BIODELIGNIFICATION OF OIL PALM TRUNK- OPTIMIZATION

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STUDENT'S DECLARATION

I declare that this thesis entitled "Biodelignification of Oil Palm Trunk- Optimization" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidate of any other degree.

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Date : 12 JANUARY 2012

DEDICATION

Special dedication to,

My parents

Mohd Fazi bin Lahaman and Hamidah bt. Ishak

My beloved brothers and sisters

Zawani, Zaiematun, Zahidah, Hamizatun, Syazana, Syafiayatun, Muhd Zuhdi, Muhd Shazwan, Muhd Zharif and Husna Naziha

and

all of my friends

for all of the supports and faith in me.

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ABSTRACT

Biodelignification is a process to remove lignin from the lignocellulosic materials to obtain celluloses. Hence this study is introduced to study the optimum value of the most affected parameters to the biodelignification process in order to maximize the removal of the lignin. Those parameters are temperature, pH, moisture content and also fungi to medium ratio. As for this research, a local type of fungi, *Pleurotus ostreatus* or commercially known as 'Oyster Mushrooms', were used as an agent of delignification and the raw material chosen are oil palm trunk wastes .In order to optimize conditions for production of lignocellulolytic enzymes by the oyster mushroom, a Response Surface Methodology (RSM) based experiment was designed by using Design Expert software. In this research, 30 runs of experiment were done. After obtaining the experimental design table, the experimental procedures were preceded and the values of the parameters were set according to the table at respective value. After that, the lignin content was analyzed by using Klason Lignin Method. With this method, the lignin content can be determined for various values of parameters. Then, the results obtained were analyzed again by using Design Expert software. At the end of this research, the optimum conditions obtained for the lignin degradation of oil palm trunk fiber are with temperature of 25 °C, pH value of 7.0, moisture content of 0.21 ml and fungi to medium ratio of 2.5 with the amount of predicted lignin degraded is 15.37%. Finally, the conformational test was done with the optimum conditions obtained and the error between the actual and predicted value is 18.7%. However, for better results, the center point in RSM should be tested before designing the experiment.

ABSTRAK

Biodelignifikasi adalah satu proses untuk menyingkirkan lignin daripada bahan-bahan lignoselulosik untuk mendapatkan selulosa. Oleh itu, kajian ini diperkenalkan bagi mengkaji nilai optimum parameter yang paling memberi kesan kepada proses biodelignifikasi bagi memaksimumkan penguraian lignin. Parameter-parameter tersebut ialah suhu, pH, kandungan kelembapan dan juga nisbah fungi terhadap media. Bagi kajian ini, sejenis fungi tempatan iaitu *Pleurotus ostreatus* atau secara komersialnya dikenali sebagai 'Cendawan Tiram' telah digunakan sebagai agen delignifikasi dan bahan mentah yang dipilih ialah sisa batang kelapa sawit. Eksperimen berdasarkan Kaedah Tindakbalas Permukaan (RSM) telah direka bentuk dengan menggunakan perisian Design Expert bagi mengoptimumkan pengeluaran enzim lignoselulolitik oleh cendawan tiram. Dalam kajian ini, sebanyak 30 percubaan telah dijalankan. Setelah mendapat jadual reka bentuk eksperimen, prosedur eksperimen telah dijalankan berdasarkan nilai dalam jadual. Kandungan lignin telah dianalisis dengan menggunakan Kaedah Klason Lignin. Kemudian, keputusan yang diperolehi dianalisis dengan menggunakan perisian Design Expert. Nilai optimum yang diperolehi untuk penguraian lignin adalah pada nilai suhu 25°C, pH 7.0, kandungan air 0.21 ml dan nisbah fungi terhadap media sebanyak 2.5 dengan nilai kandungan lignin yang dijangkakan sebanyak 15.37%. Ujian pengesahan telah dilakukan terhadap nilai optimum yg diperolehi dan didapati perbezaan antara nilai jangkaan dan nilai sebenar adalah sebanyak 18.7%. Adalah didapati keputusan kajian memenuhi objektif yang ditetapkan. Namun demikian, sebagai langkah penambahbaikan adalah dicadangkan supaya nilai titik tengah pada RSM diuji terlebih dahulu sebelum reka bentuk eksperimen dilakukan.

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LIST OF SYMBOLS

g	Gram
ml	Milliliter
°C	Degree celsius
m^2/g	Squared meter per gram
w/w	Weight over weight
%	Percentage
µm	Micrometer

LIST OF ABBREVIATIONS

AFEX	Ammonia fiber explosion
ANOVA	Analysis of Variance
CCD	Central Composite Design
CcP	Cytochrome c peroxidase
CMCase	Endo-1,4-β-D-glucanase
CO_2	Carbon dioxide
HRP	Corseradish peroxidase
LHW	Liquid hot water
RSM	Response Surface Method
YMPG	Yeast-malt-peptone-glucose

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Malaysia is one of the countries in Asia that practiced agriculture as one of its major industries of economic importance. Hence there are a lot of plantations in Malaysia especially oil palm tree and Malaysia is one of the palm oil producers around the world. Oil palm tree will start bearing fruits after 30 months of field planting and will continue to be productive for the next 20 to 30 years, thus ensuring a consistent supply of oils. After about 30 years, all of the palm tree will be felled and the trunks will be disposed. Aside from just disposing it, there will be a lot of advantages if it is processed and used in many industrial uses. This is because oil palm trunk is one of the hardwoods that rich in cellulose composition which known as lignocellulosic biomass.

The lignocellulosic biomass, which represents the largest renewable reservoir of potentially fermentable carbohydrates on earth, is mostly wasted in the form of pre-harvest and post-harvest agricultural losses and wastes of food processing industries. Lignocellulosic wastes such as oil palm trunk wastes contain cellulose, hemicelluloses and lignin. Cellulose in these wastes is very useful in many industrial purposes especially in production of bioethanol.

To remove the lignin from the waste, a method of delignification can be used. There are various methods used in the pretreatment of lignocellulosic wastes which are chemical, physicochemical, physical, and biological pretreatment and a wide variety of species are involved.

1.2 Problem Statement

Cellulose is the substance that makes up most of a plant's cell walls besides hemicellulose. Since it is made by all plants, it is probably the most abundant organic compound on earth. Aside from being the primary building material for plants, cellulose has many others uses. According to how it is treated, cellulose can be used to make paper, film, explosives, and plastics, in addition to have many other industrial uses. Those cellulose and hemicelluloses can be obtained from agricultural wastes such as oil palm trunk waste by using biodelignification. However, in past decades, the palm oil residues such as oil palm shells, mesocarp fibers and empty fruit bunch and oil palm fronds and oil palm trunk became the most abundant biomass resources in spite of Malaysia is one of the biggest producers of oil palm tree in the world. Hence, aside from disposing the wastes, the biodelignification is a method to produce more cellulose. In this process, the usage of fungi as an agent of delignification seems to be an effective method because even though it does consume a lot of time in order to remove lignin from the oil palm trunk, the energy consumption is lower and less cost compared to other pretreatment methods.

1.3 Research Objective

The main purpose of this research is

i. To study the optimization of biodelignification of oil palm trunk waste.

1.4 Scope of Research

The scopes of this study are

i. To use a type of local fungi (*Pleurotus ostreatus*) as an agent of delignification process.
ii. To optimize four factors which affect the lignin removal the most which are temperature, pH, moisture and fungi to medium ratio by using Response Surface Methodology (RSM).
iii. To determine the lignin content of the oil palm trunk using Klason Lignin method.

1.5 Rationale and Significant

By doing this study, the optimum condition for biodelignification of oil palm trunk can be determined including four factors which are temperature, pH, moisture content and fungi to medium ratio. Hence, the quantity of the cellulose obtained can be maximized when the optimum conditions are successfully determined. The process also can be done with a large amount due to the large quantity of raw materials that can be found nowadays and may produce more cellulose. However, the cost of the production can be decreased

CHAPTER 2

LITERATURE REVIEW

2.1 Biodelignification

Biodelignification is delignification process by using any biological method where delignification means the removal of the lignin from the wood by any processes. Lignin decomposition is one of the processes for delignification and it is actually a slow process whether in aerobic or anaerobic state. Biodegradation of cellulosic crop residues, agricultural solid wastes and municipal solid wastes in the absence of oxygen can be used to reduce pollution and produce biomethane as a fuel (Romana *et al.,* 2000). Common for all types of pretreatment is that they should minimize loss of sugars, consume a minimum of energy, and increase enzymatic digestibility. This is done by removing or rearranging the lignin structure, partial or full removal of hemicellulose from the fibers, and possibly altering the cellulose structure (Mette *et al.*, 2009). A variety of biological treatment is available for delignification from fungi treatment to the usage of other microbiological strains. The results obtained from a research done by Maria *et al.*, 2005 showed that the tested fungi were effectively involved in humification and lignin degradation of horticultural wastes and might be used as inoculants in a pre-treatment process before composting in order to reduce the resistance of the substrate to biodegradation.

2.1.1 Cellulose

Cellulose is a major component of the plant cell wall and different cellulose synthases perform cellulose synthesis in the primary cell wall and the secondary cell wall (Saxena and Malcolm, 2007)**.** Cellulose is a simple polymer, but it forms insoluble, crystalline microfibrils, which are highly resistant to enzymatic hydrolysis (Pierre and Jean-Paul, 1994). One of the studies from Howard *et al*. (2003) shows that the cellulose composition in the hardwood stem and softwood stem is the higher than hemicelluloses and lignin. The application of the cellulose as a precursor for chemical modifications was exploited extensively even before its polymeric nature was determined and well understood. That is why the cellulose is needed in industry. Cellulose is one of the most abundant biopolymers on earth. It exists in nature as fibrils, called microfibrils, which are only a few nanometers in diameter. Hemicellulose and lignin surround the cellulose microfibrils in plant cell walls (Masahisa *et al.*, 2010). In industry, the recovery of glucose from lignocellulosic biomass has been regarded as a powerful method to obtain valuable products such as bioethanol and various chemicals, without competing with food production (Masakazu *et al*., 2010). These products are presumably converted into true inducers by transglycosylation reactions. Several applications of cellulases or hemicellulases are being developed for textile, food, and paper pulp processing. These applications are based on the modification of cellulose and hemicellulose by partial hydrolysis. Total hydrolysis of cellulose into glucose, which could be fermented into ethanol, isopropanol or butanol, is not yet economically feasible. It is especially the rapid enzymatic decomposition of cellulose that is a key issue in the efficient recovery of glucose (Pierre and Jean, 1994).

