-woody fibres as raw materials in pulp mills (González-García et al., 2010). Amongst the several non-wood plants, cereal straw has been used extensively in many Asian, African, Eastern European and Latin American countries either for manufacturing paper pulp or for premium-quality pulps for speciality papers (Sigoillot et al., 2005; Hedjazi et al., 2009).

The aim of pulping process is cellulose fibers extraction from softwood, or hardwood trees and from non wood plants for papermaking (Wanrosli and Law, 2011). The cellulosic fibres can change significantly when formed into a wet web of paper and later subjected to such processes as pressing, drying, deinking, bleaching, printing and repulping. Microscopic observations reveal that the fibres are present in the pulp in the form of layered structure with domains having lignin and hemi-cellulose which are present to various extents depending upon the type of pulp (Shao and Li, 2006; Xu 2007).

Generally two approaches, mechanical pulping and chemical pulping, have been employed to pulping. Mechanical force is involved in mechanical pulping to separate the wood fibers and lignin is dissolved in the raw material in chemical pulping, which is used to create pulp by using chemicals (Messner and Srebotnik, 1994). Certain advances of mechanical pulping process have been made over time such as refiner mechanical pulping (RMP) and thermo mechanical pulping (TMP). The pulps obtained contain lignin; their yields are very high and suffer from low strength and low brightness (Biermann, 1996). Subsequent to pulping process, the fiber is cleaned and then bleached to obtain white paper. Several other manipulations for instance sizing, addition of fillers and colour can happen later to obtain the final paper product (Kenealy and Jeffries, 2003). Even though the strength of the paper made from chemical pulping is moderately high, the yield is by and large lower than the mechanical pulping due to exclusion of total lignin content, and degradation of some hemicelluloses and cellulose (Sulaiman et al., 2011).

As mentioned the process of paper manufacturing involves using chemicals which are emitted to the atmosphere as mixture of gaseous sulphur, nitrogen and chlorine dioxide (Gupta et al., 2012). The effluents from the bleaching process contain many substances, some of which are known to be genotoxic or mutagenic (Roaet al., 2012). As the pulp is obtained at high temperature, high pressure and using alkaline solvents, it causes a waste stream that on being discharged becomes a source for water pollution (Xie et al., 2010). The method of biodegradation of lignin can solve such problem as it has the potential to be an ecofriendly one (Huang et al., 2008).

Biotechnology is considered to be an efficient and non-waste technology as it cuts the disposal costs and resource price thus reducing the burden on environment (Müller, 1986). Utilization of biotechnology for industrial pulp was considered in 1985, after research was conducted on action of white-rot fungi for lignin degradation and subsequently characterization of these fungi was intensified

(Eriksson and Kirk, 1985). The technique applies cleaner technology to achieve high yield and is viewed as a means to expand the forest resource by saving and recycling expensive chemicals and raw materials (Das and Houtman, 2004). White rot fungi generate dominant delignifying enzyme scheme therefore their utilization appear logical and the first efforts were already under way in the 1950s and 1960s to exploit these fungi in pulp production (Messner et al., 1998).

Previously diverse studies on enzymes action, molecular degradation, pH, temperature conditions and numerous other kinds of reactions have undergone successfully (Gonzalez et al., 2002). Biotechnology is the current trend in many production processes across the world and the awareness of biotechnological process has been adopted by the researchers as instrument in the pulp and paper industries. Biotechnological processes are basically non-hazardous to the environment since delignification is a biochemical route, crucial to the earth's carbon cycle (Hatakka, 2010).

Biopulping or Biomechanical pulping (BMP) is the biological pretreatment process of wood by means of white rot fungus that is directed towards production of mechanical pulps for paper making with decreased energy requirements for fiberizing and refining and improved strength properties (Akhtar, 1997).The most important objective is to develop a technique so as to improve the efficiency of the existing pulping processes in an environmental friendly and cost-effective way. With the purpose of achieving this, the use of wood-rotting fungus containing ligninolytic enzymes has been investigated in pretreatment of wood chips. Early researchers have reported that the employment of fungi can conserve chemicals and energy in the paper-making process. The biopulping outcome is essentially dependent on the particular raw materials, microorganism and pulping conditions used (Oriaran et al., 1990; Chen et al., 1999; Isori et al., 2011). The enzymes secreted also have multiple applications like in deinking mechanical and chemical pulping and eradicate shives, besides the regular bleaching, beating and eliminating the extractives from pulp (Bajpai, 2011).

