COMPARISON OF THERMAL AND BIOLOGICAL PRETREATMENT OF OIL PALM EMPTY FRUIT BUNCH FOR LIGNIN DEGRADATION

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**BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERSITY MALAYSIA SABAH**

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I hereby declared that this thesis is based on my own work except for quotations, excerpts, citations and equations which have been duly acknowledged. I also declared of no part of this thesis has been previously or concurrently submitted for a master at any other university.

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CERTIFICATION

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ABSTRACT

Oil palm empty fruit bunches (EFB) are recoverable lignocellulosic biomass serving as feedstock for biofuel production. The major hurdle in producing biofuel from biomass is the abundance of embedded recalcitrant lignin. Pretreatment is a key step to increase the accessibility of enzymes to fermentable sugars in EFB. In this study, thermal and biological pretreatment methods were studied and compared for the degradation of lignin in EFB structure. The main objectives of this study were to optimize the operating conditions for pretreatment and characterize the effect on lignin degradations on EFB. The thermal treatment was conducted at different temperatures (150 °C to 210 °C), treatment durations (30 min–120 min) and EFB particle sizes (1 mm–10 mm). The physicochemical changes were observed using scanning electron microscope (SEM), energy dispersive X-ray (EDX) and Fourier transform infrared (FTIR) analyses. Meanwhile, the biological treatment was performed by using locally isolated lignin degraders from soils and decaying wood samples. The lignolytic ability of the isolates was screened using remazol brilliant blue anthraquinone R (RBBR) agar media and assessed via qualitative and quantitative enzymatic assays. Subsequently, the lignin degradation performance of both treatment methods was further evaluated using Klason's lignin analysis. For the thermal treatment, the characterization studies revealed some disruptions occurred on the EFB structure with removal of silica bodies and other impurities. In addition, a remarkable change on the EFB elemental contents and its functional groups was observed. Elemental analysis shows increasing of carbon compound which might indicate the decomposition of compound. Significant reduction in peak of 1225 nm and 1445 nm was observed in EFB samples treated at high temperature and using smaller EFB particles size using FTIR analysis. Smaller EFB particle sizes (1mm) were found exhibited higher lignin degradation probably due to larger surface area for bioreaction. Applying a longer duration of treatment (120 min) and higher temperature (210 °C) was enhanced lignin degradation up to 60.29 %. For the biological treatment, six fungi and eight bacterial strains were successfully isolated and screened. Both qualitative and quantitative enzymatic studies showed all isolates majorly secreted lignin peroxidase as compared to manganese peroxidase and laccase lignolytic enzymes. Apparently, isolates PWM2 (226.40 U/L) and NWM6 (201.07 U/L) show high LiP activity. Fungi isolates demonstrated superior lignolytic ability than bacterial isolates as evident by the significant RBBR color decolorization. Fungal isolates, denoted as PWM2 and PWC3 indicated a 56.28 % and 51.19 % lignin degradation respectively, whereby the highest degradation by bacterial isolate (NWM5) was determined at 52.24 %. The findings of this study show both thermal and biological treatment methods offered comparable lignin degradation ability. While the thermal treatment could be done fast, the process however required an elevated temperature (above 150 ^{o} C). On the other hand, despite a longer duration for pretreatment, the biological method could be carried out within 30 – 37 ^{o}C. These studies provide insightful findings and guideline in pretreatment of lignocellulosic materials that beneficial for green and sustainable production of biomass-derived biofuel.

