



**UNIVERSITI PUTRA MALAYSIA**

***BIODEGRADATION OF OIL PALM FIBERS USING LOCALLY ISOLATED FUNGI (*Pycnoporus sanguineus*) THROUGH PLANT BIOMECHANICS APPROACH***

**FARAH NADIA BINTI OMAR**

**FK 2018 20**



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By

**FARAH NADIA BINTI OMAR**

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfilment of the requirement for the Degree of Doctor of Philosophy**

**November 2017**

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*Dedicated to my family  
For your endless love, support and encouragement*

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in  
fulfilment of the requirement for the degree of Doctor of Philosophy

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**November 2017**

**Chairman : Azhari Samsu Baharuddin, PhD**  
**Faculty : Engineering**

Utilization of lignocellulosic OPEFB fiber has tremendously seen in Malaysia due to the cellulose and hemicellulose content. Conversion of these biopolymers into valuable products remains a challenging task with the presence of the recalcitrant lignin and scattering silica bodies on the fiber surface. Therefore, this study investigates the mechanical behaviour of the complex lignocellulosic OPEFB fiber containing silica bodies and provide an in-depth understanding of the delignification of OPEFB by fungi for further bioconversion into wide range of biomaterial applications. The microstructure of silica bodies on OPEFB fiber surface was modelled using finite element method, based on the results obtained from scanning electron microscope (SEM) images, tensile tests and X-ray microtomography (micro-CT) images. Silica body geometry, possible anisotropy/ orthotropy, debonding between the interface of the silica body and fiber, fiber thickness and presence of vascular bundle in the OPEFB were investigated through 2D and 3D models and analysed by commercial finite element software, Abaqus.

In 2D model, silica bodies contribute on integrity or strength of the fiber, however, in the 3D model, the effect of silica bodies on the elasticity of the fiber was insignificant when the thickness of the fiber is larger than 0.2 mm. In the developed representative volume element (RVE) and micro-CT models, the simulation results show that the difference of the fiber model with and without silica bodies are larger under shear than compression and tension. However, in comparison to geometrical effect (silica bodies), lignin, cellulose, and hemicellulose components of the fiber are responsible for the complex mechanical and interface behavior of oil palm fibers.

Hence, screening and isolation of lignin degrading fungi for deconstruction of lignin polymer in OPEFB was carried out. About 47 isolated fungi collected from environmental samples with six fungi were able to decolorize selective agar media, indicating possible presence of lignin-degrading enzymes; laccase and peroxidases. The highest producer of ligninolytic enzymes was identified as *Pycnoporus sanguineus* which able to utilize raw OPEFB fiber through solid state fermentation (SSF) with an increment of 1.37 folds of ligninolytic enzymes production as compared to submerged fermentation (SmF). Optimization study of different substrate pre-treatments (sodium hydroxide, Soxhlet extraction), incubation temperatures (20-40°C), ABTS concentrations (0-4%) and substrate amounts (3-15 g) on ligninolytic enzymes production was carried out. Results showed that the optimum conditions for *P. sanguineus* to produce highest laccase (15.49 U/g) with Klason lignin removal at 7.11% were using extractive-free OPEFB fiber, incubation temperature at 30°C, supplemented with 4 mM of ABTS and with 10 g of substrate loading size. Effectiveness of *P. sanguineus* for OPEFB degradation was further evaluated with the different ratio of fiber, fungi and palm oil mill effluent (POME) sludge as inoculum.

The relationship between structural OPEFB fiber degradation and delignification process by *P. sanguineus* was studied through tensile testing data, enzymatic and lignin component data, and micro-CT images. The highest total lignin loss (35.81%) and total phenolic content produced (78.03%) was determined at a condition ratio of fiber to fungi (60:40), yielding of laccase and MnP of 0.18 and 0.02, respectively while production rate of laccase and MnP were 0.98 U/g/d and 0.11 U/g/d, respectively. Micro-CT results revealed that the delignification process damaged the fiber based on the volume reduction data where 14.11% of volume reduction was observed with treated fiber while 11.21% volume reduction was achieved with untreated fiber. It is suggested that *P. sanguineus* could be a potential lignin degrader of OPEFB fiber before being manipulated for other valuable products production.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**PENGURAIAN FIBER KELAPA SAWIT MENGGUNAKAN KULAT YANG  
DIPENCILKAN KAWASAN SETEMPAT (*Pycnoporus sanguineus*)  
MELALUI PENDEKATAN BIOMEKANIK TUMBUHAN**