Oil palm is a significant commercial non woody plant that progresses in the humid tropical regions of changing density all over the world. As the demand for oils and fats has been on a continuous rise, this has resulted in a hasty expansion of the oil palm industry in the Asian region (Wanrosli and Law, 2011). Further it has lead to the alteration of enormous land areas to oil palm cultivation.

1.1 Problem Statement

The consumption of paper globally has amplified from about 300 million tonnes in 1998 to over 425 million tonnes in the year 2010 (García et al., 2008). The paper consumption directly correlates with the development of the global economy as it is known that the developed nation consumes more paper products (Xie et al., 2010). The manufacturing of chemical pulps entails a huge quantity of chemicals that might facilitate in harming the environment (Leatham et al., 1990; Berrocal et al., 2004). The chemical pulping process begins by treating wood chips at around 160 – 180^{o} C in a white liquor solution of sodium sulphide and sodium hydroxide. This treatment cleaves lignin ether bonds; dissolving upto 90% of the lignin, hemicellulose and wood extractives, though the overall yield is relatively low around 40–50% (Hataka and Hammel, 2010). Approximately 55% of the original wood is dissolved in what is now termed the "black liquor" (Hall, 1988). By-products are recovered and the liquor at evaporated phase comprises high concentration of inorganic sulphur in the form of sulphate or dithionite (Haggblom, 1990). Several volatile compounds and gases from the burnt concentrated black liquor are released to the atmosphere. Air contaminants released from pulping include particulate matter, sulphur dioxide, and total reduced sulphur compounds.

The high growth in population has led to a heavy demand of paper that has caused a steady expansion of paper industries leading to deforestation to meet the needs. Pulp and paper industry is investigating to find an apt raw material to satisfy the increasing demand of paper world wide (Wanrosli and Law, 2011). With enormous production of palm oil in Malaysia, the sum of residues produced also shows a parallel increase (Latif et al., 2004; MPOB, 2012).

Malaysia is the second largest producer in the world and holds nearly 5.038 million hectares for this crop. The type of lignocellulosic residues generated from oil palm industry includes palm kernel shells, wet shell, fiber and empty fruit bunches (EFB) from the mills, whereas oil palm fronds (OPF), oil palm trunks (OPT) are obtained from the plantation site (Chen and Danapal, 2012; RPSO, 2012).

The trunks of oil palm are available in bulk amount but there has not been any commercial achievement of its pulping (Wanrosli et al., 2007). In light of the need for industrial sustainability and clean technology the potential use of this waste in pulp and papermaking can solve most of the dumping problems and may also helps in the environmental sustainability (Obidzinski et al., 2012).

The prime benefits of biopulping are it requires mild conditions, conservation of energy and environment in comparison with other conventional methods (Koshy and Nambian, 2011). The fungal treatment is usually combined with mechanical pulping to save refining energy and its compatibility is not good with alkaline (Kraft) pulping, as the most useful white-rot fungi produce oxalic acid and other organic acids when growing in wood or straw (Akhtar et al.1998; Hofrichter et al.1999; Hakala et al., 2005) and the neutralization of these acids causes a need for extra alkali. Thus in this research four white rot fungi namely *Phanerochaete chrysosporium*, *Trametes versicolor*, *Pycnoporus sanguineus* and *Schizophyllum commune* are studied for pretreatment in combination with mechanical pulping.

1.2 Objectives

Accordingly, the main aim of this research is to study the application of innovative pretreatment on oil palm trunk. The objectives of the study are:

i. To identify optimal ligno-cellulolytic enzyme production by four white rot's *Phanerochaete chrysosporium*, *Trametes versicolor*, *Pycnoporus sanguineus* and *Schizophyllum commune* upon their growth on oil palm trunk chips.

ii. Evaluate the effectiveness of fungi's on energy consumption during mechanical refining. The general aim of the study was to encourage energy efficiency during the pulp production while improving pulp properties.

iii. To evaluate the structural changes through Scanning Electron Microscope.

iv. Contribute to the understanding of mechanisms behind the reported effects for further development of chip pre-treatment and refining processes.

Chapter 2

REVIEW OF LITERATURE

2.0 Lignocelluloses:a valuable resource

Lignocelluloses characterize a key source of organic matter as they are the chief structural constituent of forestry and agro industrial residues (Lin and Tanaka, 2006). Lignocellulosic materials are sustainable resources that are ad infinitum, as its supplies are inexhaustible as generation of new growth biomass continues (Cheng, 2002). Biomass is commonly heterogeneous and is made up of forest ecosystems, agricultural, agro-industrial residues, (e.g. wheat straw, rice straw, corn stalks) and significant share of urban solid waste (e.g. waste paper) (Grant and Long, 1981).

