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

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Thesis submitted in fulfillment of the requirements
for the award of the degree of Bachelor of
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I hereby declare that this thesis entitled "Screening of Factors for Biodelignification of Oil Palm Trunk Using *Pleurotus Ostreatus*" 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 candidature of any other degree.

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*For those who love chemistry and engineering.*

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## ABSTRACT

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 celluloses contained in the material. Unbound hemicelluloses and celluloses 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 menjadikannya 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.* Kesan 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 kemudiannya 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 sumbangan 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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## LIST OF SYMBOLS/ABBREVIATION

| | |
|---|---|
| OPT | Oil Palm Trunk |
| % | Percentage |
| °C | Degree Celcius |
| °S | Degree South (latitude) |
| °N | Degree North (latitude) |
| t | Tonnes |
| kg | Kilograms |
| ha | Hectar |
| t/ha | Tones per hectares |
| *Pl* | *Pleurotus* |
| LiP | Lignin Peroxidase |
| MnP | Manganase Peroxidase |
| L | Liter |
| rpm | Rotation per minute |
| mL | Milliliter |
| µM | micromol |

# 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 (*Metraxylon 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. sagu* 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. sagu* is mainly found in semi-cultivated stands. Irian Jaya has about 6 million ha of *M. sagu* (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 lo-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 $^{o}$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 delingnification 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 biodelignifcation 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-specifc 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.

# CHAPTER 3

# METHODOLOGY

## 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.

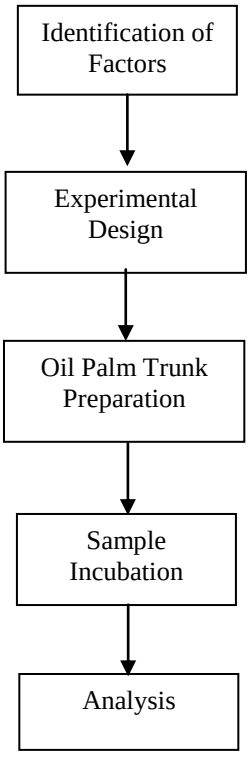


**Figure 3.1**: Research Work Flow

### 3.2 Identification of Factors and Parameters Set Up

For this research, seven factors have been identified to be tested as the factors that may affect the biodelignification process. Temperature, pH, humidity, light exposure, moisture content, fungi to medium ratio and contact time have been identified as the possible factors that can affect the process (Figure 3.2). The two-level factorial analysis was utilized using Design Expert software in order to obtain the range of the suitable parameters for the experiment. The screening was done at 1/8 fraction. The manipulated variable for factor temperature, pH, humidity, light exposure, moisture content, fungi to medium ratio, and contact time was tabulated in Table 3.1.

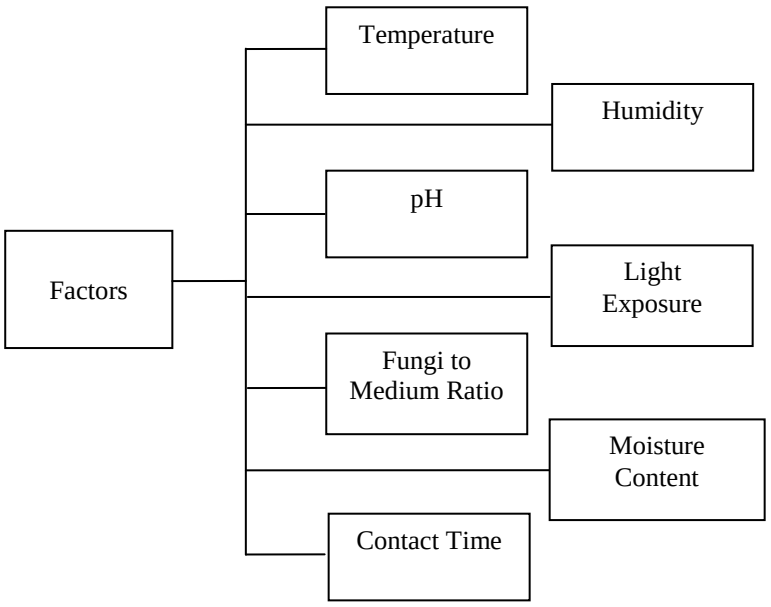


**Figure 3.2**: Possible Factors Affecting Biodelignification Process.

**Table 3.1**: Value of Each Parameter in Each Experiment Runs.

| Run | Temperature (°C) | pH | Humidity (Silica gel in grams) | Light Exposure | Moisture Content (ml per 12 hours) | Fungi to Medium ratio | Contact Time (days) |
|---|---|---|---|---|---|---|---|
| 1 | 20 | 5 | 0 | Dark | 0.5 | 0.1 | 2 |
| 2 | 30 | 5 | 0 | Dark | 5 | 0.5 | 10 |
| 3 | 20 | 8 | 5 | Dark | 0.5 | 0.5 | 10 |
| 4 | 30 | 5 | 5 | Light | 0.5 | 0.1 | 10 |
| 5 | 30 | 8 | 5 | Dark | 5 | 0.1 | 2 |
| 6 | 30 | 8 | 0 | Light | 0.5 | 0.5 | 2 |
| 7 | 20 | 8 | 0 | Light | 5 | 0.1 | 10 |
| 8 | 20 | 5 | 5 | Light | 5 | 0.5 | 2 |
| 9 | 20 | 5 | 0 | Light | 0.5 | 0.5 | 10 |
| 10 | 30 | 5 | 0 | Light | 5 | 0.1 | 2 |
| 11 | 20 | 8 | 5 | Light | 0.5 | 0.1 | 2 |
| 12 | 30 | 5 | 5 | Dark | 0.5 | 0.5 | 2 |
| 13 | 20 | 8 | 0 | Dark | 5 | 0.5 | 2 |
| 14 | 30 | 8 | 5 | Light | 5 | 0.5 | 10 |
| 15 | 20 | 5 | 5 | Dark | 5 | 0.1 | 10 |
| 16 | 30 | 8 | 0 | Dark | 0.5 | 0.1 | 10 |

### 3.3 Raw Material Preservation

In this research, oil palm trunk (OPT) was delignified using *Pleurotus Ostreatus* or also known as Oyster Mushroom locally. Both the oil palm trunk and the Oyster Mushroom were obtained locally. The oil palm trunk was obtained from an oil palm plantation near Jerantut, Pahang. Meanwhile, the Oyster Mushroom was obtained from mushroom cultivation area near Gambang, Pahang.

The oil palm trunk obtained from the plantation was squeezed to remove the sap and leaving only the fibers of the trunk. The trunk was then sealed in a plastic bag and stored in a freezer in a very low temperature to preserve the sample. The Oyster Mushroom obtained from the source area also was sealed in a plastic bag and then stored in a freezer in a very low temperature to preserve the mushroom. Before running the experiment, the oil palm trunk that stored in the freezer was removed from the freezer and dried in an oven for a day at temperature 50$^{o}$C. The mushroom was dried at room temperature.

**Figure 3.3:** Squeezed and Dried Oil Palm Trunk (OPT) Fiber

## 3.4 Experimental Set Up for Screening

### 3.4.1 Inoculum Preparation

The inoculum was prepared by cutting the Oyster Mushroom according to its spore print. The fungus was dissected and the part with high content of spore was selected. The selection of part with high content of spore was based on the fungus spore print. Then the dissected part was put into vessel for incubation.

The condition for medium in the vessel was prepared based on the two-level factorial analysis performed using Design Expert software. For the temperature manipulation, the experiment was done in an incubator with temperature of 20°C or 30°C. For pH manipulation, Citrate/Phosphate buffer solution was used to control the pH. A buffer with pH of 5 and 8 was used to control the pH of the medium.

