SCREENING OF FACTORS FOR BIODELIGNIFICATION OF OIL PALM TRUNK USING PLEUROTUS OSTREATUS

MOHD RASIDI BIN RASOL

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Faculty of Chemical and Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

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Delignification of lignocellulosic material is a crucial part in most research where it needs to be done to ensure the lignin compound are removed from the hemicelluloses and cellulosics contained in the material. Unbound hemicelluloses and cellulosics can be used further in application such as production of bioethanol. Currently known delignification method includes physical, chemical, and biological method. The biological method or biodelignification is known as a method that uses less energy and consume fewer chemicals that lead it to become the only method with environmental and cost friendly approach. Therefore, this research are conducted to study the factors that affect the biodelignification of oil palm trunk and to screen the factor that has significant effect on biodelignification of oil palm trunk using *Pleurotus ostreatus*. The effect of temperature, pH, humidity, light exposure, moisture content, fungi to medium ratio, and contact time on biodelignification process of oil palm trunk will be studied in this research. The oil palm trunk was incubated with the *Pleurotus ostreatus* in a temperature controlled incubator for a predetermined period of time. It was later cleaned after the incubation period ends and dried. The dried oil palm trunk was then analysed using the Klason-Lignin determination method where the lignin percentage in the oil palm trunk sample was obtained. The analysis done using Two-Level Factorial by Design Expert software shows that four factors are affecting with high contribution towards the biodelignification. The factors are the temperature, pH, fungi to medium ratio, and moisture content. The factors of temperature, pH, fungi to medium ratio, and moisture content contribute with 32.2%, 10.08%, 8.82% and 7.63% of contribution respectively to the biodelignification process. Besides the factor, interaction between factors also contributes to the biodelignification itself. The interaction between temperature and pH and also interaction between temperature and fungi to medium ratio gives contribution to biodelignification process with 18.29% and 8.82% respectively. The factor that found to have significant effect on biodelignification of oil palm trunk using *Pleurotus ostreatus* is temperature, pH, fungi to medium ratio, and moisture content and is suitable to be the factor for optimization experiment.
ABSTRAK

Proses pendeligninan bahan lignoselulosa adalah bahagian paling penting dalam kebanyakan penyelidikan dimana ia dilakukan untuk memastikan lignin dipisahkan dari selulosa dan hemiselulosa yang terdapat dalam bahan itu. Selulosa dan hemiselulosa yang telah terpisah boleh digunakan lebih lanjut dalam aplikasi seperti penghasilan bioetanol. Proses pendeligninan yang terkini termasuk kaedah fizikal, kimia, dan juga biologi. Kaedah biologi atau biopendeligninan adalah kaedah yang dikenali sebagai kaedah yang menggunakan rendah tenaga dan kurang bahan kimia yang meningkatkan satu-satunya kaedah yang mesra alam sekitar dan rendah kos. Oleh itu, penyelidikan ini dilakukan untuk mengkaji faktor-faktor yang mempengaruhi proses biopendeligninan batang kelapa sawit dan untuk membezakan faktor yang mempunyai kesan yang bererti kepada proses biopendeligninan batang kelapa sawit oleh *Pleurotus Ostreatus*. Kesaran suhu, pH, kelembapan udara, cahaya, kelembapan air, nisbah kulat kepada medium, dan masa terhadap proses biopendeligninan batang kelapa sawit akan dikaji dalam penyelidikan ini. Batang kelapa sawit akan diperam bersama-sama cendawan di dalam inkubator yang dikawal suhunya dalam masa yang telah ditetapkan. Ianya kemudian dibersihkan apabila pengeraman berakhir dan dikeringkan. Batang kelapa sawit yang telah dikeringkan kemudian dianalisis menggunakan kaedah Klason-Lignin di mana peratusan lignin dalam sampel batang kelapa sawit diperolehi. Analisis kemudian diteruskan dengan menggunakan analisis faktorial dua peringkat oleh perisian Design Expert menunjukkan empat faktor yang mempengaruhi biopendeligninan. Faktor tersebut adalah suhu, pH, nisbah kulat dengan medium, dan kelembapan air dengan peratusan sebanyak 32.2%, 10.08%, 8.82% dan 7.63%masing-masing. Interaksi antara suhu dan pH dan juga interaksi antara suhu dan nisbah kulat dengan medium juga memberi sumbangan dengan 18.29% dan 8.82%. Faktor yang dikesan mempunyai kesan yang bererti kepada proses biopendeligninan batang kelapa sawit oleh *Pleurotus Ostreatus* adalah suhu, pH, nisbah kulat dan medium dan kelembapan air dan sesuai untuk dijadikan faktor bagi eksperimen pengoptimuman.
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<tr>
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CHAPTER 1

