UNIVERSITI PUTRA MALAYSIA

BIODEGRATION OF OIL PALM EMPTY FRUIT BUNCH LIGNIN BY PHANEROCHAETE CHRYSOSPORIUM (BURDSAL AND ESLYN)

KOK LAI WAI

Biodegration of oil Palm Empty Fruit Bunch Lignin by Phanerochaete chrysosporium (Burdsal and Eslyn)

By

KOK LAI WAI

A Project Report Submitted in Partial Fulfillment of the Requirement
for the Degree of Bachelor of Science Bioindustry in the
Faculty of Agriculture and Food Sciences
Universiti Potra Malaysia Bintulu Sarawak Campus

2009

Dear Dad, KOK THEN SOONG

Mom, LEE MOON PIN

My Beloved Brothers KOK KEN SOONG, KOK HON BIN

and KOK YIK SOONG

Thank you for your love and support

Abstract

This study demonstrates the biodegradability efficiency of *Phanerochaete chrysosporium,* strain ATCC 34540 lignin of oil palm EFB. The fungus culture was first freeze dried into powder form. There were three treatments (different concentration of fungus culture) and one control. Treatment 1, 2 and 3 were 0.08%, 0.16% and 0.24% concentration fungus. The experiment was conducted for 80 days. Sampling was done every 10 days, the experimental design is Complete Randomize Design and data analyzed using regression. Before inoculate, the lignin content for EFB is 14.99%. After 80 DAT, treatment with concentration 0.24% decrease most (33.65%), following is treatment with concentration 0.16% (32.65%) and treatment with concentration 0.08% (11.86%). For C/N ratio test, C/N ratio decrease most in the treatment with concentration 0.24% (44.18%), following by treatment with concentration 0.16% (42.28%) and treatment with concentration 0.08% (30.11%). These shows that treatment with highest concentration of fungus culture (0.24%) is most efficiency in degrade the lignin content in EFB. The heap condition such as pH, moisture content, temperature and humidity are maintained in an acceptable level; pH 6-7.5, 50-65% percent, 29-31 ^{o}C and 90-96% respectively.

Abstrak

Kajian ini mendemostrasi kecekapan biodegradasi *Phanerochaete chrysosporium*, strain ATCC 3450 bagi lignin dalam buah tandan kosong kelapa sawit. Sebelum memulakan proses biodegradasi, kultur kulat dibeku kering kepada serbuk formula. Tiga rawatan (kepekatan yang berbeza) dan satu kontrol. Rawatan 1, 2 dan 3 ialah 0.08%, 0.16% dan 0.24% kepekatan kultur kulat masing-masing. Eksperimen ini dijalankan selama 80 hari. Sampel diambil setiap 10 hari selang kali. Reka eksperimen ialah CRD dan data dianalisi dengan mengguna regresi. Sebelum perawatan kandungan lignin bagi buah tandan kosong kelapa sawit ialah 14.99%. 80 hari selepas perawatan, rawatan dengan 0.24% kepekatan dapat didegradasi terbanyak (33.65%), kedua terbanyak ialah rawatan dengan 0.16% kepekatan (32.08%) dan paling sikit didegradasi ialah rawatan dengan 0.08% kepekatan (25.43%). Bagi kajian C/N ratio, C/N ratio bagi buah tandan kosong kelapa sawit ialah 102.12. 80 hari selepas perawatan, rawatan dengan 0.24% kepekatan dapat didegradasi terbanyak (44.18%), kedua terbanyak ialah rawatan dengan 0.16% kepekatan (42.28%) dan didegradasi tersikit ialah rawatan dengan 0.08% kepekatan (30.11%). Ini menunjukkan semakin pekat kultur kulat, semakin cekap kulat dapat mendegradasi komposisi lignin dan C/N ratio bagi buah tandan kosong kelapa sawit. Selain itu, condisi seperti pH (6.0-7.5), suhu (29-31°C) dan kelembapan (90-96%) bagi tumpukan adalah dikekal dalam kondisi yang bagus dan optimum.

ACKNOWLEDGEMENT

This work would not have been possible without the advice and support of my supervisor, Prof. Assoc. Dr. Joseph Bong Choon Fah. I would like to express my gratitude and appreciation to him. I am greatly indebted to my co-supervisor Mr. Wong Sie Chuong for his persistent and consult in my experiment conducted.

I would like to gratefully acknowledge the support of some very special individuals. They helped me immensely by giving me support and readiness to help made my work so much smoother. Their are Ch'ng Huck Ywih, Lee Hui Hung, Kon Tian Wei and Diana Kiong Siaw Bong. Last is T.C for mental support. I wouldn't be performing this work without my seniors' continual encouragement and support, Miss Hoe Pik Kheng, Miss Wong Ling Chee and Mr Lim Guan Miaw.

I cannot end without thanking my family, on whose constant encouragement and love I would like to thank my father, kok then soong who is my role model and my mother whose love is boundless. It is to them that I dedicate this work.

APPROVAL

I certify that this research project entitled "Biodegradation of Oil Palm EFB Lignin by *Phanerochaete chrysosporium* (Burdsall and Eslyn)" has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor of Science Bioindustry in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus.

_____

ASSOC. PROF. DR. JOSEPH BONG CHOON FAH
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia Bintulu Sarawak Campus
(Supervisor)

_____

PROF. DR. JAPAR SIDIK BIN BUJANG
Dean
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia Bintulu Sarawak Campus

Date: 05 JUN 2009

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

1	°C - Degree Celsius
2	% - Percentage
3	g - Gram
4	m - Meter
5	L - Liter
6	mL - Mini liter
7	mm - Mini meter
8	NaOH - Sodium hydroxide
9	H_2SO_4 - Acid sulfuric
10	H_2O_2 - Hydrogen peroxide
11	C/N - Carbon per nitrogen
12	ADL - Acid Detergent Lignin
13	PDA - Potato Dextrose Agar
14	EFB - Empty Fruit Bunch
15	*P.c - Phanerochaete chrysosporium*
16	DAT - Day After Treatment

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

Empty fruit bunch (EFB) of oil palm (*Elaeis guineensis*) can be utilized for cellulose production, papermaking, and transformation into value-added products. It is a residue of palm oil production. The oil palm production is a major agricultural industry in Malaysia. In total, about 90 million metric tones of renewable biomass (trunks, fronds, shells, palm press fiber and empty fruit bunch) are produced each year. The empty fruit bunches (EFB) represent about 9% of this total. They are residue left after the fruit bunches are pressed at oil mills and the oil extracted. (Syafwina *et al.*, 2002)

The OPEFB biomass contains cellulose, hemicellulose and lignin (Hazwani and Tuah, 2008). An estimated 10^{11}-10^{12} tone of lignocelluloses material is produced annually worldwide and lignin comprises 15-36% (Eriksson *et al.*, 1990). Lignin is the most important renewable material after cellulose. Lignin protects the plant cell from attack by microbes. It is a highly insoluble polymer of aromatic phenolic molecules that form an extensive cross linked net work within the wood and the plant cells. White-rot basidiomycetes are capable to degrade lignin more extensively and rapidly than any other group of organisms, and *Phanerochaete chrysosporium* is the most thoroughly studied species. (Theodorus *et al.*, 1998)

In the process of extraction of palm oil from oil palm fruit, a lignocelluloses material oil palm empty fruit bunch (OPEFB) is generated as a waste product. Approximately fifteen million tons of OPEFB biomass waste is generated annually throughout

Malaysia by palm oil mills. If not properly disposed this waste it will create serious environment pollution to our lake, river and others water bodies. The aquatic fauna and flora will be affected. In practice this biomass is burned in incinerators by palm oil mills which create environmental pollution problems in nearby localities.

Many researches have been conducted by using the bacteria and fungi to reduce the pollution caused by the lignocelluloses material. The white rot fungi have been of particular interest because of their ability to completely oxidize both the cellulosic and lignin components of wood to CO_2 and water. Although many microorganisms are involved in the decomposition of wood, fungi are the dominant decomposers in terrestrial systems. The three main groups of decay caused by fungi are white, brown, and soft-rot. White-rot fungi are of prime interest for use in various industrial processes utilizing lignocelluloses because of their ability to degrade lignin. Most white-rot fungi are capable to degrade cellulose and hemicelluloses to a certain level (Eriksson *et al.*, 1990). It is saprophytic on woody debris and logs, thus it has potential use in industrial applications such as biopulping (Harold and Burdsall Jr, 1998).

In this study, *Phanerochaete chrysosporium* was used to degrade the lignin content in oil palm EFB. *Phanerochaete chrysosporium* is white-rot fungi which is able to degrade lignin more extensively and rapidly than any other organism known (Kondo *et al.*, 1994).

The objectives of this study were:

a. To study the effect of *Phanerochaete chrysosporium* on lignin degradation of oil palm EFB

b. To determine the amount of lignin content in oil palm EFB after period of degradation.

CHAPTER 2

LITERATURE REVIEW

2.1 Description of *Phanerochaete chrysosporium*

Phanerochaete chrysosporium is *a* basidiomycetous white-rot fungi which is the most efficient lignin degraders (Tuomela *et al.*, 2000). This basidiomycete fungus is able to degrade complex compounds such as starch, cellulose, pectin, lignin, lignocelluloses (Asamudo *et al.*, 2005).

2.1.1 Morphology

Phanerochaete chrysosporium is a morphologically heterogeneous genus characterized by resupinate, effused fruit bodies with smooth, tuberculate to spinose hymenial surfaces, a monomitic hyphal system with primarily simple septate generative hyphae, clavate basidia, and thin-walled basidiospores (Wu, 1995).