To control the humidity in the vessel, a packed of silica gel was added in the vessel. The amount of silica gel is either 5g in a pack or no silica gel added to the vessel. As for the light exposure control, the vessel was either covered to prevent the light from entering the vessel or it was leave with no cover. The material used to cover the vessel is an aluminum foil. For controlling the moisture content in the vessel, droplets of distilled water was added to the inoculums for every 12 hours to control its moisture. The amount of droplet is either 0.5ml or 5ml for every batch of insertion.

The ratio of fungi to the medium is controlled by controlling the ratio of the Oyster Mushroom weight in the vessel with the weight of oil palm trunk. The ratio of 1:2 and 1:10 was used in controlling the parameter. For ratio of 1:2, every 1.25 grams of fungi will be added with 2.5 grams of oil palm trunk. Meanwhile, every 0.25 grams of fungi will be added with 2.5 grams of oil palm trunk for 1:10 ratio. The contact time was controlled by running the experiment for 2 days or 10 days.

### 3.4.2 Incubation

The incubation was done in an incubator with the predetermined parameter. Only four runs done in every incubator available. The experiment run was monitored every 12 hours to add the distilled water and to maintain the temperature of the incubator. If the temperature was rise beyond the temperature set point, the temperature was regulated manually by inserting a beaker of ice cube to lower the temperature. All the process was repeated until the experiment run ends.

### 3.4.3 Sample Retrieval

After the incubation period, the sample was taken out from the vessel. The Oyster Mushroom was removed from the oil palm trunk. Meanwhile, the oil palm trunk was washed with distilled water and was stored in a freezer before analyzed.

## 3.5 Analysis using Klason-Lignin Determination Method

The result of the experiment was analyzed by determining the lignin content in the sample using Klason-Lignin determination method. The oil palm trunks that have been treated with Oyster Mushroom were hydrolyzed with Sulphuric Acid for 2 hours. After that, the analyzed sample was heated in a water bath for 2 hours. The solution was then filtered and washed and then was dried. The weight was compared with the weight before it was analyzed to get its lignin percentage. The simplified detail was shown in Figure 3.4.

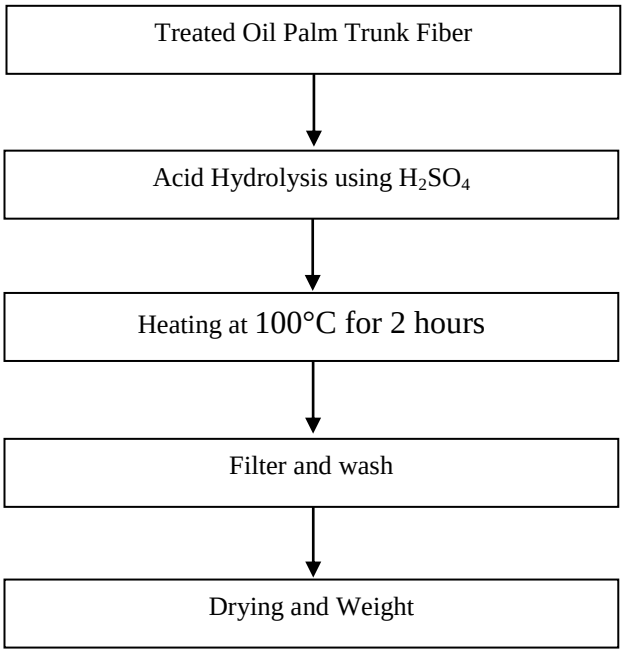


**Figure 3.4**: Work flow for Klason-Lignin Determination.

### 3.5.1 Acid Hydrolysis

The treated oil palm trunk was removed from the freezer and was dried in an oven for an hour at temperature 105°C. Then one gram of the dried oil palm trunk was weight and placed into a 1L conical flask. Then 20 mL of 72% Sulphuric Acid, $H_2SO_4$ was added to the conical flask and the solution was leave for 2 hours at room temperature.

### 3.5.2 Heating

After the treated oil palm trunk was hydrolyzed with $H_2SO_4$, 560 ml of distilled water was added to the conical flask. The solution was then heated in a shaking water bath at temperature of 100°C for two hours. Agitation was applied with speed of 25 rpm.

### 3.5.3 Filter and Wash

After 2 hours of heating in a water bath, the conical flask was removed from the water bath. The solution was then filtered using a filter funnel and filter paper. The filter paper should be dried in an oven for an overnight and the weight of the filter paper was taken before the filtering process is done. The residue of the filtering process was taken to the next step while the filtrate was discarded.

### 3.5.4 Drying and Weight

The residue obtained from filtering process was taken and dried in an oven at temperature of 40°C for a day. After that, the solid residue with the filter paper from the filtering process was transferred to desiccators to be further dried. The weight of the solid residue with the filter paper was taken daily until the weight remains constant. The data obtained was deducted with the filter paper weight to obtain the weight of lignin present in the sample. From that data, the lignin percentage and lignin reduced in percentage can be obtained.

## 3.6 Analysis From Klason-Lignin Determination

The data obtained from the Klason-Lignin Determination was analyzed by subtracting the filter paper and sample initial weight with the final weight from the drying process in Klason-Lignin determination (Figure 3.5). From there, the lignin percentage can be obtained.

$$lignin\ Percentage = \frac{(Filter\ paper + Initial\ weight) - Final\ weight}{Initial\ weight} X\ 100\%$$

**Figure 3.5:** Equation for Lignin Percentage

# CHAPTER 4

# RESULT AND DISCUSSIONS

## 4.1 Screening Results

The result obtained from the experiment was in form of lignin percentage in oil palm trunk sample after the experiment. The lignin contained in the sample obviously ranging as low as 17.8% to as high as 39.8%. Sixteen experimental run was done according to the parameters that has been predetermined by the Two-Level Factorial Analysis. The detail of the experiment setup and result can be viewed in table 4.1.

**Table 4.1**: Value for factors tested in experimental run.

| Run | Temperature (°C) | pH | Humidity (Silica gel in grams) | Light Exposure | Moisture Content (ml per 12 hours) | Fungi to Medium ratio | Contact Time (days) | Lignin Percentage in Sample (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 20 | 5 | 0 | Dark | 0.5 | 0.1 | 2 | 19.1 |
| 2 | 30 | 5 | 0 | Dark | 5 | 0.5 | 10 | 39.8 |
| 3 | 20 | 8 | 5 | Dark | 0.5 | 0.5 | 10 | 21.6 |
| 4 | 30 | 5 | 5 | Light | 0.5 | 0.1 | 10 | 23.7 |
| 5 | 30 | 8 | 5 | Dark | 5 | 0.1 | 2 | 19.6 |
| 6 | 30 | 8 | 0 | Light | 0.5 | 0.5 | 2 | 20.7 |
| 7 | 20 | 8 | 0 | Light | 5 | 0.1 | 10 | 22.1 |
| 8 | 20 | 5 | 5 | Light | 5 | 0.5 | 2 | 20.9 |
| 9 | 20 | 5 | 0 | Light | 0.5 | 0.5 | 10 | 17.8 |
| 10 | 30 | 5 | 0 | Light | 5 | 0.1 | 2 | 27.6 |
| 11 | 20 | 8 | 5 | Light | 0.5 | 0.1 | 2 | 19.0 |
| 12 | 30 | 5 | 5 | Dark | 0.5 | 0.5 | 2 | 30.2 |
| 13 | 20 | 8 | 0 | Dark | 5 | 0.5 | 2 | 20.0 |
| 14 | 30 | 8 | 5 | Light | 5 | 0.5 | 10 | 27.3 |
| 15 | 20 | 5 | 5 | Dark | 5 | 0.1 | 10 | 20.1 |
| 16 | 30 | 8 | 0 | Dark | 0.5 | 0.1 | 10 | 21.2 |

The results for the screening experiment were analyzed using Two-level Factorial Analysis by Design Expert application. The results were obtained in form of percentage of contribution to the biodelignification process of oil palm trunk. The result obtained from the screening process is as shown below. The highest percentage contribution was 32.2 % that is come from the temperature factor, followed by pH, fungi to medium ratio, moisture content, contact time, light exposure, and humidity with value of 10.08%, 8.82%, 7.63%, 3.58%, 2.05%, and 0.46% respectively. However, the interaction between the factors also provides contribution towards delignification. Two interactions seem to be contributing with high percentage that is from the interaction between factor temperature and pH and interaction between factor temperature & fungi to medium ratio. The percentage contribution for the screening process for this research is shown in Figure 4.1.

