UNIVERSITI PUTRA MALAYSIA

BIOREMEDIATION OF RAW PALM OIL MILL EFFLUENT USING MIX MICROALGAE IN MALAYSIA

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BIOREMEDIATION OF RAW PALM OIL MILL EFFLUENT USING MIX MICROALGAE IN MALAYSIA

By

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

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BIOREMEDIATION OF RAW PALM OIL MILL EFFLUENT USING MIX MICROALGAE IN MALAYSIA

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May 2016

Chairman : Hishamuddin Omar, PhD
Faculty : Science

Palm oil industry in Malaysia is one of the key players in the contribution to the economy of the country. Nonetheless, it also produces a massive amount of palm oil mill effluent (POME). It has been known that microalgae, the unicellular photosynthetic microorganisms possess the ability to reduce the number of pollutants of organic waste. Therefore, this study aims to grow mixed microalgae cultured in different concentrations of raw POME (0.5, 1, 1.5 and 2) % v/v as a biological treatment. This study is conducted on open pond cultivation system (glass tanks) in outdoor conditions. First, it is necessary to allow the mixed microalgae make an adaptation in the raw POME before the start of the actual experiment. The results showed positive effects on the biomass growth of mixed microalgae cultured in the concentration of 1% v/v raw POME. The concentration 1% v/v recorded productivity 0.09 ± 0.02 g/L. day, which significantly higher $p < 0.05$ compared with the controls and other concentrations. The findings showed that the mixed microalgae could reduce BOD, TN and TP to 78.79, 2.176, and 1.591 respectively. Finally, as many as 10 species of mixed microalgae have been identified from all the experiments, namely *Dictyosphaerium* sp., *Scendesmus* sp., *Oocystis* sp., *Monoraphidium* sp., *Nostoc* sp., *Cosmarium* sp., *Aulacoseira* sp., *Crucigenia* sp., *Cyclotella* sp., and *Asterococcus* sp.. Since, it is proven that the mixed microalgae able to grow in raw POME, it can be concluded that raw POME is a suitable medium for the cultivation of mixed microalgae without adverse effects.

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

BIOREMEDIASI EFLUEN KILANG MINYAK SAWIT MENTAH DENGAN MENGGUNAKAN CAMPURAN MIKROALGA DI MALAYSIA

Oleh

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Industri minyak sawit di Malaysia adalah salah satu penyumbang utama kepada ekonomi negara. Namun begitu, ia juga menghasilkan sejumlah besar efluen kilang minyak sawit (POME). Adalah terdahul merdapat bahawa mikroalga, iaitu mikroorganisma fotosintesis berunisel, mempunyai keupayaan untuk mengurangkan jumlah pencemar sisa organik. Oleh itu, kajian ini bertujuan meng kultur mikroalga campuran dalam kepekatan POME mentah yang berlainan (0.5, 1, 1.5 dan 2) % v/v sebagai rawatan biologi. Kajian ini dijalankan ke atas sistem kolam terbuka (tangki kaca) system terbuka. Pertama, mikroalga campuran itu perlu diaklimitasikan penyesuaian untuk POME mentah sebelum memulakan eksperimen. Hasil kajian menunjukkan kesan positif ke atas pertumbuhan biojisim pada mikroalga campuran yang telah dikultur dalam kepekatan 1% v/v POME mentah. Kepekatan 1% v/v mencatatkan produktiviti 0.09 ± 0.02 g/L /hari, yang jauh lebih tinggi ($p < 0.05$) berbanding dengan kawalan dan kepekatan lain. Kajian juga menunjukkan bahawa mikroalga campuran boleh mengurangkan BOD, TN dan TP hingga 78.79, 2.176, dan 1.591 masing-masing. Sebanyak 10 spesies alga campuran telah dikenal pasti dari semua eksperimen, iaitu *Dictyosphaerium* sp., *Scendesmus* sp., *Oocystis* sp., *Monoraphidium* sp., *Nostoc* sp., *Cosmarium* sp., *Aulacoseira* sp., *Crucigenia* sp., *Cyclotella* sp., dan *Asterococcus* sp. Kajian ini dapat membuktikan mikroalga campuran dapat bahawa membiak dan tumbuh dalam POME mentah. Sebagi kesimpulan, POME mentah adalah medium yang sesuai untuk perkembunan mikroalga campuran tanpa memberiken kesan sampingan

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I certify that a Thesis Examination Committee has met on 24 May 2016 to conduct the final examination of Inas Jasim Alwan on her thesis entitled "Bioremediation of Raw Palm Oil Mill Effluent Using Mix Microalgae in Malaysia" 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

ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
BOD_5	Biochemical oxygen demand
C	Carbon
Ca	Calcium
CH_4	Methane
Co	Cobalt
CO_2	Carbon dioxide
COD	Chemical oxygen demand
CPO	Crude Palm oil
Chll-a	Chlorophyll *a*
Cu	Cupper
D	Simpsons index
DDH_2o	Deionized water
DO	Dissolved Oxygen
DW	Dry weight
E	Evenness
EFB	Empty fruit bunches
Fe	Iron
FFB	Fresh fruit bunch
FEP	Teflon
FFB	Fresh fruit bunches
g	Gram
GHG	Green houses gases
HCO_3	Bicarbonate
HCl	Hydrochloric acid
H_2SO_4	Sulphuric acid
K	Potassium
L	Litter
Mg	magnesium
Mn	manganese
ml	mili litter
MPOB	Malaysian Palm Oil Board
mg	Milligram
mgL^{-1}	Milligram per litre
µm	Micro meter
µl	Micro litre
ln	Natural logarithm
MUFA	Mono unsaturated fatty acid
N	Nitrogen
N	Total number of individual
n	Total number of species
Na	Sodium
NADPH	nicotinamide adenine dinucleotide phosphate- oxidase
nm	Nanometre
NH_3-N	Ammoniac nitrogen
NaOH	Sodium Hydroxide

O_2	Oxygen
P	Phosphorous
PAR	Photosynthetic active radiation
PBR	Photo- bioreactor
Pi	Proportion of individuals
POME	Palm Oil Mill Effluent
ppt.	Parts per thousand
PUFA	Poly unsaturated fatty acid
rpm	Revolution per minute
R	Richness
RCBD	Randomized Complete Block Design
Sp.	Species
TN	Total Nitrogen
TP	Total Phosphorus
OD	Optical Density
UPM	Universiti Putra Malaysia
UNIDO	United Nation Industrial Development Organization
UV	Ultra violet
WHO	World Health Organisation
YSI	Yellow spring instrument
Zn	Zinc

CHAPTER 1

INTRODUCTION

1.1 Background

Nowadays, the world is facing very serious global issues related to the environment. The main problem is global warming, climate change, environmental degradation, severe drought, acid rain and haze (Lee *et al*., 2013; Naidu *et al*., 2015). Many of these problems originate from a single source, namely global warming. This phenomenon of global warming results from excessive release of CO_2 and CH_4, which form greenhouse gases that insulate heat from escaping the earth atmosphere, leading to more global warming (de Richter and Caillol, 2011; Yun *et al*., 2012; Lee *et al*., 2013). Mitigating of global warming requires multiple approaches ranging from activities in reducing CO_2 and CH_4 emission, to improving agriculture practice and agroforestry systems (Dhillon and von Wuehlisch, 2013; Crank and Jacoby, 2015). With respect to global warming, Malaysia is also in the spotlight due to palm oil industry (Singh *et al*., 2010; Madaki and Seng, 2013).

In Malaysia, one fast growing agriculture-based sector is the palm oil industry. It is revealed that this industry controls about 45 % of total palm oil production in the world (Saifuddin and Dinara, 2011; Sukumaran *et al*., 2014). In the past decade, Malaysia has risen to become a leading global producer and exporter of palm oil products. The Malaysian Palm Oil Board (MPOB) revealed that the production of crude palm oil (CPO) has recorded an increase of 2.3 % from 18.79 million tons in 2012 to 19.22 million tons in 2013 (MPOB, 2015).

In Malaysia, the most practical and feasible method for large-scale production in palm oil is the wet process in palm oil milling. As suggested in the name, this method requires a large quantity of water resources in the production of crude palm oil (CPO) from processing the fresh fruit bunches to become crude oil. This method has produced palm oil mill effluent (POME) which is considered as wastewater (Singh *et al*., 2010; Wu *et al*., 2010). According to Ahmad *et al*. (2003), about 5 - 5.7 tons of water is required to produce 1 ton of CPO. This process involves the sterilization of the palm fruit bunches and clarifying the extracted oil. However, more than 50 % of the water ends up as fresh POME. This means that for one ton of CPO obtained in the extraction process, a number of POME beings produced is 2.5 - 3.0 tons. This suggests that the amount of POME will increase as long as the production and processing of palm oil continue the upward trend to meet both domestic and global demand (Madaki and Seng, 2013).

The quality of the raw materials used and the processes of palm oil production in the mills affect the characteristics of POME (Eze *et al.*, 2013). POME as a thick brownish colloidal mixture. This composition of the mixture includes water, oil, and fine suspended solids. It has 80 - 90 ℃; the levels of both biochemical oxygen demand

