



**UNIVERSITI PUTRA MALAYSIA**

***PHYSICOCHEMICAL AND COMMUNITY PROFILES OF BACTERIAL  
ECOLOGY IN PALM OIL MILL EFFLUENT FINAL DISCHARGE AND  
POLLUTED RIVER WATER***

**SITI SUHAILAH BINTI SHARUDDIN**

**FBSB 2017 36**



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**By**

**SITI SUHAILAH BINTI SHARUDDIN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in  
Fulfilment of the Requirements for the Degree of Master of Science**

**June 2017**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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**June 2017**

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Palm oil industry is one of the major pillars of Malaysia economy. However, concurrent with the high production of palm oil, an enormous volume of palm oil mill effluent (POME) is being generated every year. Studies have shown that the discharging of treated POME into the river caused alterations of the natural properties of the receiving river, which consequently may also affect the biological balance of the ecosystem as a whole. In this study, a detailed evaluation of the impact of the treated POME final discharge on the bacterial communities with regards to their structural and functional status is discussed. The nucleic acid double staining assay based on flow cytometry was used to monitor the response of bacterial community functional status in terms of their total concentration, viability, and nucleic acid content, while the integration of PCR-denaturing gradient gel electrophoresis (DGGE) method and Illumina MiSeq approach, aided by advanced bioinformatics analysis have been used in analysing the bacterial community structures. The findings demonstrated a significant difference of bacterial community present in the upstream and downstream part, whereby the total cell concentration showed an increment in the downstream part of river water by  $9.3 \times 10^5$  to  $2.6 \times 10^7$  cells  $\text{mL}^{-1}$ . It was also shown that the upstream part of the river was dominated by non-viable bacteria ranging from 44% to 75%, while the pattern was strongly shifted towards high percentage of viable bacteria in the downstream part of the river. The shift of low nucleic acid to high nucleic acid cells was also recorded, with the increment ranging from 17% to 36%, hence, suggesting the transformation of dormant to active cells due to the induction by POME final discharge which corresponded with the significant changes of the physicochemical properties. The discharged of POME also caused the reduction of diversity level in the downstream part of the river compared to the upstream part with the values of  $9.277 \pm 0.521$  and  $9.869 \pm 0.776$ , respectively. The findings also demonstrated the shift of bacterial community composition in the downstream part of the river with the remarkable increment of *Chromatiaceae* and *Alcaligenaceae* by 35-fold and 4-fold, respectively. Hence, *Chromatiaceae* and *Alcaligenaceae* are proposed as the potential candidates for the indicator bacteria, which

can be used to complement the physicochemical monitoring in assessing the river water pollution due to POME final discharge. More importantly, a sustainable palm oil industry can be developed with a reliable and accurate assessment method in strengthening the POME pollution management. To our knowledge, this is the first study on the effect of POME final discharge on bacterial community dynamics in the effluent receiving river.



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

**FIZIKOKIMIA DAN PROFIL KOMUNITI EKOLOGI BAKTERIA DALAM PELEPASAN AKHIR EFLUEN MINYAK KELAPA SAWIT DAN AIR SUNGAI TERCEMAR**