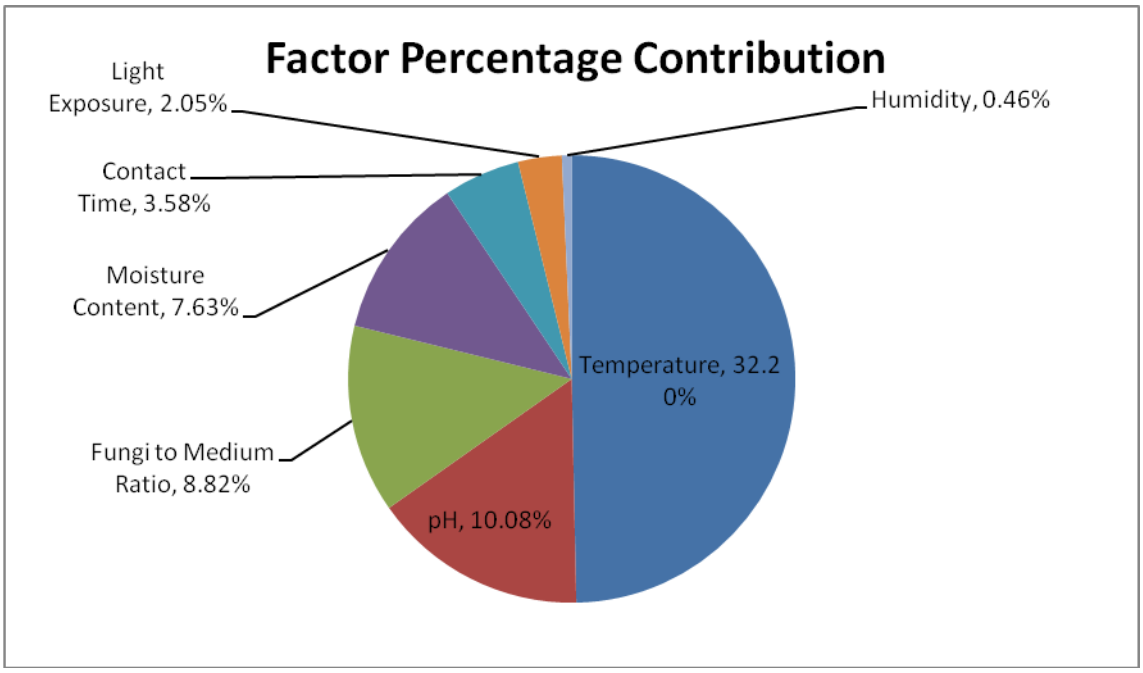


**Figure 4.1:** Percentage Contribution of the Seven Factors for Screening Process of Biodelignification of Oil Palm Trunk.

The factor that contributes most for the delignification process is the temperature with 32.2% of contribution towards delignification in this research. The temperature plays an important role to the delignification process as the cultivation of *Pleurotus*

*Ostreatus* is depending on the temperature of its surrounding (Hernandez *et al*, 2003). Thus, the production of the lignolytic enzyme will also affected by the surrounding temperature. It is known that this type of microbial species can survived in extreme temperature ranging from -18 to 121°C (Kashangura *et al, 2006*). However, selection of optimum temperature will increase the lignolytic enzyme production and will give result of high degree of degradation and therefore will minimize the amount of lignin present in the lignocellulosic material. Palmieri *et al* also reported that sample incubated at temperature 20°C are able to degrade dye color for amount of 94% from its original amount compared with sample incubated at temperature 30°C are able to degrade the dyes for amount of 86% from its original amount in an experiment of decolorisation of Remazol Brilliant Blue R dye using *Pleurotus Ostreatus.* Other than that, Liu *et al* also reported that the laccase activity of the *Pleurotus Ostreatus* increase with increase of temperature until it reach 50°C. Further increasing of temperature above 50°C resulting in sudden drop of enzyme activities showing that enzyme denaturation occurs in that temperature range.

pH is the second factor that contribute most in the biodelignification process. This is because of the enzyme production of most fungi are different at certain temperature. The *Pleurotus ostreatus* was confirmed to produce many enzyme including lignolytic enzyme, cellulolytic enzyme, and hemicellulolytic enzyme (Baldrian *et al,* 2005). Cultivation at the optimum pH will increase the production of the lignolytic enzyme and making the degree of delignification higher than the degradation of cellulose and hemicellulose. Liu *et al* shows that temperature is affecting the laccase activity produced from the *Pleurotus Ostreatus* cultivation. The activity of the laccase enzyme increases with respect to the increasing of the pH from one to four. However, increment of pH over four resulted in decrease of enzyme activity slowly until pH value of eight where it reaching zero enzyme activity. This observation supporting the fact that the laccase enzyme who is one of the enzyme responsible in biodelignification process produces by *Pleurotus Ostreatus* having an optimum pH and by doing biodelignification in optimum pH may increase the process efficiency. Thus, if confirms the contribution of pH in the process.

The factor of fungi to medium ratio contributes 8.82% for the delignification process. The amount of fungi available for certain amount of the oil palm trunk is responsible on how much the amount of lignin in the oil palm trunk will be degraded. Palmieri *et al* reported in an experiment of decolorisation of Remazol Brilliant Blue R dye using *Pleurotus Ostreatus* that sample that are incubated with 5 μM dye (small amount of medium) are showing rapid decolorisation where it reduce about 90% of the dye in duration 3 days. Compared with the sample with higher amount of medium used (5M), the sample only achieves 90% decolorisation on the sixth day of the incubation. Although this experiment is not a biodelignification experiment, the results shows that higher ratio of fungi to medium will produce high amount of lignolytic enzyme such as laccase that supports the fact that fungi to medium ratio is really contributing the biodelignification process.

The moisture content factor contributes 7.63% to the delignification process of oil palm trunk. This is because the growth of most microbial species is depending on the water availability of the species (Kashangura *et al, 2006*). Thus, the production of the enzymes from the species also depends on the water content. Dwivedi *et al* reported that the moisture content giving effect in laccase production of *Pleurotus Ostreatus.* From the range of 20% to 120% water content tested in the experiment, moisture content of 80% found out to be suitable for laccase production. Higher moisture content is inhibiting the laccase production where it interferes with the air circulation and heat transfer by biological activities meanwhile low moisture content affect the solubility of the nutrient and thus reduce their availability for growth and enzyme production (Dwivedi *et al,* 2011).