ABSTRAK

PERBANDINGAN ANTARA PRA-RAWATAN TERMAL DENGAN BIOLOGI KE ATAS DEGRADASI LIGNIN PADA TANDAN KOSONG KELAPA SAWIT

Tandan kosong kelapa sawit (EFB) adalah biojisim lignoselulosa yang boleh diperolehi dan berfungsi sebagai bahan mentah untuk penghasilan biofuel. Masalah utama dalam penghasilan biofuel daripada biojisim adalah kehadiran lignin yang rekalsitran dalam biojisim. Prarawatan adalah langkah pertama untuk meningkatkan kebolehcapaian enzim kepada gula yang boleh difermentasi dalam EFB. Dalam kajian ini, kaedah prarawatan termal dan biologi telah dikaji dan dibandingkan untuk melihat kesan degradasi lignin ke atas struktur EFB. Objektif utama kajian ini adalah untuk mengoptimumkan keadaan operasi prarawatan untuk degradasi lignin dan mencirikan kesannya terhadap struktur EFB. Rawatan haba dijalankan pada suhu yang berbeza (150 °C hingga 210 °C), dengan tempoh rawatan (30 min–120 min) dan saiz EFB (1 mm–10 mm). Perubahan fizikokimia diperhatikan menggunakan pengimbasan mikroskop elektron (SEM), analisis sinar-X penyebaran tenaga (EDX) dan analisis inframerah transformasi Fourier (FTIR). Sementara itu, rawatan biologi dilakukan dengan menggunakan mikroorganisma penghancur lignin yang diisolat daripada tanah dan sampel kayu yang mereput. Keupayaan lignolitik bagi setiap isolat disaring menggunakan media agar biru antrakuinon R (RBBR) remazol dan dinilai melalui ujian enzimatik kualitatif dan kuantitatif. Kemudian, prestasi degradasi lignin bagi kedua-dua kaedah rawatan dinilai dengan menggunakan analisis Klason lignin. Untuk rawatan haba, kajian pencirian menunjukkan beberapa perubahan berlaku pada struktur EFB seperti penyingkiran silika dan elemen lain selepas prarawatan dilakukan. Di samping itu, perubahan pada kandungan unsur EFB dan kumpulan berfungsi telah diperhatikan. Analisis unsur menunjukkan peningkatan sebatian karbon yang mungkin menunjukkan penguraian sebatian. Pengurangan ketara dalam puncak 1225 nm dan 1445 nm telah diperhatikan dalam sampel EFB yang dirawat pada suhu tinggi (210 °C) dengan menggunakan saiz EFB yang lebih kecil (1mm) menggunakan analisis FTIR. Saiz EFB yang lebih kecil (1mm) didapati menunjukkan degradasi lignin yang lebih tinggi disebabkan oleh luas permukaan yang lebih besar untuk bioreaksi. Penggunaan tempoh rawatan yang lebih lama (120 minit) pada suhu yang lebih *tinggi (210 °C) meningkatkan kadar degradasi lignin sehingga 60.29%. Untuk rawatan biologi, enam kulat dan lapan strain bakteria berjaya diasingkan. Kedua-dua kajian enzimatik kualitatif dan kuantitatif menunjukkan semua isolat menghasilkan lignin peroksidase lebih banyak berbanding dengan mangan peroksidase dan enzim laccase. Isolat PWM2 (226.40 U/L) dan NWM6 (201.07 U/L) menunjukkan aktiviti LiP yang tinggi. Kulat menunjukkan keupayaan lignolitik yang lebih baik daripada bakteria seperti yang ditunjukkan oleh penyahwarnaan warna RBBR yang ketara. Kulat, PWM2 dan PWC3 masing-masing menunjukkan 56.28 % dan 51.19 % kadar degradasi lignin, manakala kadar degradasi tertinggi oleh bakteria (NWM5) adalah pada 52.24 %. Dapatan kajian ini menunjukkan kedua-dua kaedah rawatan haba dan biologi menawarkan keupayaan degradasi lignin yang setanding. Walaupun rawatan terma boleh dilakukan dengan cepat, bagaimanapun memerlukan suhu tinggi (melebihi 150 °C). Sebaliknya, walaupun tempoh prarawatan dalam kaedah biologi lebih lama, namun boleh dilakukan pada suhu 30 °C – 37 °C. Kajian ini memberikan penemuan dan garis panduan yang bernas dalam prarawatan bahan lignoselulosa yang bermanfaat untuk pengeluaran biobahan api biojisim yang hijau dan mampan.*