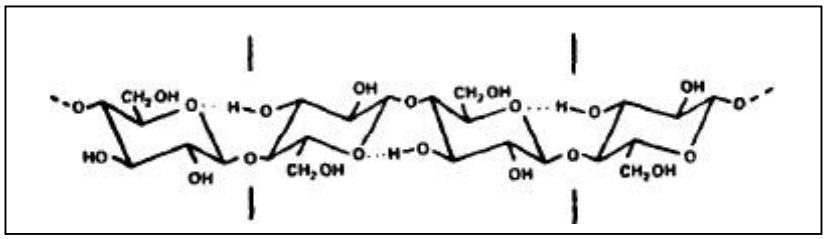

Figure 2.1: Structure of cellulose

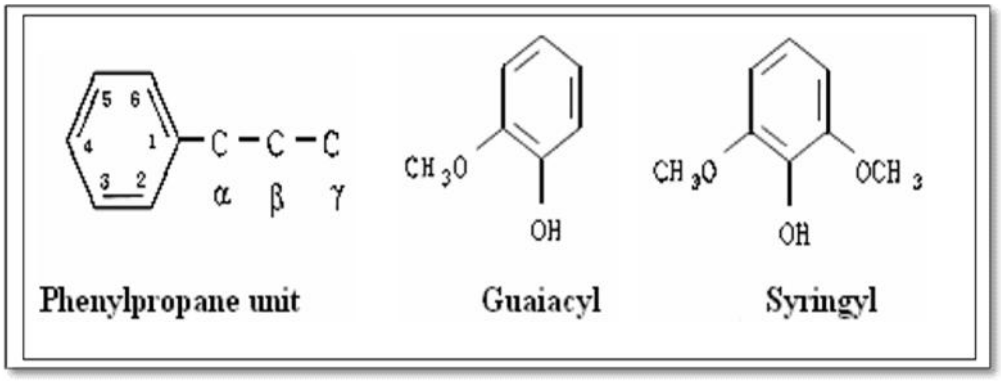

Figure 2.2: Building blocks of lignin

Source: James and Jeffrey (1997)

2.1.2 Application of Biodelignification

In the early years of application of the biological method various type of fungi such as brown-, white- and soft-rot fungi are used to degrade lignin and hemicellulose in waste materials (Schurz, 1978). Though these fungi depend on cellulose to survive, the brown-rots mainly attack cellulose alone, ignoring the lignin. The other white- and soft-rots attack both cellulose and lignin during the fungi growth.

The application of fungi in the biological method of delignification process will also likely to produce other enzymes or cellulases that can be used for the next sequencing process in the production of bioethanol. Boominathan and Reddy

(1992) discovered that white-rot fungi *P. Chrysosporium* produces lignin-degrading enzymes, lignin peroxidases and manganese-dependent peroxidases, during secondary metabolism in response to carbon or nitrogen limitation.

Other white-rot fungi also proved that these enzymes were produce during the delignification process and their presence improved the cellulose hydrolysis process (Kirk and Farrell, 1987; Waldner *et al*., 1988). Despite the benefits of the biological method such as the enzymes or cellulases that were produced and the low energy and economy cost, the biological method has a low rate of hydrolysis if it is to be used as a pretreatment process in the bioethanol production.

2.2 Raw Material (Lignocellulosic Material)

Lignocellulose is the major structural component of woody plants and non-woody plants such as grass and represents a major source of renewable organic matter. Lignocellulose consists of lignin, hemicellulose and cellulose (Betts *et al*., 1991). Of the three components, lignin is the most recalcitrant to degradation whereas cellulose, because of its highly ordered crystalline structure, is more resistant to hydrolysis than hemicellulose. The chemical properties of the components of lignocellulosics make them a substrate of enormous biotechnological value (Malherbe and Cloete, 2003). There are a lot of studies that has been done on the lignocellulosic materials. Lignocellulosic materials have two different types of surface area which is external and internal. The external surface area is related to the size and shape of the particles, while the internal surface area depends on the capillary structure of cellulosic fibers. Typically, dry cellulosic fibers have small size, about 15 to 40 μm, and therefore they possess a considerable external specific surface area, for example, 0.6-1.6 m^2/g (Taherzadeh and Karimi, 2008).

2.2.1 Banana Stem Waste

Banana is one of the important fruit and vegetable crop plants and the well-known species are abaca (*Musa Textilis*) and other wild banana plants used as a source of fibres for the paper and cordage industry (Franco *et al.,*1993). Banana plant is lignocellulosic which contain cellulose, hemicelluloses and lignin. Banana stem waste commonly contain of 41% - 57% cellulose, 8% - 12% hemicelluloses and 24% - 27% lignin (Scurlock *et al.,* 2004; Silveira *et al.*, 2007). Banana stem waste has a high organic content (83%); with 15–20% (w/w) lignin and cellulose which gives it a sheath-like texture (Kalia *et al*., 2000). The separation of lignin from lignocellulosic biomass by organic solvents can be considered as a very effective approach. According to Marcelle *et al*., (2002) the pulping process using formic acid/acetic acid/water to selectively separate cellulose, hemicellulose and lignin from banana stem at atmospheric pressure was optimised. The main residual wastes of the banana crop are leaves and pseudostems, both containing high levels of lignocellulose. These lignocellulose materials are efficient substrates for white-rot fungi, which produces lignolytic and cellulolytic enzymes that have numerous applications in industrial processes for food, drug, textile and dye use (Reddy *et al.*, 2003). However, banana stem has low lignin content and makes the delignification of banana stems appears difficult (Marcelle *et al.,* 2002).

2.2.2 Corn Stalks

Cellulose gives plants structure, and cell walls of plant cells are cellulose. Cellulose is the basis of wood, grass, and stalks of plants, and makes up 44% of all biomass. Cellulose is a structural entity, so its purpose is to provide the plant with stability. According to research published in the online edition of the American Chemical Society journal, Environmental Science and Technology by scientists at Rice University, when corn stalks and stover are going to be used for

ethanol production, using less nitrogen produces a better quality feedstock. Various kinds of solid materials containing lignin were obtained by fractionation (autohydrolysis and organosolvolysis) of corn (*Zea mays*) stalks (Manuel *et al.,* 1999). Corn Stalks remaining after it was harvested contain 43% polysaccharides consisting mainly of hemicellulose and cellulose, 29% lignin, 7% ligin, 5% ash, and 16% others (Chahal *et al*., 1990).

2.2.3 Oil Palm Trunk Waste

Oil palm (*Elaeis guineensis*) for palm oil production needs to be replanted at an interval of 20 to 25 years in order to maintain oil productivity. Oil palm trunk is categorized as hardwood. Oil palm tree has been planted since 20-30 years ago and now is the time for the replantation. That is why the amount of waste for this high processing value is estimated to be around 33 million tonnes including empty fruit bunches, oil palm trunks, fibers and shells (IMPOB, 2009b; Mohamed and Lee, 2006). In 2007, as much as 10, 827 tonnes of oil palm trunks are obtained as waste showing that these oil palm trunks are the largest contributors in waste from the agricultural industry (Goh *et al*., 2009). Like any other wood structure, hardwood is categorized under lignocellulose. The trunk, leaves and shell of the oil palm tree are lignocellulosic materials. Another study also state that oil palm is a lignocellulosic material which rich in carbohydrates in the form of starch and sugar and containing cellulose, hemicelluloses and lignin (Murai *et al*., 2009). It is an abundant waste material at replantation and harvesting sites in Malaysia and in many parts of South East Asia (Sreekala *et al.,* 1997). Large quantities of this waste are left in the field as underutilized resources. Oil palm is now considered to be one of the most promising non-wood lignocellulosic raw materials for various types of wood-based panels (Sulaiman *et al*,. 2009). All of this parts yield cellulose and monosugars such as glucose, xylose and arabinose after pretreatment. By microbial fermentation, these sugars could be used as substrate to produce renewable energy such as ethanol and hydrogen. Oil palm trunk is made up of three main structural components which

are cellulose 34.5%, hemicellulose (mainly xylan) 31.8% and lignin 25.7%. A study done by RunChang and Tomkinson (2001) stated that the chemical composition (% dry weight, w/w) of oil palm trunk fiber is the following: cellulose 41.2%, hemicelluloses 34.4%, lignin 17.1%, ash 3.4%, extractives 0.5%, and ethanol soluble 2.3%.

Table 2.1: Chemical Composition of Different Parts of the Oil Palm

Parts of oil palm	Extractives	Chemical Composition (%)		
		Holocellulose	Alpha Cellulose	Lignin
Bark	10.00	77.82	18.87	21.85
Leaves	20.60	47.7	44.53	27.35
Frond	3.50	83.13	47.76	20.15
Mid-part of trunk	14.50	72.6	50.21	20.15
Core-part of trunk	9.10	50.73	43.06	22.75
Frond	1.40	82.2	47.60	15.20
Trunk	5.35	73.06	41.02	24.51
Kenaf	-	82	-	-
Hardwood	0.1-7.7	71-89	31-64	14-34
Softwood	0.2-8.5	60-80	30-60	21-37

Source: Rokiah *et al.* (2011)

2.2.4 Selection of Raw Material Used

Most of the previous researches did not used hardwood as a source of raw material. Softwood was chose over hardwood for most of these tests mainly because it has a much weaker structure compared to hardwood. Cellulose and hemicellulose contents are more in hardwoods (78.8%) than softwoods (70.3%), but lignin is more in softwoods (29.2%) than hardwoods (21.7%)(Balat, 2009). That is why the hardwood is chosen to obtain more cellulose. Hence, for the selection of the raw material used, oil palm trunk wastes are chosen because there are a lot of oil palm trunk residues available in this country and making it as the most suitable raw material for this process compared to corn stalks and banana

stem wastes. The structural composition of various types of lignocellulosic-biomass materials are given in Table 2.2 (Demirbas, 2005).