Wood is a porous plant material made up of various types of xylem cells and is considered as a promising energy resource since it consists of abundant carbohydrates (Sun and Cheng, 2002). It can be converted to many by products at relative low costs in a sustainable manner. The forests help in soil and water conservation, biological diversity and symbolize a significant source of energy, timber and pulp (Ibisch et al., 2010). Forests are essential for human survival and well-being and they harbor two thirds of all terrestrial animal and plant species. The vegetation provides us with food, oxygen, recreation, and spiritual sustenance.

Also they are the source for over 5,000 commercially-traded products, ranging from pharmaceuticals to timber and clothing (Convention on Biological Diversity, 2009). Other benefits of forests are in shielding against soil and water conservation, biological diversity and alleviation of climate change (Motel et al., 2009).

The terrestrial plants availability is supported by the large numbers of world-wide annual lignocellulosic biomass production of about 200 billion tons that accounts for 60% of the total biomass production on the earth (Kuhad et al., 1997; Zhang et al., 2008; Sánchez and Cardona, 2008). Throwing away these wastes affect the soil as well as landfill causing harsh environmental crisis. As these wastes are rich in soluble sugars, which are easily digested by microorganisms, they are extremely suitable as raw materials in the manufacturing of industrially significant compounds by fermentation (Kumar and Kanwar, 2012).

Fermentation of lignocellulose, both by submerged or solid-state fermentation systems may produce products of concern for food, pharmaceutical and biofuels industries (Mussatto and Teixeira, 2010). Lignocellulosic biomass is a vast resource vital for the functioning of industrial societies and is significant to the growth of a sustainable worldwide economy (Maki et al., 2009). Wood and paper products have played a vital part in the evolution of civilization. Agricultural residues consisting of leaves, stems, and stalks from many sources like wheat straw, rice straw, corn fibre, corn stover, sugarcane baggasse, oil palm biomass, rice hulls, woody crops, are generated in huge amount but there commercial use is meager. These non-woody new raw stuffs have the prospective to substitute the conventional wood raw materials because they are available easily, are inexpensive and avoid shortage of forest resources (Sridach, 2010a).

The agricultural waste is usually considered the superlative substrates for the solid state fermentation (SSF) processes (Pandey et al., 2001). Previously successful industrial utilization of sugarcane bagasse, wheat and rice straws, corncobs, corn flour, banana, cassava, sugar beet pulp, coconut and mustard oil cake as substrates has been achieved (Sreedevi and Reddy, 2012).

Wood is the predominant resource of fiber supply for paper making. The lack of forest resources obliges the utilization of non-wood fibrous resource as substitute for papermaking (Ashori, 2006). The overall production of virgin pulp for paper and paperboard in 2006 was 187.6 million metric tons, out of which 17.4 million metric tons was made from non-wood fibers (Bowyer et al., 2007). The use of agro-fiber wastes in paper manufacture is beneficial in terms of environmental and socio-economic aspects.

The production of non-wood plant pulps has increased more rapidly and several non-wood fiber resources are commercially utilized to manufacture chemical pulp and paper products in countries like China, India, Latin America, Africa, Middle Eastand Turkey (Akgül and Tozluoğlu, 2009). Presently various agricultural residues like wheat and rice straws, hemp, sorghum stalks and jute are considered as suitable raw materials for pulp and paper production (Ashori, 2006, Hurter et al., 2010).

Additionally, several studies have been carried out to establish new lignocellulosic fiber resources for pulp and paper industries (Sarwar et al., 2006; Shatalov and Pereira, 2006; Tran, 2006; Wanrosli, 2007). The agricultural wastes of oil palm biomass are in steady and abundant supply in Malaysia. Amongst them the oil palm trunk is cheap lignocellulosic raw material and has qualities quite similar as compared to wood (Sulaiman et al., 2012).

The key to the most efficient use of biomass is to design a suitable and sustainable integral biopulping model to separate biomass in its major compounds in order to generate the highest value added for all fractions (Mtui, 2009).The structure of lignocellulose is depicted in the following Fig 2.1.