2.1.2 Taxonomy

Kingdom: Fungi

Phylum: Basidiomycota

Class: Basidiomycetes

Family: Phanerochaetaceae

Genus: *Phanerochaete*

Species: *Phanerochaete chrysosporium*

(Harold H. and Burdsall Jr. 1998)

2.1.3 Life cycle

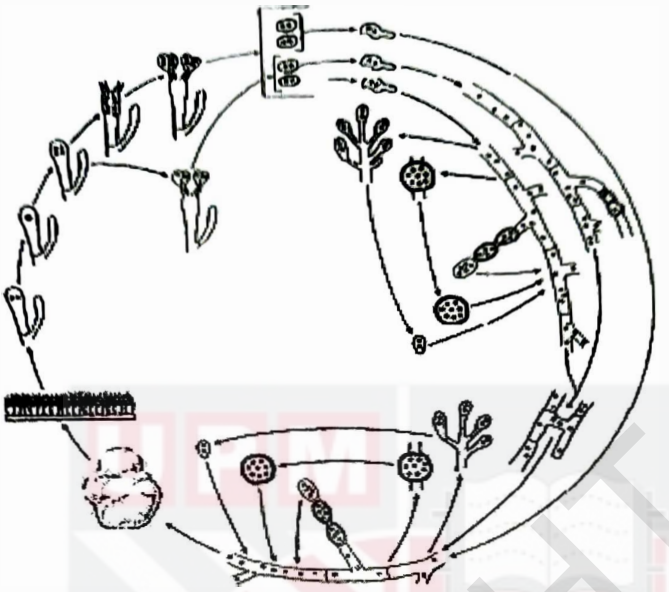


Figure1: Life cycle of *Phanerochaete chrysosporium* showing the nuclear status of each state, assuming a heterothallic incompatability system.

Most of the white-rot fungi possess a heterothallic incompatibility system. These species require that two compatible nuclei to be associated through hyphal anastomosis in order for the life cycle to continue to completion, resulting in the formation of basidia and haploid basidiospores (Harold and Burdsall, 1998).

Alic and Gold (1985) reported that their evidence indicates that *Phanerochaete chrysosporium* possesses primary homothallism. This species of white-rot fungus *Phanerochaete chrysosporium* demonstrates an unsual life cycle. When the fungus was originaly isolated from wood chips, the anamorphic (conidium forming) state was obtained. However, the sexual state, teliomorph, sometimes will formed (Harold and Burdsall, 1998).

was obtained. However, the sexual state, teliomorph, sometimes will formed (Harold and Burdsall, 1998).

2.1.4 Uses

Phanerochaete chrysosporium is capable to work in textile, polycyclic aromatic hydrocarbon (PAH), and pulp and paper mill effluent remediation. It is a basidiomycete fungus which is able to degrade complex compounds such as starch, cellulose, pectin, lignin, lignocelluloses, which are characteristics of textile effluent. It can also decolorize azo-tiphenyl methane dyes by its lignin peroxidase enzyme. (Asamudo *et al.*, 2005).

Recent studies suggest that fungal pretreatment is also effective for depicting pine wood chips (Fischer *et al.*, 1994), another benefit of fungus pretreatment is to improve dissolving pulp.

Phanerochaete chrysosporium is also involved in the bioremediation of textile effluent. In the textile manufacturing, discharge of effluent from the textile and dyestuff can cause serious environment impact in the nearby water bodies for example toxic reactive dyes and dark coloration. When the metallic effluent contaminates water, it will cause several health problems. For example, lead can interfere with enzyme activities. Besides bad impact on human, plant will also be affected. Dyes in water bodies can affect photosynthetic organisms and subsequently affect the food chain (Asamudo *et al.*, 2005).

Traditional method for cleaning up of pollutants are sedimentation, filtration, follow by chemical treatments like flocculation, neutralization and electro-dialysis. Bioremediation has emerged as the most desirable approach for cleaning up the environmental pollutants in effluents. It uses microorganism to catalyze the degradation of waste without disrupting the environment. *Phanerochaete chrysosporium* had emerged as a model system in texture, polycyclic aromatic hydrocarbon, and pulp and paper mill effluent remediation in among the microorganism. It is able to degrade the complex compound such as starch, cellulose, lignin and lignocelluloses. Enzymes involved in the degradation are manganese peroxidase and lignin peroxidase (Asamudo *et al.*, 2005).

2.1.5 Enzyme

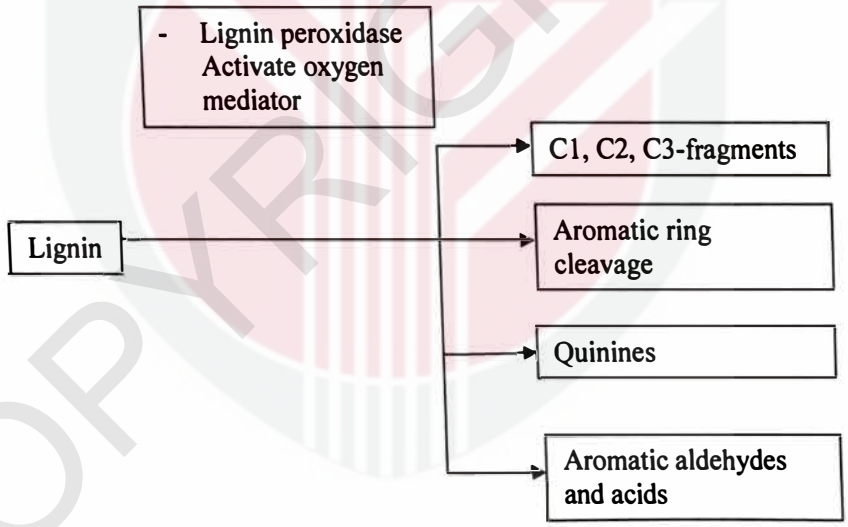
Phanerochaete chrysosporium also known as a lignin-degrading white-rot fungus which is capable to mineralize several chlorinated phenols (Reddy *et al.*, 1998); under nutrient nitrogen limiting conditions, *Phanerochaete chrysosporium* produces two extracellular haem peroxidases or lignin peroxidase (LiP) and manganese (MnP). These two enzymes play an important role in the initial degradation of the complex aromatic polymer lignin. Besides these, laccase also involve in lignin degradation. The function enzyme of laccase and manganese peroxidase is to oxidize the phenolic structure in lignin. These two enzymes play an important role in the initial degradation of the complex aromatic polymer lignin.

The lignin degradation involves several reactions such as aromatic ring cleaverage, quinine formation, acid formation and the role of oxygen in lignin peroxidase which are described by Haemmerli *et al.* (1987).

nonphenol units, acting together with lipids. In contrast, lignin peroxidase degrades only the non-phenol units and acts with the hydrogen peroxide (Hatakka, 2001).

Lignin peroxidase catalyzes a variety of oxidation, all are dependent on H_2O_2. These include C_α-C_β cleavage of lignin model compound (Tien and Kirk, 1984). This ligninolytic enzyme produced from *Phanerochaete chrysosporium* to cleave the C_α-C_β bond in the diametric lignin model.

The hypothetical scheme postulated by Leisola and Garsia (1988) for lignin degradation by *Phanerochaete chrysosoporium* is biodegradation by a combination of both oxidative and reductive conversions.



2.2 Lignin

2.2.1 Structure

Lignin is an aromatic polymer, cell wall material and complex structural component. It is synthesized by higher plants; reaching levels of 20% to 30% of the dry weight of woody tissue (D. Cullen and P.J. Kerdten, 1996). The composition and structure differ with species, type of wood and localization in the cell wall. Lignin mainly distributes in the thick secondary cell walls, but the middle lamella is found to contain highest concentration of lignin. It is a macromolecule formed by the polymerization of three phenylpropane monomer; p-hydroxy-cinnamyl alcohols p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. The aromatic rings of these alcohols are p-hydroxyphenyl, guaicyl and syringyl. The hardwoods have a lignin made up mainly of coniferyl and sinapyl alcohol, whereas for the softwoods is coniferyl alcohol (Pereira *et al.*, 2003)

The structure of lignin obtained from EFB fiber is more complex than the structure extracted from wood due to a complex arrangement of syringyl and guaiacyl units together with p-hydroxyphenyl unit in the EFB fiber (Ibrahim and Azian, 2005).

In contrast to other fungi and bacteria, white-rot fungi are capable of complete lignin degradation to carbon dioxide and water. The extracellular enzymes secreted by *phanerochaete chrysosporium* are lignin peroxidase and mangacanese peroxidase (Kersten. PJ *et al.*, 1995). These enzymes are capable to oxidize the lignin-related model compound. These enzymes can oxidize aromatic compounds containing free phenolic groups by removal of one electron from the aryl rings to form an aromatic cation radical. The radicals break down the lignin polymer. In white rot fungi, the

extracellular hydrogen peroxides required for the activity of lignin peroxidases is produced from the oxidation of glyoxal and methylglyoxal which are the metabolites secreted by the fungus.

Whereas the reason for lignin less accessible to microbial decay is the lignin polymer encrusts the cellulosic microfibrils of plants and is chemically bonded to the hemicelluloses, making these polysaccharides less accessible to microbial decay (Cullen and Kerdten, 1996).

2.3 Biodegradation

The lignin biodegradation is unusual. Firstly, the lignin differs from other biopolymers. It is a random three-dimensional structure which does not have repetitive linkages between the monomer building blocks. The second unusual is the lignin consists of high energy content but it is not used as a sole energy by the living organism. The complex structure of lignin is not readily attacked by microorganism. The most successful group of organism in lignin degradation is white-rot fungi. The remaining microorganism only shows capable to totally degrading the entire major wood component. Third, the organism and mechanisms by which the lignin macromolecule is degraded are exceptional.

Metabolism of lignin is maximizing at low concentrations (approximately 2mM) of nutrient nitrogen. It has also been study that lignin is metabolized only in the presence of an easily metabolized additional carbon source such as glucose and

cellobiose (Karl-Erik Eriksson, 1981). Glucose is utilized by virtually all fungi. For lignin biosynthesis, it is catalyzed by phenol oxidases (Freudenberg and Neish, 1968). The white-rot fungi penetrates the wood cell wall by utilizing existing holes. It can grow through the fiber cell wall by secret three different types of hydrolytic enzymes namely endo-, 4-β-glucanases which attack at random the 1, 4- β-linkages along the cellulose chain, exo-1, 4-β-glucanases which splits off cellobiose or glucose units from the non-reducing end of the cellulose and 1, 4- β-glucosidases which hydrolyze cellobiose and water soluble cellodextrins to glucose and cellobionic acid to glucose and glucono lactone (Eriksson, 1981).

