OPTIMIZATION OF OIL PLAM TRUNK CORE BIODELIGNIFICATION USING PLEUROTUS OSTREATUS

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ABSTRACT

Biodelignification is essential in recovering cellulose from any lignocellulosic material especially for glucose production. Oil palm trunk (OPT) core, a waste from logging activity, has the potential as the source of glucose production due to its high cellulose content. The presence of lignin in OPT core inhibits any reaction occurred on cellulose. This research focused on the optimization of lignin removal on OPT core using local fungi *Pleurotus ostreatus*. Chemical analysis on OPT core resulted an 18.47% of lignin content. Enzyme assay showed manganese peroxidase activity 1.6±1.5 U ml^{-1} at day 6. The screening and optimization experiments were done in 250 mL laboratory glass bottle. During the screening process, seven factors factors were screened at various values; temperature (20°C and 30°C), pH value (5 and 8), humidity (controlled by the presence of silica gel), light exposure, moisture (0.50 mL and 5.00 mL water added per 12 hour), the weight of fungi to medium ratio (1:2 and 1:10) and contact time (2 and 10 days). Design Expert 6.08 software was used for experimental design. Two-level factorial analysis with a fraction of 1/8 was used for the screening process. The lignin content was analyzed via Klason-lignin determination method. The analysis of variance (ANOVA) proved the stability of this model with the coefficient of determination (R^2) value at 0.8584. The coefficient regression for linear equation regression showed proved there was a peak point in the model that can be achieved through optimization. Four factors screened were discovered to be the highest contributors to biodelignification; temperature (32.20% contribution), pH value (10.08% contribution), the fungi to medium ratio (8.82% contribution) and moisture content (7.63% contribution). The interactions between temperature and pH value, and the interaction between temperature and the fungi to medium ratio were discovered from the screening process. These four factors were studied in the optimization process with the factors value ranging from temperature (23°C to 27°C), pH value (5.5 to 7.5), moisture content (2.0 mL to 3.5 mL water added per 12 hour) and weight of fungi to medium ratio (2:10 to 4:10). Design Expert 6.08 was used in the optimization process. A central composite design (CCD) with 32 runs and six centre points was applied. From the optimization, the ANOVA showed R^2 value was 0.8779 proving that the model was fit for regression. The optimum condition was at; temperature (25.16°C), pH value (7.54), moisture content (2.38 mL water added per 12 hour) and the fungi to medium ratio (1:3.125) with the predicted lignin content of 14.36%. Validation test was conducted to justify this optimum condition with initial lignin content of OPT at 18.47%. The final lignin content was 14.68%. This showed that 20.52% of lignin removal from OPT through biodelignification. It showed an error of 2.23% between the theoretical value and the experimental value. As an additional analysis towards the application of biodelignification, a comparison of sugar produced from treated OPT (biodelignification treatment) and untreated OPT was done via acid hydrolysis process. Glucose concentration was determined using DNS method. The analysis showed that the OPT that went through biodelignification (0.04769 g glucose/ g OPT) had a 25% higher glucose concentration compared to the untreated OPT (0.03792 g glucose/ g OPT). The results from the optimization showed that biodelignification using *P. ostreatus* was a suitable method for lignin removal of OPT. Along with the acid hydrolysis process; the biodelignification is proved to be applicable as a pretreatment method for glucose production.

