UNIVERSITI PUTRA MALAYSIA

BIOLOGICAL PRETREATMENT OF OIL PALM EMPTY FRUIT BUNCH BY A LOCAL ISOLATE OF *Schizophyllum commune* ENN1 FOR PRODUCTION OF FERMENTABLE SUGARS

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By

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Oil palm industry in Malaysia plays a major role in national socio-economic development. In line with the expansion of the industry, oil palm biomass is generated abundantly from the plantations and mills. Oil palm empty fruit bunch (OPEFB) is the most abundant lignocellulosic biomass generated from palm oil mill, composed of 25-44% cellulose, 25-28% hemicellulose and 19-27% lignin. The cellulose and hemicellulose components can be converted into fermentable sugars after being pretreated by either physical, chemical, physicochemical, biological or combination of these pretreatment methods. Currently, physicochemical pretreatment is the most common pretreatment method used to pretreat and convert OPEFB into fermentable sugars. However, this type of pretreatment utilised chemicals that lead to environmental issues and high operational cost. Therefore, biological pretreatment by fungi has been considered as an alternative to pretreat OPEFB as it is environmental friendly and requires low cost for the process. The first objective in this study was to evaluate the feasibility of *Schizophyllum commune* ENN1 in removing lignin of OPEFB through biological pretreatment. The second objective is to investigate the significant parameters affecting the biological pretreatment of OPEFB by *S. commune* ENN1 for fermentable sugars production through one-factor-at-a-time (OFAT) method.

The biological pretreatment by locally isolated fungus identified as *Schizophyllum commune* ENN1 was conducted using unwashed OPEFB without supplemented with nutrients or any moistening agents. The lignocellulosic compositional analysis showed that 53.8% of lignin was removed after biological pretreatment using *S. commune* ENN1 compared to 38.6% of lignin removal using *P. chrysosporium* UIA. The determination of residual oil content showed that *S. commune* ENN1 was able to reduce the residual oil content by 85.3% while maintaining the moisture content in the range of 51-40%. The effect of incubation time (7-28 days), temperature (25-40°C) and amount of substrate (3-9 g) were analysed in the biological pretreatment. The results also showed the highest lignin removal of 55.2% after 14 days of incubation time. This is followed with significant lignin removal by 66% at temperature 30°C. Meanwhile, the amount of substrate at 5 g gives the highest lignin removal by 71.7%. A maximum lignin removal of 67.9% was achieved at optimum conditions using 5 g of substrate after 14 days of incubation time at temperature 30°C. The highest amount of reducing sugars obtained from biological pretreatment using *S. commune* ENN1 was 230.4 ± 0.19 mg/g with 54% of hydrolysis yield in 96 h. This amount is 1.8-fold the amount obtained from untreated OPEFB (128.2 ± 0.00 mg/g) with the hydrolysis yield of 35.17%. The finding from this study showed that *S. commune* ENN1 was feasible to remove the lignin of OPEFB through biological pretreatment for fermentable sugars production.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PRA-RAWATAN BIOLOGI TANDAN KOSONG KELAPA SAWIT OLEH *Schizophyllum commune* ENN1 PENCILAN TEMPATAN UNTUK PENGHASILAN GULA TERFERMENTASI

Oleh

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Industri kelapa sawit di Malaysia memainkan peranan utama dalam pembangunan sosioekonomi kebangsaan. Sejajar dengan perkembangan industri ini, biomas kelapa sawit dijana dengan banyak dari ladang dan kilang kelapa sawit. Tandan kosong kelapa sawit (OPEFB) adalah biomas lignoselulosa paling banyak dihasilkan daripada kilang minyak kelapa sawit yang terdiri daripada 25-44% selulosa, 25-28% hemiselulosa dan 19-27% lignin. Komponen selulosa dan hemiselulosa boleh ditukar kepada gula terfermentasi selepas diprarawat samaada secara fizikal, kimia, fizikokimia, biologi atau gabungan kaedah prarawatan ini. Pada masa kini, prarawatan fizikokimia adalah kaedah prarawatan yang paling biasa digunakan untuk prarawat dan menukar OPEFB menjadi gula terfermentasi. Walau bagaimanapun, jenis prarawatan yang menggunakan bahan kimia menyebabkan isu-isu alam sekitar dan kos operasi yang tinggi. Oleh itu, prarawatan biologi oleh kulat telah dianggap sebagai alternatif untuk prarawat OPEFB kerana ia mesra alam dan memerlukan kos yang rendah untuk proses tersebut. Objektif pertama kajian ini adalah untuk menilai kebolehan *Schizophyllum commune* ENN1 dalam menyingkirkan lignin OPEFB melalui prarawatan biologi. Objektif kedua adalah untuk mengkaji parameter penting yang memberi kesan dalam prarawatan biologi OPEFB menggunakan *S. commune* ENN1 untuk penghasilan gula terfermentasi berdasarkan kaedah satu faktor pada satu masa (OFAT).

Prarawatan biologi oleh kulat tempatan yang dipencilkan dikenali sebagai *Schizophyllum commune* ENN1 telah dijalankan menggunakan OPEFB yang tidak dibasuh tanpa ditambah dengan nutrien atau mana-mana agen pelembap. Analisis komposisi lignosellulosik menunjukkan bahawa 53.8%

lignin telah disingkirkan selepas prarawatan biologi menggunakan *S. commune* ENN1 berbanding 38.6% lignin disingkirkan menggunakan *P. chrysosporium* UIA. Penentuan kandungan sisa minyak menunjukkan bahawa *S. commune* ENN1 dapat mengurangkan kandungan sisa minyak sebanyak 85.3% dan pada masa yang sama mengekalkan kandungan lembapan dalam lingkungan 51-40%. Kesan masa inkubasi (7-28 hari), suhu (25-40°C) dan jumlah substrat (3-9 g) telah dianalisis dalam prarawatan biologi. Keputusan menunjukkan penyingkiran lignin tertinggi sebanyak 55.2% selepas 14 hari masa inkubasi. Ini diikuti oleh penyingkiran lignin yang besar sebanyak 66% pada suhu 30°C. Sementara itu, jumlah substrat pada 5 g memberikan penyingkiran lignin tertinggi sebanyak 71.7%. Penyingkiran lignin maksimum sebanyak 67.9% dicapai pada keadaan optimum menggunakan 5 g substrat selepas 14 hari masa inkubasi pada suhu 30°C. Jumlah gula penurun yang diperoleh daripada prarawatan biologi menggunakan *S. commune* ENN1 adalah 230.4 ± 0.19 mg/g dengan 54% hasil hidrolisis dalam 96 h. Jumlah ini adalah 1.8 kali ganda jumlah yang diperolehi daripada OPEFB yang tidak diprarawat (128.2 ± 0.00 mg/g) dengan hasil hidrolisis sebanyak 35.17%. Keputusan dari kajian ini menunjukkan bahawa *S. commune* ENN1 adalah kulat yang sesuai untuk menyingkirkan lignin OPEFB melalui prarawatan biologi untuk penghasilan gula terfermentasi.

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Declaration by graduate student

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

OPEFB	Oil palm empty fruit bunch
OPMF	Oil palm mesocarp fibre
PKS	Palm kernel shell
OPF	Oil palm frond
POME	Palm oil mill effluent
FFB	Fresh fruit bunch
GNI	Gross national income
RM	Ringgit Malaysia
CPO	Crude palm oil
OPT	Oil palm trunk
NaOH	Sodium hydroxide
KOH	Potassium hydroxide
$CaOH_2$	Calcium hydroxide
NH_4OH	Aqueous ammonia
AFEX	Ammonia fibre explosion
LWH	Liquid hot water
CO_2	Carbon dioxide
WO	Wet oxidation
Lac	Laccase
LiP	Lignin peroxidase
MnP	Manganese peroxidase
LME	Lignin modifying enzyme
sp.	Species

NREL	National Renewable Energy Laboratory
PDA	Potato dextrose agar
SEM	Scanning electron microscope
FTIR	Fourier transform infrared
DNA	Deoxyribonucleic acid
FPU	Filter paper unit
DNS	Dinitrosalicylic acid
FPase	Filter paper cellulase
CMCase	Carboxymethyl cellulase

CHAPTER 1

INTRODUCTION

Malaysia is well known as the second largest palm oil producer and exporter in the world, which supplies 47% of world palm oil after Indonesia (Begum et al., 2013). In the year 2016 to 2020, Malaysian palm oil industry is expected to produce 15.4 million tonnes of palm oil each year (Abdullah & Sulaiman, 2013). The development of the Malaysian palm oil industry has led to the accumulation of biomass in the oil palm plantation and palm oil mill. This biomass includes oil palm empty fruit bunch (OPEFB), oil palm mesocarp fibre (OPMF), palm kernel shell (PKS) and oil palm frond (OPF) (Ying et al., 2014; Kamcharoen et al., 2014; Shariff et al., 2014). In order to address this issue, zero waste strategy has been promoted by utilising the biomass produced from the mill as a raw material for value-added products.

OPEFB is the most abundant biomass produced by palm oil mill with the total amount of 18 million tonnes per year compared to OPMF and PKS (Begum et al., 2013; Abdullah & Sulaiman, 2013). In the palm oil mill, fresh fruit bunch (FFB) undergoes steam sterilisation process and the fruits are stripped off from the bunch leaving behind the OPEFB (Law et al., 2007). Conventionally, the OPEFB was incinerated for steam and electricity generation at the palm oil mill and the ash was used as fertiliser. However, the OPEFB incineration caused incomplete combustion and released a huge amount of white smoke due to high moisture content (60%) of OPEFB (Chang, 2014; Abdullah et al., 2011). Nevertheless, OPEFB is also commonly used as soil mulch and compost in the plantation area (Geng, 2013). OPEFB is categorised as lignocellulosic biomass as it composed of carbohydrate polymers (cellulose, hemicellulose) and aromatic polymer (lignin). OPEFB consists of lignin 19-26%, hemicellulose 25-28% and cellulose 25-44% (Vandenbossche et al., 2014; Nomanbhay et al., 2013).

High cellulose content makes the OPEFB worth to be converted into fermentable sugars and other value-added products (Li et al., 2014). However, due to the intact structure of the lignocellulosic composition, the OPEFB must undergo the pretreatment process such as physical, chemical, biological and/or physicochemical pretreatment in order to alter the lignocellulosic structure (Nor et al., 2016). By combining the physical and chemical pretreatments, the physicochemical pretreatment is considered to have the highest reliability to enhance the digestibility of lignocellulosic biomass. Nonetheless, physicochemical pretreatment involves high cost of equipment and high energy requirement (Brodeur et al., 2011). The use of the chemical in the pretreatment process also leads to environmental concerns within a community (Agbor et al., 2011).

Biological pretreatment is an environmentally friendly method as it offers a mild pretreatment condition compared to another type of biomass pretreatment. Besides, this pretreatment also considered as low-cost and low energy consumption process (Mood et al., 2013). The biological pretreatment involves enzymatic action to partially degrade the lignin and expose the cellulose structure (Isroi et al., 2011). This pretreatment involved the action of fungi that are able to produce ligninolytic enzymes for lignin degradation. The white-rot fungi have been reported to be the most effective fungi for biological pretreatment (Agbor et al., 2011).