For the white-rot fungi, in the degradation and utilize lignin, they produce extracellular phenol oxidases. Quinones formed by the action of phenol oxidases. Quinones is component of cellular electron-transport system, it is highly reactive and inhibit wide range of metabolic process. Therefore reduction of quinines intermediary would be essentially.

2.3.1 Factor affecting biodegradation process

Factors that affect biodegradation process (Golueke, 1991) are micro-organisms, carbon: nitrogen (C/N ratio), particle size, aeration, moisture content, temperature and size of heap.

First is microorganism, it include bacteria, fungi and actinomycetes participate in the composting process and are responsible for the degradation of the compost materials. The fungi are the bigger and many form of filaments. On decomposed materials, fungi can be seen as furry growths or mushrooms.

Aeration is important during biodegradation espccially for the aerobic microbes which need oxygen to survive and following degrade the materials. For commercially, heap is needed to be aerated.

The temperature is another factor that wills influent the biodegradation process. The microorganism activities only functional under optimum condition, if the temperature beyond the optimum level, the fungus generally not function well.

C/N ratio is a measurement of the rate of decay in EFB. Below table show the C/N ratio and nutrient content for different materials.

Type of wastes	Nutrient (%) per dry weight basis					C/N ratio
	N	P	K	Ca	Mg	
Rice straw***	0.53	0.27	1.70	0.50	0.48	67.0
Corn stalk***	1.13	0.44	1.75	0.37	0.18	43.0
Oil palm front *	0.7	0.07	0.97	0.53	0.14	61
Oil palm empty brunch**	0.6	0.06	1.92	0.13	0.11	83***

Notes: * Aini 1992
** Aini 1994
*** Aini *et al.*, 2002

In order to provide a larger surface area for microorganism to react with the materials, the composting materials need to be shredded or ground. If the materials are larger in size, longer times need to compost it.

Moisture is necessary to support the metabolic processes of the microbes. The optimum moisture content required during composting is between 60-65%. Moisture content below 40% inhibits the microbial activity, whilst moisture more than 65% restricts air movement.

An optimum heap size needed to maintain the heat in the pile. If the heap is small, heat will easily dissipated into the air due to small internal area. The compost material will easily be dried up and not composted. The minimum heap is size is 1m X 1m X 1m high.

2.4 Empty fruit branch

2.4.1 Uses

The empty fruit bunches (EFB) are traditionally incinerated for its ash which is a very good fertilizer or soil conditioner. EFB has been shown to contain high plant nutrient and fertilizer equivalent. With the rising costs of inorganic fertilizers, some organic farmers have been introduce EFB as a fertilizer (Lim, 1991) seen it is rich in nutrient and it is also being used as mulch but due to high lignin content thus a pretreatment process is required to prepare the EFB for its efficient uses. Some researcher have conducted research to find alternative ways to beneficially utilize EFB, example is composting.

2.4.2 Nutrient content

According to Chan *et al.* (1980), nutrient of EFB is estimated at 0.33% N, 0.03% Phosphorus, 2.29% Potassium, 0.18% Calcium, 0.15% Mg similar report on the nutrient of EFB was also made by Hong and Nadaraya (1988).

Composition as a percentage of dry matter				
N	P	K	Mg	Ca
0.44	0.144	2.24	0.36	2.36

(Hong and Nadaraja, 1988)

According to Damanhuri (1998) the nutrient content of EFB was reported at 3.3 % N_2, 0.05% phosphate, 0.2% potassium, 1.0% calcium, 0.2% magnesium on a dry weight basis.

2.4.3 Chemical composition

Chemical composition (% dry weight, w/w) is the following: cellulose 42.0%, hemicelluloses 30.9%, lignin 14.2%, ash 2.8%, extractives 0.1%, ethanol solubles 2.1% (mainly on dissolved lignin), and hot water solubles 8.0% (mainly on dissolved hemicelluloses).

2.4.4 Ash and fiber content of EFB

The ash content of EFB which is rich in potassium was estimated at 30% and is usually used as fertilizer (Lim, 2000). In additional to ash component, EFB fiber is also composed of 45%-50% cellulose and about equal amount, at 25%-35% of hemicelluloses and lignin (Damanburi, 1998). The individual fiber in EFB are an average about 1mm long, 25μm wide and 3 μm thick and these fibers or bundles of fibers are physically stuck together to form vascular bundles (Deraman, 1993).

CHAPTER 3

METHOD AND MATERIAL

3.1 Culture preparation

The fungus used in this experiment was *Phanerochaete chrysosporium*, strain ATCC 34540.

3.1.1 Preparation of broth culture

A 0.72g of potato dextrose broth (PDB) was added into the conical flask and 30ml of distill water was added slowly, shaked to dissolve all the PDB powder. After sterilization, mycelium of *Phanerochaete chrydodporium* was inoculated into conical flask by using inoculating needle. Each of the conical flasks was sealed by using cotton and aluminum foil and left to stand on a sheft. A layer of mycelium mat was formed after 2 days.

3.1.2 Preparation of freeze dried culture

In order to preserve the culture and more accurate pure culture result, freeze-drying or lyophilization method was used. Labconco brand of freeze-drying machine was used. The setting modes were 0.036 mBar vapor pressure and -50 ^{o}C.

The mycelium mat was harvested from the flask and centrifuged to remove the supernatant. The mycelia sediment was repeated washed with sterile distilled water

and centrifuged 3 times before being put into a -80 °C freezer overnight to prepare for freeze-drying.

The machine was turned on for half an hour to stabilize the pressure and temperature at -50 °C prior to freeze-drying the samples.

3.1.3 Fungus culture maintenance and long term storage

The pure culture was stored on potato dextrose agar slant 8 °C. Sub-culture was done every three weeks. Long term storage culture was done by freeze-drying method.

3.2 Measurement of growth rate

The growth rate of *Phanerochaete chrysosporium* was measured using the dry cell weight.

Fourth two of 250 mL conical flask with PDB were prepared and inoculated as before. The duration studies are 14 days. Every day 3 flasks of mycelial mat were harvested for the analysis of dry cell weight. Harvesting involved filtering the flask content through a pre-weighted filter paper. Mycelium mat with the filter paper was oven-dried at 60 °C for 24 hours. The net weight of the mycelium mat then be calculated

The formulation for calculating the dry cell weight of mycelium mat is shown below:

$$Dry\ cell\ weight\ of\ mycelial\ mat =$$

$$(Mycelium\ mat\ with\ filter\ paper\ after\ oven\ dried - weight\ of\ filter\ paper)$$

3.3 Raw material preparation

The EFB were obtained from suburma oil mill in Bintulu, Sarawak. The EFB was shredded into the fibrous form before use. After that, the EFB was packed and sterilized in an autoclave. Totally 16 of 5 liter dustbin containers were used. Before that, all the dustbin containers were disinfected by using 10% Clorox. Two kg of EFB were put into each dustbin container

3.4 Biodegradation process and sampling.

After inoculating the EFB, the growth of *Phanerochaete chrysosporium* was observed. After colonization of EFB by *Phanerochaete chrysosporium*, the heap was overturned weekly and samples for analysis were taken from each treatment every 10 days (Table 2). The samples were dried at 105 °C for 16 hours before being ground to 0.5mm size using a waring blender.

Table 1: Date of observation, turning and watering.

Number	Date
1	16/11/2008
2	23/11/2008
3	30/11/2008
4	7/12/2008
5	21/12/2008
6	28/12/2008
7	3/1/2009
8	10/1/2009
9	17/1/2009
10	27/1/2009

Table 2: Date of sampling.

Number	Date
1	19/11/2008
2	29/11/2008
3	9/12/2008
4	19/12/2008
5	29/12/2008
6	8/1/2009
7	18/1/2009
8	28/1/2009

3.5 C/N ratio

The C/N ratio is the carbon content divided by the nitrogen content.

3.5.1 Total nitrogen

Extraction and analysis

A 0.5g of EFB (sieved to pass 0.5 mm) was weighed into 50 mL Kjeldhal digestion tubes. About 0.4g of salicyclic acid was added. The soil was wetted with few drops of water and 5 mL of concentrated sulphuric acid were added. Later, 1 tablet of Kjeldhal catalyst was added. The samples were shakened and allowed to equilibrate for 30 minutes. The samples were heated in the digestion block at 180 °C for one hour and then at 320 °C for 4 to 5 hours until the samples became colorless. The samples were allowed to cool down. Upon cooling, 30mL distilled water were added to make up to volume.

Ten mL of the sample and 10 mL of 40% NaOH were pipetted into distillation apparatus. The distillates were distilled and collected in 10 mL of 2% boric acid-indicator solution. The colour changed from purple to green during distillation. A 2%

boric acid was prepared by weighing 80g of pure boric acid (H_3BO_3) in a 5L flask marked to indicate a volume of 4L. About 3500 mL of mixed indicators (0.099 g bromocresolgreen + 0.066 g methyl red in 100 mL of ethanol.) were added. 0.1 M NaOH was added until the solution became reddish purple (pH 5.0). The solution was make up to 4 L with distilled water.

The 50 mL conical flask containing the distillate was removed when twice of the original volume (20mL) was obtained. The solution was titrated with 0.01 M HCl until the colour changes from green to purple (Chin, 2000).