The factor of contact time does not contribute much on the delignification. This is because when the optimum time needed to degrade the lignin has been reached, there will only a few of lignin fraction that did not degrade. Thus, if the incubation was continued, there will be significant observation will be obtained. The contact time for this biodelignification process for the oil palm trunk only contribute an amount of 3.58% from the experiment. The weight of the oil palm trunk will decrease with respect of time (Taniguchi *et al, 2005*). However, when the optimum time needed to degrade the lignin in the oil palm trunk has been reached, there will be not much different can be

observe in terms of weight change. This is supported by Taniguchi *et al* where pretreatment to degrade lignin from rice straw using *Pleurotus ostreatus* for 60 day and 72 day did not show significant different in weight of the rice straw after treatment. The duration used for the process is not the same as the sample used is different. In this research, duration of two days and ten days has been used. This shows that with the amount of OPT used with its corresponding ratios having the optimum time near the duration of two days. Besides, there is also a potential of *Pleurotus ostreatus* will degrade the amount of cellulose and hemicellulose material in the oil palm trunk. This is because instead of producing lignolytic enzymes like Mn-peroxidase and laccase, the fungi also produces cellulolytic and hemicellulolytic enzymes such as endo-1,4-β-glucanase, 1,4- β-glucosidase, endo-1,4- β-xylanase, 1,4- β-xylosidase, endo-1,4- β-mannanase and 1,4- β-mannosidase (Baldrian *et al*, 2005). Thus, by fixing the reaction time near to the optimum time would prevent of cellulose and hemicelluloses degradation.

With 2.05% of contribution, the light exposure factor does not contribute so much on the delignification of oil palm trunk. It is reported by Lee *et al* that cultivation of *Pleurotus Ostreatus* will produce an amount of L-ascorbic acid in the early progress of the cultivation with the presence of light exposure meanwhile cultivation without light exposure will produce negligible amount of L-ascorbic acid. This L-ascorbic acid can be turned into hydrogen peroxide later in the cultivation after being oxidized with heme-containing ascorbate oxidase. This hydrogen peroxide also can be used to degrade lignin contained in the oil palm trunk lignocellulosic structure. However, the amount of L-ascorbic acid in the cultivation with presence of light only maintains for a short time of six hours before the amount diminishes. Therefore, the result obtained in the cultivation with light exposure and without light exposure did not differ much on the effect to biodelignification.

Humidity giving the lowest contribution to the delignification of oil palm trunk in this research. With 0.46% of contribution, it did not giving much effect to the process. The humidity tested in this research is the humidity of the air surrounding of the fungi and the oil palm trunk itself. In this research, since the medium has already obtained an amount of water from the moisture content control factor. Controlling the humidity seems to be inappropriate to this research.

**4.2 Interaction between Factors.**

The screening process also gives results of few interactions between factors that contribute to the biodelignification process of the oil palm trunk. There are seven pairs of interaction that contribute to the biodelignification process. However, only two seems to be significantly contributes to the process. The interaction between factor temperature and pH contributes with highest percentage that is 18.29% followed by interaction between factor temperature and fungi to medium ratio with 8.82%. The other interactions are interaction between factor temperature and humidity, interaction between factor temperature and light exposure, interaction between factor temperature and moisture content, interaction between factor temperature and contact time, and interaction between factor pH and contact time with percentage of contribution of 1.62%, 1.45%, 2.19%, 1.68%, and 1.14% respectively. The fraction of the contribution of the interaction between factors is viewed in Figure 4.2. Since the interaction between factor temperature and pH and interaction between factor temperature and fungi to medium ratio is giving significant amount of contribution to the process, it will be discuss further in the next subtopic.

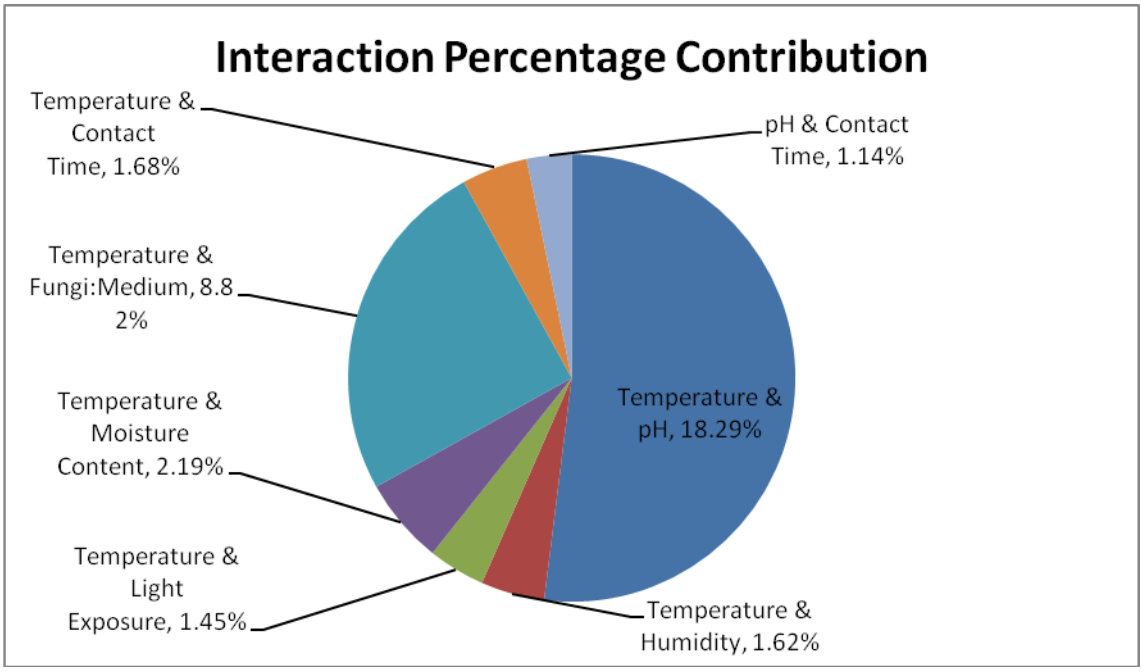


**Figure 4.2:** Percentage Contribution of the Interaction between Factors for Screening Process of Biodelignification of Oil Palm Trunk.

### 4.2.1 Interaction of Factor Temperature with pH.

The interaction between factors also contributes in the delignification process. The interaction between factor temperature and pH gives 18.29% contribution to the process which is supposedly higher than several factors alone. The interaction of factor temperature with pH can be observed in Figure 4.3. The figure shows the graph of the interaction on factor temperature and pH with response 1, where response 1 is the final lignin percentage in the sample. The interactions of the factors are because of the relation of both factors with the peroxidase enzyme activity. The pH factors contribute to the release of the peroxidase enzymes by the *Pleurotus Ostreatus* where the enzymes degrade lignin upon release. However, these enzymes only active in a certain range of temperature. This shows the interaction of the temperature and pH factor towards biodelignification. Temperature lower from the optimum temperature will result in low enzyme activity (Liu *et al*, 2009), thus biodelignification will run in low efficiency even though the pH condition is at the optimum value. Temperature higher from the optimum value will result in the enzyme denaturation. This temperature condition will inhibit the biodelignification as at the current temperature, the enzymes were denaturized.

Other than that, the temperature also found to be affecting the pH directly in buffer solution (Bergen, 2011). Bergen reported that increment of temperature will resulted in decrease in pH in buffer solution. This is because of increasing of temperature will provide energy to the molecules in the buffer solution thus, ionizes the buffer and producing more $H^+$ ions in the buffer. This condition will increase the acidity of the buffer and lower the pH. Any changes in the pH will affecting the production of peroxidase enzymes and will affect the biodelignification.