ABSTRAK

Bionyahlignin merupakan proses penting dalam perolehan selulosa dari bahan lignoselulosa dalam proses penghasilan glukosa. Teras batang pokok kelapa sawit (OPT), bahan buangan dari aktiviti pembalakan, mempunyai potensi untuk dijadikan sumber glukosa kerana kandungan selulosa yang tinggi. Kehadiran lignin dalam OPT merencatkan tindakbalas terhadap selulosa. Kajian ini tertumpu pada pengoptimuman bionyahlignin pada teras OPT menggunakan kulat tempatan *Pleurotus ostreatus*. Analisis kimia pada teras OPT menunjukkan 18.47% kandungan lignin. Asai enzim menunjukkan aktiviti mangan peroksidase pada 1.6±1.5 U ml^{-1} di hari ke-6. Eksperimen penyaringan dan pengoptimuman dijalankan pada botol kaca makmal bersaiz 250 ml. Sepanjang proses penyaringan, tujuh faktor telah dikaji pada nilai berbeza; suhu (20°C dan 30°C), nilai pH (5 dan 8), kelembapan (kehadiran gel silika), pendedahan pada cahaya, kandungan air (0.50 ml dan 5.00 ml air setiap 12 jam), nisbah berat kulat kepada medium (1:2 dan 1:10) dan tempoh tindakbalas (2 dan 10 hari). Perisian Design Expert 6.08 telah digunakan untuk rekabentuk eksperimen. Ujikaji faktorial dua peringkat dengan pecahan 1/8 digunakan pada proses penyaringan. Kandungan lignin dikaji menggunakan kaedah penentuan Klason-lignin. Analisis varians (ANOVA) membuktikan bahawa model adalah stabil dengan nilai pekali penentuan (R^2) sebanyak 0.8584. Pekali regresi bagi persamaan regresi linear membuktikan kewujudan titik maksima pada model. Empat faktor yang disaring mempunyai nilai sumbangan yang tinggi kepada proses bionyahlignin; suhu (32.20% sumbangan), nilai pH (10.08% sumbangan), nisbah kulat kepada medium (8.82% sumbangan) dan kandungan air (7.63% sumbangan). Interaksi antara faktor suhu dan nilai pH dan interaksi antara suhu dan nisbah kulat kepada medium telah dikenalpasti. Empat faktor ini dikaji dalam proses pengoptimuman dengan julat nilai; suhu (23°C ke 27°C), nilai pH (5.5 ke 7.5), kandungan air (2.0 mL ke 3.5 mL air setiap 12 jam) dan nisbah berat kulat kepada medium (2:10 ke 4:10). Perisian Design Expert 6.08 digunakan dalam proses pengoptimuman. Rekabentuk komposit berpusat (CCD) dengan 32 set eksperimen dan enam titik tengah telah digunakan. Hasil daripada pengoptimuman, ANOVA menunjukkan nilai R^2 adalah 0.8779 sekaligus membuktikan model ini sesuai untuk regresi. Nilai optimum bagi setiap faktor adalah; suhu (25.16°C), nilai pH (7.54), kandungan air (2.38mL air setiap 12 jam) dan nisbah berat kulat kepada medium (1:3.125) dengan nilai jangkaan kandungan lignin pada 14.36%. Ujian pengesahan dijalankan bagi tujuan justifikasi nilai optimum dengan kandungan awal lignin pada OPT sebanyak 18.47%. Kandungan akhir lignin adalah 14.68%. Ini menunjukkan sebanyak 20.52% lignin telah disingkirkan dari OPT melalui bionyahlignin. Ralat antara nilai jangkaan dengan nilai eksperimen adalah sebanyak 2.23%. Aplikasi bionyahlignin sebagai rawatan awal dalam proses hidrolisis asid (penghasilan glukosa) telah dijalankan. Perbandingan hasil glukosa antara OPT yang dirawat dan OPT yang tidak dirawat telah dibuat. Kepekatan glukosa ditentukan dengan kaedah DNS. Ujikaji menunjukkan OPT dengan bionyahlignin (0.04769g glukosa/g OPT) mempunyai kepekatan glukosa 25% lebih tinggi daripada OPT tanpa bionyahlignin (0.03792g glukosa/g OPT). Hasil daripada kajian pengoptimuman bionyahlignin menggunakan *P. ostreatus* menunjukkan kaedah ini sesuai untuk penyingkiran lignin dari OPT. Bionyahlignin terbukti mampu untuk diaplikasi sebagai rawatan awal untuk penghasilan glukosa.