Percentage nitrogen in EFB is calculated as:

$$\% N = \left[\frac{(V - B) \times M \times R \times 14.01}{Wt \times 1000}\right] \times 100$$

Where: V=Volume of 0.01 M HCl or H_2SO_4 titrated for the sample (mL)

B= Digested blank titration volume (mL)

M= Molarity of HCl solution (0.01M)

14.01= Atomic weight of N

R=Ratio between total volume of the digest and the digest volume used for distillation

Wt= Weight of air-dried soil (g)

3.5.2 Total carbon content

Sample was oven-dried for 16 hours at 105 °C. After that, 10g of sample was weighed (W1) and put into the crucible. The sample in crucible was put into the furnace at 300 °C for one hour. The temperature was then increased from 300 °C to 550 °C. The organic carbon was released at the temperature 550 °C. Whereas for the inorganic carbon was released at the temperature more than 550 °C. The temperature was maintained at 550 °C for 4-5 hours. After that, the samples were allowed to cool down overnight. On the next day, the sample was weighed (W2) and % carbon calculated (Chin, 2000).

$$\%\ Organic\ matter = \left[\frac{(W1 - W2)100}{W1}\right] \times 0.58$$

3.6 Lignin (acid detergent lignin) test

The ADL test was analyzed by using 2010 Fibertec® system machine from Foss Tecator AB.

3.6.1 Hot extraction (Step 1)

Crucible was placed in the Fibertec Hot Extraction unit and 100ml of Cold Detergent Solution (ADS) was added. Two to four drops of n-octanol were added to prevent foaming. The solution was heated to boiling. The heater was adjusted and allowed to boil for 60 minutes. Boiling time was measured from the time when the solution has reached the boiling point.

The solution was washed two times with hot deionized water at 30 mL each. Thirty mL portions of the water were used and sucked out as dry as possible between washings.

3.6.2 Cold extraction (Step 2)

Crucibles were placed in the Cold Extraction Unit and 25 mL acetone was added. After that, the acetone was filtered by starting the vacuum pump and turning the filtration valve to vacuum. This step was repeated once.

Twenty-five mL of 72% H_2SO_4 (pre-cooled to 15 °C) was added to the filtering crucible. The solution was stirred with glass rod at each hour interval and filtered off after 3 hours. The sample was washed with water until free from acid.

The crucibles with sample were dried at 130 °C for 2 hours and then cooled to room temperature in desiccators and weighed as W2. The samples in the crucibles were ashed at 500 °C for at least 3 hours and then cooled to room temperature in the desiccator and weighed as W3 (AOAC official method 973.18, 1997).

3.7 Maintenance of heap condition

3.7.1 pH value

The pH value indicates the degree of acidity and alkalinity of soil. Potentiometric method was used for testing the pH value for the compost.

The ratio used in this pH analysis was 1:15 which is 2 g of samples and 30 mL of water. Two g of samples was weighed in plastic vials. Thirty mL of distilled water was added, stopper it and shake at 180 rpm for 15 minutes. The solutions were left to stand overnight.

Prior to use, the pH meter was calibrated with two buffer solutions namely pH 4.0 and pH 7.0. The glass electrode was inserted in to a buffer solution of pH 7.0 and the pH meter was adjusted to read pH 7.0. The electrode was then rinsed with distilled water and placed in a buffer solution of pH 4.0 to read pH 4.0. The electrode was rinsed again with distilled water and placed in the EFB suspension. The pH was recorded. The electrode was rinsed with distilled water after measuring the pH and placed in the buffer solution of pH 7.0 (Chin, 2000).

3.7.2 Moisture content

The samples was accurately weighed at 3g (W1) and dried for at least 6 hours to constant weight at 105 ^{o}C. After that, the sample was cooled in a dessicator for 30 minutes and weighted as W2 (Chin, 2000).

Calculation for percentage of moisture content is:

$$\%\ moisture\ content = ((W1 - W2))/W1 \times 100$$

3.7.3 Humidity

The humidity in the surrounding heap was measured by using indoor thermo-hygrometer. The indoor thermo-hygrometer is placed on the heap and reading was taken after a while

3.7.4 Temperature

Temperature was measured using thermometer. Thermometer was inserted into the piles and the temperature pointed was noted.

3.8 Data analysis

Phanerochaete chrysosporium was used to degrade the lignin in oil palm EFB. The C/N ratio was calculated at every assessment. The experiment was conducted for three months. The samples were collected once every ten days, for a total of six times. Quadratic Regression was used to analyze the lignin content and C/N ratio during the period of composting. Lignin content and C/N ratio are dependent variable whereas the period of composting is independent variable. This is to analyze the change of lignin content and C/N ratio during the period of composting.

3.9 Experiment design

The experimental design was CRD with three treatments and one control, at four replication each. The treatments were three concentrations of *Phanerochaete chrysosporium* namely, 0.08% (0.2g of lyophilized fungus in 250mL liter of distill water), treatment two is 0.16% (0.4g of lyophilized fungus in 250mL liter of distill water) and treatment three is 0.24% (0.6g of lyophilized fungus in 250mL liter of distill water). Whereas, the control treatment consist of 250mL distilled water only. The culture of *Phanerochaete chrysosporium* was inoculated into oil palm EFB. For every ten days, the lignin content and C/N ratio for the pile were analyzed during the composting period. Work was carried out in the laboratory of Crop Science department.

CHAPTER 4

RESULTS

4.1 Grow curve of *Phanerochaete chrysosporium*

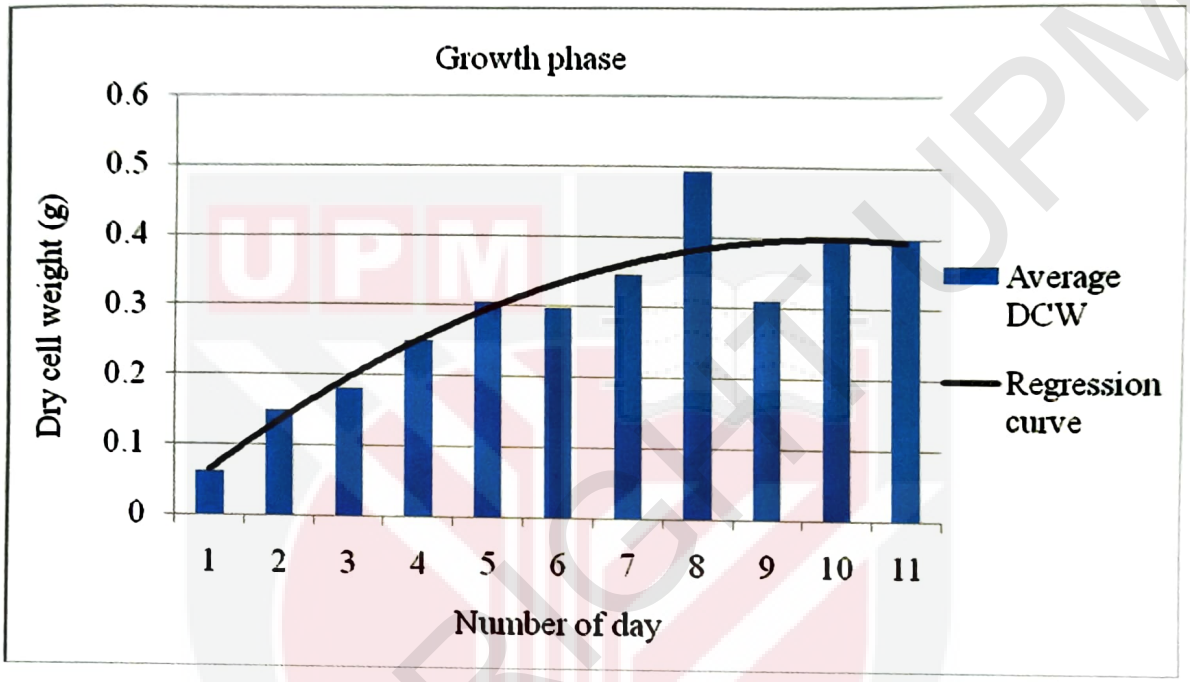


Figure 2: The growth curve of *Phanerochaete chrysosporium*

Note:
DCW: Dry Cell Weight

Description:

The growth curve of *Phanerochaete chrysosporium* was determined by measuring the dry cell weight (g). The log phase ranged from day 5 to day 8; after day 8 it was stationary phase (Figure 2). The dry cell weight was increasing until day 8, after that there was very little changing the dry cell weight.

4.2 Structure of *Phanerochaete chrysosporium* viewed under microscope.

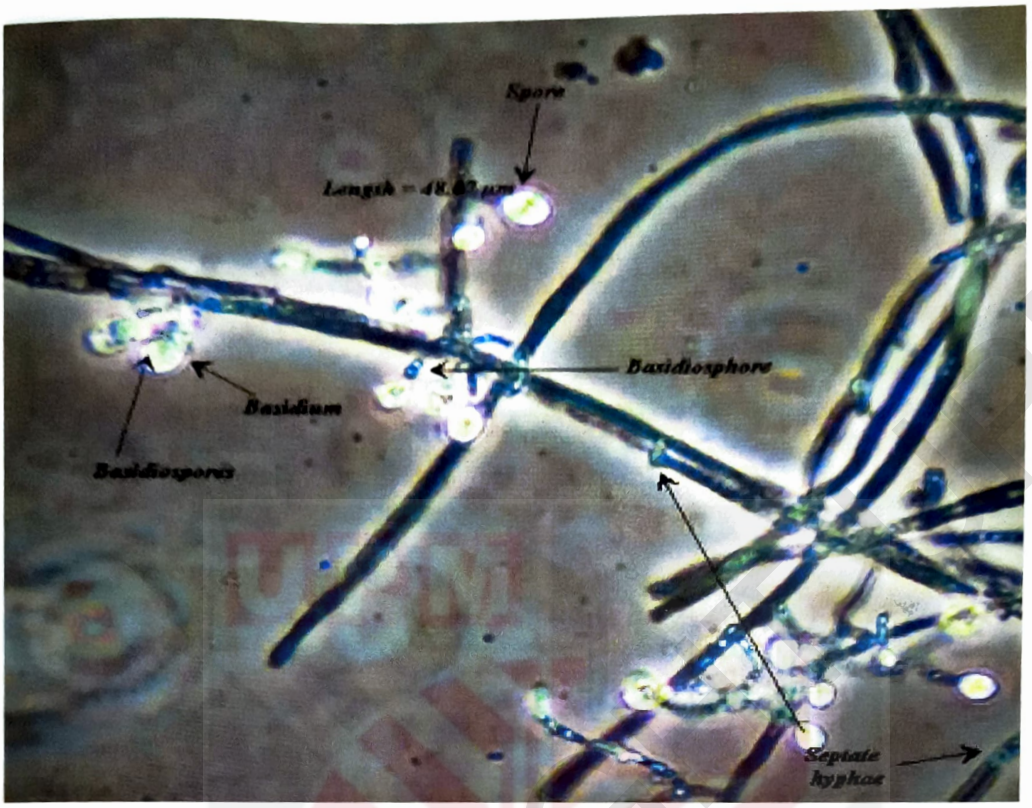


Figure 3: Morphology of *Phanerochate chrysosporium* (1000x)