INTRODUCTION

1.1 Background of Study

The oil palm tree (*Elaeis guineensis jacq.*) originates from West Africa where it grows in the wild and later was developed into an agricultural crop. It was introduced to Malaya, by the British in early 1870’s as an ornamental plant (MPOC, 2009). Today, 4.49 million hectares of land in Malaysia is under oil palm cultivation (MPOC, 2009). Every year, 9 million trees become nascent and must be cut down, with saplings planted in their stead. However anti-pollution laws introduced in the 1990s mean that the 7 million tonnes of dead wood, which is of little use for furniture making, cannot be burned. When left on the ground it has been found to make the soil infertile (NUTRA, 2000).

Each year, the oil palm industry produces more than 15 million cubic meters of oil palm trunks (OPT) during replanting. Despite their possible use as wood, the material is largely wasted. Some of the problems are a low recovery of sawn timber after seasoning, and the poor inherent physical and mechanical characteristics of the wood. Without chemical pre-treatment, oil palm lumber is generally susceptible to attack by fungi and borers. In addition, the wet wood exposed to humidity changes tends to warp and crack. The dried lumber is of low density and with poor mechanical properties. All these detract from value of the wood (Hassan *et al*., 2007).
The removal of lignin from the wood has traditionally taken place by a delignification method called the Kraft process. This name is derived from the German word for 'strong'. The mass of fibers remaining after the lignin is removed is known as pulp. The Kraft process produces stronger pulp than the methods used previously, and removes 95% of the lignin from the wood (George, 2010).

While delignification engineering has traditionally focused on wood pulp for paper and fiberboard, more recent efforts involved the use of biomass, large quantities of plant material, as a source of ethanol and an alternative to fossil fuels. This plant material must undergo delignification before it can be used for this purpose. Microbial systems are being engineered that combine the removal of lignin with the conversion of cellulose to ethanol (George, 2010).

1.2 Problem Statement

Lignin is a mixture of phenolic compounds that is intermeshed in plant secondary walls, cross-linking the cellulose carbohydrates that can be used to form paper fibers (George, 2010). Removal of lignin (delignification) from lignocellulosic biomass can allow the sugar contain in the lignocellulosic biomass to be further processed. However, typical delignification process used chemical that will produce byproduct such as hydrogen sulphide, dimethyl sulfide and dimethyl disulfide that may cause air pollution. Meanwhile, physical delignification often uses high power consumption and increase the production cost of the process. Thus, make the process not economically feasible. Delignification by biological process or biodelignification may omit the pollution hazard from the process.
1.3 Objective

The objective of this research is:

- To study the factors that affect biodelignification process of oil palm trunk
- To screen the factor that has significant effect on biodelignification of oil palm trunk using *Pleurotus ostreatus*.

1.4 Research Scope

i. To study on the effect of temperature, pH, humidity, light exposure, moisture content, fungi to medium ratio, and contact time on biodelignification process of oil palm trunk.

ii. To use *Pleurotus ostreatus* in biodelignification process.

iii. To use Klason Lignin method in determining lignin percentage in sample.

iv. To use two-level factorial analysis in manipulating parameter and analyse the sample.