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OPT	Oil palm trunk
RSM	Response surface methodology
CCD	Central composite design
hr	Hour
^{o}C	Degree Celsius
ml	Milliliter
g	Gram
%	Percent
L	Liter
wt	Weight
e.g.	Example
α	Alpha
β	Beta
P.ostreatus	*Pleurotus ostreatus*
M	Molar
mm	Millimeter
pH	Power of hydrogen
ml/min	Milliliter per minute
kg/cm^2	Kilogram per centimeter square
g/ml	Gram per milliliter
mmol/L	Millimole per liter

CHAPTER 1

INTRODUCTION

1.1 Research Overview

The palm oil production in Malaysia is a popular field. Together with Indonesia, these two countries are the main global contributors in palm oil production which is 87% of the world palm oil production (United States Department of Agriculture, 2012). Palm oil based product includes detergents, frying oil, cosmetics and even food product such as margarine. An oil palm tree has a time span of around 20 to 25 years. After this time span, the oil palm tree will lose its ability to produce sufficient amount of palm oil. Usually it is cut down for the next replantation of the new oil palm tree. These old oil palm trees are used for different purposes depending on the tree parts. Oil palm trunk (OPT) is one of the parts on the oil palm tree which are usually used to make plywood in the logging industry. However at the core of the OPT, it is actually soft and contains up to 80% of sap, making OPT core unsuitable source to make plywood (Kosugi et al., 2010). After the sap is squeezed from the trunk, the fiber wastes left after the process usually are used as fertilizer. But the usage of these wastes as fertilizer is still in question as it yields traces of the sap plus it contains a high composition of cellulose. The potential of these wastes as a raw material for cellulose-based product is high. Common application of cellulose in Malaysia includes the degradable film or bioplastic which has been used for coating purposes. These films can be made edible which is widely used in food coating with the usage of cellulose derivatives (Jafarizadeh Malmiri et al., 2011). However, with the sugar traces and the high cellulose content, glucose production outshines other potential cellulose-based product from the core of the OPT.

Glucose is one of the most common products of cellulose. It is being used regularly in Malaysia. Although cellulose is an organic compound that may serve for many other purposes, sugar or glucose remain as the most popular products as they have several potential applications. Organic forms are the best sources for glucose resources as they possess a high amount of cellulose content. As a country which is rich with agricultural activity, Malaysia has the potential in utilizing glucose as for its many benefits. Most of the agricultural wastes in Malaysia are lignocellulosic wastes. Lignocellulosic wastes are a type of material composing three main compounds; lignin, hemicelluloses and cellulose. The problem lies on the method preserving the cellulose as lignin tends to inhibit any reaction occur on cellulose. Lignin removal is a treatment which involves the removal of lignin from the structure, making cellulose exposed any reaction hence, improving glucose production subsequently.

The removal of lignin can be achieved in different methods. Various method of delignification was discovered up until today. To date, researchers were able to find out new process to remove lignin from lignocellulosic materials. Method used for the process is usually determined by depending on the type of feedstock used. Sánchez and Cardona (2008) have summarized the methods that have been used for the removal of lignin which are the physical methods such as mechanical comminuting. Another known method of lignin removal is the chemical method. By applying the dilute-acid hydrolysis procedure, lignin was able to be removed from the cellulose. Other types of similar hydrolysis method do share the same concept of degrading the lignin structure. The most common method used is the chemical pulping process (Wanrosli et al., 2007). Chemical pulping process can be varied according to the chemical used which depends on the raw material used. Soda pulping is a method of chemical pulping which emphasizes on the usage of alkaline pulping of lignocellulosic material.

However, the most environmental friendly method is the biological method which involves the degradation of lignin by other organic form. The biological method uses other microorganism as an agent for the delignification process such as fungi (Sun and Cheng, 2002). Known fungi types which have shown positive results on the delignification are the white-rot and brown-rot fungi (Ohkuma *et al*., 2001). These types of fungi are known for their high sustainability to grow on without restricted conditions. Biodelignification was also proved to be having low energy consumption and mild environmental conditions (Sánchez and Cardona, 2006). Another benefit of

biodelignification is that it can serve as a great pretreatment method in improving mechanical and chemical pulping (Breen and Singleton, 1999). The lignolytic enzymes produced during the fungal growth of white-rot fungi is necessary for lignin degradation. These enzymes have the tendency to oxidize lignin hence degrading it from the structure itself. Laccase, lignin peroxidase and manganese peroxidase are the common enzymes produced during the growth of white-rot fungi. Although biodelignification method is not new in the lignin removal scene, it has a quite low popularity due to its mysterious and unknown factors. The factors affecting biodelignification seems to be very random depending on the climate and location.