Oleh

**FARAH NADIA BINTI OMAR**

**November 2017**

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Penggunaan sisa lignoselulosa gentian tandan kelapa sawit kosong (OPEFB) sangat banyak di Malaysia kerana kandungan selulosa dan hemiselulosa. Penukaran biopolimer ini kepada produk bernilai telah terhalang oleh kehadiran lignin dan partikel silika di permukaan gentian. Oleh itu, kajian penyelidikan tingkah laku mekanikal gentian OPEFB kompleks yang mengandungi partikel silika dijalankan dan memberi kefahaman proses penguraian lignin dalam OPEFB oleh kulat untuk seterusnya melalui proses biopenukaran kepada pelbagai jenis aplikasi biomaterial. Mikrostruktur partikel silika di gentian OPEFB telah disimulasi dengan menggunakan kaedah '*finite element*' berdasarkan keputusan dari gambar imbasan mikroskop elektron (IME), ujian tegangan dan mikro-tomografi (mikro-CT). Geometri partikel silika, kebarangkalian *anisotrophy/ orthotropy*, peleraian ikatan antara permukaan partikel silika dan gentian, ketebalan gentian, dan kehadiran bukaan "*vascular bundle*" telah dikaji menggunakan model 2D dan 3D didalam perisian komersil "*finite element*", Abaqus.

Dalam model 2D, partikel silika menyumbang kepada integriti gentian, manakala dalam model 3D kesan partikel silika kepada kekenyalan gentian tidak signifikan pada ketebalan gentian melebihi 0.2 mm. Dalam model '*representative volume element*' (RVE) dan model mikro-CT, keputusan simulasi menunjukkan perbezaan model gentian dengan dan tanpa partikel silika adalah besar di bawah mod ricih berbanding mampatan dan tegangan. Walau bagaimanapun, jika dibanding dengan kesan geometri (partikel silika), komponen lignin, selulosa dan hemiselulosa bertanggungjawab kepada tingkahlaku mekanikal dan antara permukaan yang kompleks pada gentian kelapa sawit.

Oleh itu, penyaringan dan pemencilan kulat pengurai lignin dijalankan untuk menguraikan polimer lignin dalam OPEFB. Sebanyak 47 kulat dikutip dari kawasan sekitar dan enam kulat mampu menyah-warna agar media saringan menunjukkan kehadiran enzim pengurai lignin; *laccase* dan peroksida. Kulat yang menghasilkan enzim tertinggi dipilih dan dikenali sebagai *Pycnoporus sanguineus* yang mampu menguraikan gentian OPEFB dan menghasilkan enzim yang tinggi melalui fermentasi fasa pepejal iaitu peningkatan sebanyak 1.37 kali ganda berbanding dengan fermentasi fasa terendam. Kajian pengoptimuman menggunakan pelbagai pra-rawatan substrat (natrium hidroksida, pengestrakan Soxhlet), suhu pengeraman (20-40°), kepekatan ABTS (0-4%) dan jumlah substrat (3-15 g) kepada penghasilan enzim ligninolitik dijalankan. Keputusan menunjukkan keadaan optimum penghasilan enzim *laccase* tertinggi (15.49 U/g) dengan penyingkiran Klason lignin sebanyak 7.11% oleh *P. sanguineus* adalah menggunakan gentian OPEFB bebas ekstraktif, suhu pengeraman 30°C, dibekalkan 4 mM ABTS dan dengan menggunakan 10 g substrat.