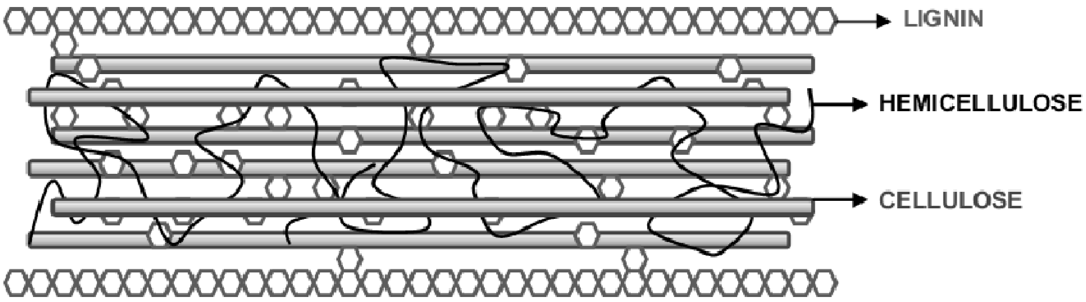

Fig 2.1: Structure of lignocellulose illustrating cellulose, hemicellulose and lignin fractions (Mussatto and Teixeira, 2010).

2.1 Significance of using oil palm

Oil palm tree is a perennial monoecious plant, and is the produced by 42 countries for good economic gains. Oil palm is botanically known as *Elaesis guineenis*, Jacq, which is derived from the Greek *elaion* meaning oil and the specific name of *guineensis* signifies its origin from the Equatorial Guinea coast (Nordin et al., 2004). Palm oil is the world's principal vegetable which is obtained from mesocarp and the kernel of its fruit (Hashim et al., 2012).

The oil palm industry in Malaysia is vast with 6 million hectares of plantation, generating over 11.9 million tons of oil and 100 million tons of dry lignocellulosic biomass (Abdul Khalil et al., 2010a). On an average the amount of biomass created inclusive of the oil and lignocellulosic materials, is 231.5 kg dry wt yr^{-1} (Abdul Khalil et al., 2010b). Malaysia is the world's leading producer and exporter of the oil palm, accounting for around 60% of the world's oil and fat production (Abdul Khalil et al., 2012). Morphologically the oil palm is a single stemmed and grows erect up to 20m tall with pinnate leaves of length 5m. The tree is without branches and trunks enlongating towards the crown.

In general there are 41 wide leaves or fronds on each mature palm. Corley and Gray (1976) reported that the trunk remains covered by old leaf bases until the palm is about 11 to 15 years old. The trunk of an old palm is usually completely free of leaf bases, except just below the crown. Oil palms are felled after their economic life-span of 25 years leaving the trunks behind and make residual wood debris causing severe problems of environment (Wanrosli and Law, 2011).

Oil palm industries generate enormous amount about millions of tons of biomass each year (Rozman et al., 2005) this waste can create substantial environmental problems when simply left on the plantation fields (Bazmi et al., 2011). Oil palm biomass (OPB) is lignocellulosic residues that contain 50% cellulose, 25% hemicellulose, and 25% lignin in their cell wall (Alam et al., 2009). The abundant lignocellulosic residues produced from oil palm industries are oil palm fronds (OPF), oil palm trunks (OPT) and empty fruit bunches (EFB), presses fruit fibre (PFF), kernel shell and palm oil mill effluent (POME). Oil palm fronds accounts for 70% of the total oil palm biomass produced, while the EFB accounts for 10% while OPT accounts for only about 5% (Ratnasingam, 2011).

With the oil palm industry generating immense amount of lignocellulosic rich material, it also should be prepared to utilize the available biomass in the best possible manner (Basiron, 2007). Several researchers have stated that a large amount of oil palm residues resulting from the harvest can be utilized as by–products. Researchers carried out an extensive study on utilization of OPB as a source of renewable materials (Sumathi et al., 2008).

Their fibres offer exceptional properties and have potential as outstanding reinforcing fillers in the matrix. They can be used as an alternative material for bio-composites, hybrid composites, pulp and paper industries (Abdul Khalil et al., 2009). Research on OPT recognizes it as a valuable residual with great potential for useful by products thus research and development activities focused on its utilization are continuing (Hashim et al., 2012).

Earlier composite panels, laminated veneer lumber, medium-density fiberboard, particleboard and plywood from frond and trunk have been successfully made (Chew and Ong, 1985; Laemsak and Okuma, 2000). The production of block board as well as furniture (Mohamad et al., 1985) from OPT lumber have also been investigated with promising potentials.