1.2 Problem Statement

In most glucose production involving lignocellulosic material, the lignin content of the material is crucial as it may inhibit reactions to the cellulose. This simply proves that lignin removal is essential as a pretreatment in any glucose production process. The physical method, chemical method and biological method is the three major lignin removal method existed to date. The physical method, while simple, requires high cost equipment making the capital cost of the method quite high (Papatheofanous et al., 1998). Certain proper equipment expertise and manpower are also required for this method. Without the proper conduct and supervision, this will lead to low cellulose yield or even other unexpected incidents (Lynd et al., 2002; Sun & Cheng, 2002). The chemical method is one of the common methods used in the industry. While the method yield better results than physical method, there are still problems with the low inhibitors formation and cellulose swelling (Lynd et al., 1999). Its high lignin removal would end up with the cost of damaging the cellulose itself over time. This would result in a lower quality glucose produced. The process of lignin removal by using local fungi is a biological delignification method which can remove lignin without damaging the source of glucose. This method emphasizes on cellulases and lignin-degrading enzymes activities (Tengerdy & Szakacs, 2003). During fungal growth, it is known that the enzymes released affect the delignification. Three enzymes that play some crucial role in biodelignifcation are lignin peroxidase, manganese peroxidase and laccase (Yang et al., 2013). However, there is yet little information about the optimum conditions of these fungi as agent of delignification. The candidate of the suitable fungi for this

process is also still in the dark. There are several types of fungi that proved to be able to degrade lignin well but the best has yet to be discovered. By nature, these fungi require different growth condition depending on the type of the fungi, the environment and the lignocellulosic biomass used (Giles et al., 2012). This proved that there are certain factors that affect the biodelignification of a biomass. The analysis and optimization of the factors affecting biodelignification would be a lucrative development in this area. In this study, a type of fungus was used as an agent of delignification and the optimum conditions of the process were determined. Several factors that affect the delignification process were chosen from literature study. These factors require their respective optimum condition for the biodelignification to achieve its maximum potential. These factors were studied and controlled at certain value to optimize the lignin removal. The usage of fungi as an agent of delignification seems to be an interesting method. Although it may be time consuming in order to remove lignin from the oil palm trunk, it's less consumption of energy and less risk compared to other pretreatment method proved how lucrative this method may become in the future.

1.3 Research Objective

The main purpose of this research was to remove lignin from oil palm trunk using the fungus *Pleurotus ostreatus* as an agent of delignification. Hence the research objectives were:-

a) To characterize the OPT core and fungi used in the biodelignification process
b) To analyze the factors which affect the biodelignification of oil palm trunk
c) To optimize the biodelignification process

1.4 Scope of Study

As a follow-up to the objectives, the scopes of this study were:-

i. To use a type of local fungi as an agent of biodelignification process

ii. To analyze the chemical characteristics of OPT core before and after the biodelignification process

iii. To analyze the enzymes released by the fungi during biodelignification process

iv. To screen and analyze the following parameters which affect biodelignification process using two-level factorial method:-

   a. Temperature
   b. pH value
   c. Humidity
   d. Darkness
   e. Fungi to medium ratio
   f. Moisture
   g. Contact time

iii. To optimize the value of factors which affect biodelignification process using Response Surface Method

iv. To utilize the Design Expert software in both factor analysis and optimization process

v. To determine whether the biodelignification process enhances the glucose production from OPT core

1.5 Organization of the thesis

The important information of this research was presented in five chapters of this thesis. An introduction of glucose production, oil palm biomass potential as a glucose source and its current status were outlined in Chapter 1.