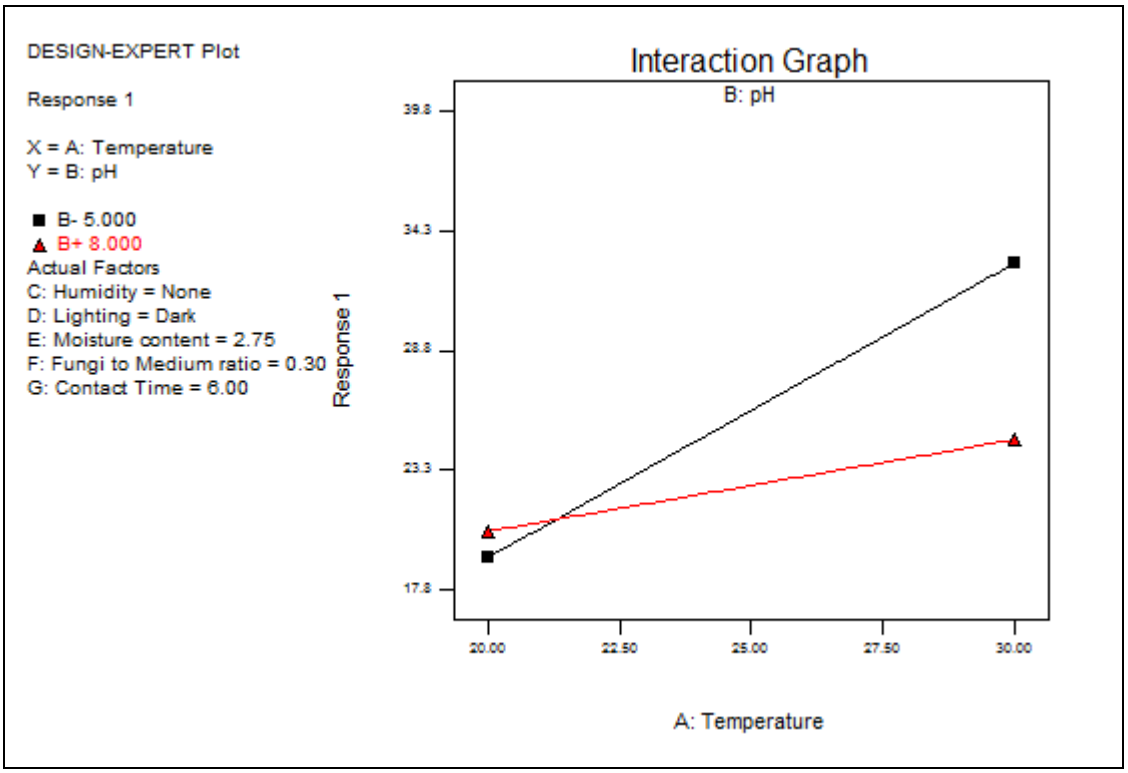


**Figure 4.3:** Graph for Interaction of Factor Temperature with pH.

### 4.2.2 Interaction of Factor Temperature with Fungi to Medium Ratio.

The interaction of factor temperature with fungi to medium ratio factor contributes 8.82% to the biodelignification process. The interaction of factor can be observed from Figure 4.4. The figure shows the graph of the interaction on factor temperature and fungi to medium ratio with response 1, where response 1 is the final lignin percentage in the sample. Higher fungi to medium ratio means there is more amount of fungi in a certain amount of oil palm trunk, which producing more

peroxidase enzymes by *Pleurotus ostreatus*. Regardless of how many enzymes produce by the fungi, the biodelignification still depend on the temperature condition of the process (Palmieri *et al*, 2004). Thus, this shows a clear interaction between the factors. Besides, increment of temperature above 40°C causing the fungi to become inactive or dead (Cristensen, 1975). When this happened, the amount of fungi available will become less, thus changing the ratio of the fungi to medium and reduces the amount of peroxidase enzyme produced.

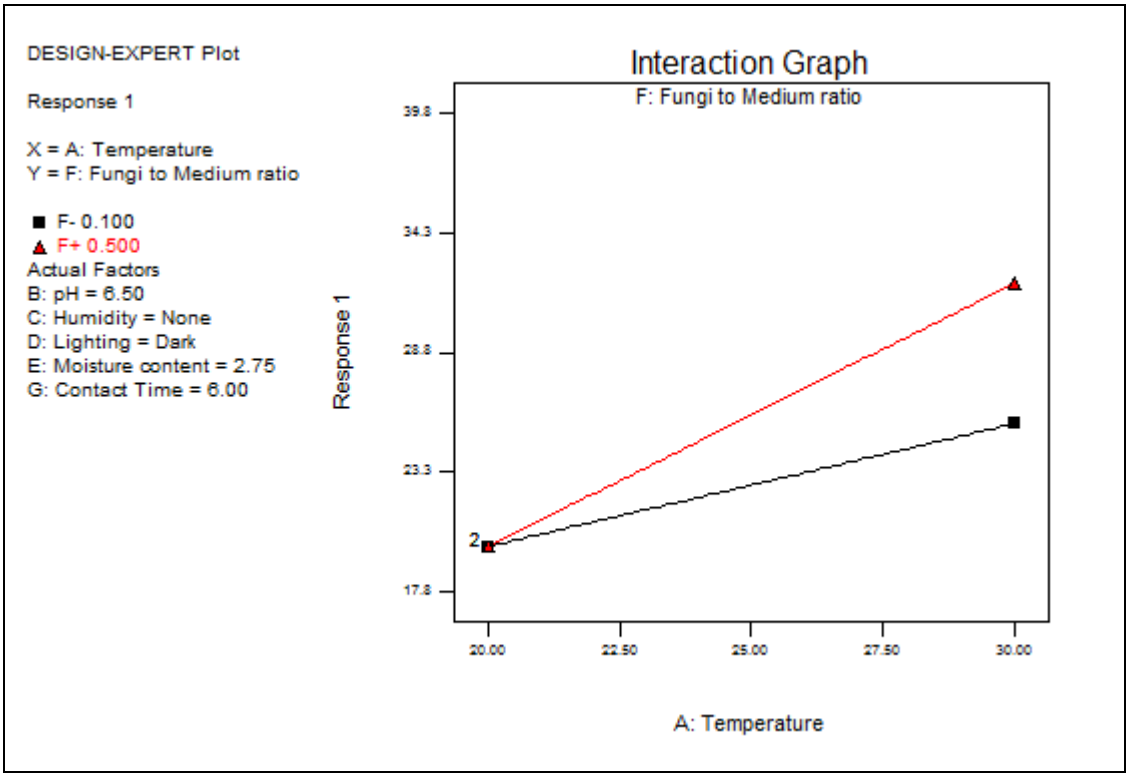


**Figure 4.4:** Graph for Interaction of Factor Temperature with Fungi to Medium Ratio.

### 4.3 Non-Interacting Factors.

There were total of 14 pairs of factors that did not interact with each other. The factors are as listed below.

- Factor of pH and humidity
- Factor of pH and light exposure
- Factor of pH and moisture content
- Factor of pH and fungi to medium ratio
- Factor of humidity and light exposure
- Factor of humidity and moisture content
- Factor of humidity and fungi to medium ratio
- Factor of humidity and contact time
- Factor of light exposure and moisture content
- Factor of light exposure and fungi to medium ratio
- Factor of light exposure and contact time
- Factor of moisture content and fungi to medium ratio
- Factor of moisture content and contact time
- Factor of fungi to medium ratio and contact time

The factor of pH does not interact with factor humidity, light exposure, moisture content, and fungi to medium ratio. As for the pH with humidity, the interaction is not possible because of humidity is the measurement of water molecules in the air, while the pH is the measurement of acid dissociation in the buffer solution. Therefore, the manipulation of either one factor will not affecting the other one. For pH with light exposure the factor did not interact because the pH measurement is not dependent with light. Any manipulation of light exposure will result zero effect to the pH. For the pH and moisture content, as the moisture content is the measurement of water in a material, changes in the moisture content will not affect the pH. For pH with fungi to medium ratio, the interaction is not possible because of changes of pH will only affect the activity of the enzymes, not the fungi itself (Cristensen, 1975). Thus, the changes of pH will not affecting the fungi to medium ratio like temperature factor.