Description:

The basidiospore which is an exogenous sexual spore is borne on a basidium. The basidiosphore is modified hyphae that support the basidium. (Wu, 1995). The mycelium of *Phanerochaete chrysosporium* consists of septa hyphae

4.3 Lignin (acid detergent lignin) test

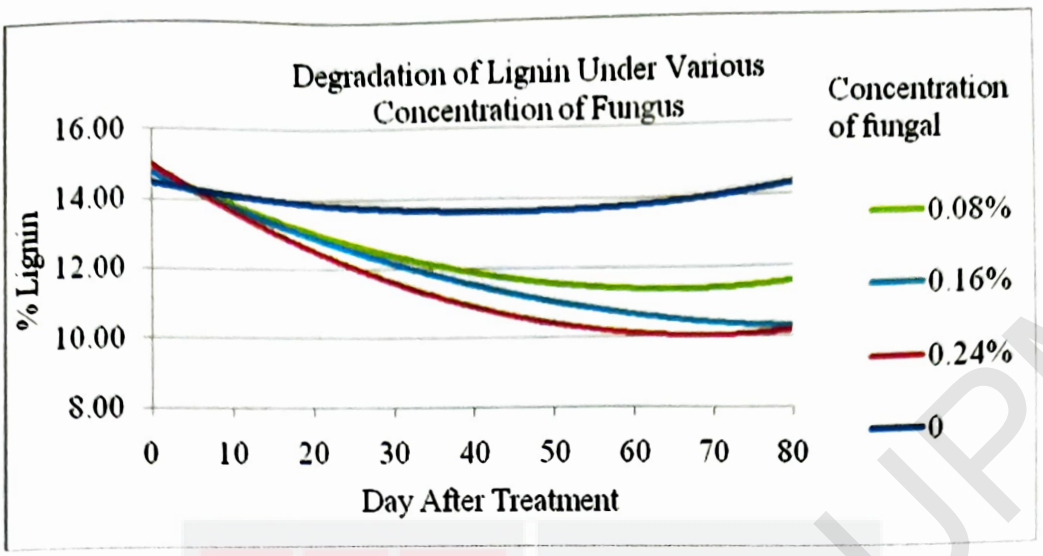


Figure 4: Biodegradation of lignin until 80 DAT with different concentration of culture.

Table 3: Percentage of net lignin decrease until 80 DAT

Treatment	Concentration of *P.c* (v/v)	Net of decrease in lignin
1	0.08%	25.43
2	0.16%	32.08
3	0.24%	33.65
4	0	5.98

As an overall, results for all the treatments show a decrease in lignin content from 10 DAT to 80 DAT. Highest concentration culture (0.24%) shows the highest decrease in the lignin content (33.6%), followed by 0.16% concentration and 0.08% concentration with 32.08% and 25.43% decrease respectively. All the values were higher than the control (5.98%).

4.4 C/N ratio test

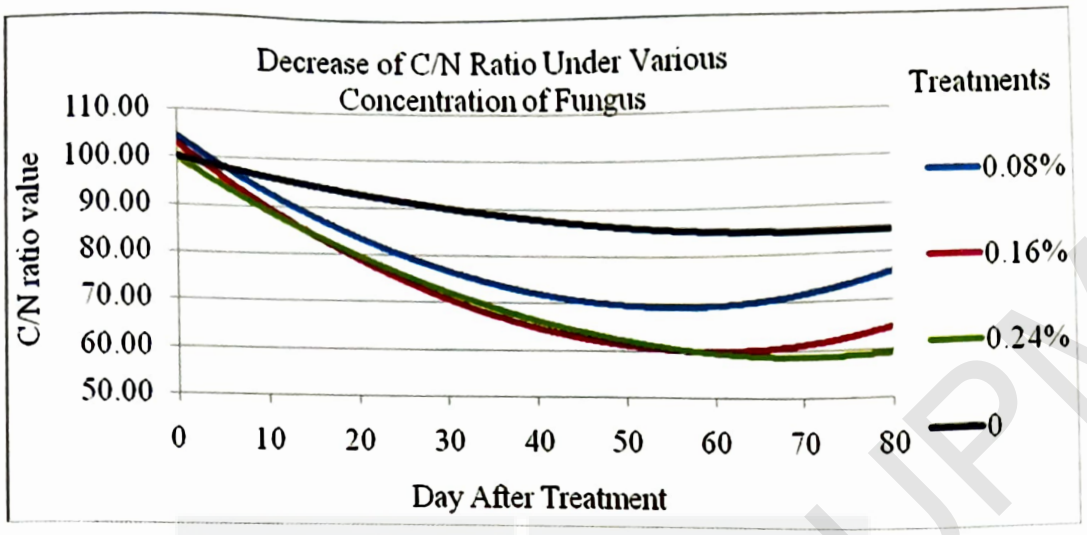


Figure 3: Quadratic regression graph for the C/N ratio. It indicates that three treatments (different concentration of fungus culture) with control and the C/N ratio analyzed 10 day interval.

From the quadratic equations shown in figure, there was negative relationship between the C/N ratio and DAT. All treatments showed decrease of C/N ratio as time progressed. The C/N ratio decreased most during the range of 10-40 DAT for all the treatments.

Table 4: Percentage decrease of net C/N ratio after 80 DAT.

Treatments	Concentration of *P.c* (v/v)	Net % decrease of C/N ratio
1	0.08%	30.11
2	0.16%	42.28
3	0.24%	44.18
Control	0	21.02

Overall, the C/N ratio decreased across 60 DAT. C/N ratio decreased most in the treatment with 0.24% concentration (44.18%) which is the highest concentration of fungus culture, followed by treatment with 0.16% concentration (42.28%) and treatment 0.08% concentration (30.11%). The control decreased the least among the treatments which was 21.02% (Table 4).

4.4 C/N ratio test

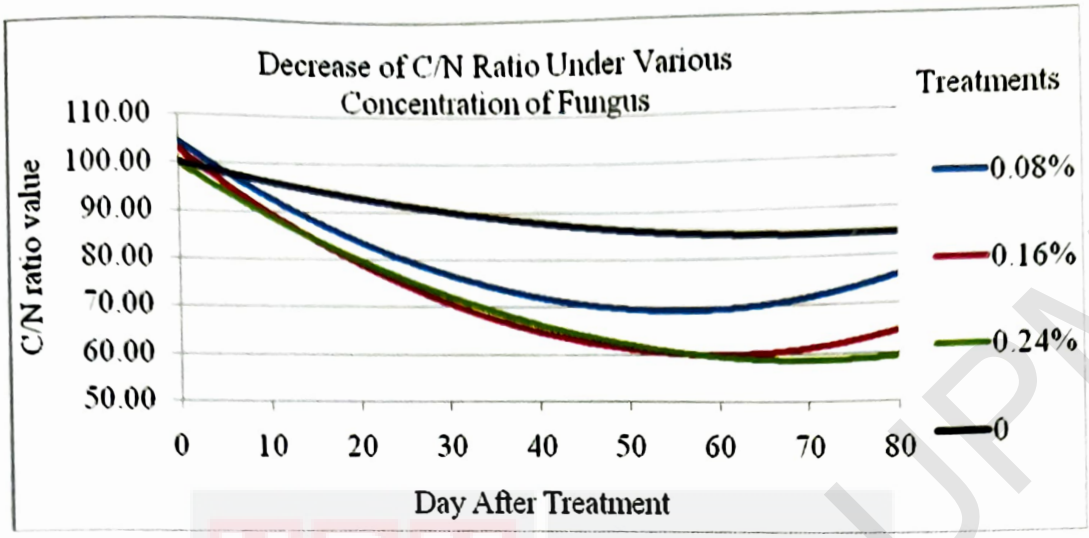


Figure 3: Quadratic regression graph for the C/N ratio. It indicates that three treatments (different concentration of fungus culture) with control and the C/N ratio analyzed 10 day interval.

From the quadratic equations shown in figure, there was negative relationship between the C/N ratio and DAT. All treatments showed decrease of C/N ratio as time progressed. The C/N ratio decreased most during the range of 10-40 DAT for all the treatments.

Table 4: Percentage decrease of net C/N ratio after 80 DAT.

Treatments	Concentration of *P.c* (v/v)	Net % decrease of C/N ratio
1	0.08%	30.11
2	0.16%	42.28
3	0.24%	44.18
Control	0	21.02

Overall, the C/N ratio decreased across 60 DAT. C/N ratio decreased most in the treatment with 0.24% concentration (44.18%) which is the highest concentration of fungus culture, followed by treatment with 0.16% concentration (42.28%) and treatment 0.08% concentration (30.11%). The control decreased the least among the treatments which was 21.02% (Table 4).

4.5 Identification of contaminants

During the study, four types of contaminants have been identified in the heap.