Extensive investigations are carried out in best possible ways so as to evaluate the possibilities of OPB for commercial and environmental purposes (Sulaiman et al., 2012). Currently with the restricted supply of raw materials from forests and rubber tree plantations, the biomass of oil palm mainly EFB, OPT and OPF can be processed further for the manufacturing industrially viable products (Hashim et al., 2012). The estimated use of OPB as an unconventional raw material for pulp and paper industries can be one of the alternative materials for wood-based industries in Malaysia. The oil palm tree is one material that has been identified as an alternative raw material, which is locally available for the furniture manufacturers.

Thus in this research we studied OPT for their suitability for pulp and paper manufacture. This research considers the potential and challenges of using oil palm trunk biomass for pulp and paper making. Despite all these extensive research work the study on utilization the OPT for production of pulp and paper could not be traced in the literature.

2.1.1 Anatomy of oil palm trunk

Killman and Lim, (1985) investigated and concluded that the oil palm lacks cambium, secondary growth, annual growth rings, ray cells, sapwood, heartwood and branches. The cross sectional view of the OPT, depicts three main parts are the cortex, peripheral region and the central zone. The trunk comprised of long vascular bundles, encrusted in parenchyma ground tissue. In general the expansion in diameter of the stem is due to cell division and cell enlargement in the ground tissues of parenchyma (Noorbaini, 2009).

The epidermal layer of the trunk is the bark or known as cortex which is approximately 3-3.5 cm thick. The cortex consists of ground parenchyma with plentiful strands of small and irregular fibrous strands and vascular bundles. Also narrow layers of parenchyma which is filled up of vascular bundlesmake this layer. The key function of the epidermis is in providing mechanical support for the palm stem (Noorbaini, 2009). The larger central zone is made up of diverse kinds of widely scattered vascular bundles.

Oil palm trunks have several extraordinary distinctive features, prime one is that it holds high moisture content, roughly around 1.5-2.5 times the weight of the dry matter (Husin et al., 1986). The high moisture content around 40- 50% indicates the huge amount of sap present in it (Kosugi et al., 2010). Secondly the content of cellulose and lignin are moderately lesser, and higher contents of water-soluble and NaOH-soluble compounds compared to rubber wood and baggasse (He and Terashima, 1990; Husin, 1985). Lim and Khoo, (1986) reported a regular increase in moisture content along the stem height and towards the central region, as the outer and lower zones having lower content.

The compositions of the main components of OPT are cellulose (45%), hemicelluloses (25%) lignin (18%) and extractives (10%). All the components can be fractionated, isolated and purified to obtain useful products (Anis, 1999). Law et al. (2007) studied chemical and physical characteristics of fibres from OPT and found that when bleached to the desired brightness, OPT fibre is suitable to substitute the hardwood Kraft component in printing and writing grades.

Anis et al. (2000) conducted analysis on the sugar components of hemicelluloses, and concluded that xylose is main sugar in each fraction, however glucose and arabinose is the minor constituent. From the evaluation, it was found that there is a good potential for the use of hemicelluloses as a food ingredient such as dietary in food formulation.

The density of OPT varies from 230 to 520 kg m^{-3} the average density being 370 kg m^{-3}. There is a density gradient between the central core and the peripheral zone, which is reflected in the clear dissimilarity observed in hardness and weight between the outer and inner portions of the trunk (Husin et al., 1986). The density distribution could be because of the different morphological structure from other palms, change of pattern with age and size, movements of starch deposits in parenchyma cells up to the top of palm and larger amount of fibrous bundles in top core than bottom core (Corley and Gray, 1976).

2.1.2 Mechanical properties of oil palm fibres

The mechanical properties of wood are measures of its resistance to exterior forces, which tend to deform its mass. In contrast to metals and other materials of homogenous structure, wood exhibits different mechanical properties in axial, radial and tangential growth directions, making it mechanically anisotropic (Tsoumis, 1991). According to the study by Killman and Lim, (1985) regular features in OPT are prominent decline from periphery to pith on all levels of trunk height for modulus of elasticity (MOE), modulus of rupture (MOR), compression and hardness. The mechanical properties of the OPT are fairly reduced therefore it's not suitable for construction or for flooring and framing. Table 2.1 illustrates the data for mechanical properties of fibres. Mechanical properties such as tensile strength and modulus related to the composition and internal structure of the fibers.