The factor of humidity does not interact with factor of light exposure, moisture content, and fungi to medium ratio. The humidity is the measurement of water in air. For humidity and light exposure, changes in the light exposure is not affecting the humidity because of the experiment is done in a close container. Therefore, no matter how much energy (light) was exposed to the container, the humidity will remain the same. The same idea also valid for moisture content. For humidity and moisture content with fungi to medium ratio, the fungi to medium ratio are the ratio between the *Pleurotus Ostreatus* and the oil palm trunk. If any changes happen to the humidity or the moisture content, the ratio will remain the same. For humidity with moisture content, moisture content is the measurement of water in medium while humidity is the measurement of water in air. If one of the factor changes, the other will not have the same effect as it was not related. The contact time is not interacting with humidity, light exposure, moisture content, and fungi to medium ratio. This is because changes of contact time does not affecting the other factor because the experimental design was in a close container.

There is no interaction between light exposures with fungi to medium ratio. This is because the light exposure does not causing either the fungi or the oil palm trunk losing mass. Therefore, there is no interaction.

# CHAPTER 5

# CONCLUSION AND RECOMMENDATIONS

## 5.1 Conclusion

Oil palm trunk is recognized as a material that has potential to be the feedstock for bioethanol production. However, like other lignocellulosic material, the oil palm trunk also did not escape from the problem that its lignin content in the material is inhibiting the process of fermentation to produce the bioethanol. *Pleurotus Ostreatus* has shown its credibility in degrading the lignin in oil palm trunk and other lignocellulosic material. The process of biodelignification is a process that promises economically feasible process because of low energy consumption and fewer chemicals involve in the process made it environmentally friendly. The effect of factor temperature, pH, humidity, light exposure, moisture content, fungi to medium ratio, and contact time on biodelignification process was studied in this research. Out of seven factors tested in this research, four of them were found to be important and significant to the process. The factors are the temperature factor, pH factor, fungi to medium ratio factor, and moisture content factor with percentage of contribution to the biodelignification process 32.2%, 10.08%, 8.82%, and 7.63% respectively. There also two interactions of factors that found to be significant to the process that is interaction of temperature factor with pH and interaction of temperature with fungi to medium ratio with 18.29 and 8.82% of contribution respectively.

## 5.1 Recommendations

In order to improve this research, there were few amendments can be made. Addition on study on the interaction of factor temperature with another six factors could help in understanding further in the biodelignification of oil palm trunk by *Pleurotus Ostreatus* as the temperature factor and the interaction of the factor is greatly contribute to the delignification process. The factor temperature does interact with the other factor. Some with high contribution, while some with low contribution. If further study was conducted on this factor, perhaps more observation and optimization ways could be found out.

Other than that, the screening of factor on the biodelignification of oil palm trunk by *Pleurotus Ostreatus* could be improved further if addition of another factor such as the addition of growth medium to enhance the growth of the fungi. If the growth of the fungi could be increase, the contact time could be shortened and a more effective process could be discovered.

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# APPENDIX A

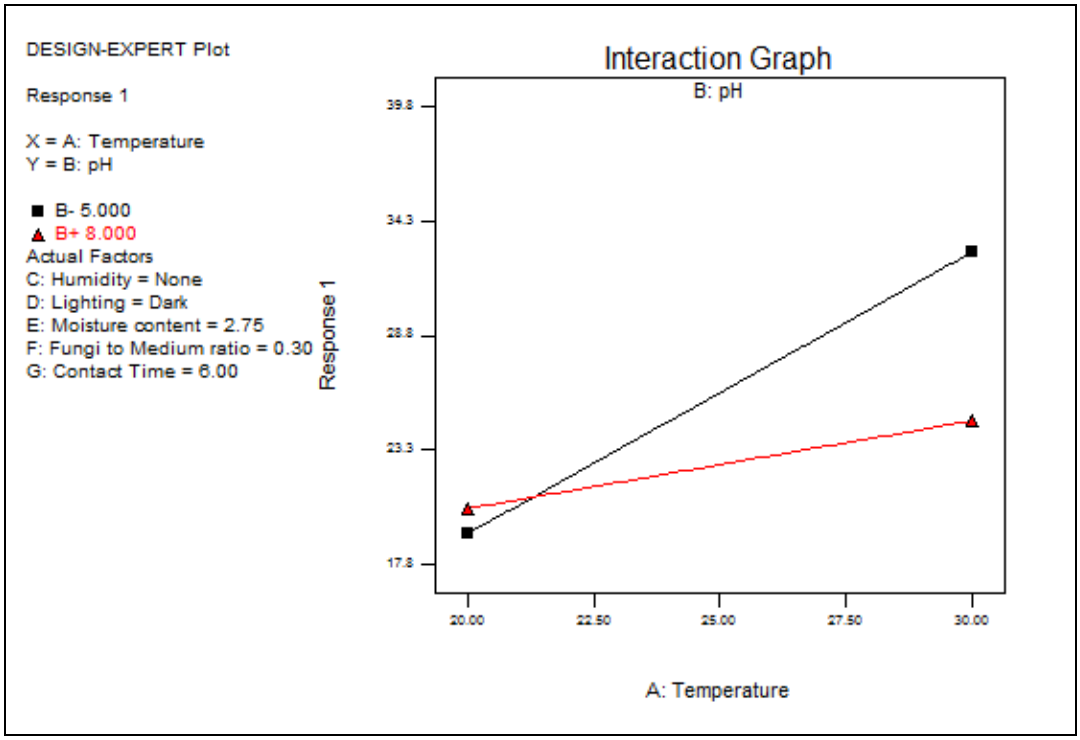
## EXPERIMENT PROCEDURE

### Klason-Lignin Determination

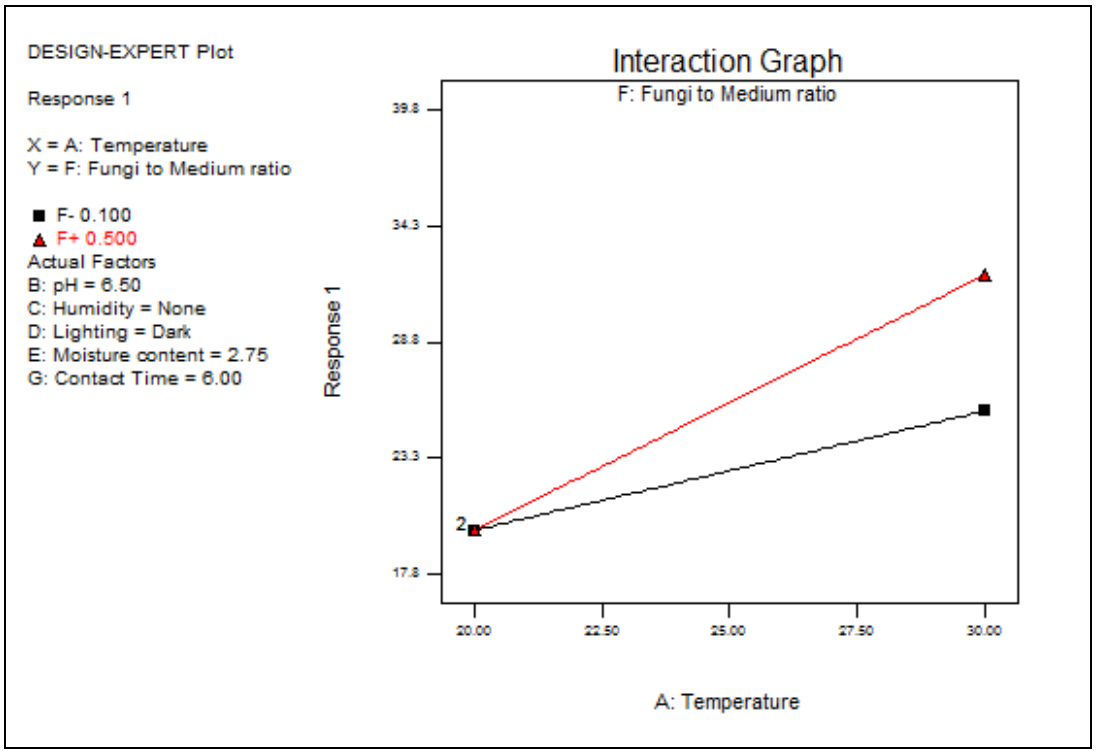
1) Dry the oil palm trunk in an oven at 105 $^{o}$C for one hour.
2) Take out one gram of sample and put into 100 mL beaker.
3) Add 20 mL of 72% Sulphuric acid into the sample and leave for two hours at room temperature.
4) After two hours, transfer the sample into a 1 L conical flask with the suphuric acid solution. Add up 560 mL of distilled water and heat the solution at 100 $^{o}$C in a shaking water bath for two hours.
5) Dry a filter paper in an oven at temperature 50 $^{o}$C. Weigh the filter paper. Put it back into the oven. Repeat step 5 until the weight is constant.
6) After two hours, filter the solution with a filter paper. Wash the residue in filter paper with distill water. Repeat three times.
7) Dry the sample with filter paper in an oven at 105 $^{o}$C for one hour.
8) Weight the sample.
9) Leave the sample in dessicator for 12 hours.
10) Weight the sample again.
11) Repeat step 9 and 10 until the weight is constant.