Table 2.2: Composition of various types of lignocellulosic-biomass Materials (% dry weight)

Material	Cellulose	Hemicelluloses	Lignin	Ash	Extractives
Algae(green)	20-40	20-50	-	-	-
Cotton, flax, etc.	80-95	5-20	-	-	-
Grasses	25-40	25-50	10-30	-	-
Hardwoods	45±2	30±5	20±4	0.6±0.2	5±3
Harwood barks	22-40	20-38	30-55	0.8±0.2	6±2
Softwoods	42±2	27±2	28±3	0.5±0.1	3±2
Softwood barks	18-38	15-33	30-60	0.8±0.2	4±2
Cornstalks	39-47	26-31	3-5	12-16	1-3
Wheat straw	37-41	27-32	13-15	11-14	7±2
Newspapers	40-55	25-40	18-30	-	-
Chemical pulps	60-80	20-30	2-10	-	-

2.3 Methods of Biodelignification

A large number of microorganisms have attracted particular attention for their potential ability of lignin degradation (Hatakka, 2001). In the early years of application of the biological method various type of fungi such as brown-, white- and soft-rot fungi are used to degrade lignin and hemicelluloses in waste materials (Schurz, 1978). White-rot fungi are known as the most efficient lignin degraders, in which the representative species *Phanerochaete chrysosporium* has been most extensively studied due to the ability to degrade a wide range of organic substrates (Wen *et al*., 2009; Yu *et al*., 2009). Fungi are able to break down resistant materials such as cellulose, gums, and lignin. They dominate in acidic, sandy soils

and in fresh organic matter. In natural decomposition of lignocellulosic matter, both fungi and aerobic bacteria play an important role in degrading holocellulose and lignin to lower-molecular-weight products, some of which are then further metabolized by facultative and obligate anaerobic soil bacteria and actinomycetes (Maccubbin and Hodson, 1980). Biological pretreatment involves microorganisms such as brown-, white- and soft-rot fungi that are used to degrade lignin and solubilize hemicellulose. Among the three types of the fungi, white-rot fungi are the most effective biological pretreatment of lignocellulosic materials (Taherzadeh, 2008). The advantages of biological pretreatment include low energy requirement and mild environmental conditions.

2.3.1 Soft-rot Fungi

In soft-rot decay mainly cellulose and hemicelluloses are degraded, while lignin degradation is restricted. Soft-rot is characteristic to wood in wet environments such as railway slippers, poles, piles, ship and boat wood. In buildings soft-rot is found in moist window frames. Both softwoods and hardwoods are attacked by soft-rot fungi. Characteristic species are *Chaetomium Globosum, Richurus Spiralis* and *Phialophora Mutabilis* (Schultz *et al*., 2003).

2.3.2 Brown-rot Fungi

Brown-rot fungi utilize carbohydrates and lignin only slightly affected (Fan *et al.,* 1987). Brown-rot fungi produced high levels of hydrolytic activities and no phenoloxidase activity. An example of brown-rot fungi, *Laetiporeus sulfurous* demonstrated a very limited degradative capacity, contrasting with *Wolfiporia cocos,* which induced an effective decay. However, the fungi providing the highest values of lignin loss were also responsible for the highest values of polyoses removal (Angela and Ferraz, 2001). Brown-rot is a type of wood decay

caused exclusively by members of the Basidiomycotina. In brown-rot cellulose and hemicelluloses are broken down in the wood substrate, while lignin remains preserved in a slightly modified form (Rayner & Boddy, 1988). In contrast to white rot fungi, most brown rot fungi lack extracelluar phenoloxidases.

2.3.3 White-rot Fungi

Lignin degradation by white-rot fungi has received considerable attention as a means for reducing accumulation of lignocellulosic wastes in the environment. The stimulatory effect of surfactants on fungal lignocellulose bioconversion also has attracted wide interest (Yun-Shan *et al,* 2000). Further studies on the white-rot fungi ability to degrade lignin are tested and it is proved to be the most effective basidiomycetes for biological pretreatment of lignocellulosic materials (Fan *et al*., 1987). White-rot fungi such as *Phanerochaete chrysosporium* and *Coriolus Versicolor* are the most efficient ligninolytic organisms described to date. Their ability to degrade lignin and a wide variety of aromatic compounds is due to a nonspecific extracellular enzyme system, which involves lignin peroxidases, laccases and manganese-dependent peroxidases as well as hydrogen-producing oxidases (Kirk and Farrell, 1987; Reddy, 1995). However, white-rot fungi produced hydrolytic and ligninolytic enzymes, but low levels of hydrolytic activities (Angela and Ferraz, 2001). The capacity of white-rot fungi for wood degradation (determined as weight and component losses) seemed to be correlated with the levels of oxidative activities only after long biodegradation periods. Other than those studies, Lee *et al.,* (2007) also did a study that evaluated biological pretreatment of Japanese red pine (*Pinus densiflora*) using three white-rot fungi (*Ceriporia lacerata, Stereum hirsutum,* and *Polyporus brumalis*). Pretreatment with *S. hirsutum* resulted in selective degradation of the lignin rather than the holocellulose component. White-rot fungi are the most effective basidiomycetes for biological pretreatment of lignocellulosic materials (Sun and Cheng, 2002).

2.3.3.1 *Pleurotus Ostreatus* (Oyster Mushroom)

Pleurotus ostreatus and *Phanerochaete chrysosporium* are two model lignin-degrading basidiomycetes (Francisco *et al.*, 2007). Oyster mushroom (*Pleurotus ostreatus*) is an edible mushroom having an excellent taste and flavour. It belongs to the class Basidiomycetes, subclass Hollobasidiomycetidae, and order Agricales. It grows wild in the forest and is cultivated in the temperate and sub tropical regions of the world (Shah *et al*., 1981). *Pleurotus ostreatus* were investigated for their ability to produce various lignolytic and cellulolytic enzymes such as laccase, lignin peroxidase, xylanase, endo-1,4-β-D-glucanase (CMCase) and exo-1,4-β-D-glucanase (FP activity) on banana agricultural waste (leaf biomass and pseudostems) at solid substrate fermentation (Reddy *et al*., 2003). A study on five different strains on corn stalk showed that *Pleurotus v. florida* and *Pleurotus. sp.* were the best effective strains for cellulose and hemicellulose degradation (Hamza *et al*., 2000). Many wood decomposing fungi utilize lignocelluloses efficiently and this characteristic is related to their ability to metabolize lignin (Kirk, 1971). *Pleurotus sp.* are found to be one of the most efficient lignocellulose solid state decomposing types of white rot fungi. Thus, many agricultural and industrial wastes can be utilized for production of *Pleurotus sp.* as a substrate (Zadrazil and Brunnert, 1981).

2.3.3.2 *Phanerochaete Chrysosporium*

One of nature's most important biological processes is the degradation of lignocellulosic materials to carbon dioxide, water and humic substances. This implies possibilities to use biotechnology in the pulp and paper industry and consequently, the use of microorganisms and their enzyme to replace or supplement chemical methods is gaining interest. Lignin and manganese peroxidases are secreted by the basidiomycete *Phanerochaete chrysosporium* during secondary metabolism. These enzymes play major roles in lignin degradation. The active site amino acid sequence of these lignin degrading

peroxidases is similar to that of horseradish peroxidase (HRP) and cytochrome c peroxidase (CcP). Lignin degrading peroxidases are able to catalyze the oxidation of substrates with high redox potential. This unique ability is consistent with a heme active site of low electron density, which is indicated by high redox potential (Cai and Tien, 1993).

2.3.4 Selection of Method of Biodelignification

The usage of fungi treatment for delignification process has been done by some research group such as Sun and Cheng (2002) and Tengerdy and Szakacs (2003). These researches shows 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 costs a lot more money and energy. Oyster mushroom is relatively easy to grow (Gibriel *et al*., 1996). The oyster mushroom, *Pleurotus ostreatus*, which is a type of white-rot fungi showed maximum lignolytic activity and thus it is used in the study for the treatment of lignocellulosic materials. White-rot fungi are the most important fungi in delignification process. Brown-rot and soft-rot fungi, though they degrade lignin, but to a limited extent only because they lack the complete array of ligninotytic enzymes (Kirk & Highley, 1973). Besides that, oyster mushrooms are primary decomposers of hardwood trees and are found worldwide (Kong, 2004) hence making it the best choice to degrade the lignin of oil palm trunk which is a type of hardwood.

2.4 Optimization

2.4.1 Factors

2.4.1.1 Temperature

In a method of keeping Termitomyces (example of mushroom) sample for researches, the fungi were kept in laboratory nest boxes in complete darkness at a constant temperature of 24 – 25 °C and watered occasionally to prevent colonies from drying out (De Fine Licht *et al*., 2007). *Pleurotus ostreatus* strain IE8 was obtained from the Institute of Ecology of Jalapa, Veracruz, México and grown on malt extract agar (MEA) at 25 °C (Oscar *et al*., 1999). *Pleurotus ostreatus* has high cellulolytic as well as lignolytic activity at 25-30 °C temperature and at neutral pH (Bharati, 2010). Other than that, temperature optima were found to vary (between 25–37 °C) amongst the isolates done (Read *et al*., 2001). For the optimization process, most of the values tested were at a small range. Surrounding temperature will affect the growth of the fungi hence will give effect to the delignification process. This is because it will affect the production of the lignin degrading enzymes. The temperatures itself will be controlled by running the experiment in an incubator at respective temperature.