Table 2.1: Properties of oil palm trunk fiber (Abdul Khalil et al., 2012)

Properties	Values
Tensile strength	300 – 600 N/mm
Lignin	23.03%
Alpha-cellulose	46.58%
Holocellulose	72.12%
Bulk density	1100 kg m^{-3}
Modulus of Elasticity	15-32 GPa

Aji et al. (2009) reported that the tensile strength and young's modulus of plant fibre is raised with rising cellulose content of the fibres. The trunk of oil palm tree can also be processed to form a good source of fiber. The process of fiber extraction does not require a complicated engineering process like those required by synthetic fiber. The previous researches on fibers of OPT, suggests that medium density fiber board (MDF) was stronger with better fiber-to-fiber strength recorded than the frond and empty fruit bunch (EFB) MDF (Lionel, 1996). Oil palm trunk fibre found to be suitable as reinforcement because it possesses high tensile strength (300-600 MPa) in compared with other natural fibre (Ahmad et al., 2010).

The cellulosic fibers properties are strongly influenced by chemical composition, fibre structure, micro fibril angle, cell dimensions and defects. It differs from different parts of a plant as well as from different plants (Dufresne, 2008). The thicker walled fibre is likely to produce an open and bulky sheet with low burst/tensile strength and high tearing resistant (Mishra et al., 2004). The fibres are hard and tough, and found to be a potential reinforcement in polymer composites (Jawaid and Abdul Khalil, 2011).

Law et al. (2007) considered physico-chemical characteristics of fibres from OPT and found that when bleached to the desired brightness, it is suitable to supplant the hardwood kraft component in printing and writing grades. The oil palm fibres have been focus of study in Malaysia and around the world but still don't hold much economic value (AbdulKhalil et al., 2012).

Oil palm biomass has shown potential to be used as a raw material for paper and paperboard production. Since the 1980s, the suitability of this raw material for papermaking has been explored using a variety of pulping methods (Choon and Wan, 1991; Kamarudin et al., 1991; 1997). Likewise, Sulaiman et al. (2011) analyzed the production of pulp from oil palm trunk (OPT) using *Aspergillus* species and a few white rot fungi. They reported that screened pulp yield, kappa number and paper strength increased on exposure to fungi between 9 to 36 days.

The presence of high degree of α–cellulose content in the raw material has no direct influence on the pulping properties; but overall it may affect the amount of pulp yield (Shimada et al., 1994). The large amount of α-cellulose content present in the raw material is a great indicator of its potential as a papermaking raw material (Rodríguez et al., 2008). The hemicellulose should also be retained as much as possible to preserve higher pulp yield and to get better paper property as its existence makes it capable to improve interfibre bonding (Hocking, 2005).In papermaking, preserving the carbohydrate from total degradation during pulping and bleaching processes is essentially an important factor, since carbohydrate is the main component of the pulp yield and the strength property of paper.

2.2 Structural composition of lignocellulose

Lignocelluloses biomass is most abundant energy resource existing on the earth (Lin and Tanaka, 2006). It has a complicated internal structure which is comprised of a number of chief components that also have complex structures. The chemical composition of lignocellulosic biomass differs considerably and is influenced by a number of factors (Harmsen, 2010).

The augmentation of the excellence and manufacturing effectiveness of products from lignocellulosic biomass has been obstructed by the lack of clarity of composite structures and chemical compositions of the materials. Cellulose, hemicellulose and lignin are the major constituents of lignocellulosic materials; these polymers are intimately associated with each other constituting the cellular complex of the vegetal biomass (Deobald and Crawford, 1997).

Mainly, cellulose forms a skeleton which is bounded by hemicellulose and lignin. Apart from these primary polymers, plants comprise other structural polymers for e.g. waxes and various glycoproteins (Showalter, 2001). The chemical composition of plants varies considerably as is depicted in Table 2.2 and is also influenced by environmental and genetic factors. The following Table 2.3 shows the chemical composition of some lignocellulosic materials.

Table 2.2: Chemical composition of various lignocelluloses (Sixta, 2006)

Raw material	Lignin (%)	Cellulose (%)	Hemicellulose %)	Extractive (%)
Hardwoods	18-25	45-55	24-40	2-8
Softwoods	25-35	45-50	25-35	1-5
Grasses	10-30	25-40	25-50	0.6
Bagasse	20	40	30	10
Cotton	1	95	2	0.4
Wheat straw	50	30	15	5
Hemp	6	70	22	2
Jute	13	71	14	2

Table 2.3: Chemical compositions of some woods and non woods (Sixta, 2006)