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&	-	And
:	-	Ratio
α	-	Alpha
β	-	Beta
γ	-	Gamma
+	-	Positive
-	-	Negative
^{0}C	-	Degree Celcius
%	-	Percentage
nm	-	Registered trademark
®	-	Nanometer
cm	-	Centimetre
µl	-	Microlitre
µg	-	Microgram
g	-	Gram
m	-	Meter
min	-	Minute
M	-	Molar
mL	-	millilitre
mg	-	milligram
h	-	Hour
rpm	-	Revolution per minute
U/µl	-	Unit per microlitre
U/L	-	Unit per litre
psi	-	Pounds per square inch
[O]	-	Oxidation

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Malaysia is a second world palm oil producer with oil palm production estimated at around 19.14 million tons in 2020 which contributes to total 31.4 % of total oil and fat products worldwide (MPOC, 2021). Although the total production of oil palm was plummet in 2020 (19.14 million tons) compared to previous year (19.86 million tons) due to Pandemic Covid-19 (MPOC, 2021), the average annual production of oil palm in recent five years shows a positive trends which indicates the efficacious development of palm oil industry in this country that could strengthen the economic value in agriculture as well as the average income of the society. However, despite their benefits, there are several residues are associated with improper disposals such as oil palm trunk (OPT), an oil palm frond (OPF), oil palm empty fruit bunch (EFB), palm pressed fibres (PPF), palm shells and palm oil mill effluent (POME) which commonly burnt to the air or left as residues and subsequently led to another environmental problem and health issues (Novianti *et al.,* 2014). The accumulations of these residues arise from the increasing number of agricultural, forestry, municipal and other human activities that resulted in an enormous amount of waste being dumped or burnt into the surrounding and eventually cause health problems and environmental issues. Therefore, proper management of these wastes is highly pursued (Adejumo & Adebiyi, 2020).

Lignocellulosic biomass is one of the most abundant organic bio-resource and renewable carbon compounds that could be found worldwide. In accordance to Ravindran & Jaiswal, (2016), due to its large quantities, easily available and fairly distributed around the world, it becomes the cheapest source of carbohydrates which can be utilized for the production of valuable products such as biofuels, biofertilizers and enzyme production. As fossil fuels and oil gas has been depleted, the use of lignocellulosic biomass has been seen as one of the promising alternative sources for sustainable biofuels production such as bio-ethanol to meet the increasing demand for energy consumption (Ahorsu, Medina & Constanti, 2018). Lignocellulosic biomass is composed of polymers such as cellulose, hemicellulose and lignin which are strongly interconnected with each other which contribute to their recalcitrance properties against enzymatic hydrolysis, in turn, lowering the enzyme digestibility of these materials (Sun *et al.,* 2016). Like other lignocellulosic biomass, EFB contains about 23% - 65% of cellulose, 20.58% - 33.52% of hemicellulose and 14.1% - 30.45% of lignin which made up from the phenyl propane unit with aromatic backbone and acts as the protective layer of the plant (Chang, 2014; Harmsen *et al*., 2010). Both cellulose and hemicellulose can be converted into glucose or fermented to produce ethanol. However, the strong covalent or non-covalent bonds, as well as the cross-linking between these three polymers, make it impossible to separate individual polymers for direct fermentation (Rabemanolontsoa & Saka, 2016; Gellerstedt, 2015).

Therefore, eradication of lignin through delignification is necessary before EFB can be used as substrate or feedstock. According to Sun *et al.* (2016), pretreatment able to overcome the recalcitrance properties of the lignocellulosic biomass by altering the chemical composition and structural properties of the lignocellulosic matrix that will subsequently enhance the enzymatic accessibility. In addition to that, Zheng *et al*. (2014) stated that the optimal condition and method of pretreatment can be obtained regarding the types of lignocellulose present in the biomass. Apart from that, several factors might influence the efficiency of the pretreatment and should be considered before any pretreatment method is chosen (Ravindran & Jaiswal, 2016; Zheng *et al.,* 2014). In brief, pretreatment can be applied on EFB to modify the lignocellulosic matric components to enhance the enzyme accessibility before they can be used as sugar substrate or feedstock in the fermentation process.