Oleh

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Industri minyak sawit adalah salah satu daripada tonggak utama ekonomi Malaysia. Walau bagaimanapun, seiring dengan pengeluaran minyak kelapa sawit yang tinggi, efluen minyak kelapa sawit (POME) turut dijana dengan kadar yang tinggi setiap tahun. Kajian telah menunjukkan bahawa pelepasan POME yang dirawat ke dalam sungai menyebabkan perubahan sifat semula jadi ekosistem sungai, yang seterusnya juga boleh menjejaskan keseimbangan ekosistem biologi secara keseluruhan. Dalam kajian ini, penilaian terperinci kesan POME yang dirawat terhadap komuniti bakteria berhubung status struktur dan fungsi mereka telah dibincangkan. Asai pewarnaan berganda asid nukleik berdasarkan sitometri aliran telah digunakan untuk memantau tindak balas komuniti bakteria dari segi jumlah kepekatan, kebolehhidupan dan kandungan asid nukleik, manakala integrasi PCR- gel elektroforesis penyahasian kecerunan (DGGE) dan Illumina MiSeq, dibantu oleh analisis bioinformatik telah digunakan dalam menganalisis struktur komuniti bakteria. Hasil kajian menunjukkan perbezaan yang signifikan dalam populasi bakteria di bahagian hulu dan hilir sungai yang tercemar, dengan kepekatan sel di hilir sungai menunjukkan peningkatan sebanyak  $9.3 \times 10^5$  kepada  $2.6 \times 10^7$  sel  $\text{mL}^{-1}$ . Kajian turut menunjukkan hulu sungai didominasi oleh bakteria mati dalam lingkungan 44% ke 75%, dan corak ini beralih ke peratusan bakteria hidup yang tinggi di hilir sungai. Peralihan asid nukleik rendah kepada asid nukleik tinggi juga turut direkodkan dengan kenaikan di dalam lingkungan 17% ke 36% yang mencadangkan transformasi sel-sel dorman kepada sel-sel aktif yang disebabkan oleh pelepasan akhir POME yang juga turut menyebabkan perubahan ketara sifat-sifat fizikokimia. Pelepasan POME yang dirawat juga menyebabkan pengurangan tahap kepelbagaian komuniti di hilir sungai berbanding hulu sungai dengan nilai  $9.277 \pm 0.521$  dan  $9.869 \pm 0.776$ . Hasil kajian juga menunjukkan peralihan komposisi komuniti bakteria di bahagian hilir sungai dengan kenaikan yang luar biasa bagi *Chromatiaceae* dan *Alcaligenaceae* dengan peningkatan 35-kali lipat dan 4-kali lipat. Oleh itu, *Chromatiaceae* dan *Alcaligenaceae* dicadangkan sebagai calon berpotensi bakteria penunjuk, yang boleh digunakan untuk menambahbaik kaedah pemantauan fizikokimia

dalam menilai pencemaran air sungai akibat daripada pelepasan akhir POME. Lebih penting lagi, industri minyak sawit yang mapan boleh dibangunkan dengan kaedah penilaian yang boleh dipercayai dan tepat dalam mengukuhkan pengurusan pencemaran POME. Setakat yang diketahui, kajian ini dipercayai merupakan yang pertama tentang kesan pelepasan akhir POME terhadap dinamik komuniti bakteria dalam sungai yang menerima efluen.



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I certify that a Thesis Examination Committee has met on 2<sup>nd</sup> June 2017 to conduct the final examination of Siti Suhailah Binti Sharuddin on her thesis entitled Physicochemical and Community Profiles of Bacterial Ecology in Palm Oil Mill Effluent Final Discharge and Polluted River Water 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 Master of Science.

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## LIST OF ABBREVIATIONS

APHA	American Public Health Association
APS	Amminoum Persulphite
BOD	Biological Oxygen Demand
Bp	Basepair
CaCl <sub>2</sub>	Calcium Chloride
CO <sub>2</sub>	Carbon Dioxide
COD	Chemical Oxygen Demand
CPO	Crude Palm Oil
ddNTPs	Dideoxy Nucleoside Triphosphates
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Dinucleotide Acid
DO	Dissolved Oxygen
DOE	Department of the Environment
EFB	Empty Fruit Bunch
FeCl <sub>3</sub>	Ferric Chloride
FFB	Fresh Fruit Bunch
g	Gram
HNA	High Nucleic Acid
IC	Inorganic Carbon
kg	Kilogram
LNA	Low Nucleic Acid
m <sup>3</sup>	Cubic Metre
mg/L	Milligram per Litre
MgSO <sub>4</sub>	Magnesium Sulphate
min	Minutes
mL	Millilitre
mm	Millimetre
MPOB	Malaysian Palm Oil Board
MPOC	Malaysian Palm Oil Council
ng	Nanogram
nm	Nanometer
NGS	Next Generation Sequencing

NSTIs	Nearest Sequenced Taxon Indexes
O <sub>2</sub>	Oxygen Gas
OPF	Oil Palm Frond
PCR	Polymerase Chain Reaction
PKC	Palm Kernel Cake
PKO	Palm Kernel Oil
POME	Palm Oil Mill Effluent
rRNA	Ribosomal Ribonucleic Acid
TAE	Tris-Acetate-EDTA
TEMED	Tetraethylmethylenediamine
TOC	Total Organic Carbon
TSS	Total Suspended Solids
VSS	Volatile Suspended Solids
wt/vol	Weight per Volume
μL	Microlitre

## CHAPTER 1

### INTRODUCTION

Rapid industrialization is inevitable as it is a fundamental aspect in developing countries. However, the development in a global economy not just resulted in a significant increase of industrial activities, but also generates a huge amount of undesirable wastes. Consequently, the environment is becoming more polluted due to various wastes discharged from a wide range of industrial applications (Ado et al., 2015). In Malaysia, palm oil production is regarded as one of the main economic-driven industries and is ranked as a second largest producer of palm oil in the world (Ibrahim et al., 2012). The oil palm crop (*Elaeis guineensis*) currently planted in Malaysia is from Tenera hybrid which yields about 40 tonnes of palm oil per ha. It has been widely planted all around Malaysia and covers approximately 5,642,943 ha land areas (MPOB, 2015). Nevertheless, while palm oil industry is recognized for its contribution to the remarkable rise in the nation economy, it is also commonly associated with environmental issues due to the generation of an enormous quantity of wastewater known as palm oil mill effluent (POME) (Wu et al., 2010).

