SUSTAINABLE PRODUCTION AND CHARACTERIZATION OF MICROALGAE USING AQUAPONICS SYSTEM FOR BIOFUEL APPLICATION

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SUPERVISORS' DECLARATION

I hereby declare that I have checked the thesis and in my opinion, this thesis is adequate in terms of scope and quality of the award of the degree of Bachelor of Applied Science (Honor) Industrial Biotechnology.

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own and not plagiarized except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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ABSTRACT

This thesis consists microalgae production using aquaponics system. Building of this system involves the piping and connection to integrate several components. It starts with the fish tank where fish were to be fed and secrete wastes into the water. The waste water then pumped up to the hydroponic tank containing hydroton, or known as clay balls for microbes and plants attachment. The waste water should be converted to nutrient water by nitrogen-fixing bacteria and directed (drained) to other tank for microalga culture. Lastly, the water flow now will pass through a filter cartridge to harvest algae and giving clean water back to the aquarium. In term of characterization of microalgae, firstly the growth of microalgae are determined using spectrophotometric and gravimetric analysis. Specific growth rate of microalgae in aquaponics system I can reach up to 0.127g of wet mass/day, or 0.141g of dry mass/day and 0.14 absorbance value/day. As compared to system I, system II achieved relatively higher rate, with 0.171g of wet mass/day, 0.194g of dry mass/day or 0.145 absorbance value/day. Throughout this research, microalgae were harvested 3 times, at the 21st day, 35th day and 77th day, and the highest microalgae biomass productivity calculated for system I and II are 0.0799g/L of culture per day and 0.1095g/L of culture per day respectively. Besides that, microscopic identification of microalgae species present in the culture by referring to available literature sources. Some dominant species present in the sample microalgae population identified are *Desmodesmus sp.*, *Micractimium sp.*, *Chlorella sp.*, *Staurodesmus sp.*, *Coelosphaerium sp.;* and *Frustulia sp.* On the other hands, for the biofuel application, conventional liquid-liquid extraction with chloroform-methanol was used to extract microalgal lipid, the highest total lipid obtained in the experiment was 21.73% and 18.82% for system I and II respectively. Then, the production of FAME, using alkali-catalysed transesterification are quantified and analyzed using GC-MS. The most abundant FAME biofuel analyzed was methyl formate and acetic acid methyl ester, with up to 9.03% of peak area. Lastly, the biofuel yield calculated was 3692.08g/hectare and 4646.37g/hectare for system I and II respectively. In addition, nile red staining technique was also used to obtain the fluorescence intensity for microalgae lipid quantification. The lipid concentration recorded was 749.50mg/l and 3389.77mg/l for system I and II respectively.

ABSTRAK

Tesis ini mengandungi sedikit sebanyak tentang penghasilan mikroalga dengan menggunakan sistem akuaponik sementara mengenal pasti ciri-cirinya. Pembinaan sistem ini melibatkan sambungan paip untuk mengepam air sisa ke bekas tumbuhan hidroponik yang dipasang di posisi atas. Ia mengandungi bebola tanah liat sebagai sokongan kepada mikrob dan tumbuh-tumbuhan. Seterusnya, sistem sifon membolehkan tangkungan air dalam bekas tumbuhan dan mengalirkannya ke tangki mikroalga sebelum dialirkan ke penapis untuk penuaian alga dan pembersihan air. Proses ini akan berulang dan berterusan. Dari segi usaha yang diguna untuk mengenal pasti mikroalga, pertumbuhan mikroalga diukur dengan menggunakan analisis spektrofotometri dan gravimetrik untuk mendapatkan kuantiti dan biomasnya. Graf akan diplot untuk mengira kadar penumbuhan. Kadar pertumbuhan spesifik mikroalga dalam sistem aquaponics I boleh mencecah sehingga 0.127g jisim basah sehari, atau 0.141g jisim kering setiap hari dan 0.14 nilai kuantiti setiap hari. Berbanding dengan sistem I, sistem II mencapai kadar yang lebih tinggi, dengan 0,171 g jisim basah setiap hari, 0.194g jisim kering sehari atau 0.145 nilai kuantiti setiap hari. Kemudian, sepanjang kajian ini, mikroalga dituai 3 kali, pada hari ke-21, hari ke-35 dan hari ke-77, dan produktiviti mikroalga paling tinggi untuk sistem I dan II adalah 0.0799g setiap Liter sehari dan 0.1095g setiap Liter setiap hari. Selain itu, pengenalan spesies alga juga dilakukan dengan cara mikroskopik dan rujukan sumber-sumber sastera yang sedia ada. Spesis dominan di dalam populasi sampel mikroalga yang dikenal pasti ialah *Desmodesmus sp., Micractimium sp., Chlorella sp., Staurodesmus sp., Coelosphaerium sp., Selenastrum sp., Frustulia sp.* Di samping itu, konvensional pengekstrakan cecair dengan menggunakan kloroform-metanol untuk mendapatkan lipid mikroalga, jumlah lipid yang paling tinggi diperolehi dalam eksperimen adalah 21.73% dan 18.82% bagi sistem I dan II. Kemudian, pengeluaran biofuel atau dikenali sebagai FAME akan dihasilkan dengan cara transesterification dalam situasi alkali. Produk akan diukur dan dianalisis menggunakan GC-MS. FAME yang paling banyak dianalisis adalah formate metil dan asid asetik metil ester, dengan nilai 9.03% daripada kawasan puncak. Akhir sekali, hasil biofuel yang dikira adalah 3692.08g sehektar dan 4646.37g sehektar untuk sistem I dan II. Di samping itu, teknik pewarnaan lipid mikroalga dengan menggunakan 'nile red' dapat mengetahui kandungan lipidnya dengan bacaan penyinaran isyarat pendarfluor yang ditunjukkan secara kuatitatif. Kepekatan lipid dicatatkan adalah 749.50mg/l dan 3389.77mg/l untuk sistem I dan II.