(BOD) and chemical oxygen demand (COD) are very high. The presence of organic acids in complex forms makes it acidic with a pH of around 4.5. This makes the decomposition process of POME difficult to achieve due to their cost effective processing (Habib *et al*., 1998; Igwe and Onyegbado, 2007; Wu *et al*., 2010). While POME is considered a waste, it is considered a load rather than a part of the production process of palm oil. Nowadays, the biggest challenge faced by the palm oil industry is related to the disposal of large quantities of POME (Igwe and Onyegbado, 2007; Madaki and Seng, 2013). One of the most common and environmentally friendly methods for the disposal of wastes palm produced from the oil mill industry is the discharge of raw or partially treated POME into the waterways. Nevertheless, this method may cause serious environmental threats since discharge of POME not only pollutes fresh water bodies, but its retention can also be harmful to the environment (Wu *et al.*, 2010). This is because during the retention period in a series of oxidation ponds take up to 155 days before it is being discharged into the rivers. During the oxidation process, POME undergoes fermentation that releases CO_2 and CH_4, which contribute to the increase in greenhouse gasses (Igwe and Onyegbado, 2007; Singh *et al*., 2010; Madaki and Seng, 2013). Due to exceptionally high BOD levels in POME, it has been recognized as one of the largest source of pollutants into the rivers in Malaysia (Habib *et al*., 1998; Singh *et al*., 2010; Madaki and Seng, 2013). On the other hand, the high organic matter present in POME, which includes carbohydrates, protein, phosphorus, potassium, magnesium and calcium make it as a vital nutrient resource (Habib *et al*., 1998; Madaki and Seng, 2013).

Based on these findings, many research studies have started the search for alternative solutions on the POME conversion into useful forms such as animal feed, biofertilizer and a source of green energy, for the benefits of mankind including a decrease in environmental pollution Foo and Hameed, (2010);Rupani *et al*., (2010); Lam and Lee, (2011); Chin *et al*.,(2013).

There are many suggestions to utilize POME such as convert this waste product into beneficial substance namely feedstock and organic fertilizers using the biological method of vermicomposting (Rupani *et al*. 2010). Also, POME can be treated by using the physical method that uses membrane ultrafiltration. It is an excellent way to treat wastewater into the best sample quality (Wah *et al*. 2002). Furthermore, through the use of anaerobic thermophilic digestion process POME can be converted to release biogas(Madaki and Seng, 2013). The other method relies on the use of chemical substances. For example, the flocculation process involves the removed of a substance such as oil, alum, aluminum chlorohydrin, aluminum sulfate and chitosan (Saifuddin and Dinara, 2011).

Recently, microalgae and unicellular photosynthetic microorganisms have been highlighted as attractive sources of renewable energy due to their incredible abilities; in rapid production of biomass and a significant reduction for pollutants (Lam and Lee, 2011). In general, microalgae absorb solar energy, while consuming organic compounds to fix carbon substances, besides producing biomass (Vairappan and Yen, 2008; Wolkers *et al*., 2011; Sukumaran *et al*., 2014). It is reported that microalgae grow 100 times faster than land-based plants, so they have the ability to double biomass production in less than a day (Lam and Lee, 2011). Another feature of microalgae is their ability to utilize organic pollutants, and subsequently improve water quality (Priyadarshani *et al*., 2011; Othman *et al*., 2014).

Therefore, many research has adopted the suggestion of converting the treatment of POME, from disposed waste to beneficial activities (Azimatun Nur and Hadiyanto, 2014; Nwuche *et al.*, 2014; Shaha *et al*., 2014; Sukumaran *et al*., 2014). In line with this suggestion, many research works used microalgae in the bioremediation of POME. The researchers utilized POME as a low cost. However, most of these research works focused on specific types of microalgae, resulting in various shortcomings. One of the reasons is variation in the composition of POME from different sources which be favorable the growth of certain species of microalgae. Another reason is the pre-determination of the optimum concentration of POME and the cultivation environment for that particular species (Ponraj and Din, 2013; Azimatun Nur and Hadiyanto, 2014; Nwuche *et al*., 2014). Therefore, the use of more than one specific species of microalgae in the form of mixed microalgae would be a more efficient bioremediation method for the treatment of POME (Pandey and Tiwari, 2010; Kshirsagar, 2013; Shaha *et al*., 2014).

Hence, this research study proposes the use of different species of microalgae in a form of mixed microalgae that might offer the better ability to remediate the raw POME than the use of a single species of microalgae. Also, the use of microalgae could reduce environmental pollution with the potential to be a great source for many biofuels and renewable green energy. As such, this research study attempts to use raw POME, which is considered a waste pollutant, as a cheap and vital source of nutrient to grow the mixed microalgae in order to determine its physical, chemical and productivity characteristics as an important source of green energy.

1.2 Problem Statement

While POME is considered a waste,it is considered a load rather than a part of the production process of palm oil. Therefore, the choice for the cheapest method of disposal is the continuous discharge of POME into nearby rivers. But, this of method of disposal increases the risk of environmental pollution. Many research used microalgae in the bioremediation of POME. However, most of the research works focused on specific types of microalgae. This research study processes the use of mixed microalgae to remediate the raw POME rather than the use of single species microalgae. This Proposal not only reduces environmental pollution, but it is also a great source for many biofuels and renewable green energy.

1.3 Objectives

The main objective of this research study is to determine the extent of the ability of the use of mixed microalgae to remediate raw POME and consequently to show a decrease in pollution and an enhancement of water quality. Thus, the main objectives of this study are :

1. To characterize raw POME at different concentrations and compare it before and after the cultivation of mixed microalgae.
2. To determine the productivity and species diversity of mixed microalgae in different concentrations of raw POME.

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