Kecekapan *P.sanguineus* untuk menguraikan gentian OPEFB dikaji lebih mendalam dengan menggunakan pelbagai nisbah gentian, kulat dan enap cemar dari sisa efluen kilang sawit (POME) sebagai inokulum. Hubungan antara penguraian struktur gentian OPEFB dan proses penguraian lignin oleh *P. sanguineus* dikaji melalui data ujian tegangan, enzim dan data komponen lignin, dan imej mikro-CT. Penyingkiran tertinggi lignin (35.81%) dan jumlah kandungan fenolik yang terhasil (78.03%) dikenalpasti pada keadaan nisbah gentian kepada kulat (60:40) menghasilkan *laccase* dan MnP masing-masing 0.18 dan 0.02, manakala kadar penghasilan *laccase* dan MnP masing-masing adalah 0.98 U/g/d dan 0.11 U/g/d. Keputusan mikro-CT menunjukkan proses penguraian lignin telah merosakkan gentian berdasarkan data pengurangan isipadu di mana 14.11% pengurangan isipadu telah diperolehi dari gentian terawat manakala 11.21% pengurangan isipadu diperolehi dari gentian yang tidak terawat. Ini membuktikan *P. sanguineus* berpotensi untuk menjadi kulat pengurai lignin gentian OPEFB sebelum gentian ini dimanipulasi untuk kegunaan pembuatan produk berharga.



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I certify that a Thesis Examination Committee has met on 20 November 2017 to conduct the final examination of Farah Nadia binti Omar on her thesis entitled "Biodegradation of Oil Palm Fibers using Locally Isolated Fungi (*Pycnoporus sanguineus*) through Plant Biomechanics Approach" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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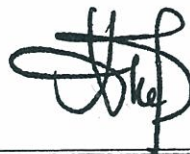
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## LIST OF ABBREVIATIONS

ABTS	2,2'-azinobis-(3-ethyl- )benzothiazoline-6-sulphonate
MnP	Manganese peroxidase
LiP	Lignin peroxidase
OPEFB	Oil palm empty fruit bunch
POME	Palm oil mill effluent
SSF	Solid state fermentation
SmF	Submerged fermentation
FEA	Finite element analysis
TPC	Total phenolic compound
KL	Klason lignin
Micro-CT	Micro computed tomography
Si	Silica bodies
SiO <sub>2</sub>	Silica oxide
H <sub>2</sub> SO <sub>4</sub>	Sulphuric acid
NaOH	Sodium hydroxide
RVE	Representative volume element



## LIST OF SYMBOLS

$^{\circ}\text{C}$	degree celcius
$\text{g/L}$	gram per litre
$\%$	percentage
$\text{rev/min}$	revolutions per minute
$\beta$	beta
$\text{O}_2$	oxygen
$\text{CO}_2$	carbon dioxide
$\text{H}_2\text{O}$	water
$\text{h}$	hour
$\text{min}$	minutes
$\mu\text{m}$	micrometer
$\text{mm}$	milimeter
$\text{Pa}$	Pascal
$\text{U/g}$	unit per gram

## CHAPTER 1

### INTRODUCTION

#### 1.1 Lignin degradation of OPEFB

Malaysia is blessed with valuable oil palm tree plantation where it has been as one of the major exporter of palm oil in the world where total oil palm planted area reported was 5.39 million hectares where it covered more than 73% of agricultural land that makes oil palm as a potential renewable biomass to be exploited for better use (Awaluddin et al., 2015). About 95.38 million tonnes of fresh fruit oil palm bunches were processed and the estimation of oil palm biomass generated from its process was 40.55 million tonnes (Loh, 2017). Oil palm empty fruit bunch (OPEFB) alone were contribute about 7.34 million tonnes where current practice of OPEFB manipulation involves incineration of OPEFB to produce bunch ash and further applied as soil conditioner and soil fertilizer and straight dumping on the field as soil mulching agent (Zainudin et al., 2012).

Some researchers have maximized the usage of OPEFB fibers as part of biocomposite materials in construction industries (Hassan et al., 2010) while some researchers use OPEFB as the main feedstock in bioconversion process into value added products such as fermentable sugars (Abu Bakar et al., 2012; Zainudin et al., 2012), biofuel (Sudiyani and Hermiati, 2010; Nieves et al., 2011), organic acids (Akhtar et al., 2014) and others. The cellulose and hemicelluloses is the most intriguing materials in the utilizing of the OPEFB as potential feedstock for the production of biofuel (Jeon et al., 2014; Kim and Kim, 2013), biochemicals (Reeb et al., 2014; Katinonkul et al., 2012). However, the utilization of cellulose and hemicellulose is hindered with the high content of lignin.