4.5.1 *Syncephalastrum racemosum*

Microscopic morphology of *Syncephalastrum racemosum* showed terminal vesicle, finger-like merosporangia and sporangiospores. It has abundantly branched erect conidiophores (sporangiophores), branched; tips enlarged bearing a head of rod-shaped sporangioles, each produring a row of nearly spherical spores, resembling a chain of conidia.

4.5.2 *Monilia* sp.

The mycelium in PDA plate and colonization of *Monilia* sp on EFB showd gray color. Under the view of microscope, the morphology of *Monilia* sp show 1-celled, short cylindrical to rounded catenulate and formed acropetally conidiophores branched.

4.5.3 *Penicillum* sp.

The colonization of *Penicillum* sp. showed greenish colour in PDA culture. Under microscope the morphology showed that conidiophores arised from the mycelium singly, branched near the apex to form a brush-like, conidia-bearing apparatus; ending in phialides, the conidiophores is terminated by a swollen vesicle bearing flask-shaped phialides.

CHAPTER 5.0

DISCUSSION

5.1 Preparation fungus culture

For the preparation of fungus culture, freeze-drying method was used rather than using bioreactor nor hemocytometer, because after freeze-dried the culture became powder form and thus could be evenly spread on the EFB. Moreover this method can accurately and precisely measure the concentration of fungus because the freeze drying method involves removal of water or other solvent from the culture by a process called sublimation. Thus it can estimate the fungus concentration. The other advantage was that the freeze-dried culture can be store long term.

The volume and configuration of the fungal culture to be freeze-dried often determine how the material is freeze-dried. From observation during freeze-drying process, the greater the ratio of the surface area to the volume of the suspension, the faster drying the occurs. This was due to a greater area for the water molecules to leave the culture compared to the distance they have to travel to reach the surface of the frozen matrix (Labconco Laboratory Manual, 1998).

5.2 Preparation of raw material

The EFB was ground into fibrous form before starting the decomposition process. Material in fibrous form or grinding of material helps speed up decomposition by increasing the surface area available for microbial action, and providing better aeration.

Generally, successful composting depends on a number of factors that have both direct and indirect influence on the activities of the microorganisms. It includes the type of raw material being composted, its nutrient composition, moisture content, temperature, acidity or alkalinity and aeration. Microorganisms prefer work on condition that is plenty of oxygen and moisture (Taiwo and Oso, 2004).

5.3 Growth curve (dry cell weight) of *Phanerochaete chrysosporium*

The growth curves were used as estimates the growth rate of *Phanerochaete Chrysosporium* in nutrient medium to visualize small changes in growth rates during growth curve and to determine the time point after the growth rate changes significantly.

The growth curve was developed by measuring the dry cell weight. Based on the figure 2, the log phase which is the growth curve reached the maximal slope at day 7 to day 8. The maximum growth rate for *Phanerochaete Chrysosporium* was at the 8th day after inoculation. Thus, the culture was harvested on the 7th day after inoculation which was during the log phase. The fungus grew actively during the log phase.

After day 8, the growth curve decreased. The last phase was the stationary phase which was day 10 and day 11, where there were no changes or negative slopes on the growth curve (Meletiadis *et al.*, 2000).

5.4 Lignin (Acid Detergent lignin) test

ADL is the mass fraction of insoluble fibers. A complex indigestible substance contained in the woody parts of plant, such as cob, hulls and fibrous portion of stems and leaves (AOAC official method 973.18, 1997).

Lignin molecules are very large and complex, consisting of hundreds of interlinked phenolic ring subunits. Because the linkage among these structures are so varied and strong, only a few microorganism mainly white-rot fungi is capable to break down. Initially, decomposition proceeded very slowly at first (Figure 4). It showed that from the 0 DAT to 10 DAT, decrease in % lignin was very little. Treatment with concentration 0.08% decreased by 3.24%, treatment with 0.16% decrease 4.10% and treatment with concentration 0.24% is 6.48%. Once the subunit or monomers of complex lignin molecules were separated, the degradation of lignin was faster (Buck, 2002). During 10 DAT to 40 DAT lignin decreased very fast; treatment with concentration 0.08% decreased 15.64%, treatment with 0.16% concentration decrease 20.65% and treatment with concentration 0.24% decrease 19.45%.

Treatment with the most concentrate of inoculums (0.24%) showed the highest lignin degradation level (33.65%) and colonization rate compared to the others two treatments; treatment with 0.16% concentration was 32.08% and treatment with

0.08% concentration was 25.43%. Based on the observation done for every week, the rate of colonization with the higher concentration of culture was faster to coverage the whole pile than the dilute concentration of fungus culture (refer to the appendix 9). Higher cell density will have higher ligninolytic enzyme activity for white-rot fungi. This broke down lignin in the plant cell wall more efficiency in order to access the ligno-cellulose which the fungus degrades to cellobiose and ultimately glucose (Evans *et al.*, 1994). The production of lignin-degrading enzymes, particularly lignin peroxidase and Manganase peroxidase are secreted in increased quantities by the fungus during ligninolytic activity. As for the control, these were not much changes but the net lignin still decreased a little (5.98%). This was due to natural degradation by other microorganisms from the environment as the experiment materials has conducted in the natural environment. More over, some cross contamination of *Phanerochaete* sp. in control may have occurred during the conducted the experiment (sampling and overturning the piles). In addition, all treatments were placed at the same site to create a uniform environment.

Based on the result, both C/N ratio and lignin content decreased until 80 DAT. Berg and Soderstrom (1979) stated that white-rot fungi produces high amounts of ligninolytic enzymes in the presence of high nitrogen and carbon concentrations due to increased biomass yields and a positive correlation between the amount of imported nitrogen and fungal biomass increase. There was a relationship between lignin content, nitrogen and carbon. Nitrogen in the substrate is used for cellular growth and metabolism, and stimulates enzyme production. When the nitrogen level is high, subsequence carbon and lignin will be low. High nitrogen content can

stimulate more ligninolytic enzyme to break down the lignin and also increase the rate of microorganism metabolism (Lindahl and Boberg, 2008)

5.5 C/N ratio test

The ratio of total carbon (C) to nitrogen (N) is called C/N ratio. It is used as an indicator of compost stability and N availability. Analysis of C/N ratio for EFB helps to determine the rate of decay in EFB. The total carbon is a direct measurement of all organic and inorganic carbon. Carbon is used by the microorganism for both energy and growth, while nitrogen is essential for protein production and reproduction. Thus the C/N ratio typically decreases during composting and N is lost during the composting process.

There are negative relationships between the C/N ratio and DAT in all the treatments (Figure 5). It means that the C/N ratio is decreasing over the day after treatment. The control show not much changes. There are few reasons that explain the decrease in C/N ratio on the oil palm EFB. The organic carbon compounds are enzymatically oxidized to produce carbon dioxide, water, and energy and decomposer biomass. Since the EFB is a plant material, it composes a large amount of carbon and hydrogen and under well-aerated condition the oxidation of the organic compounds will occur. During the organic matter decomposition, oxygen is consumed and carbon dioxide (CO_2) is release. As the C/N ratio decrease, most of the carbon is released during the initial rapid breakdown of this EFB residue is converted to carbon dioxide, with a smaller amount of carbon converted into fungus synthesized compound (biomass).

EFB consists of high lignin content, which decomposes slowly. The lignin is complex compound with multiple ring-type or phenol structure. This phenolic compound can dramatically slow the rates of both nitrogen mineralization and carbon oxidation (Samudro and Hermana, 2007). The lignin acts as a protective layer from the decaying and degradation process of others microorganisms. Once the lignin was utilized, rate of decomposition of other organic matter increased. From 0 to 10 DAT the C/N ratio decrease very slow and after that from 10 to 40 DAT the decrease of C/N ratio was faster (Figure 4). During the decomposition, nitrogen is enzymatically released from decomposing EFB tissue, but immediately absorbed by the mycelium in the piles. The utilization of organic nitrogen, nitrogen-containing compound as a source of energy, leaves ammonium as a by-product. The nutrients will be translocated throughout their entire mycelium (Lindahl and Boberg, 2008).

In overall, the C/N ratio for all the treatments were decreasing but there was a slightly increase in C/N ratio for treatment with 0.16% concentration and control on 50 DAT. This could be due to the sampling problem. The heap were turned on 49 DAT which was one day before sampling. The colonization of fungus might not be even after overturning. Moreover the turning of the compost pile encouraged the forming of the hyphal due to aeration enhancement. The newly formed hyphal will start to metabolize the carbon and mineralize the nitrogen. Thus the decrease of C/N ratio was less even.

For the control, C/N ratio decreased 21.02%. Control was not inoculated with *Phanerochaete chrysosporium* culture. The decrease in C/N ratio might be due to the

presence of indigenous microflora activity and natural degradation processes. For the control, *Penicilium* sp. and *Phanerochaete* sp. were isolated from the compost pile. *Penicilium* sp. can be transmitted through spore and it was present in air. Cross contamination of *Phanerochaete* sp. in the control was suspected to be due to sampling and overturning the piles. When sampling and overturning the piles, spore can be transmitted through the air, thus cross contamination occurred. Action to be taken after that was to place all the control to another site to avoid cross contamination.

Treatment with the highest concentration of inoculums (0.24%) showed highest decrease in C/N ratio (44.18%), following by treatment with 0.16% (42.28%) and treatment with concentration 0.08% (30.11%) (Table 4). It can be concluded that highest concentration of fungal is more efficient in decreasing the C/N ratio as the rate of colonization was faster than lower concentration treatments. The rate of decomposition of the organic matter was faster for the highest concentration of fungal treatment.

5.6 Heap condition

5.6.1 pH

The pH measurement for the samples was using Mettler Toledo Seven Easy pH meter. All the heaps were maintain in an optimum condition. The pH range from 6.00 to 7.50. The most suitable pH condition for piles is in the range of pH 6.0 to 8.0 (Sullivan and Miller, 2001). Thus, lime or elemental sulfur was not necessary added. The pH value at 5.5 or 9.0 is likely be less effective for the microorganism to carry their activity than it is at a pH near neutral (pH 7). A high pH of above 8.5 encourages the conversion of nitrogen compounds into ammonia gas, resulting in nitrogen loss from the compost. The pH can be adjusted by adding an acidifying agent, such as superphosphate or elemental sulfur.