Analysis (%)	Extractives Ash	Alcohol Benzene %	1% NaOH	Hotwater %	Lignin	Holo-cellulose	α-cellulose	Hemi cellulose
EFB (Wanrosli et al. 2013)	5.4	2.3	N/a	N/a	17.2	82.5	60.6	21.9
Wheat straw (Deniz et al., 2004)	4.7	7.8	40.6	14	15.3	74.5	38.2	36.3
Bagasse (Jahan et al., 2002)	1.8	8.0	17.8	12.9	41.0	49.4	N/a	N/a
E. globulus (Rodríguez et l.,2008)	0.6	1.2	12.4	2.8	20.0	80.5	52.8	27.7
P. pinaster (Rodríguez et l.,2008)	0.5	2.6	8.0	2.0	26.2	69.6	55.9	13.7
Kenaf core (Latifah et al., 2007)	6.4	2.1	28.4	5.5	14.3	80.0	54.5	25.5
Bamboo (Jahan et al., 2002)	2.5	6.2	27.3	9.4	28.0	75.5	N/a	N/a

Also mentioned are the physical properties of each of the components and how each of these components adds to the behavior of the intricate structure as a whole. The study is leaning towards breaking down the compound of lignocelluloses utilizing the WRF to produce paper after pre-treatment. To attain an apparent view of the material an analysis of the structure of each major component is made in this section.

2.2.1 Lignin

The term lignin is originated from the Latin word for wood *lignum* and is the most plentiful raw material with an aromatic ring structure. Lignin is a macromolecule with a three dimensional structure lacking stereo regularity (Dashtban et al., 2009). It is located in the middle lamellae and secondary cell walls of higher plants and its prime function is to give strength and resistance to environmental stress (Ralph et al., 2007).

Lignin is a hydrophobic optically inactive biopolymer and made up of units of phenyl propane that acts as its principal building blocks. Lignin provides the resistance for collision, compression and bending to the cell and helps in its development. Additionally it functions in the transfer of water and metabolites within the plant cell while acting as binder among cells creating a composite material that has a notable.

The monomeric units of phenyl propane are oxidatively coupled through diverse types of ether, ester bonds and carbon-carbon linkages (Lin and Lin, 2002; Ralph et al., 2007). The phenyl propanoid subunits are p-hydroxy (H-type), phenyl guaiacyl (G- type) and syringyl (S-type) units. Their covalent bonding comprises of more than 10 different types of randomly distributed bonds, most frequent being the β-aryl ether (β-O-4) bond (Argyropoulos and Menachem, 1997).

The higher plants are divided into two categories, softwood (gymnosperm) and hardwood (angiosperm). The lignin as of softwood comprises of over 90%

coniferyl alcohol, while the remaining being p-coumaryl alcohol units. As mentioned earlier in Table 2.2, the lignin content in softwoods is from 24-35%, in hardwoods from 19-28% (Dence and Reeve, 1992). All together the lignin in hardwood is made up of changing ratios of coniferyl and sinapyl alcohol units (Kirk-Otmer, 2001).

The enormities of lignin show a broad discrepancy depending on the plant type, its age and part. Klason lignin is the acid soluble lignin and is reported to be present in the range of 8-22% for non-woody crops and between 19-30% for woody plants (Hatakeyama and Hatakeyama, 2005). The constitution of lignin is dissimilar not only between species, but also relating different tissues of an individual plant (Besombes and Mazeau, 2005). It’s complex and heterogeneous nature makes the enzymatic hydrolysis exceedingly complicated.

Amongst other cellulose-containing material the lignin content is lower and ranges from below 3% in cotton and 6% in extracted flax or hemp bast fibres, to around 11-15% for jute. In perennial grasses such as cereal straws, bamboo or oil palm biomass the lignin content ranges from 15-25% (Bagby et al., 1971). Though lignin has its distinctive characteristics and has high degree of chemical and biophysical roles, it is mostly under exploited and is viewed as a low quality waste material with limited commercial applications (Gosselink et al., 2004).

Lignin and cellulose has developed during evolution for construction and preservation purposes (Call and Mücke, 1997). Their degradation for pulping and bleaching processes is essential for the making paper products. Lignin degradation occurs gradually in nature via the action of bacteria and fungi that causes breaking of lignocellulose using a pool of oxidative and hydrolytic enzymes (Chen and Dixon, 2007). The enzymes cause cell wall decay and provide greater access to the digestible plant cellulosic material (Fuhr et al., 2011; Badhan et al., 2014).