In the second chapter, the in-depth information of the study was presented. Briefly, Chapter 2 was segregated into seven subchapters; biodelignification, lignocellulosic material, delignification methods, roles of enzyme in biodelignification, factors affecting biodelignification, optimization methods, and cellulose application. The in-depth information on biodelignification was explained in the first subchapter. Biodelignification is a known lignin removal method since a long time but it was not favored in the industry compared to its other counterfeit such as the chemical pulping method. The raw material used for this study was introduced in the second subchapter. The core of the OPT was the raw material used for this study. This part contained high sugar concentration which fits the requirement as the raw material. White-rot fungus (*Pleurotus ostreatus*) was used as an agent of biodelignification method for its ability to preserve cellulose. The next subchapter explained the roles of enzyme affecting biodelignification process. Three enzymes were discussed on their effect to biodelignification. As for the factors affecting the process, various parameters were discussed. The most affecting factors were selected for the next step. The optimization method selection was presented in the next subchapter. In this subchapter, the selection of the suitable optimization method available was selected and discussed. In the final part of the chapter, the analysis method was clarified. The method used to determine lignin content was covered in this subchapter. The optimum condition was used to justify the viability of biodelignification as pretreatment for glucose production via acid hydrolysis.

Chapter 3 focused on the methodology of this study. This chapter emphasized on the sequencing step or procedure required to achieved each of the study scopes. It was divided into nine subchapters where the materials, general method, characterization of OPT core, fungi characterization, fungi preparation, factor analysis process,

optimization process, analysis method and the application of biodelignification were described. Most of the procedures in this chapter were explained thoroughly in Appendix A. The last subchapter concluded the methods used in achieving the objectives of this study.

Chapter 4 discussed all the acquired results and findings. It was divided into six subchapters. The proximate analysis of OPT core by calculation data was discussed in the first subchapter. This subchapter covered the characterization of the OPT core. In the second subchapter, the enzyme assay results were shown and discussed. The selection of variables through the factor analysis method was discussed on the next subchapter. Each of the factors screened were discussed for its effect on biodelignification. These factors were selected as the factors for optimization. The following subchapter focused on the results of optimization. In this subchapter, the optimum conditions for each factor were revealed. The application of biodelignification process in glucose production was discussed on the fifth subchapter. In this subchapter the viability of biodelignification as a pretreatment method for glucose production was discussed. The last subchapter concluded the results and discussions obtained from this study.

In the last chapter, the conclusions of the objectives and some recommendation for future research were revealed. These conclusions were based on the objectives of the studies and according to the findings in Chapter 4. Based on conclusion, recommendations for future work were suggested.

CHAPTER 2

LITERATURE REVIEW

2.1 Biodelignification

Lignin removal is an essential process in the production of glucose. The recovery of cellulose from a lignocellulosic material that could occur using physical, chemical and biological method would affect the glucose produced. Lignin removal process also occurs naturally with the one most noticeable, fungi growing on other biomass.

2.1.1 Description

Delignification is a process to remove the lignin from the cellulose and hemicellulose in the lignocellulosic material. This process reduces the cellulose crystallinity, and increases the porosity in order to improve the hydrolysis of cellulose (McMillan, 1994). Delignification method varies from the physical method to the biological method. The selection of the method is usually was determined depending on the type of feedstock used. Sanchez and Cardona (2008) have summarized the methods that have been used for the removal of lignin. Such methods are; the physical methods (mechanical comminuting), the physical-chemical method (steam explosion), chemical methods (dilute-acid hydrolysis, concentrated-acid hydrolysis) and biological methods (fungal treatment and bioorganosolv treatment). Bioorganosolv is a biological pulping technique that emphasize on organic solvent to solubilize lignin and hemicellulose.

Lignin decomposition is one of the processes for biodelignification and it is actually a slow process whether in aerobic or anaerobic state. Lignin is the last component to decompose when an organic matter is under degradation. The optimum

breakdown temperature of lignin is at 37°C for mesophilic microflora. Younger hardwood tissue degrades more rapid compared to older hardwood. The usage of fungi treatment for delignification process was done by some research group such as Sun and Cheng (2002) and Tengerdy and Szakacs (2003). These researches showed that the usage of fungi is actually a good solution in lignin removal as these fungi are low on cost compared to other method. Chemicals and high pressure processes would cost highly economy wise. A variety of biological treatment is available for delignification from fungi treatment to the usage of other microbiological strains. Aside from removing lignin, delignification must meet the certain conditions in order to improve cellulose reaction which is: (1) the degradation or any means of losing carbohydrate must be avoided during the process; (2) improve the formation of the structure in favor of cellulose reaction; (3) the formation of any byproducts that may affect the latter processes must be avoided; and (4) the process has to be cost-effective (Sanchez and Cardona, 2008).