Lignin makes up of 15-40% of the dry matter of woody plant gives the rigidity and strength to cell walls and resilient towards degradation (Naseem et al., 2016). It is a highly stable biopolymer made of three cross-linked phenylpropane units and it present interlocking the cellulose and hemicelluloses polymers with strong ether bonds (C-O-C) and normal hydrogen bonds (C-C). Degradation of lignin in lignocellulosic biomass has been reported using various methods; 1) physical pretreatment such as by high pressure steam (Baharuddin et al., 2013); 2) chemical pretreatments by sodium hydroxide (Palamae et al., 2017; Zulkiple et al., 2016; Muryanto et al., 2015); and 3) biological pretreatment by fungal and ligninolytic enzyme. A number of fungi (white and brown rot) and some bacteria are effective as a lignin

degrader due to their ability to produce lignolytic enzymes. Lignolytic enzymes can be categorized as peroxidases (lignin peroxidase, manganese peroxidase, versatile peroxidase) and oxidative enzymes (laccase) could depolymerize the lignin polymers into smaller compounds through oxidative and electron transfer process (Bugg and Rahmanpour et al., 2015). Lignin can be precipitated as droplets on the surface of cellulose and hemicellulose, making them less accessible to enzymes attack. Hence, lignin removal is crucial in further utilization of cellulose and hemicellulose as it tends to adsorb the hydrolytic enzymes more easily and consequently reduce the effectiveness of the hydrolytic enzymes to access the cellulose and hemicellulose sites (Mishra et al., 2017; Li et al., 2009).

In addition, OPEFB fiber has some distinct features on its fiber surface, where random scattering protrusion silica bodies are found. These silica bodies are embedded half-way through the fiber surface, and it is made of silica oxide ( $\text{SiO}_2$ ). It has been reported that silica bodies play a big role in providing mechanical support, strength and rigidity of the plant (Neethirajan et al., 2009; Ma and Yamaji, 2006). The presence of silica bodies in plant has been numerously studied especially on fermentable sugar production (Nurul Hazirah et al., 2016; Shamsudin et al., 2012). However, to this date, there are limited studies investigates the role of silica bodies in providing strength and rigidity towards plant particularly for oil palm tree. This issue, however, will be addressed and explained in this thesis focusing the presence of silica bodies on OPEFB fiber.

## **1.2 Oil palm fiber biomechanics**

Micromechanics is a study of materials by understanding the interaction between constituent materials at microscopic level. Theoretically, it helps to compute and predict the behavior, properties and failure mechanisms of the materials. The main idea of micromechanics is to replace the original material with imaginary microscopic material so that the analysis of the original material could be understand and simplified (Yu, 2016). Micromechanics study have been used widely in building of materials as such each properties and behavior of the building material will be simulated and the overall performance of the material will be evaluated. A simple way to witness the micromechanics study is when natural fiber is used as reinforcement to other composite materials. The behavior of fibers will be simulated at various conditions and barriers and success and failure mechanisms of the overall materials will be evaluated. The micromechanics study of natural fibers like woody and plant cells have been well established (Hayot et al., 2012; Burgert and Dunlop, 2011). However, very limited studies are available in the literature that involves the study of the silica bodies on OPEFB fiber and their contributions to the mechanical behavior of OPEFB fiber. Only recently, there

are studies on micromechanics of oil palm fiber performed by a research group in UPM. Hanipah et al. (2016) and Xiang et al. (2015) utilized numerical approach of micromechanics of oil palm fiber and revealed the viscoelastic properties as evident from stress relaxation curves. Likewise, in another study conducted by Wang et al. (2014), finite element analyses study of royal palm at tissue level was performed where cellular structure of the palm was reconstructed with polynomial area weighted tessellation model in order to simulate the vascular tissue behavior and area ratio and parameter ratio of adjacent cells were calculated and compared.

Deeper understanding and investigations of oil palm fiber cellular and tissue structure could be performed with both numerical and analytical micromechanics approaches. The behavior, properties, response and failure mechanisms could be understood and explored. This is essential especially if the utilization of oil palm fiber in composites or any other purposes are required if one aims to utilize it in its most possible way.