The pH was lower in the initial stage compared to samples 50 DAT and 60 DAT. This was due to the release of organic acids from decaying organic matter. Over time, the pH rose as organic acids were broken down, and the pH usually approaches neutrality (pH 7) during the maturation phase. Based on the the result on 60 DAT, the pH value was about 6.80 to 7.26.

5.6.2 Moisture

The moisture content ranged from 50-65% which was in the acceptable level and optimum condition for the microorganism activity. The most important precautionary measures to be taken are to ensure that the EFB does not dry out. Moisture was lost during the process, thus extra water should be added. It is important to measure the moisture content regularly because the milled EFB reduced the size of the EFB to increase the surface are should not reduce the moisture content by too much. The

available moisture within the EFB provides a favorable environment for microbiological activity.

5.6.3 Temperature and humidity

The temperature for the piles was maintained at 29-30 °C whereas the humidity was around 90% to 94% in the bin. The temperature and humidity in the bin is another factor that influences the growth of fungi on piles (Huai and Y. Susumu, 1999).

Growth of fungus was maximum under condition of constant temperature and constant humidity. From the result, the temperature and humidity were in the acceptable level. It is important that to maintain the temperature and humidity for the piles because the varying temperatures will yield minimum growth of fungus since it is disadvantageous for fungal growth during this period. The piles were incubated in approximately 90% humidity and dark condition which were suitable condition for *Phanerochaete chrysosporium* (Blanchette *et al.*, 1992).

5.7 Identification of contaminants

Some contaminants were presents but as whole colonization of *Phanerochaete chrysosporium* was the dominant colonizer on the heaps. Some of the contaminants isolated from the heaps were *Syncephalastrum racemosum, Monilia* sp. and *Penicilium* sp.

CHAPTER 6

CONCLUSION

From the experiment conducted, *Phanerochaete chrysosporium* showed the ability to degrade lignin in oil palm EFB and able to metabolize the carboneous compound. Higher concentration of fungal culture showed better ability to degrade the lignin. A 0.24% fungal culture was capable of degrading 32.37% of lignin and metabolizes the carboneous compound since the C/N ratio also decreased by 43.29%. In addition, there was a correlation between carbon and nitrogen and lignin content. High nitrogen content can stimulate more ligninolytic enzyme to break down the lignin and also increase the rate of microorganism metabolism. As the percentage of lignin decrease, the C/N ratio also decreases.

REFERENCES

Aini, Z. 1992. Effect of compost from cocoa pods on tomato. Teknol. Koko- Kelapa **8**: 29-33

Aini, Z. 1994. Commercial and small scale composting. Teknol. Horticulture **10**:33-90

Aini, Z. Mageswaran, T. Abdullah, N. and Sivapragasam, A. 2002. Research experiences in developing an organic vegetable farm. Proc. International Conference on Organic Farming for Sustainable Development. 6-7Aug. 2002, Kuching, Sarawak. p. 85-89. Kuching: Sarawak Development Institute/ SEDC Sarawak, MAFI

Asamudo. 2005. Bioremediation of textile effluent using *Phanerochaete chrysosporium*. African Journal of Biotechnology. **4 (13)**:1548-1553

AOAC official method 973.18. 1997. Fiber (acid detergent) and lignin in animal feed, in official methos of analysis of AOAC international, 16 edition. **4**: 28-29 AOAC international, Arlington.

Berg and Soderstrom. 1979. Fungal Biomass and Nitrogen in Decomposing Scots Pine Needle Litter. In *Soil Biology and Biochemistry*. **11**: 339-341.

Buck. 2002. Soil organic matter. In the nature and properties of soils thirteenth edition, ed. Nyle c. brady and ray r. well, p. 498-542.

Boberg and Lindahl. 2008. Distribution and Function of Litter Basidiomycetes in Coniferous Forests. In *Ecology of Saprotrophic Basidiomycetes* ed. Lynne Boddy, Juliet C. Frankland and Pieter Van West, p. 183-196. Elsevier: Academic Press.

Chin, S.P. 2000. Laboratory Manual of Methods Soil Analysis. Department of Agriculture, Sarawak. p. 15-22

Cullen and Kerdten P.J. 1996. The Mycota III Biochemistry and Molecular Biology. Enzymology and Molecular Biology of Lignin Degradation. Springer-Verlag Berlin Heidelberg. p. 295-306.

Christov, L.P., Akhtar, M. and Prior, B.A. Biobleaching in dissolving pulp production. In: Biotechnology in the pulp and paper industry: Recent Advances in Applied and Fundamental Research (Messner, K. and Srebotnik, E.,eds), Facultas Univeritasverg, Vienna. p. 625- 628.

Dan, M. Sullivan, Robert, O. and Miller. 2001. Compost Quality Attributes, Measurements, and Variability. In Compost Utilization In Horticultural Cropping Systems, Ed. Peter J. Stoffella and Brian A. Kahn, p.95-120. New York: CRC Press.

Damanhuri, M.A. 1998. Hands-on experience in the production of empty fruit branches (EFB) compost. Paper presented at the CETDEM Malaysian organic Farming Seminar. Petaling Jaya, Selangor, Malaysia.

Deraman, M. 1993. Carbon pellets prepared from fibers of oil palm empty fruit branches: 1.A quantitative X-ray diffraction analysis. PORIM Bull. Palm Oil Res. Inst. Malaysia. p. 26-30.

Eriksson, K.-E. L., Blanchette, and P. Ander. 1990. Biodegradation of hemicellulose. In T. E. Timell (ed.), Microbial and enzymatic degradation of wood and wood components. Springer-Verlag. p. 181-224.

Evans and Christine, S. 1985. Laccase activity in lignin degradation by Coriolus versicolor: in vivo and in vitro studies. FEMS Microbiology Letters, **27**: 339-343

Golueke, C.G. 1991. Principles of Composting. In: The Biocycle Guide to the Art and Science of Composting. p. 14-37. Emmaus, Pennsylvania: JG Press Inc.

Haemmerli, S.D., Leisola, Schoemaker, H.E., Schmidt, H.W.H., Leisola, M.S.A. 1987. Oxidation of Veratryl Alcohol by the Lignin Peroxidase of *Phanerochaete chrysosporium*. Involvement of Activated Oxygen. FEBS Letter. **220**: 149-154

Hatakka, A. 1994. Lignin – modifying enzymes from selected white – rot fungi: production and role in lignin degradation. FEMS Microbiol. Rev. **13**: 125 – 135

Harold, H. Burdsall, Jr. 1998. Environmentally Friendly Technologies for the Pulp and Paper Industry. In Taxonomy of Industrially Important White-Rot Fungi, ed. Raymond A. Young and Masood Akhtar, p. 260. John Wiley and Sons, Inc.

Hazwani, C. and Tuah, P.M. 2008. Isolation and Characterization of Lignocellulose-degrading Thermophilic Bacteria for Biocomposting of Oil Palm Empty Fruit Bunch. Ecological and environmental quality studies. ICERT. p. 503-506.

Huai, P.T and Susumu, Y. 1999. Influences of temporal variations in environmental temperature and humidity on the fungus growth rate. Biocontrol science. **5(1)**: 51-55

Hoong, H.W. and Nadaraja, M.N. 1988. Mulching of Empty Fruit Bunchesof Oil Palm in S.L.D.B. In *SLDB/PORIM Workshop on palm oil milling technology*, p. 38-50. Kota Kinabalu, Sabah.

Ibrahim, M and Azian, H. 2005. Extracting Soda Lignin from The Black Liquor Of Oil Palm Empty Fruit Bunch. *Jurnal Teknologi*, **42(C)**: 11–20

Kondo, R., Kurashiki, K. and Sakai, K. 1994. In vitro bleaching of hardwood kraft pulp by extracellular enzymes excreted from white rot fungi in a cultivation system using a membrane filter. Appl Environ Microbiol. **60**: 921–926

Kersten, P.J. 1995. *Phanerochaete chrysosporium* glyoxal oxidase is encoded by two allelic variants: structure, genomic organization and heterologous expression of glx1 and glx2. J Bacteriol. **177**:6106-6110.

Labconco Laboratory Manual. 1998. A Guide to Freeze Drying For The Laboratory. U.S.A.

Leisola, Haemmerli, SD. Waldner, R. Schoemaker, HE. Schmidt, HWH. Fiechter, A. 1988. Metabolism of a lignin model compound, 3,4-dimethoxybenzyl alcohol by phanerochaete chrysosporium. Cellulose chemistry technology. **22**: 267-277

Lim, K.C. and Zahirah, A.R. 2000. Decomposition and N and K release by oil palm empty fruit branches under mature palms. *Journal of Oil Palm Research*. **12**: 55-65

Lim, KH. 1991. Compositing and utilization of oil palm empty fruit bunches. Paper presented at seminar on developments in palm oil milling technology and environmental management organized by PORIM. p. 167.

Meletiadis, Jacques F. G. M. Meis, Johan W. Mouton and Paul E. Verweij. 2001. Analysis of Growth Characteristics of Filamentous Fungi in Different Nutrient Media. Journal of Clinical Microbiology. **39(2)**: 478-484

Uribe, A. and Bernal, G. 1989. Utilization of EFB ash as fertilizer in Oil Palm Growth. *Journal of Palm Oil in The Peat Soil* **3**: 147-427

Pereira, Graca, J. Jose, C. and Rodrigues. 2003. Wood Quality and Its Biological Basis. Wood Chemistry In Relation To Quality, ed. John R. Barnett and George Jeronimidis, p. 53-86. Blackwell: CRC Press.