2.2.2 Cellulose

Cellulose is the most abundant natural polymer and is synthesized by a great diversity of living organisms (Brown, 1990). It is the chief constituent of plant cell walls and represents up to the 50% of the dry weight of woody biomass. It is chemically composed only of glucose monomers and is linked through α-1, 4-linkage arranged in a microcrystalline structure regularly that makes it hard to hydrolyze under natural conditions. The glucan chains have a large attraction for one another than they do for the aqueous solvent.

There is a distinctive number of glucose units in cellulose, like in the primary cell wall cellulose polymers have about 8000 glucose units per chain while the secondary wall cellulose has a higher degree of polymerization (DP), up to 15,000 (Brown, 1985). The characteristic of bond among the glucose molecules is (β-1, 4 glucosidic), which permits the polymer to be organized in elongated straight chains. The arrangement of the molecule is associated with the fact that the hydroxides are consistently distributed on both sides of the monomers. This allows for formation of hydrogen bonds connecting the molecules of cellulose as they consecutively help in the establishment of a compound that consists of several parallel chains (Faulon and Carlson, 1994).

The adjacent cellulose polymers interact through hydrogen bonds, forming highly stable structures that contain both amorphous and crystalline regions (McCann and Carpita, 2008). The micro fibrils are stabilized by intra and intermolecular hydrogen bonds, while the mannans and xylans are linked to cellulose by covalent and hydrogen bonds (Heredia et al., 1995). These covalent bonds are extremely resistant to chemical and biological hydrolysis.

On the other hand, amorphous regions within the cellulose crystalline structure have a heterogeneous composition characterized by a variety of different bonds. Ultimately, this asymmetrical arrangement, which characterizes amorphous regions, is crucial to the biodegradation of cellulose. The accessibility of cell wall polysaccharides from the plant to microbial enzymes is stated by the degree to which they are associated with phenolic polymers (Kuhad et al., 1997).

Cellulose is hygroscopic material and absorbs 8-14% water under normal atmospheric conditions (25°C, 60% relative humidity). Also it is insoluble in water and in dilute acid and alkaline solutions at low temperature. The solubility of the polymer is strongly linked to the degree of hydrolysis attained. At higher temperatures the energy supplied is enough to break the hydrogen bonds that hold the crystalline structure of the molecule, cellulose becomes soluble.

Cellulose is synthesized by a cellulose synthase complex that is located within the cytoplasmic membrane of plant cells. The cellulose synthase complex contains many enzymes that include 36 cellulose synthase enzymes assembled as rosette structure (Taylor et al., 2000). The hydrolysis of cellulose requires the collective action of three enzymes: (1) endo-glucanases to randomly cleave inter-monomer bonds; (2) exoglucanases to remove mono and dimers from the end of the glucose chain; and (3) β-glucosidase to hydrolyze glucose dimers (Deobald and Crawford, 1997; Tomme et al., 1995). The actions of these enzymes are essential for complete hydrolysis and consumption of cellulose. The rate-limiting stage is the ability of endoglucanase to reach amorphous regions and create new chain ends, which exo-cellobiohydrolases are capable to attack (Zhang et al.,2013).

2.2.3 Hemicellulose

The term hemicellulose is a collective term that represents family of hetero polysaccharides, which are chemically and structurally similar to cellulose though there is a variation in the type and amount of monosaccharide. They are considered as the second most abundant renewable biomass, accounting for approximately 25-35% of total wood dry weight (Scheller and Ulvskov, 2010).

The hemicelluloses backbones are composed of β-1, 4-linked sugars and have low molecular weight and exists in amorphous condition. In the plant cell wall sugars such as arabino-xylans, gluco-mannans, galactans are present which have different composition and structure depending on their source and the extraction method.

The specific sugar composition of hemicellulose depends on the source of the polysaccharide (Zhang et al., 2012). While cellulose is a homopolymer and is non branched, hemicellulose vary in degree of branching averaging between 100 and 200 and have a lesser crystallinity Rowell, (2005). The monosaccharide can be classified into hexoses (mannose, galactose), pentoses (xylose, arabinose), and glucoronic acid. Hemicelluloses fasten with bundles of cellulose fibrils to form micro fibril that augments the steadiness of the cell wall. They create a complex network of bonds with lignin, which provides strength and resistance against microbial decay (Ladisch et al., 1983; Lynch, 1992).

Xyloglucan is the main hemicellulose of primary cell walls and consists of β-1, 4-linked glucose backbone that is decorated with xylose, galactose andsometimes fucose branching sugars (Hayashi and Kaida, 2011). The most dominating component belonging to the hemicellulose family of polysaccharides is xylan