### **1.3 Problem statements**

Micromechanics study of silica bodies on OPEFB fiber and its contribution on the fiber integrity has yet been studied, where this information could provide the fundamental background on its behavior, properties as well as other mechanisms. Up to date, there are no detailed models available that discuss the mechanics of the oil palm fiber specifically with silica bodies. Hence, development of model through numerical and analytical methods of micromechanics is essential to predict the behavior of the fiber by providing an in depth understanding of the effects of silica bodies physiologies and structures towards the fiber strength and therefore, may contribute to the decision of the degree of pretreatment for silica bodies removal needed especially in industries with natural fibers utilization like biocomposites and fiber bioconversion process. By knowing this information, it would minimize the energy, time and money spent on the silica bodies removal treatment process. This studies also provides deeper understanding of silica bodies and role of OPEFB fiber as a bioresources material, and the models could also be used for other natural fiber modelling as well.

Unnecessary compound and by-products formation from lignin degradation could be eliminated through biological treatments as it is substrate specific and involve no harsh chemicals. However, involvement of biological treatments usually lead to delayed response and achievements. Therefore, microbes with high production of lignin-degrading enzymes is preferred for lignin degradation to occur effectively. Removal of lignin is important as such it intensely being incorporated into emergent lignocellulose biorefineries.

Additionally, the mechanism of structural degradation between fungi and OPEFB during lignin degradation process is an intriguing subject of research and up to this date, there are no comprehensive studies conducted and discussed in the literatures. The OPEFB biodegradation studies is important not only to solve the solid waste disposal in Malaysia, but also to prepare the fiber for holocellulose utilization which would later on would be greatly useful for numerous valuable products generations such as biosugars, carboxymethyl cellulose etc.

To fill in the gaps mentioned above, a study on micromechanics study on silica bodies on OPEFB fiber was conducted and the degradation of lignin by fungi was evaluated through structural and physico-chemical data analyses. The objectives of this study therefore are:

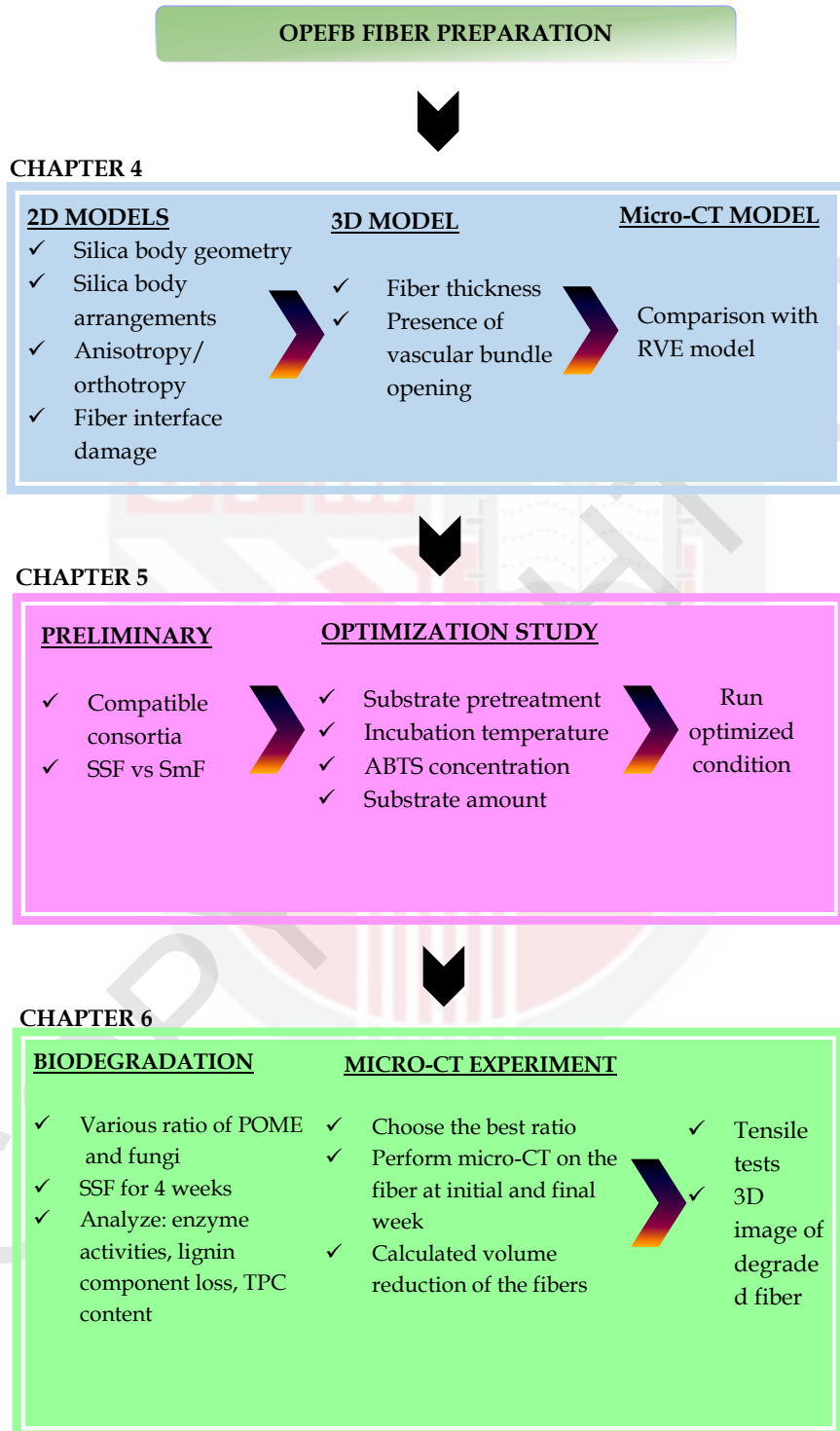
1. To determine the effect of silica bodies on OPEFB fiber integrity through solid mechanics approach.
2. To optimize the environmental conditions for laccase production from local isolated white rot fungi, *P. sanguineus*.
3. To study the relationship between structural and physico-chemical behavior of OPEFB after degradation process by *P.sanguineus*.

