CULTIVATION OF SELECTED GREEN ALGAE FOR BIOMASS AND REMEDIATION OF PALM OIL MILL EFFLUENT (POME)

By

WAIS MOHAMMAD NAVID

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

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DEDICATION

To my beloved father Mohammad Kazim Wais,
My beloved mother Mastora Wais,
&
Strong supporters from my siblings,
Mohammad Khalid Wais,
Zuhal Wais,
Mohammad Edrees Wais,
And
Mohammad Shahid Wais

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

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Chairman :Shahrizim Zulkifly, PhD
Faculty :Science

The palm oil industry in Malaysia is one of the key players in contributing to the economy of the country. One of the major byproducts of the oil extraction process is the palm oil mill effluent (POME) and must undergo mandatory treatment before releasing the waste near rivers or water bodies. Studies on the remediation treatments is essential to prevent contamination to the surrounding aquatic environments. A massive amount of (POME) released from the industry is an ideal source of nutrients to cultivate micro and macroalgae. Micro and macroalgae are unicellular photosynthetic organisms that have the capability to reduce and assimilate contamination from wastewater and produce yield biomass for industrial applications. Therefore, this study aimed, to culture micro and macroalgae in different concentrations of POME (20%, 40%, 60%, and 80%) for microalgae and (5%, 10%, 15%, and 20%) for macroalgae as biological treatment. Furthermore, the species of micro and macroalgae were identified using microscopic and 18S rRNA gene sequences. The two species of microalgae (*Chlorella vulgaris, Tetradesmus bernardii*), mixed microalgae and macroalgae *Pithophora roettleri* were chosen for this research study. All the cultivation for micro and macroalgae were carried out in 250mL Erlenmeyer flasks containing 200mL medium with continuously light illumination 2000 Lux, pH 7-7.8, and room temperature of 25±1℃ for 21 days. The results showed positive effects on the growth, biomass production with 20% POME was optimal concentration for microalgae and 10% POME for macroalgae. Mixed microalgae revealed the highest growth rate with a mean value of (1.8677 ± 0.0560), followed by *Chlorella vulgaris* (1.7960 ± 0.0773) and *Tetradesmus bernardii* (1.7607 ± 0.0290). The lowest growth was found in 80% POME for *Tetradesmus bernardii* with a mean value of (0.7970 ± 0.2358) followed by *Chlorella vulgaris* (0.8927 ± 0.0363) and mixed microalgae (1.0413 ± 0.0644) respectively. And the highest biomass productivity was observed in 20% POME for mixed microalgae (with a mean value of 0.1733 ± 0.0057), followed by *Chlorella vulgaris* (0.1633 ± 0.0057) and *Tetradesmus bernardii* (0.1603 ± 0.0020). The lowest biomass was found in 80% POME for *Tetradesmus bernardii* (with a mean value of 0.0407 ± 0.0045) followed by *Chlorella vulgaris* (0.0447 ± 0.0055) and

mixed microalgae (0.0440 ± 0.0.0069), and for macroalgae, the highest biomass (fresh weight) was observed in 10% POME with a mean value of (0.8903 ± 0.0237). The lowest biomass (fresh weight) was found in 20% (with a mean value of 0.5417±0.0124). Similarly, the highest nutrient removal (COD, TN, TP, N, P, and NH_4) was observed in 20% POME for mixed microalgae with the highest percentage of COD (66%), TN (86%), TP (68%), N (80%), and P (64%) and lowest (in 80% POME) for COD (13%), TN (20%), TP (18%), N (29%), and P (31%). This is followed by *Chlorella vulgaris* at 20% POME with the highest removal of COD (64%), TN (79%), TP (49), N (72%), and P (59%) and at 20% POME, the lowest removal of COD (12%), TN (17%), TP (13%), N (26%), and P(19%) respectively. Next is *Tetradesmus bernardii* at 20% POME with highest removal of COD (61%), TN (72%), TP (35%), N (62%), and P (53%), and at 80% POME, the lowest removal at for COD (13%), TN (8%), TP (8%), N (21), P (17%). Lastly for macroalgae at 20% POME with the highest percentage of removal COD (70%), TN (70%), TP (80%), N (81%), and P (72%) and at 80% POME for lowest removal COD (16%), TN (16%), TP (38%), N (31%), and P (22%) respectively. In the future, POME has the prospect of an alternative medium for the cultivation of micro and macroalgae to achieve yield biomass for future industrial applications.