1.5 Rationale and Significant

The usage of fungi in biodelignification process is more environmental friendly compared to typical delignification process such as Kraft process that use chemical to remove the lignin. The byproduct of the Kraft process such as hydrogen sulfide, dimethyl sulfide, and dimethyl sulfide may cause air pollution meanwhile using physical method is economically not feasible. Other than that, biodelignification by *Pleurotus ostreatus* can produce a new delignification process with lower cost of operation as *Pleurotus ostreatus* is abundantly found in Malaysia. The process use less equipment, does not need a high temperature and pressure, and use chemical in a least amount. Thus, it is very economical and has high potential in industry. It can also benefit to our local society by making profit from selling the felled down oil
palm trunk that usually being thrown away. Environmentally, this process can avoid the soil from loses its fertility when the felled oil palm trunk is no longer left on the ground to degrade after replanting process.
CHAPTER 2

LITERATURE REVIEW

2.1 Hardwood Waste

2.1.1 Oil Palm (Elaeis Guineensis) Trunk Waste

Oil palm is one of the most significant plantation crops in Malaysia partially covering a total area of approximately 3.8 million hectares and producing about 13.35 million tonnes of palm oil per year. Each year, Malaysia produces about 90 million tonnes of oil palm biomass and out of this, 40 million tonnes are in the form of oil palm frond, empty fruit bunches and oil palm trunk (Sulaiman et al., 2007).

With such a large area of cultivation, there is a large amount of oil palm biomass available, especially after replanting (which normally takes place after 25 years of growth). During the replanting process, the trunks and fronds are usually either left to rot or burnt down in the field, resulting in a huge amount of lost oil palm biomass that could be converted into high value added product (Hashim et al., 2009). This oil palm biomass could also be the causes of severe environmental pollution (Sulaiman et al., 2007).

This industry is one of the major contributors to the lignocellulosic-rich, solid waste materials, generated in the field and the oil mill. The main residues in the field are the pruned fronds removed during harvesting and the trunk and fronds removed at replanting activity. The mill residues include mesocarp fiber, shell, palm kernel cake, boiler ash, empty fruit bunches, palm oil mill effluent and bunch ash. Except for the palm kernel cake, boiler and bunch ash and palm oil effluent, all of the residues contain
high percentage of lignocellulose and therefore useful to be used as a source of carbon (Hussein et al., 2002).

Oil palm trunk contains 41.2% cellulose, 34.4% hemicelluloses, 17.1% lignin, 3.4% ash, 0.5% extractives, and 2.3% ethanol soluble (Sun et al., 2001) The presence of a large proportion of non-condensed syringyl, a small amount of guaiacyl and fewer p-hydroxyphenyl units indicated that the fractions can be considered as straw or grass type lignin. This is helpful as more information is available on these lignins compared with that for oil palm.

Syringaldehyde is the predominant phenolic component, which comprised 65.6–68.5% of the total phenolic monomers in the oxidation mixtures. This basic result may explain the high degree of biodegradability of oil palm as this unit is more susceptible compared with guaiacyl containing wood lignin (Schwarze, 2007). Vanillin was the second major phenolic component. The presence of syringaldehyde and vanillin resulted from the degradation of non-condensed syringyl and guaiacyl units, respectively. The lower yields of alkaline nitrobenzene oxidation of these lignin fractions indicated a higher degree of condensation of the isolated lignins compared with the corresponding yields of hardwood lignins.

2.1.2 Sago Palm (Metroxylon Sagu) Trunk Waste

The genus Metroxylon is found from 17°S to 15–16°N latitude ranging from Thailand, peninsular Malaysia and Indonesia, to Micronesia, Fiji, and Samoa. The palms are generally found at low elevations in swamps. M. sago is by far the most important economic species and is now grown commercially in Malaysia, Indonesia, the Philippines, and New Guinea for production of sago starch and/or conversion to animal food or fuel ethanol. In many countries of South East Asia, except Irian Jaya, M. sago is mainly found in semi-cultivated stands. Irian Jaya has about 6 million ha of M. sago (McClatchey et al., 2006).
The sago palm is 6-14 m tall and hapaxantic - that is, it flowers once and dies shortly thereafter. Just before flowering, the plant converts its stored nutrients into starch, which tills the trunk. Sago is now only a minor crop in Peninsular Malaysia, occupying less than 1% of the total agricultural land. The largest sago-growing areas in Malaysia are to be found outside the Peninsula, in the state of Sarawak, which is now the world’s biggest exporter of sago, exporting annually about 25,000 to 40,000 t of sago products to Peninsular Malaysia, Japan, Taiwan, Singapore, and other countries (Abd-Aziz, 2002).

Sago palm is exploited as a staple and cash crop in Southeast Asia because the trunk contains a large amount of starch (150-250 kg/trunk) (Kuroda et al., 2001). The production capacity of the sago palm varies from 2-5 t of dry starch/ha in the wild to 10-25 t/ha in the case of cultivated plants. The non-pith parts of the sago palm trunk are utilized in a variety of ways (4, 5): as an excellent building material for local and urban houses, sheds, or other buildings; as a resource for composting (biofertiliser); as a resource for gasification and energy production; and as an animal feed. The pith consists mainly of starch, which has to be separated from the cellulosic cell walls of the trunk. The residue from starch extraction is a very strong pollutant because of its cellulosic fibrous material (Abd-Aziz, 2002).

Its high starch content, ease of cultivation in swampy areas, and high productivity has increased the production of sago starch. Interest has also grown in utilizing sago palm as a new energy resource and for industrial raw materials. Although no high added value applications have been found for the palm so far. With increasing production of sago starch, however, huge amounts of fibrous residues are left over in the starch mills. The residues and the trunk bark pollute the environment as well as the waste water. From the determination by acidic sodium chloride, the sago palm trunk consists of 64.4% cellulose, 25.1% hemicellulose, and 10.5% lignin (Kuroda et al., 2001).
2.1.3 Rubber Tree (*Hevea Brasiliensis*) Trunk Waste

*Hevea brasiliensis* was first found in the Amazon basin. The rubber trade became a mainstay of the Brazilian economy, providing at its height almost 40% of its export revenues. It was not long before the idea was conceived of domesticating rubber. However, Brazil was not the site of the successful commercialization of rubber. Rubber cultivation was, instead, transferred to Southeast Asia. Soon abundant and cheap, rubber was put to thousands of uses. Its reduced cost was an important factor in the emergence of a mass market of automobiles; from two-thirds to three-quarters of the demand for rubber soon came from the makers of tires and tubes for motor vehicles. After tires, latex products, footwear, belts and hoses, and wire cables are the most important uses for rubber. Rubber is harvested in Africa, Central and South America, and in Asia, the latter accounting for greater than 90% of production (Raintree Nutrition Inc, 2010).

At the age of 22 to 29 years, latex production becomes uneconomic and the trees are then cut and replanted. Thus the rubber plantation is a sustainable source of rubber as well as timber, contributing positively to the environment. (Rubber Board, 2002) The lumber was obtained from cut down a 25 to 30 year old plantation grown rubber tree and usually used as raw material for furniture production (Matan et al., 2008). The wood consists of 77.8% cellulose, 17.8% lignin and 3.4% extractive (Simatupang *et al*., 1992).
2.1.4 Selection of Oil Palm (*Elaeis Guineensis*) Trunk Waste As Raw Material

The selection of oil palm trunk waste as raw material based on few criteria that it inferiors to another hardwood waste. In overall, the oil palm trunk waste have highest amount of lignin percentage present in the trunk composition. By possessing high amount of lignin in the trunk, the efficiency of lignin removal in the trunk can be monitored in higher accuracy compared to the other sample. Although the lignin content in rubber wood is slightly higher than in oil palm trunk, it was not chosen because of rubber wood was used as raw material for furniture in Malaysia. Other than that, the relatively abundant amount of oil palm trunk available in Malaysia can be another factor to choose the trunk as raw material. High resources will promises uninterrupted supply and will promise a good raw material for industrial scale production.

2.2 Pretreatment Process of Lignocellulosic Biomass

2.2.1 Physical Pretreatment

Lignocellulosic biomass has high potential for fermentation. However, to convert its structural polysaccharides into simple sugar is a big problem due to existence of lignin molecule in its structure. Lignin acts as a cementing material, which together with hemicelluloses, forms an amorphous matrix in which the cellulosic fibrils are embedded and protected against chemical or enzymatic degradation (Himmel *et al.*, 2007). Therefore, for a highly efficient fermentation process, a pretreatment process must be included.

Many methods were extensively used in the world in order to remove lignin from lignocellulosic biomass molecule. There were physical, chemical and biological processes. The purpose of pretreatment is to remove lignin and hemicellulose, reduce crystallinity, and increase the porosity of the materials (Sun *et al*., 2001). Pretreatment must improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis, avoid the degradation or loss of carbohydrate, avoid the
formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes, and be cost-effective (Sun et al., 2001).

Mechanical comminution and pyrolysis is an example of physical pretreatment. Waste materials can be comminuted by a combination of chipping, grinding and milling to reduce cellulose crystallinity. The size of the materials is usually 10–30 mm after chipping and 0.2–2 mm after milling or grinding (Sun et al., 2001). Vibratory ball milling has been found to be more effective in breaking down the cellulose crystallinity of spruce and aspen chips and improving the digestibility of the biomass than ordinary ball milling. The power requirement of mechanical comminution of agricultural materials depends on the final particle size and the waste biomass characteristics (Sun et al., 2001). Pyrolysis is when the material is treated in temperature greater than 300 °C (Sun et al., 2001). The cellulose are rapidly decomposes to produce gaseous products and residual char.

2.2.2 Chemical Pretreatment

There are many chemical pretreatment technique used in the industry nowadays. The techniques are such as ozonolysis, acid hydrolysis, and alkaline hydrolysis. Ozonation has been widely used to reduce the lignin content of both agricultural and forestry wastes (Balat, 2010). Ozone can be used to degrade lignin and hemicelluloses in many lignocellulosic materials such as wheat straw, bagasse, green hay, peanut, pine, cotton straw, and poplar sawdust (Sun et al., 2001). The advantage of ozonolysis is it effectively removes lignin, it does not produce toxic residues for the downstream processes, and the reactions are carried out at room temperature and pressure. However, a large amount of ozone is required, making the process expensive (Sun et al., 2001).

Acid hydrolysis uses concentrated acid such as sulphuric acid and hydrochloric acid to treat lignocellulosic materials. Although they are powerful agents for cellulose hydrolysis, concentrated acids are toxic, corrosive and hazardous and require reactors that are resistant to corrosion. In addition, the concentrated acid must be recovered after hydrolysis to make the process economically feasible (Sun et al., 2001).
Alkaline hydrolysis uses bases to treat lignocellulosic materials and the effect depends on the lignin content of the materials (Sun et al., 2001). The mechanism of alkaline hydrolysis is believed to be saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components, for example, lignin and other hemicellulose. The porosity of the lignocellulosic materials increases with the removal of the crosslinks (Sun et al., 2001). Dilute NaOH treatment of lignocellulosic materials caused swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure (Sun et al., 2001). The disadvantage of alkaline hydrolysis is it require long residence time, it form irrecoverable salts and incorporated into biomass (Balat, 2010).

2.2.3 Biological Delignification (Biodelignification) Process

Biological pretreatment of lignocellulosic material or in other word, biological delignification is a process that uses microorganism to degrade lignin and hemicelluloses in waste material (Sun et al., 2001). Microorganism such as brown, white, and soft-rot fungi has been used in degrading lignin in waste material (Sun et al., 2001). The potential of biological pretreatments has been explained by the ability of certain microbes to disrupt the plant cell wall by partial breakdown of the lignin/carbohydrate complex (Gupta et al., 2010).

*Pycnoporus cinnabarinus*, a selective lignin degrading white rot fungus, has been reported to produce laccase, degrade lignin and to transform lignin derived compounds (Gupta et al., 2010). The advantages of biological pretreatment include low energy requirement and mild environmental conditions (Balat, 2010).

2.2.4 Selection of Biodelignification Process as Selected Process

Pretreatment has been viewed as one of the most expensive processing steps within the conversion of biomass to fermentable sugar (Balat, 2010). Thus, a best
pretreatment process is a process that can maximize the lignin degradation and in the other side, give a cheap operating cost. Biodelignification can be one of the most effective pretreatment processes as it uses only simple equipment and require low energy (Balat, 2010).

2.3 Biodelignification Process

2.3.1 Biodelignification using Oyster Mushroom (*Pleurotus Ostreatus*)

The white-rot fungus *Pleurotus (Pl.) ostreatus* is an edible basidiomycete known for its ability to degrade agro-industrial lignocellulosic wastes, which are mainly composed by cellulose, hemicelluloses, and lignin. It is generally cultivated on wheat straw, but other lignocellulosic substrates, such as cotton stalks, have proved adequate for its growth. The enzymatic activity of *Pl. ostreatus* is greatly affected by the cultivation media. For instance, when grown on cotton stalks, it mainly degrades lignin. While, during the colonization of wheat straw, it also reduces the hemicellulose content significantly (Locci et al., 2007).

From Wu *et al*., among five strains that was tested in degradation of lignin in pulp mill wastewater by white-rot fungi on biofilm, among the five strains tested, *P. chrysosporium, P. ostreatus* and S22 demonstrated higher capabilities for degrading lignin than Lentinus edodes and Trametes versicolor. The initial degradation of lignin was fast, reaching 54% for S22 and 52% for *P. ostreatus* on day 4. Thereafter, the degradation of lignin slowed down, achieving 60% removal efficiency on day 7. This result shows that *Pleurotus Ostreatus* have high efficiency in degrading lignin without using too much time.

2.3.2 Biodelignification using *Pycnoporus cinnabarinus*

Basidomycetes have been used extensively in biological delignification process as the microorganism for breaking the plant cell wall and to remove lignin. Among the basidomycetes, white rot fungi have shown most promising ability to degrade lignin efficiently. *Pycnoporus cinnabarinus* is an example of white rot fungi that have the ability to perform the biological delignification. *Pycnoporus cinnabarinus* reported to
produce laccase, degrade lignin and transform lignin derived compounds (Gupta et al., 2010). However, the application of P. cinnabarinus for delignifying the lignocellulosic materials and subsequently its effect on enzymatic hydrolysis of the fermented substrate (mycosubstrate) has scarcely been studied (Gupta et al., 2010).

2.3.3 Biodelignification using Ganoderma australe

White rot fungi have shown high efficiency in degrading lignin compound in wood. Ganoderma australe is a white-rot fungus that causes biodelignification in some hardwoods found in the Chilean rainforest (Mendonça et al., 2008). In order to degrade lignin, these fungi produced extracellular oxidative enzymes such as laccase, lignin peroxidase (LiP), manganese peroxidise (MnP) and also low molecular mass compounds that mediate the action of these enzymes (Mendonça et al., 2008).

This non-specific oxidative system makes white-rot fungi useful for a wide range of biotechnological applications, for instance, in the pulp and paper industry for pitch control, biopulping or biobleaching and to degrade recalcitrant compounds such as aromatic hydrocarbons, aromatic dyes and other pollutants from cellulose and textile industries (Mendonça et al., 2008). The limitation of this fungus is this selected lignin degradation process seems to be restricted to some native wood species under specific climate conditions occurring in the south of Chile (Elissetche et al., 2001)

2.3.4 Selection of Oyster Mushroom as Selected Fungi

Compared with other fungi, Pleurotus Ostreatus have much advantage to be selected as the used fungus. Besides from the abundance amount in Malaysia, it is also have high efficiency in degrading lignin. Other than that, the fungi can also be found with cheap price, thus it can contribute to decrease the raw material cost.
3.1 Overview of Research Methodology

This research consists of five main parts, which are factor identification, experimental design, sample preparation, incubation and analysis. During incubation the oil palm trunk (OPT) was incubated with the Oyster Mushroom in a vessel. All manipulated variable was tested during the incubation period. The sample was incubated according to the data manipulated from the seven factors affecting the biodelignification process. To analyze the data, Klason-Lignin determination was used to calculate the lignin percentage contain in the sample. Therefore, the percentage of lignin degraded can be obtained.

Figure 3.1: Research Work Flow