#### **1.4 Scope of research and thesis structure**

This study is principally concerned about the micromechanics study of OPEFB and its relationship with biodegradation of OPEFB fiber by local isolated white rot fungi. During this research, and in depth study has been performed in studying the feedstock, OPEFB fiber in terms of the micromechanics behavior and modeling of the silica bodies onto the surfaces of the fiber. A 2D model was adopted to explore the effect of silica bodies' arrangement and spiked geometry of silica bodies. 3D models were later developed in order to further investigate with the contribution of silica bodies towards fiber integrity. On the other hand, white rot fungi was isolated and the ability to perform the degradation on OPEFB was evaluated. The performance of the fungi was evaluated in both submerged and solid state fermentation. Based on the lignocellulosic content and phenol content, the fungi show some potential in depolymerizing the lignocellulosic content in OPEFB. Finally, the relationship between structural and physico-chemical behavior of degraded OPEFB fibers were reported and discussed in detail.

In this thesis, there are 5 chapters will be included in which each chapter will explain independent topics. In the Chapter 1, a brief introduction of the overall research was written together with objectives of the study and the scope of the research. In the Chapter 2, extensive literature review was written covering

current available knowledge on the micromechanics of natural fiber OPEFB, fermentation strategies and biodegradation of lignin process through fungal degradation. In Chapter 3, the first objective of the study was elaborated in which to study the micromechanics modeling of the silica bodies on the OPEFB fibers where constitutive material behavior (stress-strain), 2D and 3D modelling were performed in order to investigate the oil palm fiber behavior. In the Chapter 4, the second objective of the research was explained in which to explore the potential of the local isolate white rotting fungi for OPEFB biodegradation in solid state fermentation. In the Chapter 5, the third and final objective was well intricate in which to study the utilization of the micro computed tomography in the microstructure behavior of the degraded OPEFB fibers and the relationship between structural and physico-chemical behavior of degraded OPEFB was discussed. In the final chapter, chapter 6, final conclusions and some of recommendations were mentioned. Appendix and references used in this entire study was listed at the back of the thesis. The overview of the experimental design reported in the thesis is provided in Figure 1.1.



**Figure 1.1: Overview of the overall experimental design**

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