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

PENGKULTURAN ALGA HIJAU TERPILIH UNTUK BIOJISIM DAN REMEDIASI EFLUEN KILANG KELAPA SAWIT (POME)

Oleh

WAIS MOHAMMAD NAVID

May 2022

Pengerusi : Shahrizim Zulkifly, PhD
Fakulti : Sains

Industri minyak sawit di Malaysia merupakan salah satu penyumbang utama kepada ekonomi negara. Salah satu hasil sampingan utama proses perahan minyak ialah efluen kilang kelapa sawit (POME) dan perlu menjalani rawatan mandatori sebelum sisa itu dilepaskan berhampiran sungai atau badan air. Kajian mengenai rawatan pemulihan adalah penting untuk mengelakkan pencemaran dalam persekitaran akuatik di sekeliling. Mikro dan makroalga adalah organisma unisel fotosintetik yang mempunyai keupayaan untuk mengurangkan dan mengasimilasikan pencemaran daripada air sisa dan menghasilkan biojisim untuk aplikasi di dalam industri. Oleh itu, kajian ini bertujuan untuk mengkultur mikro dan makroalga dalam kepekatan POME yang berbeza (20%, 40%, 60%, dan 80%) untuk mikroalga dan (5%, 10%, 15%, dan 20%) untuk makroalga sebagai rawatan biologi. Mikroalga campuran (*Chlorella vulgaris* dan *Tetradesmus bernardii*), *Chlorella vulgaris*, dan *Tetradesmus bernardii*, dan makroalga, *Pithophora roettleri* telah dipilih untuk kajian ini. Kesemua pengkulturan mikro dan makroalga telah dijalankan di dalam kelalang Erlenmeyer 250mL yang mengandungi 200mL medium dengan pencahayaan cahaya berterusan 2000 Lux, pH 7-7.8, dan suhu bilik 25±1℃ selama 21 hari. Hasil kajian menunjukkan kesan positif terhadap pertumbuhan dan pengeluaran biojisim dengan kepekatan optimum untuk mikroalga adalah 20% dan untuk makroalga, 10%. Mikroalga campuran menunjukkan kadar pertumbuhan tertinggi dengan nilai min (1.8677 ± 0.0560), diikuti oleh *Chlorella vulgaris* (1.7960 ± 0.0773) dan *Tetradesmus bernardii* (1.7607 ± 0.0290). Pertumbuhan paling rendah didapati dalam 80% POME untuk *Tetradesmus bernardii* dengan nilai min (0.7970 ± 0.2358) diikuti oleh *Chlorella vulgaris* (1.7960 ± 0.0773) dan mikroalga campuran (1.0413 ± 0.0644). Produktiviti biojisim tertinggi diperhatikan dalam 20% POME untuk mikroalga campuran dengan nilai min (0.1733 ± 0.0057) diikuti oleh *Chlorella vulgaris* (0.1633 ± 0.0057) dan *Tetradesmus bernardii* (0.1603 ± 0.0020). Pertumbuhan paling rendah didapati dalam 80% POME untuk *Tetradesmus bernardii* dengan nilai min (0.0407 ± 0.0045) diikuti oleh *Chlorella vulgaris* (0.0447 ± 0.0055) dan mikroalga campuran (0.0440 ± 0.0069), dan untuk makroalga biojisim tertinggi (berat basah) diperhatikan

dalam 10% POME. Nilai (0.8903 ± 0.0237). Biojisim terendah (berat basah) didapati dalam 20% dengan nilai min (0.5417 ± 0.0124). Begitu juga, penyingkiran nutrien tertinggi (COD, TN, TP, N, P, NH_4) diperhatikan dalam 20% POME untuk mikroalga campuran telah dengan peratusan COD tertinggi (66%), TN (86%), TP (68%), N (80%), dan P (64%) dan terendah (dalam 80% POME) untuk COD (13%), TN (20%), TP (18%), N (29%), and P (31%). Ini diikuti oleh *Chlorella vulgaris* pada 20% POME dengan penyingkiran COD tertinggi (64%), TN (79%), TP (49), N (72%), and P (59%) dan terendah (dalam 80% POME) untuk COD (12%), TN (17%), TP (13%), N (26%), and P(19%) masing-masing. Seterusnya *Tetradesmus bernardii* pada 20% POME dengan penyingkiran COD (61%), TN (72%), TP (35%), N (62), and P (53%), dan pada 80% POME, penyingkiran terendah COD (13%), TN (8%), TP (8%), N (21), P (17%). Akhir sekali, untuk makroalga pada 20% POME, dengan penyingkiran tertinggi COD (70%), TN (70%), TP (80%), N (81%), and P (72%) dan pada 80% POME dengan penyingkiran COD terendah (16%), TN (16%), TP (38%), N (31%) dan P (22%).Mikro dan makroalga telah terbukti berkesan dalam remediasi POME secara. Pada masa hadapan, dapat disimpulkan bahawa POME merupakan prospek sebagai medium alternatif untuk pengkuluran mikro dan makroalga bagi mencapai hasil biojisim untuk aplikasi industri.