Reddy G.V.B., Sollewijn, G.M.D. and Gold,M.H. 1998. Degradation of 2,4,6-trichlorophenol by Phanerochaete chrysosporium: involvement of reductive dechlorination. J Bacteriol **180**: 5159-5164

Samudro, G. and Hermana, J. 2007. Denitrification Efficiency In Compost Bed With Various Carbon and Nitrogen Contents. *Journal of Applied Sciences in Environmental Sanitation*, **2(2)**: 57-62

Syafwina, Y. Honda, T. Watanabe and Kuwahara, M. 2002. Pre-treatment of Oil Palm Empty Fruit Bunch by White-rot Fungi for Enzymatic Saccharification. Wood Research. **89**: 19-20

Theodorus, H. De Koker, J. Zhao, D. Cullen and Janse, B.J.H. 1998. Biochemical and Molecular Characterization of South African Strains of *Phanerochaete chrysosporium*. Characterization of *Panerochaete chrysosporium* From South Africa. **102 (1)**: 88-92

Tuomela, M., Vikman, M., Hatakka, A., Ita¨ vaara, M., 2000. Biodegradation of lignin in a compost environment: a review. Bioresource Technology **72**: 169–183

Tien, M and Kirk, TK. 1984. Lignin-depending Enzyme from *Panerochaete Chrysosporium*: Purification, Characterization and Catalytic Properties of A Unique H_2O_2-Oxygenase. Procnatl Acad Sci USA, **81**: 2280-2284

Taiwo and Oso. 2004. Influence of composting techniques on microbial succession, temperature pH in a composting municipal solid waste. *Africa journal of biotechnology* **3(4)**: 239-242

Wu, S.H. 1995. A study of the genus Phanerochaete (Aphyllophorales) with brown subicular hyphae. Mycotaxon **54**: 163-172

APPENDICES

Appendix 1

Average dry cell weight of *Phanerochaete chrysosporium*

Date	Average DCW (g)
15.8.08	0.0616
16.8.08	0.1497
17.8.08	0.18
18.8.08	0.2495
19.8.08	0.306
20.8.08	0.299
21.8.08	0.346
22.8.08	0.4945
23.8.08	0.3115
24.8.08	0.403
25.8.08	0.4015

Appendix 2:

Result of percentage lignin with different treatments (concentration of culture) and the Day After Inoculate (DAT).

DAT	Treatments			
	1	2	3	C
0	14.9900	14.9900	14.9900	14.9900
10	14.5066	14.3784	14.0215	13.7167
20	12.0167	10.9500	12.2167	13.0500
30	11.6794	12.8787	10.9948	14.0718
40	12.2378	11.4093	11.2944	14.0361
50	12.0126	11.9443	10.5696	13.3899
60	11.8603	10.3802	10.1399	14.0117
70	11.4641	10.4198	10.2924	14.0965
80	11.1798	10.183	9.9477	14.0966

Appendix 3:

Table 3: C/N ratio for different treatment with the timeframe 10 DAT until 60 DAT

DAT	Treatments			
	1	2	3	C
0	102.1270	102.1270	102.1270	102.1270
10	103.6158	97.9197	88.4652	103.8904
20	75.9553	64.5852	76.6015	76.4313
30	72.9842	80.1163	72.2055	91.2128
40	62.6747	58.5052	59.0843	84.5369
50	82.2322	62.2604	73.3461	89.7882
60	69.8581	59.8928	57.9187	96.2383
70	74.4826	68.9381	59.5833	81.2527
80	71.3736	58.9497	57.0114	80.6591

Appendix 4:

R-square value and the equation for all the treatments for lignin test.

Treatments	R^2	Equation
1	85.70	$Y= 14.8312 -0.1065 day +0.0008 day^2$
2	69.68	$Y= 14.7436 -0.1053 day +0.0006 day^2$
3	96.41	$Y= 15.9857 -0.1441 day +0.0011 day^2$
c	32.51	$Y= 14.4761 -0.0411 day +0.0005 day^2$

Appendix 5:

Quadratic equations for C/N ratio test and R^2 value.

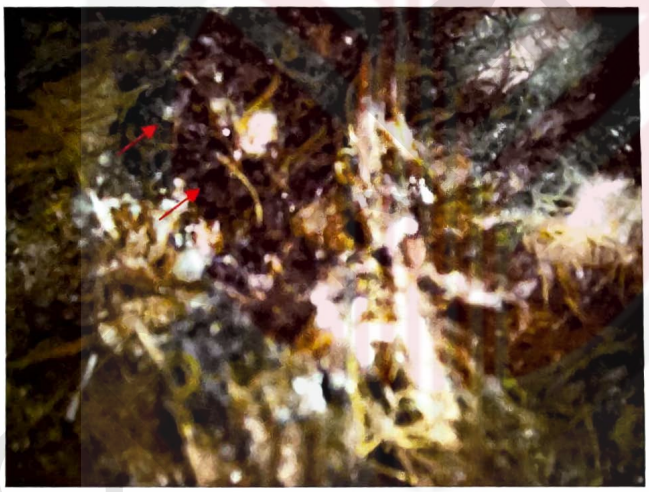
Treatments	R^2	Equation
1	71.05	$Y= 104.2681 -1.2613 day +0.0113 day^2$
2	78.33	$Y= 102.7431 -1.4235 day +0.0118 day^2$
3	89.54	$Y= 99.7148 -1.1636 day +0.0082 day^2$
c	32.78	$Y= 99.9529 -0.4220 day +0.0029 day^2$

Appendix 6:

Colonization of *Phanerochaete chrysosporium*

Appendix 7:

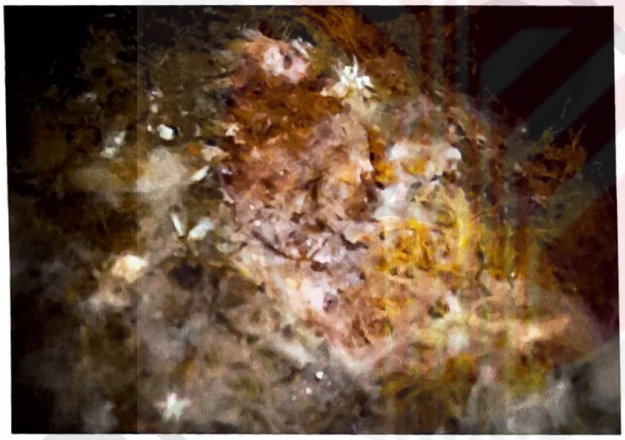
Colonization of *Monilia* sp. on EFB



Appendix 8:

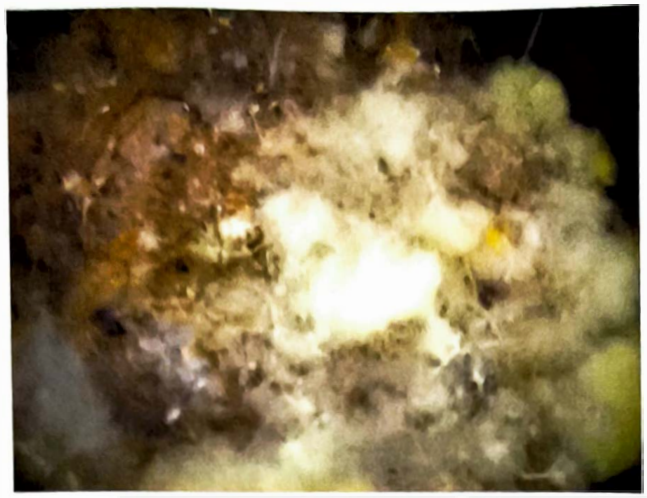
Colonization of Penicilium sp. on EFB

Appendix 9:



Colonization of *Phanerochaete chrysosporium* on 10 DAT

Appendix 10:

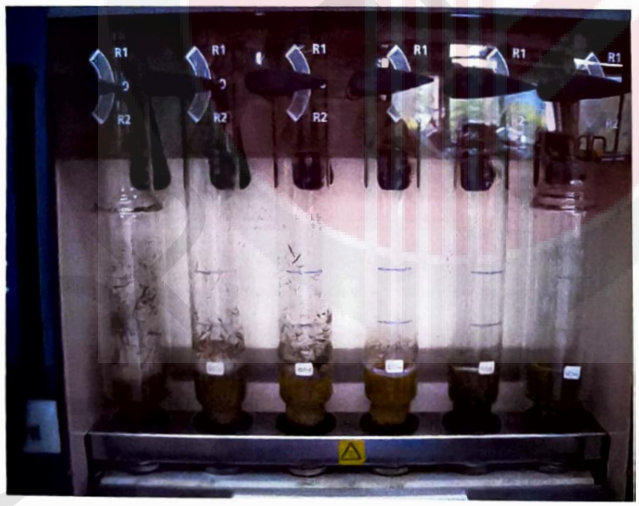


Colonization of *Phanerochaete chrysosporium* on 20 DAT

Appendix 11:

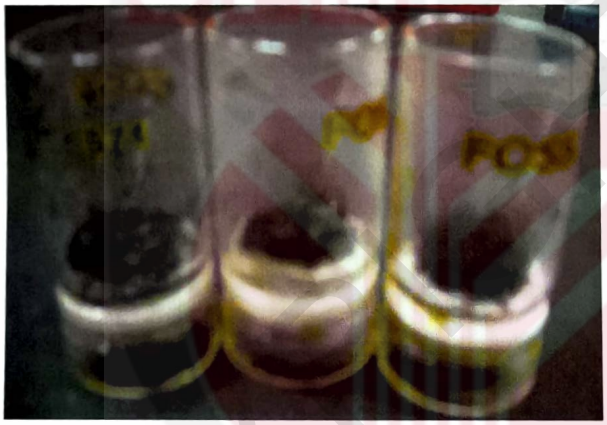
Procedure in lignin test:

11.1 Hot extraction



11.2 Cold extraction

11.3 Oven-dried



11.4 Ignite

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "**Biodegradation of oil palm EFB lignin by *Phanerochaete chrysosporium* (Burdsall and Eslyn)"** by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus and published in the form approved by the Faculty.

_____

KOK LAI WAI

Date: