UNIVERSITI PUTRA MALAYSIA

EFFECT OF PALM OIL MILL FINAL DISCHARGE ON THE PHYSICOCHEMICAL CHARACTERISTICS AND BACTERIAL COMMUNITY OF RIVER WATER

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Thesis Submitted to the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, in fulfilment of the requirement for the Degree of Bachelor Science (Hons.) Biotechnology

2015

FAKULTI BIOTEKNOLOGI DAN SAINS BIOMOLEKUL

UNIVERSITI PUTRA MALAYSIA

Date: 29 JUNE 2015

LETTER OF PERMISSION

It is thereby to state that I, WAN NOR SALMIAH BINTI TUN MOHD SALIM (Matric No: 161982) have completed a final year project entitled **"Effect of palm oil mill final discharge on the physicochemical characteristics and bacterial community of river water"** under the supervision of Dr. Norhayati Ramli from the Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

I hereby give permission to my supervisor to write and prepare manuscript from the results of this research to be published in any form, if I do not do so within the period of six (6) months from the date above, in condition that my name is also added as one of the article's authors. The arrangement of the names of credited authors in the manuscript depends on the supervisor herself.

Yours sincerely,

……
(WAN NOR SALMIAH BINTI TUN MOHD SALIM)

FAKULTI BIOTEKNOLOGI DAN SAINS BIOMOLEKUL

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APPROVAL SHEET

This thesis entitled **"Effect of palm oil mill final discharge on the physicochemical characteristics and bacterial community of river water"** is submitted by WAN NOR SALMIAH BINTI TUN MOHD SALIM (161982) in fulfilment of the requirement for the Degree of Bachelor of Science (Hons.) Biotechnology in Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

Approved by,

…….. Date:

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Project Supervisor
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ABSTRACT

Abstract of thesis presented to the Faculty of Biotechnology and Biomolecular Sciences in fulfilment of the requirement for the Degree of Bachelor Science (Hons.) Biotechnology

EFFECT OF PALM OIL MILL FINAL DISCHARGE ON THE PHYSICOCHEMICAL CHARACTERISTICS AND BACTERIAL COMMUNITY OF RIVER WATER

By:

WAN NOR SALMIAH BINTI TUN MOHD SALIM

June 2015

Supervisor : Norhayati Ramli, PhD

Faculty : Faculty of Biotechnology and Biomolecular Sciences

Palm oil industry plays a major role in Malaysia due to its contribution as a main exporter of palm oil throughout the world. Production of palm oil from fresh fruit bunch (FFB) involves several mechanical processes such as pressing, sterilizing and stripping which require large amount of water. However, huge water consumption leads to the formation of palm oil mill effluent (POME) and up to 50% of this wastewater will be discharged to the environment. Nowadays, most of the palm oil mills in Malaysia have been practicing the direct discharge of raw or partially treated POME into the river. This is due to the cheapest and easiest method for disposal since POME is a high strength wastewater and very difficult to manage. However, excessive amount of POME discharge will contribute to high organic content in the water stream. This condition needs high dissolved oxygen to be consumed by the microbes for degradation of organic matter. Thus, it will interrupt the environment of aquatic life. Besides, water pollution can cause serious health problems and potentially threaten both life quality and public health due to contamination of drinking water. Therefore, this study was carried out to investigate the physicochemical properties and bacterial community structure of the polluted river caused by palm oil mill final effluent in comparison to the

unpolluted river. The water and sediment samples from Marong River, Negeri Sembilan, Malaysia which located near to the Felda Pasoh Hilir Palm Oil Mill were collected once a month started from November 2014 until January 2015. Seven physicochemical parameters were determined which were pH, temperature, biological oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), total suspended solid (TSS) and volatile suspended solid (VSS) by using standard methods. In addition, bacterial community structure was analyzed by denaturing gradient gel electrophoresis (DGGE) of PCR-amplified region of 16S rDNA from two different points of Marong River and palm oil mill final discharge. The upstream of Marong River was considered as unpolluted river as it was free from POME contamination, meanwhile the downstream and final discharge points were proposed as polluted river. From the results, the unpolluted river showed lower pH (6.38 - 7.13) compared to polluted river (7.27 - 8.27). Meanwhile the recorded temperature for both unpolluted and polluted river showed no significant difference, which was in the range of 26°C - 29°C. In addition, COD (31 mg/L - 107 mg/L), BOD (4.36 mg/L - 17.44 mg/L) and TOC (8.02 mg/L - 32.91 mg/L) of polluted river were higher compared to those of unpolluted river throughout three months sampling. The results showed similar characteristics profile with the sediment samples whereby the sediment in polluted river showed higher percentage of TOC which was in the range of 0.36% - 2.31% compared to the unpolluted river. Besides, the polluted river contained higher amount of TSS (53.33 mg/L - 70.00 mg/L) and VSS (33.33 mg/L - 66.67 mg/L) in comparison to unpolluted river. Based on the DGGE fingerprinting obtained, the changes of microbial community structure have been observed between polluted and unpolluted river. The study showed that shift in the bacterial community composition in the river water was affected by palm oil mill final discharge.

ABSTRAK

Abstrak tesis yang dikemukakan kepada Fakulti Bioteknologi dan Sains Biomolekul sebagai memenuhi sebahagian daripada keperluan untuk Bacelor Sains (Kepujian) Bioteknologi

KESAN PELEPASAN AKHIR KILANG MINYAK KELAPA SAWIT TERHADAP CIRI FIZIKOKIMIA DAN KOMUNITI BAKTERIA DALAM AIR SUNGAI

Oleh:

WAN NOR SALMIAH BINTI TUN MOHD SALIM

June 2015

Penyelia : Dr Norhayati Ramli

Fakulti : Fakulti of Bioteknologi dan Sains Biomolekular

Industri minyak sawit memainkan peranan utama di Malaysia kerana sumbangannya sebagai pengeksport utama minyak kelapa sawit di seluruh dunia. Pengeluaran minyak kelapa sawit daripada buah tandan segar (FFB) melibatkan beberapa proses mekanikal seperti menekan, pensterilan dan pelucutan yang memerlukan jumlah air yang banyak. Walau bagaimanapun, penggunaan air yang banyak membawa kepada pembentukan efluen kilang minyak sawit (POME) dan sehingga 50% daripada air sisa ini akan dilepaskan ke alam sekitar. Pada masa kini, kebanyakan kilang minyak kelapa sawit di Malaysia telah melepaskan POME yang mentah atau sebahagiannya dirawat ke dalam sungai. Ini adalah kerana cara ini adalah yang paling murah dan mudah untuk pelupusan kerana POME adalah air sisa yang tinggi kepekatan dan sangat sukar untuk diuruskan. Walau bagaimanapun, jumlah pelepasan POME yang berlebihan akan menyebabkan kandungan organik yang tinggi dalam aliran air. Keadaan ini memerlukan banyak oksigen yang terlarut untuk digunakan oleh mikrob bagi degradasi bahan organik. Oleh itu, ia akan mengganggu persekitaran kehidupan akuatik. Selain itu, pencemaran air boleh menyebabkan masalah kesihatan yang serius dan berpotensi

mengancam kedua-dua kualiti kehidupan dan kesihatan awam akibat pencemaran air minuman. Oleh itu, kajian ini dijalankan untuk mengkaji sifat fizikokimia dan struktur komuniti bakteria di dalam sungai yang tercemar disebabkan oleh pelepasan akhir daripada kilang minyak kelapa sawit berbanding dengan sungai yang tidak tercemar. Sampel air dan sedimen dari Sungai Marong, Negeri Sembilan, Malaysia yang terletak berhampiran Kilang Minyak Kelapa Sawit Felda Pasoh Hilir dikumpulkan sebulan sekali bermula dari November 2014 hingga Januari 2015. Tujuh parameter fizikokimia telah ditentukan iaitu pH, suhu, permintaan oksigen biologi (BOD), permintaan oksigen kimia (COD), jumlah karbon organic (TOC), jumlah pepejal terampai (TSS) dan pengewapan pepejal terampai (VSS) dengan menggunakan kaedah standard. Di samping itu, struktur komuniti bakteria dianalisis dengan DGGE di bahagian amplifikasi PCR daripada 16S rDNA dari dua bahagian Sungai Marong yang berbeza dan pelepasan akhir oleh kilang minyak kelapa sawit. Hulu Sungai Marong dianggap sebagai air sungai yang tidak tercemar kerana bebas daripada pencemaran POME manakala hillir Sungai Marong dan bahagian pelepasan akhir kilang dianggap sebagai air sungai yang tercemar. Daripada hasil yang diperolehi, sungai yang tidak tercemar menunjukkan pH yang lebih rendah (6.38 - 7.13) berbanding dengan sungai yang tercemar (7.27 - 8.27). Manakala, suhu yang telah direkodkan untuk kedua-dua sungai yang tidak tercemar dan tercemar menunjukkan tiada perbezaan yang ketara dalam linkungan 26.0°C - 29°C. Selain itu, COD (31 mg/L - 107 mg/L), BOD (4.36 mg/L - 17.44 mg/ L) dan TOC (8.02 ppm – 32. 91 ppm) sungai tercemar adalah lebih tinggi berbanding dengan sungai tidak tercemar sepanjang tiga bulan persampelan. Hasil kajian menunjukkan ciri-ciri yang serupa dengan profil sampel sedimen di mana sedimen di dalam sungai tercemar menunjukkan peratusan TOC yang lebih tinggi iaitu dalam linkungan 0.36% - 2.31% berbanding dengan sungai yang tidak tercemar. Selain itu, sungai tercemar mengandungi jumlah TSS (53.33mg/L - 70.00 mg/L) dan VSS (33.33 mg/L - 66.67 mg/L) yang lebih tinggi berbanding dengan sungai yang tidak tercemar. Berdasarkan pencapjarian DGGE yang diperoleh, perubahan struktur komuniti mikrob telah diperhatikan antara sungai tercemar dan tidak tercemar. Kajian ini menunjukkan bahawa perubahan dalam komposisi komuniti bakteria di dalam air sungai dipengaruhi oleh pelepasan akhir daripada kilang minyak kelapa sawit.

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Alhamdulillah, praise to Allah SWT, whom with His willing I was able to complete this final year project. A heartfelt thanks goes to all that have involved directly or indirectly in completing my final year project. This research project would not have been possible without the support of many people.

First and foremost, I take this opportunity to express my profound gratitude to my project supervisor, Dr. Norhayati Ramli for introducing this topic and providing guidance, criticism and support for every single time throughout this project period. I especially thank her for her patience and kindness when dealing with me and the problems I faced during the project. Her constant encouragement helped me a lot during the project period and writing this thesis. I hope my project thesis meet your expectations.

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree celcius
AN	Ammoniacal nitrogen
APS	Ammonium persulfate
BOD	Biological oxygen demand
CDM	Clean Development Mechanism
CER	Certified Emission Reduction
CH_4	Methane
$C_8H_5KO_4$	Potassium hydrogen phthalate
CO_2	Carbon dioxide
COD	Chemical oxygen demand
CPO	Crude palm oil
CPKO	Crude palm kernel oil
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DOE	Department of Environment
EFB	Empty fruit bunch
FFB	Fresh fruit bunch
G	Gram
GNI	Gross National Income
HCL	Hydrochloric acid
H_3PO_4	Phosphoric acid
HRT	Hydraulic retention time

IC	Inorganic carbon
1NWQS	Interim National Water Quality Standards
kWh/m^3	Kilowatt hour per cubic metre
L	Litre
m^3	Cubic metre
MBR	Membrane bioreactor
mg	Milligram
mg/L	Milligram per litre
mm	Millimetre
MPIC	Ministry of Plantation Industries and Commodities
$NaHCO_3$	Sodium bicarbonate
Na_2CO_3	Sodium carbonate
OER	Oil extraction rate
OLR	Organic loading rate
OPF	Oil palm fronds
OPT	Oil palm trunks
PCR	Polymerase chain reaction
PKC	Palm kernel cake
POME	Palm oil mill effluent
POMS	Palm oil mill sludge
rRNA	Ribosomal ribonucleic acid
sp	Species
SIAN	SubIndex NH_3-N
SIBOD	SubIndex BOD
SISS	SubIndex SS
SS	Suspended Solids

TAE	Tris-acetate-EDTA
TC	Total carbon
TEMED	Tetramethylethylenediamine
TOC	Total organic carbon
TSS	Total suspended solid
VSS	Volatile suspended solid
WQI	Water Quality Index