# APPENDIX B

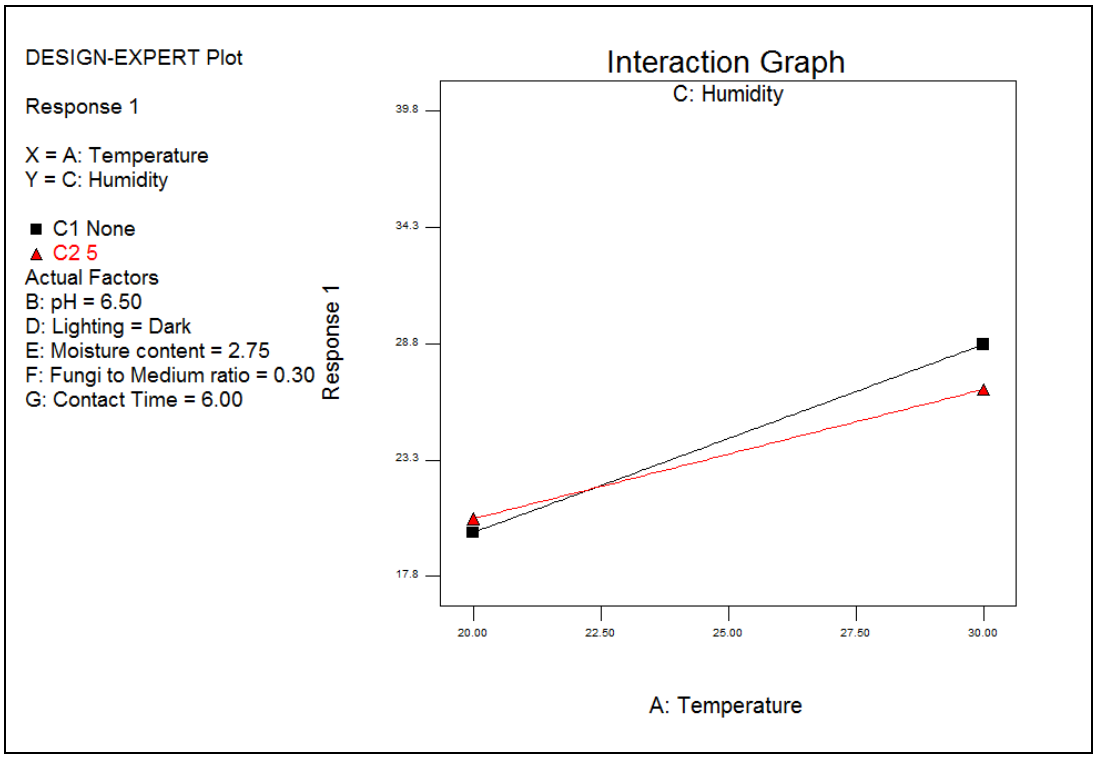
## INTERACTION GRAPHS



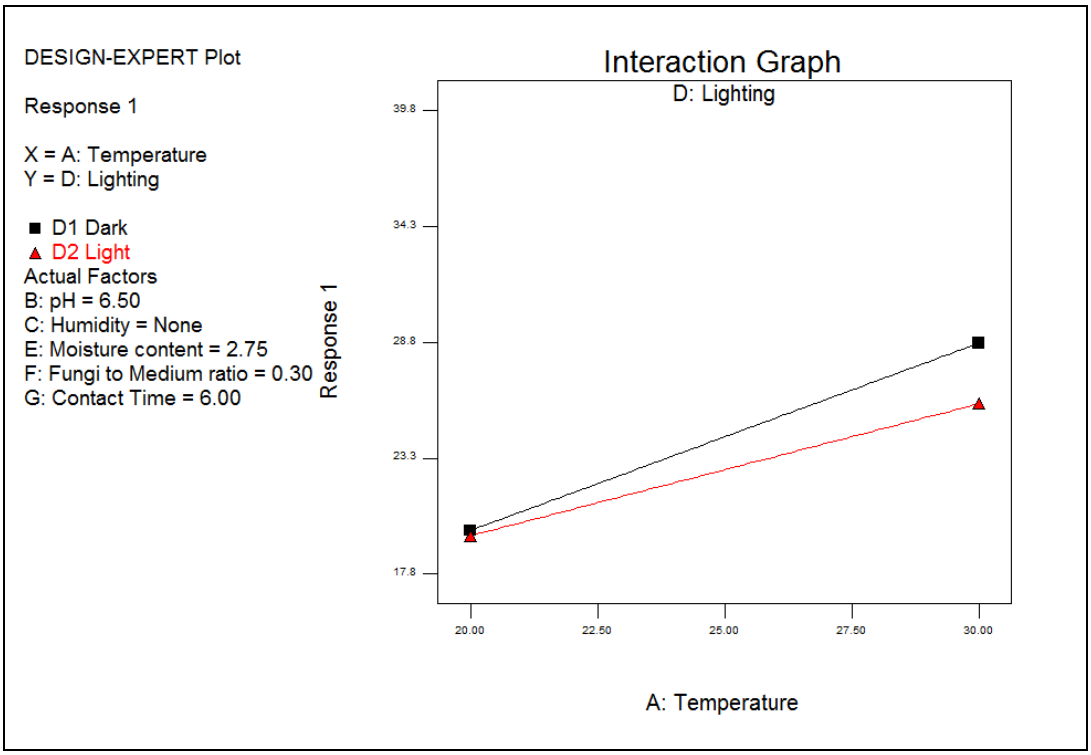

**Appendix B 1:** Graph for Interaction of Factor Temperature with pH.



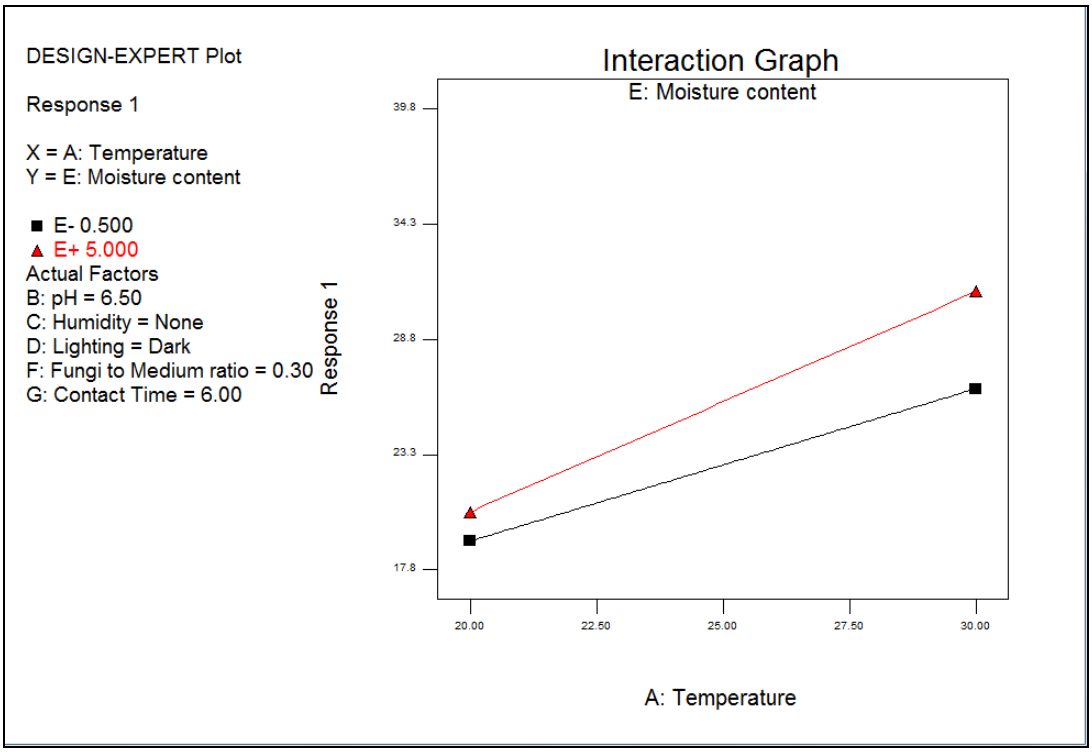

**Appendix B 2:** Graph for Interaction of Factor Temperature with Fungi to Medium Ratio.



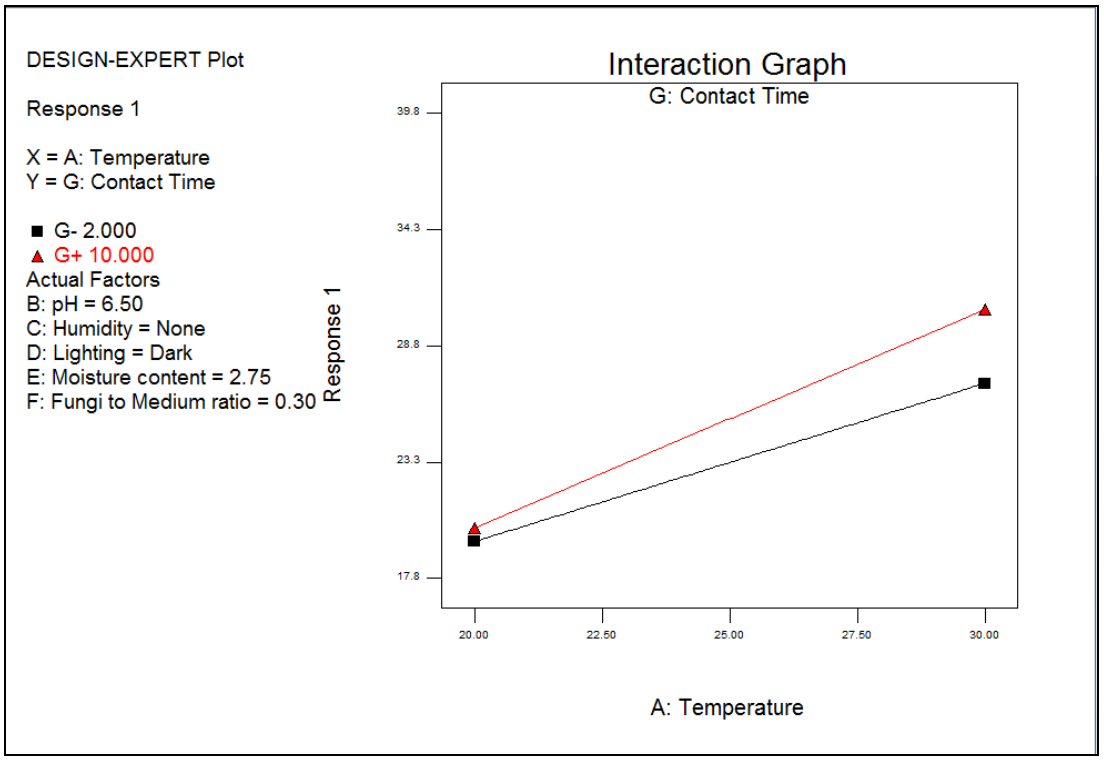

**Appendix B 3:** Graph for Interaction of Factor Temperature with Humidity.



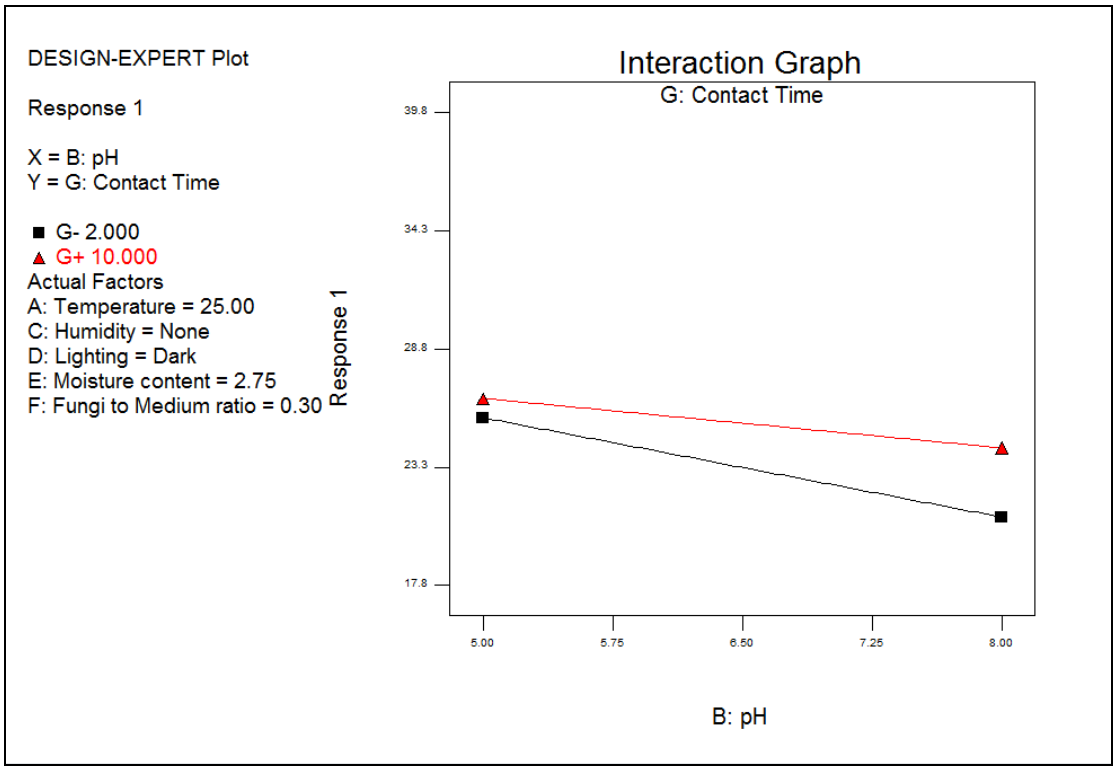

**Appendix B 4:** Graph for Interaction of Factor Temperature with Light Exposure




**Appendix B 5:** Graph for Interaction of Factor Temperature with Moisture Content




**Appendix B 6:** Graph for Interaction of Factor Temperature with Contact time




**Appendix B 7:** Graph for Interaction of Factor pH with Contact time