2.1.2 Biodelignification Methods

Lignin removal is crucial in the production of glucose from lignocellulosic material. Cellulose holds a variety of applications from cloth to alternative energy resource and the most common application of cellulose is glucose. Lignocellulosic material consists of three compounds; cellulose, lignin and hemicelluloses. The composition of each compound depends on the type of the material itself. However, because of this structure, the presence of lignin is making the access of cellulase enzyme to cellulose difficult (Sun and Cheng, 2002). Any reaction occur to the cellulose are inhibited by lignin compound. This results in products with lower yield and various compounds. Figure 2.1 shows a brief schematic of biodelignification or pretreatment to biomasses. With the lignin degraded, the cellulose is exposed to any reaction that may occur hence simplifying the next subsequent process such as glucose production.

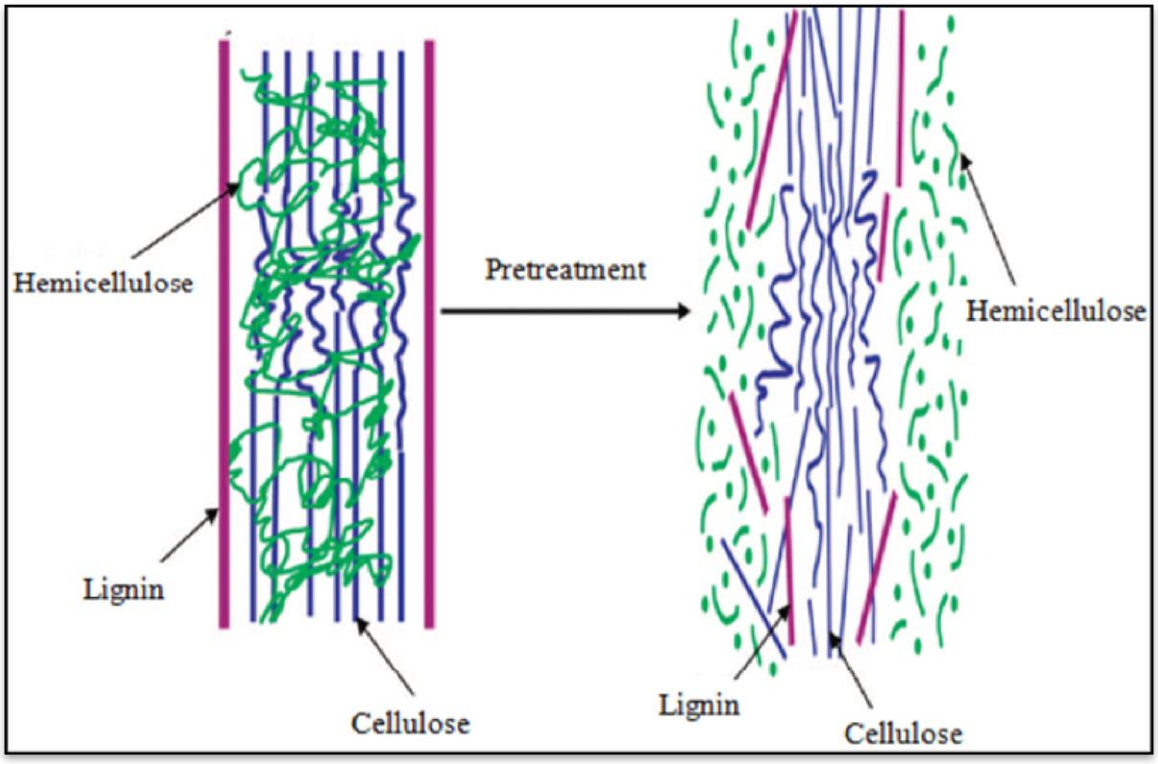

Figure 2.1: A brief schematic of pretreatment or biodelignification (Kumar et al., 2009)