Whilst POME is not the only waste produced during processing of fresh fruit bunch, it gains the most attention due to the costly treatment system which is needed to treat the large volumes of POME. As a result, raw POME or partially treated POME is still being discharged into the environment including the river water and nearby lands, in order to reduce the waste management cost (Rupani et al., 2010). Typically, 1 tonne of crude palm oil production requires approximately 5 to 7 tonnes of water which over 50% of it ends up as POME. The untreated or improperly treated POME could become a major contributor to serious pollution problems as a consequence of their high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) (Wang et al., 2015a). Owing to these, most palm oil mills are still unable to comply with the discharge limit of 100 mg/L of BOD requirement imposed by the Department of Environment (DOE) of Malaysia (Madaki and Seng, 2013).

Previously, the scientific community was mainly focussed on assessing the influence of POME final discharge on the physicochemical properties of the receiving ecosystem (Awotoye et al., 2011; Edward et al., 2015), since the monitoring of these parameters is crucial in order to meet the standard regulations imposed by the authority. However, we are neglecting the impact of the disturbance from POME final discharge towards the bacterial community residing in that particular area. The impact of POME final discharge on the receiving-river water, for instance, may be insidious whereby the immediate consequences may not be apparently visible. The long-term discharging of POME may adversely alter the natural composition of the bacterial community in the river, which may pose a threat to human health and disrupt the balanced ecosystem as a whole.

Thus, the lack of detailed knowledge about the relationship between bacterial community structure and hydrogeochemistry at effluent receiving-river water impeded the monitoring process and the understanding of the severity level of pollution caused by POME. Several studies have shown that bacterial population is highly dynamic and can differ strongly in their response to resource availability such as the concentration of organic carbon, nitrogen, and phosphorus in a particular environment (Gözdereliler et al., 2013). Therefore, to ensure a sustainable industrial practice, it is essential to have more knowledge on their population, diversity and how they are related to the process

performance and severity of pollution. The current conventional culturing methods used in analysing the bacterial community structure is inadequate as most of the important environmental bacterial species are unculturable (Lu et al., 2015; Rani et al., 2008). However, with the application of molecular phylogenetic methods and the emergence of next generation sequencing, it is now possible to obtain a view on the bacterial community that was previously inaccessible (Tan et al., 2015).

Flow cytometric DNA analysis serves as a powerful tool to monitor the response of bacterial community towards the introduction of POME final discharge in terms of their viability and functional status. Flow cytometer in conjunction with fluorescent probes or specific dyes that bind nucleic acid allows the application of rapid and automated methods in the study of bacteria in their natural aquatic environment (De Roy et al., 2012; Matos and Lopes Da Silva, 2013). Meanwhile, the integration of PCR-denaturing gradient gel electrophoresis (DGGE) approach and high-throughput MiSeq serves as a valuable tool for the study of bacterial community composition and structure (Yu et al., 2015). The databases containing information regarding the relative abundance and activity of bacterial communities provide thorough insights on the influence of POME final discharge towards bacterial community structure. The use of these approaches also allows the determination of subtle differences present within different groups of microorganisms from different areas that can subsequently be used to identify the potential indicator bacteria capable of indicating contamination by pollutant in a particular area (Guo et al., 2016).

Hence, by using the mentioned approaches, it is expected that we will be able to monitor and forecast the impact of POME final discharge on the structural and functional status of the bacterial community in the receiving river water, which later can be used to determine potential indicator bacteria. This effort is important in order to establish a more reliable and specific pollution assessment method to indicate the contamination caused by POME in the river water in which to our knowledge, has not been documented. Thus, the objectives of this study were:

- i) To assess the shift of bacterial community functional status in relation to the changes of physicochemical properties in the palm oil mill effluent-receiving river water.
- ii) To determine the changes of bacterial community structure profiles for the identification of potential indicator bacteria in the polluted river caused by palm oil mill effluent final discharge.

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