CULTIVATION OF MICROALGAE USING PALM OIL MILL EFFLUENT FOR LIPID PRODUCTION

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To my lovely husband

Reski Mai Candra

Very thankfull and grateful of the strong support.....

To my beloved parents ibu and ayah,

Roslaini and Syamsuri

Thanks for your valuable sacrifice and compassion.....

To my dearest sister and brother

Efni Uslinda, Efrizal Hendri, Eva Mardiyah, Efniza Sulsiyah, Elfi Mulziyah

To my brother in law : Bahrul Anif, Donni, Eko Widianto

To my cute nephew and niece : Fawwaz, Haziq and Najwa

Thanks for your support and always there for me in happiness and sadness.

I am very proud to have all of you

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ABSTRACT

Microalgae are reported as the potential resources to produce lipid from their biomass cell. Lipid is generally a group of organic compound that important as primary biofuel raw material, and also as component for foods, cosmetic products, fertilizers, animal feed, etc. As the resources of lipid production from synthetic media are costly, therefore the derivation of cheap sources from waste is useful in massive scale. Therefore, the study is emphasized on the effectiveness of industrial wastewater such as palm oil mill effluent (POME) as main carbon source to maintain the growth of microalgae and simultaneously increase the lipid content. In addition, glucose ($C_6H_{12}O_6$) is also used to compare the effectiveness of their cultivations. Furthermore, investigation of five selected strains of green microalgae are applied namely *Chlorella vulgaris* (Korean Collection for Type Cultures (KCTC) Biological Resource Center (BRC)), *Chlorella pyrenoidosa* (POME), *Chlorella sorokiniana* (UTEX 1602), *Botryococcus sudeticus* (UTEX 2629), and *Tetraselmis sp* (UTEX 2767). All cultivation of microalgae were initially carried out in 250 mL erlenmeyer flask containing 100 mL medium under ± 30^oC of temperature with continuous illumination (± 14 µmol/m^2/s) and up to 20 days of cultivations. The study demonstrated that *Chlorella sorokiniana*, is the predominant species for specific growth rate (µ), biomass productivity and lipid content in diluted POME with the value 0.099/day, 8.0 mg/L.day, 2.68 mg lipid/mg Cell Dry Weight (CDW), respectively. However, *Chlorella sorokiniana* showed that there was about one and half times more lipid productivity when the biomass cells utilized $C_6H_{12}O_6$ as carbon source, compared to POME. The optimization condition was determined with various carbon-to-total nitrogen (C:TN) ratio and light/dark (L:D) cycles, respectively. As a result, the highest lipid content achieved when the condition controlled at C:TN (100:7) and continuous light duration (24 hr), with recorded value of 17 mg lipid/mg CDW. These results conclude that *Chlorella sorokiniana* had highest growth rates and lipid production in diluted POME compared to other strains of microalgae. Finally, the study suggested several improvement of the experiment to achieve higher lipid production at steady - state condition by manipulating the ratio of carbon-to-total nitrogen and the medium of light intensity.

ABSTRAK

Mikroalga dilaporkan sebagai sumber yang berpotensi untuk menghasilkan lipid daripada sel biojisim. Lipid secara umumnya merupakan satu kumpulan sebatian organik yang penting sebagai bahan mentah utama biofuel, dan juga sebagai komponen untuk makanan, kosmetik, produk baja, pemakanan haiwan, dan lain - lain. Sumber penghasilan lipid daripada media sintetik adalah mahal, oleh kerana itu penghasilan sumber yang murah daripada sisa bahan buangan adalah berguna untuk skala yang besar. Kajian ini memberi penekanan kepada kesan air sisa industri seperti buangan kilang kelapa sawit (POME) sebagai sumber karbon yang utama untuk mengekalkan pertumbuhan mikroalga dan meningkatkan kandungan lipid. Di samping itu, glukosa ($C_6H_{12}O_{6)}$ digunakan sebagai perbandingan pengkulturan yang berkesan. Selain itu, lima jenis mikroalga hijau yang dipilih untuk kajian adalah seperti *Chlorella vulgaris* (Koleksi dari Korea untuk jenis kultur (KCTC), Pusat Sumber Biologi (BRC), *Chlorella pyrenoidosa* (POME), *Chlorella sorokiniana* (UTEX 1602), *Botryococcus sudeticus* (UTEX 2629) dan *Tetraselmis sp* (UTEX 2767). Semua pengkulturan mikroalga dilakukan di dalam 250 mL kelalang yang mengandungi 100 mL medium di bawah suhu kawalan ± 30^{o}C dengan pencahayaan yang berterusan (± 14 µmol/m^2/s) dan dikultur sehingga 20 hari. Kajian ini menunjukkan bahawa *Chlorella sorokiniana* adalah spesies yang berpotensi dan paling dominan kerana ia mencatatkan bacaan yang lebih baik untuk kadar pertumbuhan tertentu (µ), biojisim, dan kandungan lipid dalam cairan POME dengan nilai yang direkodkan masing – masing sebanyak 0.099/hari, 8.0 mg/L.hari dan 2.68 mg lipid/mg berat kering sel (CDW). Namun, *Chlorella sorokiniana* mempunyai produktiviti lipid kira - kira satu setengah kali lebih apabila sel – sel biojisim menggunakan $C_6H_{12}O_6$ sebagai sumber karbon, berbanding POME. Keadaan yang optimum didapati sebagai variasi nisbah karbon terhadap pelbagai jumlah nitrogen dan cahaya/gelap (L:D) kitaran, masing – masing. Hasilnya, kandungan lipid tertinggi diperolehi pada keadaan yang dikawal C:TN (100:7) dan pencahayaan yang berterusan dengan nilai sebanyak 17 mg lipid/mg CDW. Keputusan ini menunjukkan bahawa pertumbuhan *Chlorella sorokiniana* mempunyai kadar pertumbuhan dan pengeluaran lipid tertinggi dalam POME yang dicairkan, jika dibandingkan dengan jenis mikroalga yang lain. Akhir sekali, kajian ini mencadangkan beberapa penambahbaikan untuk menjalankan eksperimen bagi mencapai penghasilan lipid yang lebih tinggi pada keadaan stabil dengan memanipulasikan nisbah karbon kepada jumlah nitrogen dan keamatan cahaya yang sederhana.