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Shahrizim bin Zulkifly, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Mohd Hafiz bin Ibrahim, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIF, PHD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 14 December 2023

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
µL	Microliter
mg	Milligram
g	Gram
kg	Kilogram
DO	Dissolved oxygen
OD	Optical density
POME	Palm oil mill effluent
COD	Chemical Oxygen Demand
TN	Total Nitrogen
TP	Total Phosphorous
N	Nitrogen
P	Phosphate
CPO	Crude Palm oil
Cm	Centimeter
CO_2	Carbon di Oxide
L	Liter
GNI	Gross national income
pH	Potential of hydrogen
mg/L	Milligram per liter
µg/L	Microgram per liter
ppt	Parts per thousand
hr	Hour

FFB	Fresh fruit bunches
BOD	Biological Oxygen Demand
PBRs	Photobioreactors
NH_4	Ammonia
ATP	Adenosine triphosphate
NCBI	National center biotechnology information
TDS	Total dissolved solids

CHAPTER 1

INTRODUCTION

1.1 General introduction

Oil palm (*Elaeis guineensis* Jacq) is a South African species. It thrives in all tropical climates and has become a popular evergreen tree (Alam et al., 2015; Mohammed et al., 2011) The British introduced the African oil palm to Malaysia as an evergreen plant in the early 1870s. Henri Fauconnier, a Frenchman, was in charge of the first industrial oil palm cultivation in 1917 at Tennamaram state in Selangor (Alam et al., 2015). Palm oil is an essential component of the worldwide economic chain. (*Elaeis guineensis*) is able to be cultivated greater in moisture and hot tropical weather than other crops (Alam et al., 2015; Mohammed et al., 2011). It is a valuable crop that contributes to food security, and in several decades it expanded in tropical areas (Khatun et al., 2017). Crude palm oil and soyabean oil is the most extensively used vegetable oil worldwide. Oil palm produces almost half of all vegetable oil and fat, the fatty acid of CPO includes 13 specifics of fatty acids such as (lauric, myristic, pentadecanoic, palmitic, palmitoleic, margaric, stearic, oleic, vaccenic, linoleic, α-linolenic, arachidic, and gondoic) (Gonzalez-Diaz et al., 2021). Furthermore, this oil consists of several usages in food, cooking, oil industry, margarine, vegetable ghee, confectionery fat, icer cream, non-dairy cream, salad dressing, chocolate, supplements, vitamins, oleochemicals, cosmetics, agrochemicals, toilet soap, industrial cleaning, printing ink, biofuel, biodiesel, jet fuels, and biogas (Reijnders & Huijbregts, 2008). The palm oil (*Elaeis guineensis* Jacq.) is one of the most valuable and extensively grown tropical tree crops. Over the last few years, the palm oil sector has developed rapidly in Southeast Asia, particularly Indonesia and Malaysia (Tan et al., 2022).

The palm oil is the most significant oil-producing evergreen crop, which covers 73% of agriculture area, and produce 13 million tons annually (Ahmad et al., 2016). Malaysia has become the second-largest palm oil producer globally with estimated energy production of 5.53 million tons, 11% oil and fat export, and providing labor for one million people (Hazman et al., 2018). The palm oil expansion has contributed to job creation, income development, and poverty alleviation, contributing a large portion of the country's Gross National Income (GNI) with 47% global production (Rowland et al., 2022). Meanwhile, the palm oil industry has been recognized for its contribution to economic prosperity and quick expansion. It has also assisted in environmental contamination due to the huge volumes of by-products produced during the oil extraction (Ahmad et al., 2016).

The origin of wastes in the form of liquid produced during extraction and processing of oil and solid wastes include the leaves, trunks, decanter cake, empty fruit bunches, seed shells, and fibre from mesocarp. (Singh et al., 2010). Furthermore, the wastewater produced by the production of palm oil, known as palm oil mills effluent (POME), contains a variety of dissolved and suspended contaminants consisting of 95 - 96% water, 4 - 5% total solids, including 2 - 4% suspended solids, and 0.6 - 0.7% oil (Kamyab et al., 2015). POME consists of a high level of contaminants such as chemical oxygen demand (COD), biological oxygen demand (BOD), Nitrogen, Phosphorous that, exceeding the level of these nutrients will cause contamination to the aquatic zone (Khalid et al., 2016). Currently, several methods are used to treat POME in Malaysia, including anaerobic, aerobic, and facultative ponding systems (Hazman et al., 2018). In these POME treatment ponds, microorganisms such as bacteria, fungi, and green microalgae (*Chlorophyta*) are used to remediate wastewater (Mohd Udaiyappan et al., 2020). Ponding systems assist in producing methane, carbon dioxide (CO_2), and greenhouse gases after the decomposition of organic substances by bacteria (Hariz et al., 2018).