2.4.1.2 pH

In order to make sure that the growths of the fungi are optimum, the pH of the medium also should be considered. At different pH value, fungi tend to release different enzymes that will very much likely affect the delignification process. According to Reddy *et al.* (2007) enzymes were extracted from 5 g of sample with 20 ml cold 0.05 M acetate buffer (pH 6.5). Another study showed that pH value of in between 4.5 to 10.5 with 6.5 having the best growth result. Besides that, *Pleurotus ostreatus* has high cellulolytic as well as lignolytic activity at neutral pH (Bharati, 2010). Optimum pH is needed for the fungi to produce the lignin

degrading enzyme such as laccase. The example of white-rot fungi, *Trametes trogii* (MYA 28-11) is an outstanding producer of laccase. The impact on enzyme production of three quantitative variables, namely pH, copper and nitrogen concentrations, was investigated by using a wood-based solid medium supplemented with malt extract. Optimization was aimed at simultaneously minimizing cellulose activity and maximizing ligninolytic enzyme production. Such condition is pH 4.5.

2.4.1.3 Moisture Content

Moisture content also important to the growth of the fungi**.** According to Keiko *et al.* (2010), the supplementation of glucose to the media as well as the increase of moisture content favored the production of biomass. Besides that Wang *et al.* (2000) suggest that the optimum moisture content of the spent grain substrate for cultivation of *Pleurotus ostreatus* is 70%.

2.4.1.4 Fungi to Medium Ratio

The medium used in this research are oil palm trunk wastes and the fungi are *Pleurotus Ostreatus*. Fungi to medium ratio commonly used are 2g: 10g – 4g: 10g. That's mean 2g fungi are used in 10g medium of oil palm trunk waste. The quantity of the fungi used in a medium should be sufficient for the production of the several enzymes to degrade the lignin of the wastes.

2.4.2 Response Surface Methodology (RSM)

Response Surface Methodology (RSM) which invented by Box and Wilson, is a collection of mathematical and statistical techniques for empirical model building. The effect of the composition of a mixture containing, oat straw (OS), oat bran (OB) and copra cake (CC), on the mycelial growth of *Pleurotus ostreatus* was studied using mixture and response surface methodologies (Oscar *et*

al., 1999). Response surface methodology (RSM) is defined as a tool to analyze the effect of a selected response of independent variables and for modeling of complex systems. It's also software that able to map a response surface over a particular region of interest (RunChang and Tomkinson, 2001).

RSM is a useful approach for analyzing biological processes and has been used widely in food science and technology, microbiology and enzyme applications. Mixtures methodology and RSM could be associated by studying the physical characteristics of the measured response surface such as the shape, slope or the highest point (Martínez-Carrera, 1997). RSM is a well known up to date approach for constructing approximation models based on either physical experiments, computer experiments (simulations) (Box *et al*., 1974) and experimented observations. By careful design of experiments, the objective is to optimize a response (output variable) which is influenced by several independent variables (input variables). An experiment is a series of tests, calledruns, in which changes are prepared in the input variables in order to recognize the reasons for changes in the output response (Montgomery & Runger, 1994). RSM involves two basic concepts. The first one is the choice of the approximate model and the second one is the plan of experiments where the response has to be evaluated (Raissi *et al*., 2009). One of the most popular response-surface designs is the central-composite design (CCD), due to Box and Wilson (1951).

2.5 Conclusion

The optimization of biodelignification process by using oil palm trunk waste can be done by using white-rot fungi as the agent of biodelignification. The stimulation done by using Response Surface Methodology (RSM) to obtain the optimum conditions for the degradation.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Process Flow Chart

The brief process method for this research is shown in the flowchart below. The response surface methodology is also run for the optimization part. Below is a brief flow chart for the oil palm trunk delignification process.

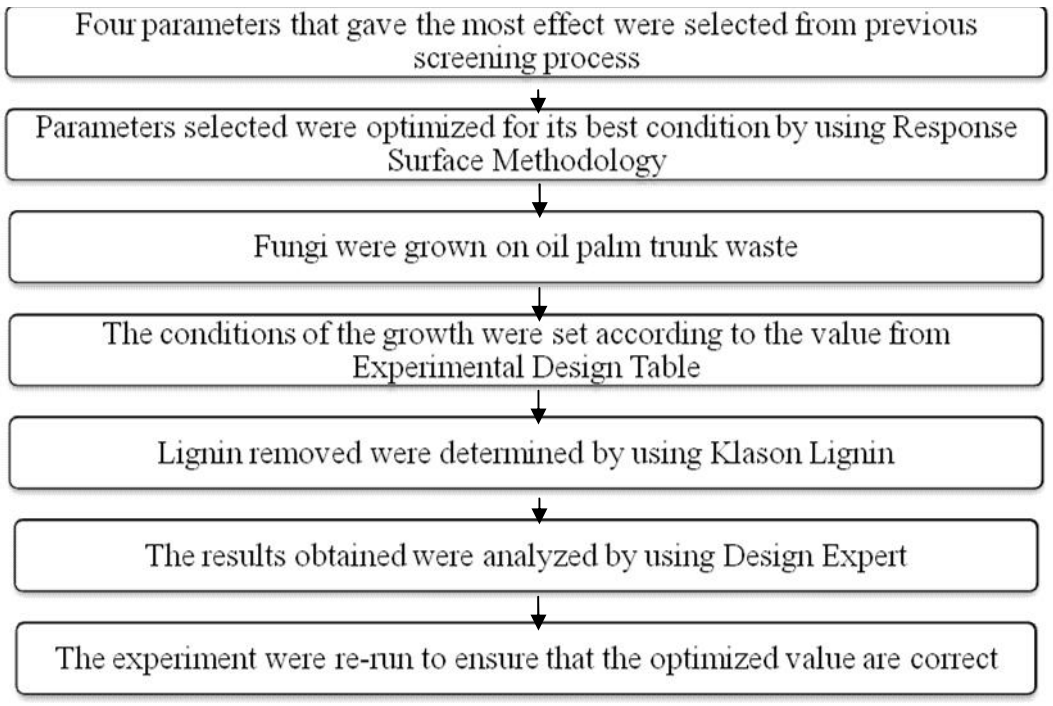

Figure 3.1: Process flow chart

This method uses a type commercialized local fungi, *Pleurotus Ostreatus,* or also known as the oyster mushroom, as the agent of delignification. From the flow chart, we can see that the optimization method with four most effective parameters selected from previous study was used. Experimentally, the fungi will be first grown on the oil palm trunk. During the growth, all of the four parameters were controlled during the experiment. The ranges of the factors that will be controlled are from the table below.

Table 3.1: Ranges of optimization for all factors

Factor	Ranges
Temperature	23°C – 27 °C
pH	5 – 8
Moisture Content	2.0 mL- 3.5 mL
Fungi to Medium Ratio	2g:10g – 4g: 10g

3.2 Response Surface Methodology (RSM)

To get the respective value of the parameters to be controlled, the response surface methodology (RSM) is also run to get the experimental design table. Central composite design is choose over other design because of its stability for building a second order (quadratic) model. This design is known for the ability to analyze the response variable without using three-level factorial experiment. The optimization method with four most effective parameters was used. In this research, 30 runs of experiment were done. Hence, the experiments were done by referring to the table respectively and all of the four parameters were also controlled during the experiment.

Table 3.2: Experimental Design Table

Std	Run	Block	Factor 1 A:Temperature (°C)	Factor 2 B:pH value	Factor 3 C:Moisture Content (mL of water added per 12 hours)	Factor 4 D: Fungi to Medium Ratio
1	4	Block 1	24	6.0	2.375	0.25
2	19	Block 1	26	6.0	2.375	0.25
3	8	Block 1	24	7.0	2.375	0.25
4	18	Block 1	26	7.0	2.375	0.25
5	2	Block 1	24	6.0	3.125	0.25
6	13	Block 1	26	6.0	3.125	0.25
7	14	Block 1	24	7.0	3.125	0.25
8	12	Block 1	26	7.0	3.125	0.25
9	17	Block 1	24	6.0	2.375	0.35
10	20	Block 1	26	6.0	2.375	0.35
11	16	Block 1	24	7.0	2.375	0.35
12	5	Block 1	26	7.0	2.375	0.35
13	9	Block 1	24	6.0	3.125	0.35
14	10	Block 1	26	6.0	3.125	0.35
15	1	Block 1	24	7.0	3.125	0.35
16	6	Block 1	26	7.0	3.125	0.35
17	3	Block 1	25	6.5	2.750	0.30
18	15	Block 1	25	6.5	2.750	0.30
19	7	Block 1	25	6.5	2.750	0.30
20	11	Block 1	25	6.5	2.750	0.30
21	26	Block 2	23	6.5	2.750	0.30
22	23	Block 2	27	6.5	2.750	0.30
23	21	Block 2	25	5.5	2.750	0.30
24	22	Block 2	25	7.5	2.750	0.30
25	24	Block 2	25	6.5	2.000	0.30
26	25	Block 2	25	6.5	3.500	0.30
27	27	Block 2	25	6.5	2.750	0.20
28	29	Block 2	25	6.5	2.750	0.40
29	30	Block 2	25	6.5	2.750	0.30
30	28	Block 2	25	6.5	2.750	0.30

3.3 Oil Palm Trunk Delignification Preparation

Biological treatment or microbial treatment has been chosen as the method delignification method. This method uses a type commercialized local fungi, *Pleurotus ostreatus,* or also known as the oyster mushroom, as the agent of delignification. Various techniques are used to prepare substrate for the cultivation of oyster mushrooms (Geml *et al*., 2001; Villa-Cruz *et al.*, 1999). First of all, 2.5 g of oil palm trunk was weighed and put into different bottles. After that, all of the bottles were autoclaved for 2 hours. Then, to continue with the delignification process, the fungi which are the oyster mushroom were cut to get the spores and weighed (Parisa *et al.*, 2010). Each bottle was added with oyster mushroom with respective value from design table. This process has been done in laminar flow cabinet to reduce contamination. Buffer solutions also was prepared and added into the samples with respective pH. This part also has been done in laminar flow cabinet. Distilled water was dropped into the bottle with respective value and the bottles were placed in the incubator at respective temperature.