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LIST OF ABBREVIATION

ATP	-	Adenosine Triphosphate
BBM	-	Bolds Basal Medium
BOD	-	Biochemical Oxygen Demand
B. sudeticus	-	*Botryococcus sudeticus*
C. sorokiniana	-	*Chlorella sorokiniana*
C. pyrenoidosa	-	*Chlorella pyrenoidosa*
C. vulgaris	-	*Chlorella vulgaris*
C : TN	-	Carbon to total nitrogen ratio
Ca	-	Calcium
CDW	-	Cell Dry Weight
cells/mL	-	Cell per mili liter
CO_2	-	Carbon dioxide
COD	-	Chemical Oxygen Demand
DNA	-	Deoxyribonucleic Acid
Fe	-	Ferrite
g/L	-	Gram per liter
H_2O	-	Hidrogen Oxide
$H_2PO_4^-$	-	Dihydrogen Phosphate Ion
HPLC	-	High-Performance Liquid Chromatography
HPO_4^{2-}	-	Hydrogen Phosphate Ion
K	-	Kalium
kJ/g	-	Kilo Joule per gram
L: D	-	Light : dark
Mg	-	Magnesium
mg/L/day	-	Mili gram per liter per day

MLSS	-	Mixed Liquor Suspended Solid
MLVSS	-	Mixed Liquor Volatile Suspended Solid
N	-	Nitrogen
NADP+	-	Nicotinamide Adenine Dinucleotide Phosphate
NH^{4+}	-	Ammonium
NO_2^-	-	Nitrite
NO_3^-	-	Nitrate
OD	-	Optical Density
P	-	Phosphorus
POME	-	Palm Oil Mill Effluent
R^2	-	Variance accounted
RNA	-	Ribonucleic Acid
So	-	Substrate concentration
T_{opt}	-	Temperature optima
TAGs	-	Triacylglycerols
TSS	-	Total Suspended Solids
VSS	-	Volatile Suspended Solids
Zn	-	Zinc

LIST OF SYMBOLS

μ	-	specific growth rate
μ_m	-	maximum specific growth rate
A	-	weight of filter +dried residue
B	-	weight of filter
C	-	weight of residue + dish before ignition
D	-	weight of residue + dish or filter after ignition
e	-	exponential
K_i	-	dissociation constant
K_s	-	half saturation constant
L	-	width of cuvette
t	-	time
τ_d	-	doubling time of cell mass
τ'_d	-	doubling time based on the replication rate
V_1	-	volume of extract
V_2	-	volume of sample
X	-	concentration at beginning
X’	-	growth of microalgae concentration
X'_0	-	value of growth concentration
X_0	-	concentration at beginning
α	-	alpha
β	-	beta
γ	-	gamma
ε	-	epsilon

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Nowadays, palm oil industry is growing rapidly and becoming a significant agriculture-based industry in Malaysia. The number of palm oil mills has increased tremendously, at starting with 10 mills in 1960 climbed to 410 operated mills in 2008. At least 44 million tones of POME was generated and are expected to rise every year in Malaysia (Wu *et al*., 2010), particularly because of the initiative of the government to promote palm oil industry. Furthermore, it is necessary to proper address in Palm Oil Mill Effluent (POME) treatment so as not contribute to human health hazards and environmental pollution.