Algae are photosynthetic organisms due to availability of chlorophyll have the capability of changing sun light into chemical energy through the photosynthesis process. Algae come in variety shapes and sizes. Macroalgae the length is estimated in some species to 60 meters, and the microalgae is visible through magnification equipment (Ameen et al., 2021). Algae can assist in treating different wastewater, including municipal and industrial, with benefiting from nutrient removal (Ding et al., 2016). Algae require, a culture medium that includes inorganic elements such as nitrogen, phosphorous, iron, and silica, light energy for photosynthesis, and water for their growth. Wastewater is a considerable medium and source of water with less cost for growth, biomass production, and eco-friendly (Jasni et al., 2020). Meanwhile, microalgae are ideal because of the potential to absorb CO_2 via photosynthesis. It has been suggested as a substitute for the biological treatment of POME to eliminate the residual contaminants from palm oil mill effluent (POME) (Khalid et al., 2016). Furthermore, microalgae have the capability to grow in wastewater and a possible resource for renewable energy generation and provide economical bioethanol, biogas, and by-products (Kamyab et al., 2017).

The Usage of microalgae and macroalgae for wastewater remediation is ideal for reducing the cost and commercializing the algae-based biofuel, which is extremely eco-friendly and sustainable for the environment (Cheah et al., 2018). Micro and macroalgae have been used extensively for biofuel manufacture (Anto et al., 2020). Previous studies showed that *Chlorella* sp. was used for treating POME and CO_2 fixation (Hariz et al., 2018). whereas micro-macro algal mixture was used for treating POME and as a source of feeding for animals (Kamyab et al., 2014). Furthermore, green macroalgae also have the ability to eliminate the contaminants and heavy metals from polluted water bodies. In addition, To remediate the different wastewater, green macroalgae is a promising microorganism that has the ability to uptake the dissolved nutrient for their growth (Sode et al., 2013). Previously showed four species of macroalgae (*Oedogonium* sp., *Cladophora* sp., *Spirogyra* sp., and *Klebsormidium* sp.) have the high ability to uptake the nutrient from municipal wastewater (Lawton et al., 2021). Whereas macroalgae *Ulva rigida* were used for treating sugar industry wastewater for biogas and methane production (Karray et al., 2017). Moreover the other studied showed macroalgae *Cladophora glomerata* as a bio sorbent of toxic metals removal from wastewater (Michalak et al., 2019). Therefore, it can be a solution in which nutrients in wastewater can be recycled and transferred to biomass and contribute to ecological sustainability while assisting in food production, by-products, and bioenergy (Cole et al., 2016).

1.2 Problem statement and justification

Palm oil mill effluent (POME) is wastewater generated by oil palm processing, which causes contamination into the waterbodies. Manufacturing of palm oil requires much water, which one ton of palm oil needs 5-7 tons of water, and 50% finishes us as POME and 2.5 m^3 of POME produced form every ton of crude palm oil processing. Palm oil manufacturing is expected to raise production by 25 million tons in 2035. POME consist of a huge amount of chemicals such as COD, BOD, TP, TN, suspended solids. Additionally, it contains various heavy metals for instance; iron (Fe), Zinc (Zn), and manganese (Mn). Furthermore, the release of untreated POME can cause a crucial impact on the diversity of phytoplankton, disorder in reproductive and physiological system of fishes in aquatic ecosystem. Meanwhile, algae have the ability of removal nutrient from waterbodies due to their simple cell structure, and availability in different aquatic environments.

Micro and macroalga required water and nutrients for their growth which wastewater consist of nutrients is a suitable medium for the growth, through the photosynthetic process, algae transfer the light and CO_2 into valuable biomass. Algae biomass rich in organic and inorganic carbon is visible for different industrial purposes such us biofuel, biodiesel, biohydrogen, bioethanol, biomethane. On the other hand, algae-based bioenergy is renewable, biodegradable, low release of CO_2 into environments. Along with bacteria and fungi, microalgae are naturally available in the treatment ponds, especially green algae (Chlorophyta) have the capability to drive under photosynthetic process and assimilate the nutrient for their growth and produce profitable industrial biomass. Therefore, algae is a considerable microorganism to remediate the amount of effluent is released every day into the rivers, that negatively impact the aquatic environments and needs to find a conventional treatment process to meet the regulation set by the Department of Environment (DOE).

1.3 Objectives

The objectives of this study are:

1. To identify selected green algae species using 18S rRNA gene sequencing.
2. To cultivate selected green algae in artificial media infused with different concentrations of POME.
3. To evaluate selected green algae biomass from artificial media infused with POME.
4. To evaluate nutrient removal efficiency of selected green algae.

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