Biological delignification or biodelignification is not quite demanding in Malaysia compared to chemical pulping method. Similar at the global stage, biodelignification in Malaysia is shrouded with the clouds of unknown and possibilities. Kumaran et al. (1997) stated that an edible mushroom, *Pleurotus sajor-caju* has been instrumental in his research of observing the laccases, cellulases and xylaneses activity on sago *hampas*. By 12 days, the cellulose to lignin ratio has increased significantly during a solid state fermentation which is from 2.74 to 3.3. This shows that the fungus reaction to the sago increased the cellulose to lignin ratio. During a process of phenol reduction using *Pleurotus spp.*, it is discovered that the activity of the fungi used in the research at 15^{th} day result in 73% of phenol reduction which is the optimized value of the research (Aranda et al., 2006). These researches proved that biological delignification is a time consuming process compared to its other method of lignin removal. Besides the fungi used in the process, the process duration is highly affected by rate of delignification which will depend on the lignin concentration and monomer composition (Paterson et al., 2008). These two factors will affect the delignification process regardless the method it is done. The time consuming factor was a problem in

biodelignification for some time as this is one of the major drawbacks of this method. As another way of looking at this drawback, this is a good point in improving biological delignification. Shorter process duration with improved result is such a bonus in the biological delignification area as it will save up more cost in both energy and economic way.

It has been a struggle to find the right fungi for biological delignification process. Almost all fungi families have the ability to degrade lignin but only few proved to be able to preserve cellulose during the process. Cellulose preservation is very important during biodelignification as it would affect the glucose production. Common fungi families capable of degrading lignin are the white-rot fungi, brown-rot fungi and soft-rot fungi. White-rot fungi proved to be a better candidate than brown-rot and soft-rot fungi as it degrades less cellulose compare to the latter (Sanchez and Cardona, 2008). However not all white-rot fungi is efficient in lignin degrading. A research on the factor analysis of the best white-rot fungi for delignification was done by Ruqayyah et al. (2011) shows that among twelve white-rot fungi obtained from Gombak area (Malaysia), seven of the fungi studied have the tendency of producing lignin peroxidase enzymes but some of these fungi also released other enzymes which may affect the preserved cellulose. The main microorganisms that produce lignin degrading enzymes (cellulases and hemicellulases) were one of the factors towards a fungus-based biodelignification (Lee et al.,1997; Kang et al., 2004). Some fungus such as the *Phanerochaete chrysosporium* proved to possess the ability to degrade lignin of a biomass in a process involving separate fermentation processes (Zhang, 2006). The *Ceriporiopsis subvermispora* was also proved to be having the ability to degrade lignin in a sufficient duration time. At the end of the study, the fungi network was decomposed (Itoh et al., 2003). There are also other similar fungi that have such ability. Taniguchi et al., (2005) stated that among four white-rot fungi tested in a delignification of rice straw (*P. chrysosporium*, *Trametes versicolor*, *C. subvermispora*, and *Pleurotus ostreatus*) the sample with *P. ostreatus* proved to be selective towards lignin rather than cellulose. Other fungi showed reaction to cellulose and hemicelluloses content. This simply proved that not all white-rot fungi were suitable for preserving cellulose. The problem lies when it comes to choosing the best fungi to be used. There are more fungi in this world that have not being tested for their capability for delignification. There

may be better fungi out there which can improve biological delignification method which may be a good challenge for this research area.

2.2 Lignocellulosic Material

Lignocellulosic material consumes of a variety of material available around our environment. It is known to be the most abundant biopolymer on earth. It is also considered comprising around 50% of world biomass (Claassen *et al*., 1999). Lignocellulosic material covers from grasses to animal solid waste. In general, the lignocellulosic materials which are used for bioethanol production can be divided into six main groups: crop residues, hardwood, softwood, cellulose wastes, herbaceous biomass, and municipal solid wastes (Sanchez and Cardona, 2008). This type of material consists of three general parts which are lignin, cellulose and hemicelluloses. The composition of each part is however differs depending on the type of the lignocellulosic material. However, the availability of the material is something that matters the most. It doesn't matter how high the yield or cellulose content of a material if its availability is low. Table 2.1 is a composition breakdown on various lignocellulosic materials.

Table 2.1. The contents of cellulose, lignin and hemicelluloses in various common sources.