3.3.1 Temperature

In this research, incubators were used to control the temperature of the samples respectively. Based on previous study, the optimum temperature is between 25- 37 ºC (Read *et al*., 2001). However, according to the table of optimization parameters, there are temperatures between 23 to 26 ºC. The incubators temperature was set with the respective value and has been observed frequently to avoid temperature changes.

3.3.2 pH Value

Phosphate-citrate buffer was used to get the variation pH value. The buffers need to be prepared first according to the required pH for each process. This

buffer was chosen because the chemical needed is available and easy to match the variety of pH value. The calculation of the buffer preparation was attached in the Appendix A.

3.3.3 Moisture Content

Fungi requires wet environment to grow (Keiko *et al.*, 2010). Hence, the moisture of the medium is also one of the factors that affect fungi growth. However, commercialized fungi were kept wet and were watered occasionally. There was no exact amount of water required for fungi growth. Medium moisture content was controlled by the frequency of water drops into the sample which was done every 12 hours by referring to the amount in the optimization design table by using dropper.

3.3.4 Fungi to Medium Ratio

Fungi to medium ratio are a variable or parameters to check how much amount of fungi are required to remove lignin on a certain amount of medium. The amount of fungi is added according to the Table3.2 respectively. In this process, for example, if the value of the ratio that been researched is 0.25, the amount of fungi used is 0.625 g where the amount of the oil palm trunk (medium) is fixed to be 2.5 g.

3.4 Klason Lignin

The analysis of the lignin degraded is determined by the Klason Lignin determination method (Lopez *et al*., 2010), where the lignin content of the treated oil palm trunk is compared with the oil palm trunk that was not treated with the delignification method. With this method, the performance of the delignification can be determined at various values of parameters.

For the Klason Lignin methods, firstly the samples were dried at 60 °C for 12 hours or until constant weight. Meanwhile, sulfuric acid (72%) was prepared. Then 20 ml of sulfuric acid was added with 1 gram of sample and leaved for two hours. After that, distilled water was added as much as 560 ml into the solution and heated in the water bath for two hours. After two hours, the sample was and washed. To make sure that the filtration process is effective and not time consuming, the vacuum filtration was used. It makes the experiment can be finished faster. The residue was taken and dried again with temperature of 105 °C for one hour in the oven. The sample was leaved in the dedicator for two days or until the weight is constant and the sample was weighed. The Klason Lignin method was repeated by using the sample that is not reacted with fungi as blank.

3.5 Final Procedures

After finishing the methods, the data collected were added into RSM and analyzed. Then, the experimental steps were done again to compare and confirm the optimum values obtained.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Factors in Biodelignification Process

In biodelignification process, there are some factors that influenced it effectiveness which are temperature, pH, moisture content and fungi to medium ratio.

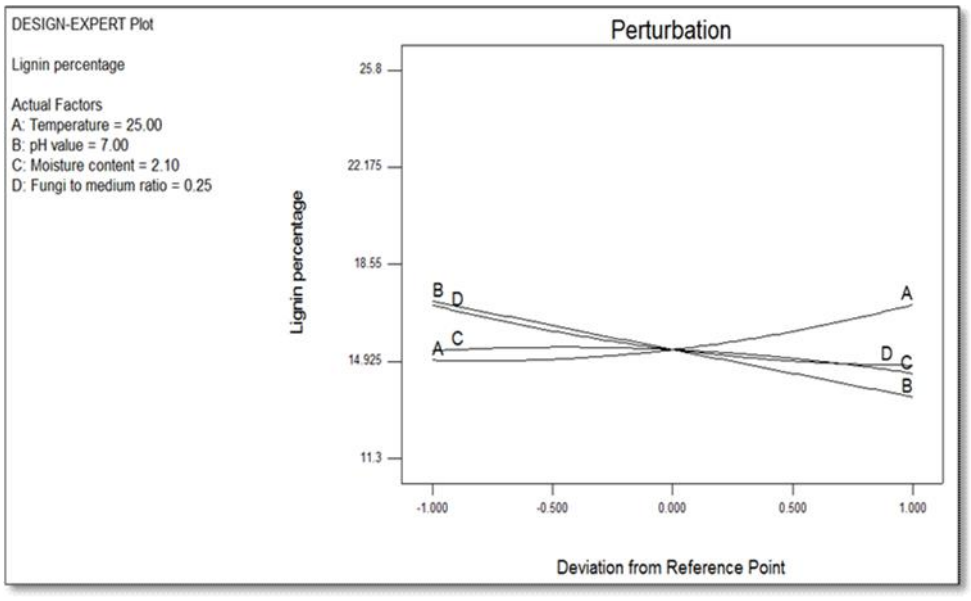

Figure 4.1: Perturbation plot

Figure 4.1 shows the perturbation plot obtained from Design Expert software containing all of the factors related to the study.

4.1.1 Effect of Temperature

The temperature set in this study is between 23 – 26 °C which is obtained from the results of screening. From Figure 4.1, it shown that the line pattern of Factor A (temperature) is different from other factors where the temperature is directly proportional to the lignin percentage. That is meaning that the lower temperature is better for the lignin degradation and can be seen that if the temperature is too high, the degradation process will decreased. This is because the rate of biodelignification is influenced by the growth of fungi. Most wood destroying fungi grow best and their growth curves show well-defined maximum temperature from 20-35 °C (Ungger, 1995). The enzymatic reactions of fungi are strongly influenced by temperature and the temperature dependence of fungal activity follows reaction rate theory (Zabel and Morrel 1992).

There are three cardinal growth temperatures for each species of fungi which are the minimum temperature where the growth begins, the optimum temperature for best mycelial growth and the maximum temperature where the growth ceases, or stop. For white-rot fungi, the room temperature should be kept 20-24 °C at the first stages of mycelium growth, and the moisture 80 – 90%. At this time, the temperature should never get above 27-28 °C, as the insides of the sacks will be 2-3 degrees hotter than the outside. At 30 degrees, mycelium growth slows and at 32 °C and more, it dies. Similarly, around 13 degrees the growth slows and mycelium dies at 0 degrees. As we can see, the temperature should always be controlled. According to a research done by Buah *et al*. (2010), the growth of oyster mushroom requires high temperature (25-30 °C) for the vegetative growth called spawn running and lower temperature (18-25 °C) for fruit body formation (Viziteu, 2000). There are also other studies proved that the optimum temperature

for the growth of the oyster mushroom is about 25 °C. Even though there are some variations in growth of the mycelium according to the strains in a species, *P. ostreatus*, *P. florida*, *P. sajor-caju* and *P. eryngii* reach their optimum growth at 25 °C. The optimum temperature for the growth of the fungi is significant in optimizing the lignin degradation. Hence, the temperature should not be set too high in order to avoid the increasing of the percentage of lignin left after the degradation process and because reaction temperature is one of the three major parameters were found to have significant effects to the biodelignification treatment (Chun *et al.,* 2011).

4.1.2 Effect of pH

The susceptibility of wood to attack by fungi also depends on its hydrogen ion concentration (pH value). Most wood species are weakly acidic with pH value of approximately 5. Nwokoye *et al*. (1999) stated that the optimum pH for the growth of the oyster mushroom is pH 9. However in this study, there are no pH 9 tested where the maximum pH value is 8. By referring to the perturbation plot, by lowering the pH, the lignin left in the sample will decreased. That's mean, the lignin degraded is increasing when the pH value is lower where more lignin was removed. For brown-rot and white-rot fungi, they reach optimum growth at pH values of 5-6, the total growth range. The reason why the lower pH is better for the degradation is the mushroom need to live in acidic medium. From Chang and Miles (2004), pH optimum for the mycelial growth of *Pleurotus ostreatus* is between 5, 4-6, while Gyurko (1979) determined the pH optimum to 5,5.5, and 8. From a research done by RunChang and Tomkinson (2001), the results showed that the quantity of lignin separated from the lignucellulosic material is decreasing when the pH is lower. It same goes to the results obtained in this study.

4.1.3 Effect of Moisture Content

Lignin decomposition significantly increased at higher medium temperatures and moisture contents. However, it was found that the medium moisture was more important in affecting the lignin degradation than either temperature or pH (Donnelly *et al*., 1990). For moisture content which is Factor C, the lignin percentage is inversely proportional to the quantity of moisture added into the sample. A larger quantity of moisture present in the sample resulting in the higher percentage of lignin degraded. This is because the fungi need optimum water content to degrade the lignin and referring to a research by Mahler (1992) the moisture content should be maintained between 80-90% for the mycelia growth. Another research done also proved that the growth of oyster mushroom requires high humidity which is about 80 to 90 % (Buah *et al*., 2010). On the other hand, Hernandez *et al.* (2003) stated that the moisture content of 70% is the optimum condition for the degradation by oyster mushroom. The moisture should also be controlled and kept around 85- 90%. If the room isn't moisturized enough, the compost will start to dry and the efficiency will drop. But moisture levels higher than 90% is very suitable for a great number of problems.

4.1.4 Effect of Fungi to Medium Ratio

Figure 4.1 shows that the fungi to medium ratio also inversely proportional to the percentage of lignin left. Rationally, when the volume or quantity of the fungi is higher, the reaction or degradation process also will be higher. This is because more fungi can involve in the degradation of the lignin and increase the percentage of the lignin removed from the oil palm trunk fiber. That is why the degradation of lignin is higher when the fungi to medium ratio are higher. As in a book written by Steven (1981), it stated that a wide range of media are used for growing fungi. Fungi need a wide media for sporulation process and enhance the degradation of the lignin. This is because in the media, there are nutrients such as glucose and nitrogen source that are needed by the fungi to grow.

Oil palm trunk contains quite significant amount of organic nutrients such as nitrogen, phosphorus, potassium, and magnesium and calcium (Khan, 2004). However, in the biodelignification process, the nutrient in the oil palm trunk that affecting the growth of the fungi is the carbon and nitrogen sources. The nutritional requirements for fungal growth and sporulation vary among different fungi for example, nematophagous fungi are greatly influenced by nutrients and culture conditions (Coscarelli and Pramer, 1962). Elson *et al.* (1998) concluded that higher carbon to nitrogen (C:N) ratios or higher carbon concentrations reduced sporulation. Leite *et al*. (2003) found that the nitrogen components had more impact than carbohydrates on the growth of the fungi.

4.1.5 Interaction between Factors

Among the factors in the biodelignification, there are interactions happened between them that seem to improve the process. In this study, there is only one interaction discovered which are shown in Figure 4.2. Referring to the Figure 4.2, it shows that there exist an interaction between Factor A (temperature) and Factor C (moisture content) where the lines of moisture content of 1.85 and 2.35 are intercepting at a point which have a same temperature.

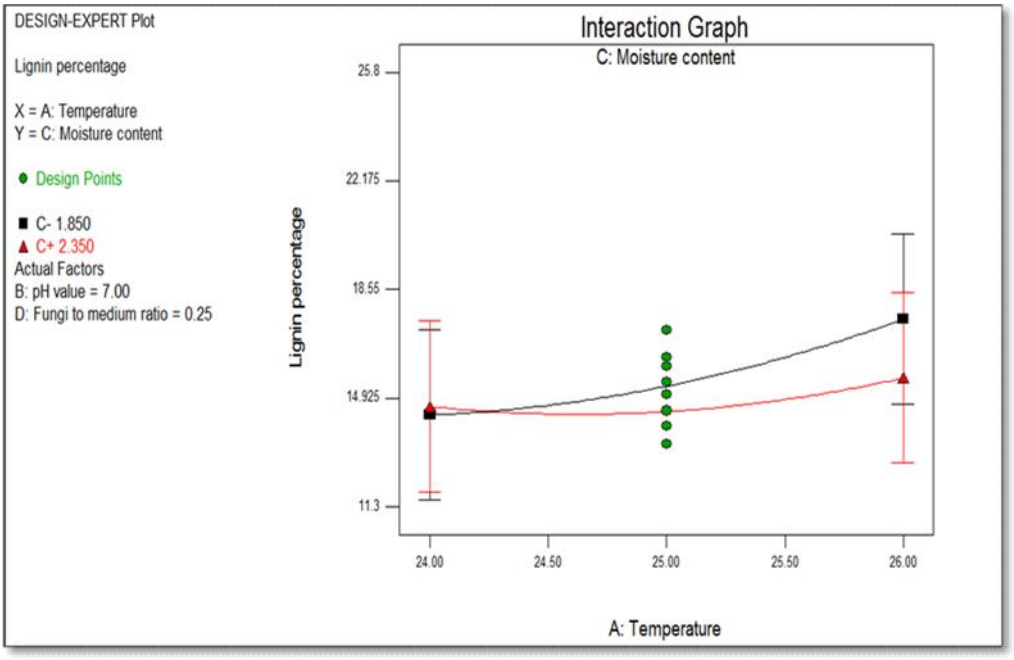

Figure 4.2: The interaction between Factor A, temperature and C, moisture content

In Figure 4.2, the interaction shows that, before the interception point, the lignin percentage for the moisture content of 1.85 is increasing with temperature. Meanwhile, at moisture content of 2.35, the lignin percentage is decreasing when the temperature increased. However, after the interception, the lignin percentages for both moisture contents are increasing with the increment of temperature.

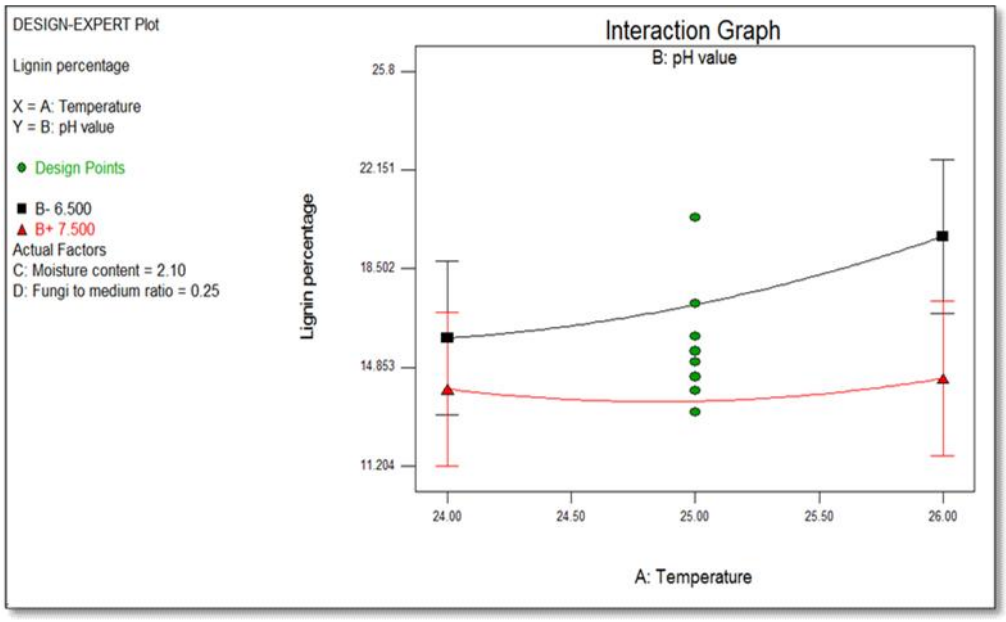

Figure 4.3: The interaction between Factor A, temperature and B, pH value

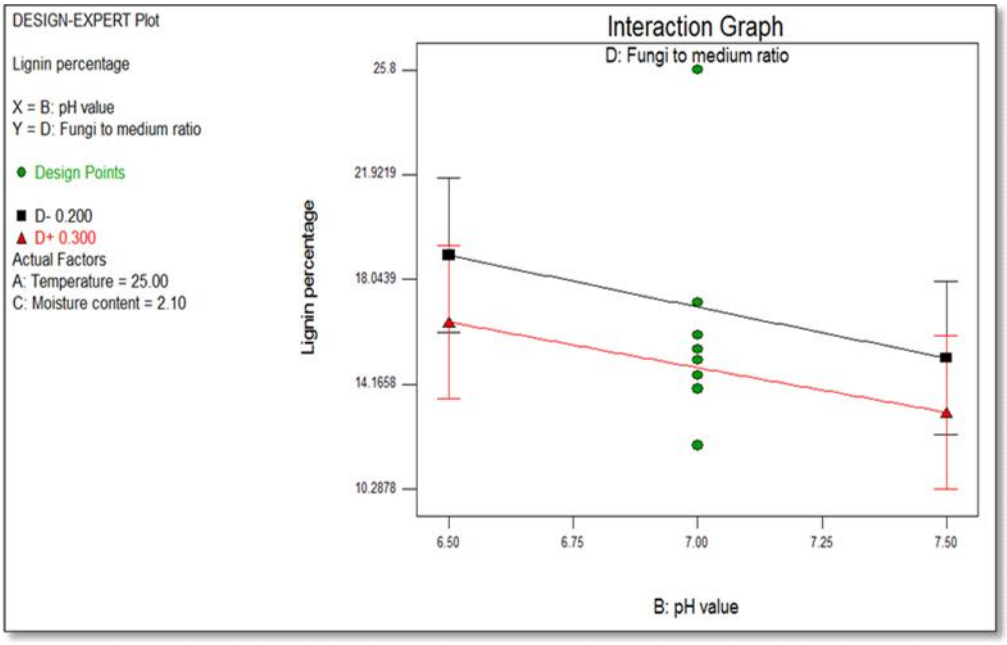

Figure 4.4: The interaction between Factor B, pH value and D, fungi to medium ratio

However, for the other plots, there are no interaction occurs as shown in Figure 4.3 to Figure 4.7. Referring to Figure 4.3, there is no interaction occurs between temperature and pH value. It shows that the lignin percentage for both of the pH value is increasing when the temperature increased.

In Figure 4.4, there are also no interaction occurs between Factor B (pH value) and Factor D (fungi to medium ratio). It is clearly showed that at the fungi to medium (F:M) ratio of 0.2 and 0.3, when the pH is increasing, the lignin percentage is significantly decreased for both of the ratio.

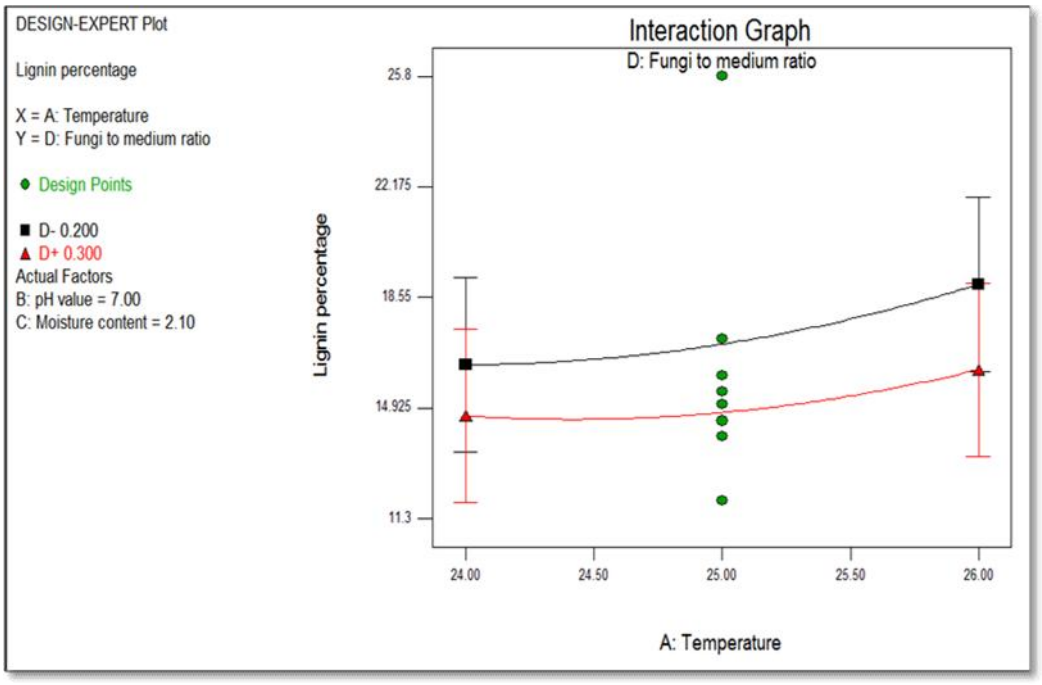

Figure 4.5: The interaction between Factor A, temperature and D, fungi to medium ratio

Fungi to medium ratio influenced the competition between fungi to grow. Temperature did not affect the competition of the growth because it is wholly supplied. Basically, for Figure 4.5 it can be seen that lignin percentage is directly

proportional to the temperature for both of the F:M ratio. It is because the lignin left is increasing for the F:M ratio of 0.2 and 0.3 when the temperature was increased.