In order to break the polymeric network of lignin, several methods have been employed to disrupt the lignocellulosic structure of biomass including chemical, biological and physical pretreatment. The usage of alkaline or acid chemicals in chemical pretreatment, unfortunately, causes toxic accumulation and environmental damages (Kang *et al.,* 2020). Physical pretreatment, on the other hand, has received great interest as an environmentally friendly technology for not involving hazardous chemicals (Montgomery and Bochman, 2014). Besides, the technology is an easy technique to perform at short treatment duration under controlled thermal conditions. The most common technique used in physical pretreatment is such as grinding, chipping, milling as well as drying of lignocellulose biomass (Kumar & Sharma, 2017). The entire process of making a bulk amount of biomass into their reduced particle size required less chemical, hence less chemical waste is produced (Basile & Delana, 2019). According to Norfadhilah *et al.* (2019), most of the thermal treatment was focused on developing bio-ethanol and bio-fuel from biomass especially wastes from palm oil plantations such as EFB. Recent technology has come up with more advanced physical pretreatment which only employed liquid hot water without a chemical catalyst to disrupt the structure of lignocellulose biomass (Yao *et al.,* 2018). However, the thermal treatment needs a higher pretreatment temperature which leads to high energy consumption and requires high-end equipment with necessary skilled operating officers (Sidhu *et al.,* 2016).

Meanwhile, biological pretreatment offers an environmentally friendly pathway by utilizing bacterial and fungal enzymes for pretreatment of lignocellulosic biomass with low energy requirement than the physical approach and low formation of toxic material (Narayanaswamy *et al.,* 2013). Biological pretreatment can be employed using enzymes or lignin-modifying enzymes secreted by microorganisms such as bacteria and fungi which involve minimal energy input and often rely on the incubation of the substrate with the selected microorganism to modify the composition of biomass (Chen *et al.*, 2010; Montgomery and Bochman, 2014). Microorganisms such as white rot fungi, brown rot fungi, yeast and bacteria have been reported as lignin degraders which commonly can be isolated from the environment. These microorganisms are capable to secrete ligninolytic enzymes such as manganese peroxidase, lignin peroxidase, laccase, versatile peroxidase and other oxidative peroxidases, which allowed the conversion of polymers into their monomeric unit (Falade *et al.,* 2017; Casciello *et al.*, 2017). Microorganisms such as *Ceriporia lancerata*, *Cyathus stercolerus*, *Phanerochaeta chrysoporium*, *Ceriporiopsis subvermispora* and *Pleorotus ostreaus* can produce lignolytic enzymes like manganese peroxidase, laccase and lignin peroxidase which allows delignification of various type of lignocellulose biomass (Begum *et al.,* 2020). However, the expensive processes for enzyme production and purification have been recognized as the major hurdle for large scale application (Chauhan, 2020).

Thermal and biological pretreatment methods are both considerably a green technology with regards to the absence of chemicals during pretreatment. Despite their respective advantages, nevertheless, both technologies inherit some disadvantages which may raise concern among researchers and technologists. There is little reported literature on the comparison of these pretreatment methods in lignin degradation of EFB. Therefore, in this study, the effectiveness of the physical pretreatment at optimized operating conditions and biological pretreatment by using the locally isolated lignin degraders for lignin degradation of EFB were evaluated and compared.

The important parameters in both techniques were investigated. The effect of elevated temperature ranging from 150 ^{o}C to 210 ^{o}C, effect of pressure, pretreatment duration (30 min – 120 min) size of a particle subjected for thermal treatment (1mm – 10mm) were employed to maximize lignin degradation. The changes that occurred during the treatment were observed using a scanning electron microscope (SEM), Energy Dispersive Xray (EDX) and Fourier transform infrared (FTIR) analyses. Meanwhile, best lignin degraders from Sabah local diversity were isolated, screened and characterized. Their lignin-degrading ability was qualitatively and quantitatively evaluated through series assays such as remazol brilliant blue R (RBBR) assays, manganese peroxidase assays, lignin peroxidase assays and laccase assays. Finally, the lignin degradation ability of the thermally-treated EFB under optimized pretreatment condition and biologically-