Lignocellulosic Material	Cellulose (%)	Lignin (%)	Hemicellulose (%)
Hardwood stem	40-55	18-25	24-40
Softwood stem	45-50	25-35	25-35
Nut shell	25-30	30-40	25-30
Corn cob	45	15	35
Grass	25-40	10-30	35-50
Paper	85-99	0-15	0
Leaves	15-20	0	80-85

Sources: Reshamwala et al. (1995); Cheung and Anderson (1997); Boopathy (1998); and Dewes and H€unsche (1998)

2.2.1 Softwood

Plants with leaves that have needle-bearing conifers fall under softwood. Softwood does not necessarily means that this type of wood has to be soft. The difference between softwood and hardwood are the anatomy of the wood itself. As hardwoods are known for their angiosperms type, softwoods have fewer cell types compared to hardwood. Common softwood includes pines, cedars and cypress trees.

A variety of softwood has been compiled by Angles *et al*. (2001), revealing the average composition of the selected softwood. The research shows that the mixture of softwoods have an average of 25% of lignin, 38% of glucose, 12% of mannose, 7.4% of hot water extractives, 6.6% of arabinose, 5.9% of xylose, 4.1% of galactose, 3.3% of toluene extractive and 0.4% of ash. This was a result based on 100 grams of dry solid basis.

Although softwood may offer the prospect of a potential glucose source with its considerable cellulose content, most of the common softwoods stated before are not common in Malaysia. None of the softwood family is durable in the tropical forest in Malaysia. Softwood in Malaysia is also known to be the contributor to furniture production. The waste from this process is not significant to be treated with biodelignification as the amount is quite low. Another matter to take note of is that softwood is commonly treated with acidic hydrolysis for the lignin removal process (Jeong *et. al*., 2012), leaving the option to treat softwood with biodelignification a bit more competitive.

2.2.2 Hardwood

Hardwoods are one of the most easy to obtain wood in Malaysia. A majority of lands in Malaysia are covered by forests which consist of hardwood varieties. From the logging industry to the oil palm industry, some parts of hardwood are even considered waste as it has no use in the certain industry. Sawdust from hardwood is likely to be used as fuel. There are also other parts of a log that are unwanted in the logging industry such as the inner part of the oil palm trunk (OPT) which contains high sugar

concentration, making it not suitable for logging but a great candidate as a source of glucose.

Hardwood falls under the category of angiosperms plants which are also known as flowering plants. The subclasses of hardwood trees are monocotyledon and dicotyledon. Each seed of the monocotyledon plant has one leaf attached to it. This leaf-seed attribute is also known as cotyledon which gives out the name itself. Commercially known monocotyledons are such as bamboo and rattan. As for dicotyledons, each of the seed is attached to two leaves. In countries with four seasons, most dicotyledons lose their leaves during autumn season.

Sun and Cheng (2002) compiled the composition of most lignocellulosic materials from hardwood stems.They stated that hardwood stems contained a composition of cellulose as much as 40% to 55%, hemicelluloses 24% to 40% and lignin 18% to 25% in the structure. It is clear that hardwood is a potential source of glucose because a majority of its part consists of cellulose. However the structure of hardwood itself prevented any reactions to occur in the cellulose as lignin usually inhibit these reactions.

The OPT falls under the hardwood category. The interest in the utilization of oil palm and oil palm biomass in Malaysia for biofuel production has been increased lately. An amount of larger than 40 million tons of waste ranging from empty fruit bunches (EFB), OPT and oil palm fronds (OPF) are generated annually (Abdul Khalil and Rozman, 2004). This large amount led to the utilization of such raw material into becoming one of the suitable candidates for biodelignification. Most oil palm trees will lose their ability to produce palm oil after 25 years. After that time span, the oil palm trees were taken down. The soil will be used for the plantation of new oil palm trees. Since the previous plantation of oil palm trees were established in the early seventies, the oil palm tree biomasses are available around this decade for the time being (Azmalisa *et. al.*, 2010). Figure 2.2 shows the OPT that were taken down during the replantation process in a local oil palm estate. In plywood production, manufacturers were only using 40% of the OPT to produce plywood while the rest was classified as waste (Wan Asma et al., 2010). During the replantation, a large quantity of cellulosic