Small scale and economically viable technologies that combine wastewater treatment and energy production can treat the industrial effluents and enhance the availability of the energy simultaneously (Lansing *et al.*, 2008). The feasible way that is more attentions in the present time is the use of microalgae, which is known to have the potential to treat wastewater (Tarlan *et al.*, 2002) such as remove CO_2 and NO_x (Jin *et al*., 2008), high capacity of nutrient uptake (Park, 2009). The idea of using microalgae in wastewater treatment has been investigated since 1950s, by Oswald (1957).

Microalgae appear to be an attractive renewable energy source especially for biodiesel production (Travieso *et al*., 2006; Chisti, 2007; Griffiths and Harrison, 2009; Converti *et al*., 2009; Gao *et al*., 2010; Feng *et al*., 2011). Its due to their have rapid growth rate: 100 times faster than land based plant and they can double their biomass in less than a day. Furthermore, microalgae are able to divide once every 3-4 h, but mostly divide every 1-2 days under favorable growing conditions (Griffiths and Harrison, 2009; Huang *et al*., 2010; Lam and Lee, 2011).

Actually, the interest in microalgae for biodiesel production is due to the high lipid content of some species, and to the fact that lipid synthesis, especially of the non-polar TAGs (*triacylglycerols*), which are the best substrate to produce biodiesel, can be modulated by varying growth conditions. The total content of lipids in microalgae may vary from about 1–85% of the dry weight, with values higher than 40% being typically achieved under nutrient limitation. Moreover, view of factors such as temperature, irradiance and, most markedly, nutrient availability have been shown to affect both lipids composition and content in many microalgae (Rodolfi *et al*., 2009).

1.2 Problem Statement

Because agro-industrial wastewater consist of large amounts of organic compounds and heavy metals are hazardous to environmental health, microalgae have been suggested as very good candidate to remove these pollutants and breakdown the organic compounds present (Munoz and Guieysse, 2006).

On the other hand, culturing microalgae in wastewater offers an inexpensive alternative to conventional forms of wastewater treatments. At the same time microalgae can utilize the nitrogen and phosphorus compound in wastewater to generate microalgae biomass for lipids production as well as biofuel production (Huang *et al*., 2010). Moreover, since the late 1900's, the fuel have been tremendously used for transportation, power plants, heating, and cosmetics, then increasing fossil fuel prices and the contribution of petroleum to air pollution make fossil fuel untenable as the predominant source of energy.

Therefore, an alternative of energy and fuel source, especially from the renewable resource could make us be less dependent on the conventional fuel energy. Hence, microalgae come up as alternative technologies to resolve this problem. This study was undertaken with the aim to evaluate the potential of POME as carbon source for microalgae to produce high lipid.

1.3 Objectives of the Study

i. To identify a suitable strain of microalgae species, which could effectively grows in POME.

ii. To compare the ability of microalgae species growth and survive in POME and glucose as carbon sources.

iii. To quantify the lipid content from suitable microalgae based on optimum condition of carbon to total nitrogen ratio (C: TN), and cultivation period.

1.4 .Scope of the Study

The study is to emphasize on the production of microalgae using POME as substrate with varying operating parameter (e.g optical density (OD), Chlorophyll content, Mixed Liquor Suspended Solid (MLSS), Mixed Liquor Volatile Suspended Solid (MLVSS), and Cell Dry Weight (CDW)). POME was collected from facultative ponds in Kahang Palm Oil Mill, Johor Bahru, Malaysia. This study start under different concentration, i.e. 0, 250, 500, 1000 mg COD/L with *Chlorella vulgaris* applied.

The appropriate concentration would be used in subsequent experiments. First, to investigate five strain of green microalgae such as *Chlorella sorokiniana, Chlorella pyrenoidosa, Botryococcus sudeticus, Tetraselmis sp* and also *Chlorella vulgaris*, which were detected as high lipid content. Next, comparison between POME and glucose as carbon source has been identified to examine the effect of mixed carbon compound for the specific growth rate (μ) of microalgae. Finally, optimization of lipid content from chosen microalgae strain based on suitable conditions including, carbon to total nitrogen ratio and photo light period have been investigated.

1.5 Significance of the Study

A number of researchers used wastewater as growth medium for microalgae cultivation, biomass productivity and test the capability of microalgae to remove nitrate, reduce levels of phosphate, ammonium and nitrate. Kind of wastewater commonly used comes from domestic wastewater, municipal wastewater, agricultural wastewater, artificial wastewater and industrial wastewater.

Furthermore, culturing microalgae in wastewater offers an inexpensive alternative to conventional forms of biological wastewater treatments and at the same time to utilize the nitrogen and phosphorus compounds in wastewater. Here, this study is employing industrial wastewater comes from palm oil mill effluent in order to evaluate the potential of microalgae growth, biomass productivity, and lipid production. Therefore, the study is significant to prove that POME as potential carbon source for microalgae growth in producing lipid, which are useful for finding an alternative energy and fuel source particularly from the renewable sources.

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