CHAPTER 1

INTRODUCTION

Palm oil industry is considered as one of the main agro-industries in Malaysia. Malaysia now ranks second of palm oil exporter in the world after Indonesia (Ibrahim *et al.*, 2012). Palm oil which is from the mesocarp consist mainly of palmitic acid (C16:0) and oleic acid (C18:1) which are the most common fatty acids in natural oils and fats. Palm oil plantation had increased at a rapid pace since 1960. In 1985, 1.5 million hectares were planted with palm tree, and it had increased to 4.3 million hectares in 2007. As of 2011, the total planted area was 4.917 million hectares. After reaching as high as 16.66 million tonnes of palm oil production in 2010, Malaysia exports have seen a growing trend with 17.99 million tonnes of palm oil were exported in 2011 (MPOB, 2011). With the innovation of palm oil residue into a highly valuable end commodity, there has been a steadily growing interest in this research field. The installation of efficient fractionation and refining facilities at the industry were done as the value addition to the crude palm oil.

Generally, there are two types of oils produced from oil palm which are crude palm oil from the fibrous mesocarp and palm kernel oil from the palm kernel. In order to extract the crude palm oil from fresh fruit bunches, the mechanical process is applied. In Malaysia, wet milling process is the standard way to extract the palm oil, which consists of several stages such as stripping or threshing, sterilizing, pressing and purifying processes. During digestion and purification process, large amount of water is required. Thus, more than 50% of water will be discharged to the environment during the palm oil production, which is known as palm oil mill effluent (POME) (Ahmad *et al.*, 2003). POME usually defined as colloidal suspension originating from mixture of sterilizer condensate, separator sludge and hydro cyclone wastewater (Wu *et al.,* 2010). In 2004, about 26.7 million tonnes of solid biomass and 30 million tonnes of POME were generated from 381 palm oil mills in Malaysia and the amount of POME generated is increasing every year (Yacob *et al*., 2006).

The quality of the raw material and palm oil production processes in palm oil mills influence the characteristics of the POME discharge. The fresh POME is hot (80°C - 90°C), acidic with pH around 4.7 and consists of suspended cellulosic materials such as palm fibre, fat, oil and grease residue, as well as water soluble components of palm fruits. In addition, POME have high concentration of biological oxygen demand (BOD) (25,000 mg/L), chemical oxygen demand (COD) (50,000 mg/L), oil and grease (4,000 mg/L) and high amount of total solid (40,500 mg/L) (Ma, 2000). In Malaysia, the characteristics of POME must follow the standard discharge limit that has been set by the Department of Environment before the effluent can be legally discharged into the water ways. Therefore, one of the biggest challenges for palm oil mills in Malaysia is to treat POME efficiently before being discharged into the river water in order to conserve the environment while maintaining the sustainability of the economy.

Palm oil industry has been identified as one of the largest contributors to river water pollution due to the discharge of the raw or partially treated POME from the palm oil mills into the river or stream (Ibrahim *et al*., 2012). The effluent discharge into the nearby rivers is considered as the easiest and cheapest method for disposal. However, POME must be well treated before being discharge into the river water to prevent water pollution. The discharge of partially treated or raw POME into the river water will lead to depletion of oxygen in the water body, suffocate aquatic life and despoil the rivers. Based on literature, the raw or partially treated POME contain high organic load. Even though the effluent from palm oil mills have been set to follow the standard discharge limit, the natural ecology of the river is still affected if POME is discharged in the huge amount and in uncontrolled flow rate (Gopalkrushna, 2011).

In addition, river is an important natural resource that many living things need to survive. Among all living organisms, bacteria are known to carry out large number of small scale processes that underlie many environmentally important functions. According to Sun *et al*. (2012), microorganisms are very sensitive to the changes of river's environmental factor and the communities of bacterial can be sensitive indicators for contaminants stress. It is believed that the microbial community structure in polluted river water and sediment may change due to the introduction of POME.

In this study, the overall objective is to assess the effect of palm oil mill final discharge on the physicochemical characteristics and bacterial community of river water. The specific objectives are:

1) To characterize the physicochemical properties of the polluted river caused by palm oil mill final discharge in comparison to unpolluted river water.

2) To assess bacterial community profiles in polluted river caused by palm oil mill final discharge in comparison to unpolluted river water.

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