Commonly, OPEFB needs to undergo substrate preparation process before any pretreatments to remove oil residue on the surface of untreated OPEFB. The presence of oil residue on the surface of untreated OPEFB may inhibit the growth of non-indigenous fungus. However, the indigenous fungus found on the pile of OPEFB naturally grown on the oily surface of OPEFB. Thus, the biological pretreatment using indigenous fungus (*Schizophyllum commune* ENN1) is a new approach to pretreat the OPEFB by omitting the substrate preparation process and nutrient supplied throughout the biological pretreatment.

To the best of our knowledge, there are limited literature studies on biological pretreatment of OPEFB using *Schizophyllum commune*. The factors such as incubation time, temperature and amount of substrate are crucial factors in biological pretreatment that could influence the efficiency of lignin removal during the pretreatment. Furthermore, a different type of fungi and substrates used in biological pretreatment may have a different performance at favourable conditions. Therefore, optimum conditions for biological pretreatment of OPEFB was investigated in this study in order to obtain pretreated OPEFB that is suitable as a feedstock for fermentable sugars production.

The objectives of this study are:

1. To evaluate the feasibility of *Schizophyllum commune* ENN1 for lignin removal of OPEFB through biological pretreatment.
2. To investigate the effects of incubation time, temperature and amount of substrate in biological pretreatment of OPEFB using *Schizophyllum commune* ENN1 for fermentable sugars production.

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BIODATA OF STUDENT

Enis Natasha Noor Binti Arbaain was born in Alor Setar, Kedah Darul Aman on September 23, 1992. She attended her elementary school at Sekolah Kebangsaan Guar Napai for 6 years from 1999 to 2004. She continued her secondary education at Sekolah Menengah Kebangsaan Jitra, Kedah (2005 – 2007) and MARA Junior Science College Pendang, Kedah (2008 – 2009). She obtained her pre-university education at Penang Matriculation College, Penang from 2010 to 2011 for one-year matriculation program. In the year of 2011 until 2015, she enrolled as a degree student in Bachelor of Chemical Engineering Technology (Industrial Biotechnology), a four-year program at Universiti Malaysia Perlis (UniMAP), Perlis. She was accepted for six-month internship program (February-July 2015) in Department of Plant Functional Genomics at Sime Darby Technology Centre Sdn. Bhd. During her final year of Bachelor's Degree, she managed to complete a final year project, entitled as "Phase equilibrium studies of biodiesel derivatives based on soybean methyl ester for engine technology". In 2016, she further her study in degree of Master of Science in Environmental Biotechnology. She was attached to Kyushu Institute of Technology starting from August until October of 2017 under sponsorship of Japan Student Services Organization (JASSO).

LIST OF PUBLICATIONS

Paper publication:

Enis Natasha Noor Arbaain, Ezyana Kamal Bahrin*, Mohamad Faizal Ibrahim, Yoshito Ando and Suraini Abd-Aziz (2019). Biological pretreatment of oil palm empty fruit bunch by *Schizophyllum commune* ENN1 without washing and nutrient addition. *Processes*, 7(7). (Published)

Enis Natasha Noor Arbaain, Ezyana Kamal Bahrin*, Nurshakinah Mohd Noor Mohamad Faizal Ibrahim, Norhayati Ramli and Suraini Abd-Aziz. Chemical-free pretreatment of unwashed oil palm empty fruit bunch by using locally isolated fungus (*Schizophyllum commune* ENN1) for delignification. *Food and Bioproducts Processing*. (Accepted)

Abstract in conference/symposium:

Enis Natasha Noor Arbaain, Ezyana Kamal Bahrin, Suraini Abd-Aziz and Mohamad Faizal Ibrahim. In the 5th SAES - International Symposium on Applied Engineering and Sciences (SAES2017) UPM - Kyutech, UPM, Selangor, Malaysia. (Poster presenter)

Enis Natasha Noor Arbaain, Ezyana Kamal Bahrin, Suraini Abd-Aziz and Mohamad Faizal Ibrahim. In the Wood and Biofibre International Conference 2017 (WOBIC2017), Putrajaya, Selangor, Malaysia. (Poster presenter)