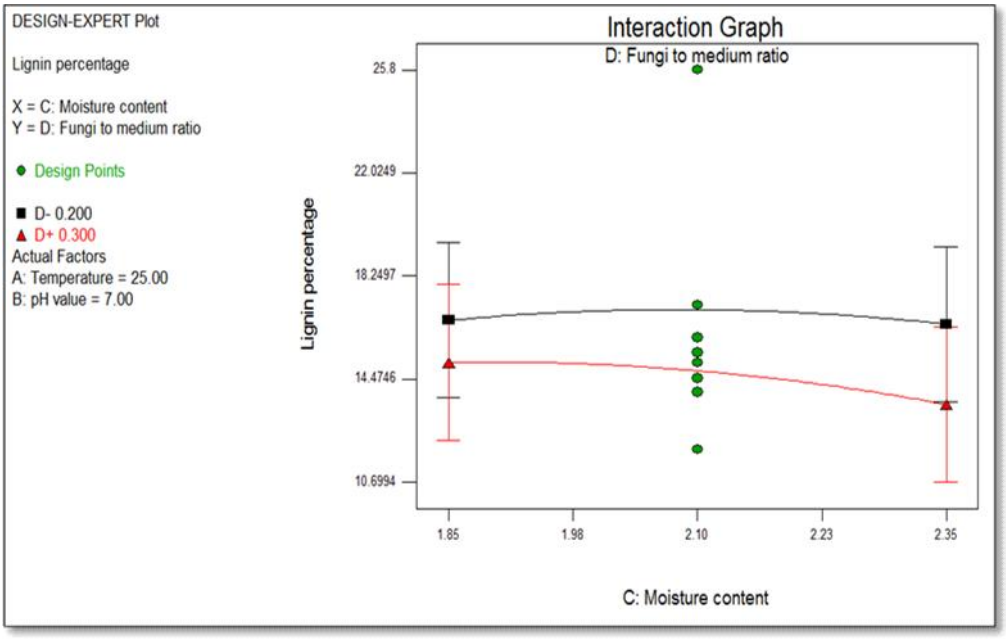

Figure 4.6: The interaction between Factor C, moisture content and D, fungi to medium ratio

For Figure 4.6, the case is the same with the case in Figure 4.5 where fungi to medium ratio will influenced the competition to get the nutrients required for growth. Meanwhile, the function of moisture content is for supplying the humidity to the fungi. Hence, these two factors would not influence each other because of their different function. It can be said that, at a specific fungi to medium ratio, there must be a specific amount of moisture content in order to obtain a specific value of lignin degraded. The plot showed that the lignin percentages are quite constant for both fungi to medium ratio when the moisture content increased but slightly decreased when the moisture content is too high for the F:M ratio of 0.3.

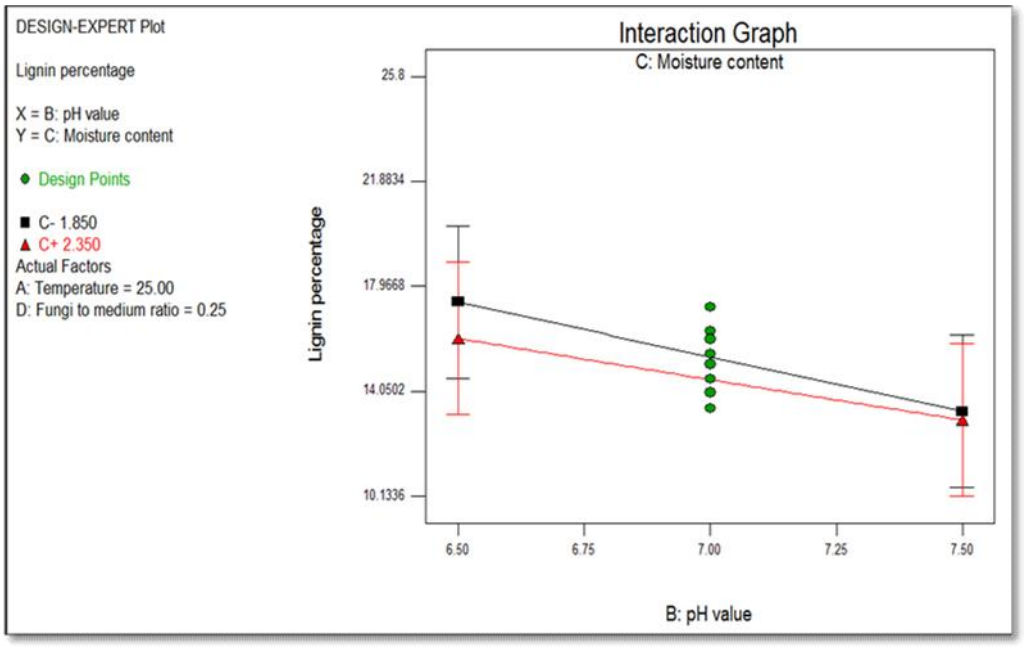

Figure 4.7: The interaction between Factor B, pH value and Factor C, moisture content

Here, in Figure 4.7, the interaction shows that the percentage of lignin left in the sample is inversely proportional to the pH value for both moisture contents. At the end of the graph, it can be seen that an interaction is almost occur between the two factors. Its mean that the pH value does affect the lignin percentage for both moisture contents of 1.85 and 2.35.

4.2 Analysis of Lignin Degraded by Klason Lignin

Klason lignin is a standard method in determining the content of the lignin in the sample (Hashim *et al.*, 2011). Lignin content was measured using the procedure of Kirk and Obst (1988).

Table 4.1 Percentage of lignin left

Run	Block	Temperature (°C)	pH	Moisture Content	Fungi to Medium Ratio	Lignin percentage (%)
1	Block 1	27	7.0	2.10	0.25	24.30
2	Block 1	25	7.0	2.10	0.15	25.80
3	Block 1	25	7.0	1.60	0.25	16.30
4	Block 1	25	6.0	2.10	0.25	20.40
5	Block 1	25	7.0	2.10	0.25	14.00
6	Block 1	24	6.5	1.85	0.30	15.20
7	Block 1	26	7.5	2.35	0.20	11.30
8	Block 1	24	6.5	2.35	0.20	14.80
9	Block 1	26	6.5	2.35	0.20	21.40
10	Block 1	24	7.5	2.35	0.20	14.30
11	Block 1	26	7.5	2.35	0.30	13.90
12	Block 1	26	6.5	1.85	0.30	22.90
13	Block 1	25	7.0	2.10	0.35	11.90
14	Block 1	24	7.5	1.85	0.30	14.08
15	Block 1	24	7.5	1.85	0.20	13.30
16	Block 1	26	6.5	2.35	0.30	13.00
17	Block 1	25	7.0	2.10	0.25	15.99
18	Block 1	24	6.5	1.85	0.20	16.76
19	Block 1	26	7.5	1.85	0.20	15.03
20	Block 1	25	7.0	2.10	0.25	14.50
21	Block 1	24	7.5	2.35	0.30	15.09
22	Block 1	23	7.0	2.10	0.25	14.27
23	Block 1	25	7.0	2.10	0.25	15.06
24	Block 1	25	7.0	2.60	0.25	13.40
25	Block 1	25	8.0	2.10	0.25	13.19
26	Block 1	26	7.5	1.85	0.30	12.24
27	Block 1	26	6.5	1.85	0.20	16.07
28	Block 1	25	7.0	2.10	0.25	17.20
29	Block 1	25	7.0	2.10	0.25	15.45
30	Block 1	24	6.5	2.35	0.30	18.20

In order to compare the content of the lignin in the oil palm trunk with treatment and without treatment with *Pleuratus ostreatus*, the Klason Lignin method was also done to the untreated oil palm trunk fiber. After the test, the final weight obtained is 18.7 g. The percentage of the lignin left after bidelignification process is shown in Table 4.1. By referring to the table, the value of the lignin left is in the range of 11 to 26 %. Halimahton and Abdul Rashid (1991) reported that the Klason lignin content of oil-palm trunk averaged 20.6% of the dry sample weight and was quite evenly distributed throughout the trunk. The initial value of lignin percentage in the sample in this study is 18.7% which is quite small. However, it is an acceptable value because according to Beijing Forestry and Parks Department of International Cooperation, the oil palm trunk basically contains about 18-21% of lignin.

Values obtained by previous researchers on some parts of oil palm, hardwood, softwood and other non-wood were tabulated in Table 4.2 for comparison. From the table, for the hardwood, the lignin degraded is about 26% which means that the lignin left in the sample is 76%. It is significantly higher than the lignin left in this study. Even though after 30 days of treatment, the highest lignin loss is only about 46%. It took a longer period to have that amount of degradation.

Table 4.2 Percent losses of different components of the hardwood and softwood pretreated with *Enchinodontium Taxodii*

Pretreatment time (day)	**Selectivity Value**	**Component Loss (%)**			
		Weight	**Lignin**	**Cellulose**	**Hemi-cellulose**
Hardwood (Chinese Willow)					
30	28.9	16.0 ± 0.3	26.0 ± 1.5	0.9 ± 0.9	31.0 ± 1.5
60	3.0	22.1 ± 0.1	35.5 ± 3.7	11.7 ± 1.0	35.2 ± 2.0
90	2.0	30.7 ± 0.7	41.7 ± 4.9	20.9 ± 0.3	44.8 ± 0.6
120	1.7	32.5 ± 1.7	45.6 ± 2.0	26.7 ± 0.2	50.8 ± 1.8
Softwood (China-fir)					
30	7.1	15.1 ± 0.3	23.3 ± 1.0	3.3 ± 1.9	21.6 ± 1.6
60	5.2	19.6 ± 0.1	31.9 ± 1.8	6.1 ± 1.4	25.8 ± 2.7
90	3.7	23.4 ± 0.8	38.8 ± 1.3	10.6 ± 2.7	24.9 ± 2,5
120	3.2	24.1 ± 0.9	39.8 ± 1.2	12.6 ± 0.1	31.4 ± 2.7

According to Jiebing *et al.* (2009), the lignin loss from the hardwood after pretreatment is 86% when they used a physicochemical method of degradation. The physicochemical used is steam explosion. The percentage is quite similar with the percentage of lignin degraded in this study which can reach 88.7% in run 7 and 88.1% in run 13 where the percentage of lignin left in the sample is 11.3% and 11.9% respectively. Hence, it can be concluded that although this study only used simple biological method, the percentage of lignin degraded can be as much as the lignin degraded in their study.

However, biological method is much better than the physicochemical method. The most successful physicochemical pretreatments include thermochemical treatments such as steam explosion or (steam disruption), liquid hot water (LHW), ammonia fiber explosion (AFEX) and CO_2 explosion (Sun and Cheng, 2002). In these processes, chipped biomass is treated with high-pressure saturated steam, liquid ammonia or CO_2 and then the pressure is swiftly reduced, making the materials to undergo an explosive decompression. Physicochemical method will require a lot of money where the method used more complicated and using chemicals. In biological method, which is in this study, the method used is much simpler where it needs mild environment conditions and also has low energy requirements. Besides that, no chemical and high required for this process and making the cost for this biodelignification process is lower than physicochemical process. Hence, the biological treatment is better than physicochemical treatment

However, there are also studies that using biological methods in the pretreatment such as a research done by Lopez *et al*. (2006). In their research, they used basidiomycete which is *P.flarida-alba* as the lignin degrader and grown on yeast-malt-peptone-glucose (YMPG) agar for two weeks. This treatment manages to get the maximum degradation of 46% and this value is lower than the value of maximum degradation in achieved in this study. Hence, using *Pleuratus ostreatus* is much better than using *P.flarida-alba* because it can degrade more lignin and the duration of the process also shorter which is only 6 days.

Other than that, Lee *et al*. (2007) also did a research on biodelignification by using white-rot fungi (*S.hirsutum*). The medium used in their study is softwood which is Japanese red pine, *Pinus densiflora*. Basically, in this study about 14.6% of lignin degraded where it is also significantly low even though the medium is softwood which is known to be easier to degrade compared to oil palm trunk (hardwood). Furthermore, it is time consuming where it needs 8 weeks to get this value of lignin loss and all of these disadvantages proved that choosing oyster mushroom as the agent of delignification is the best idea.

4.3 Analysis Using Response Surface Methodology (RSM)

After done with all of the experiments, the data collected were analyzed by using ANOVA (Analysis of Variance) in RSM and these results were tabulated in Table 4.3 for the comparison of the actual and predicted value. The actual values were obtained from the experiments, meanwhile the predicted values were calculated from the final equation below;

$$\text{Lignin percentage} = +15.37+1.04*A-1.78*B-0.42*C-1.12*D+0.64*A^2+0.021*B^2-0.47*C^2+0.53*D^2-0.84*A*B-0.56*A*C-0.28*A*D+0.26*B*C+0.11*B*D-0.35*C*D \quad \textbf{(4.1)}$$

where,

A = Temperature

B = pH value

C = Moisture content

D = Fungi to medium ratio

R^2 is the relative predictive power of a model and a descriptive measure between 0 and 1. The closer it is to one, the better is the model or the ability to predict is higher (Simon, 2008). In this research, R^2 obtained is 0.4936 which is very low. This value of R^2 indicates that the data points are scattered away from the

values predicted by the multiple regression equation and that the independent variables are a poor predictor of the dependent variable.

Rationally, the value of R^2 is very low due to the selection of the center point in the RSM. It might be the center point chosen is wrong or not accurate enough. That's why the error is large where the actual values are not as predicted as tabulated in Table 4.3.

Table 4.3: Comparison between actual value and predicted values

Std Order	Run Order	Actual Value	Predicted Value	Error (%)
1	18	16.76	16.74	0.14
2	27	16.07	22.17	37.94
3	15	13.3	14.10	5.99
4	19	15.03	16.17	7.58
5	8	14.8	17.19	16.15
6	9	21.4	20.38	4.78
7	10	14.3	15.60	9.07
8	7	11.3	15.43	36.53
9	6	15.2	15.52	2.12
10	12	22.9	19.84	13.36
11	14	14.08	13.34	5.25
12	26	12.24	14.30	16.84
13	30	17.48	14.58	16.60
14	16	13	16.65	28.11
15	21	15.09	13.44	10.90
16	11	13.9	12.16	12.50
17	22	14.27	15.87	11.19
18	1	24.3	20.01	17.64
19	4	20.4	19.02	6.78
20	25	13.19	11.88	9.90
21	3	16.3	14.35	11.98
22	24	13.4	12.66	5.50
23	2	25.8	19.74	23.47
24	13	11.9	15.27	28.29
25	28	17.2	15.37	10.66
26	20	14.5	15.37	5.98
27	29	15.45	15.37	0.54
28	17	15.99	15.37	3.90
29	5	14	15.37	9.76
30	23	15.06	15.37	2.04

4.4 Optimum Conditions

The experiment was done by referring the conditions from the experimental design table obtained from Design Expert software respectively. A total of 30 runs were done to obtain the optimum conditions of four factors that influencing the biodelignification of oil palm trunk by using *Pleurotus ostreatus* as the agent of degradation.

Table 4.4: Comparison of predicted and actual value of optimization

Value of Parameters		Predicted Value	Actual Value
A: Temperature (°C)	25.00	15.3667 %	12.500%
B: pH value	7.00		
C: Moisture content	2.10		
D:Fungi to medium ratio	0.25		

It can be seen that there are differences between the actual and predicted value from the table shown. The error can be calculated as below;

$$\text{Error} = \frac{\text{Actual value} - \text{predicted value}}{\text{Actual value}} \times 100\%$$

$$= [(15.3667-12.500)/ 15.3667] \times 100\%$$

$$= 18.7\%$$

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, the objective of this experiment is to study the optimization of biodelignification of oil palm trunk waste is successfully achieved. The oyster mushroom was used to degrade the lignin of oil palm trunk and the percentage of lignin degraded was determined by using Klason Lignin methodology.

This study demonstrated that RSM was used in designing, analyzing, finding the optimum point and assessing the effects of factors leading to a higher biodelignification of oil palm trunk by using *Pleurotus ostreatus* (oyster mushroom). The optimum conditions for the lignin degradation of oil palm trunk fiber are as follows: temperature = 25°C, pH value = 7.0, moisture content = 0.21 and fungi to medium ratio = 2.5. However, the conditions are optimum, but not extremely optimum due to the value of R^2 which is very low.

From conformational run, the optimum lignin degraded obtained is12.500 % which is lower than the predicted value which is 15.3667%. The error is about 18.7%.

5.2 Recommendation

In this study, the lignin degraded by using oyster mushroom is high enough for simple biological method used. However, there are several ways to increase the lignin degradation.

Firstly, during the experiment, nitrogen sources can be supplied to the media to increase the degradation. This is because nitrogen source stimulate the lignin degradation of wood (Reid, 1983).

This research showed that the optimum conditions for the biodelignification of oil palm trunk were obtained. However, the errors in this study also big. In order to reduce the error, a new experimental design is needed and center point resulting from the screening process must be tested before designing the project.

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APPENDIX A

MATERIALS AND METHODOLOGY

Buffer Preparation

To prepare buffers for various pH for the growth of fungi in this research, the materials used are Na_2HPO_4 (0.2 M) and Citric acid (0.1 M)

The requirements for 100 ml of each pH values are shown in table below;

Table A1: Quantity of Na_2HPO_4 and Citric Acid Required for Various pH

pH value	Na_2HPO_4(ml)	Citric Acid(ml)
5.5	55.75	44.25
6.0	62.52	37.48
6.5	69.30	30.70
7.0	82.00	18.00
7.5	90.55	9.45
8.0	95.80	4.20

Figure A1: Citic acid (0.1M)

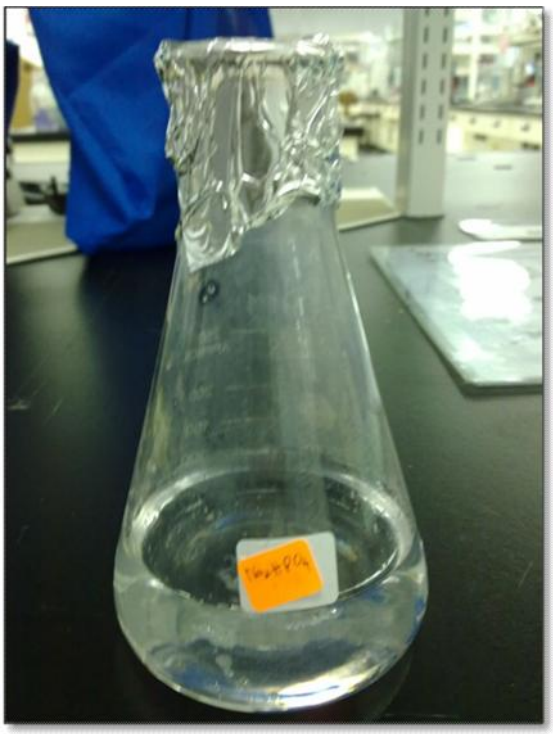

Figure A2: Na_2HPO_4 (0.2 M)

The biodelignification process was done in the bottle as the reactor containing the media (oil palm trunk fibre).

Figure A3: Reactor for the biodeligfication process

Figure A4: Shaking water bath for Klason Lignin

Figure A5: Laminar flow hood for transferring fungi into the reactor