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LIST OF SYMBOLS

~	Approximately
&	And
e.g.	For example
r	Specific growth rate
k	Doubling time
x_m	Mass of empty falcon tube
y_m	Mass of centrifuged pellet in falcon tube
z_m	Mass of dried pellet in falcon tube
s	Standard deviation
$\bar{X}$	Mean
x	X-value of sampling data
R^2	Regression coefficient
$\sum$	Summation
n	Number of sampling data

LIST OF ABBREVIATIONS

GHG	Greenhouse gas
DO	Dissolved oxygen
Temp.	Temperature
Cond.	Conductivity
TDS	Total dissolved solid
FAME	Fatty acid methyl ester
GC-MS	Gas chromatography- Mass spectrometer
FFA	Free fatty acid
GAP	Glyceraldehyde phosphate
Acetyl-coA	Acetyl coenzyme A
ASTM	American Society for Testing and Materials
ACCE	Acetyl-coA carboxylic enzyme
FAS	Fatty acid synthase
MDH	Malate dehydrogenase
GPDH	Glycerol-3-phosphate dehydrogenase
TO	Triolein
PC	Phosphatidylcholine
TG	Triglyceride
BFT	Bioflocs technology
BMM	Bristol's Modified Medium
UV-Vis	Ultraviolet-Visible Light
sp.	Species
BNS	Batch nitrogen starvation

ANOVA	Analysis of variance
SE	Standard error
CV	Coefficient of variation

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF THE RESEARCH

The vital role of microalgae as the alternative source for biofuel production is getting more focused due to the need for sustainable production of renewable energy. This is because the world energy crisis is being aggressively addressed by global researchers and developers since the exhaustion of fossil fuel resources is getting viral. In addition, the release and accumulation of Green House Gas (GHG) like nitrous oxide (N_2O) and methane (CH_4) as the consequence of fuel combustion has put our earth in jeopardy. The agriculture, transportation and energy sector had emitted 29% to 69% of GHG, which lead to the occurrence of global warming. Absorption of one third of GHG by ocean has also increased the water pH, and indirectly affected aquatic and marine ecosystem balance (Mata *et al.*, 2009). It is proven that biofuel application from microalga is renewable and carbon-neutral with CO_2 separation or removal. Microalga produce non-toxic and highly biodegradable biofuel.

One of the cultivation technologies used for microalga is the open pond systems. The population of microalgae are cultivated in open ponds with design called raceway pond. Open ponds can be built and operated very economically in large scale, with relatively easy harvesting. However, the productivity cannot be manipulated due to the water lost via evaporation and contamination by foreign species. Raceway ponds are more expensive to build as with extra and stable infrastructure to overcome faster flow rates as well as existing geographical resistances. Besides that, the closed bioreactor design which saves input usage like water, energy and chemicals, thus it requires low cost. It also boosts productivity with adjustable volume and optimized condition like axenic condition, ambient environmental condition. However, setup cost for high

technology and equipment are required, with the necessary of expertise with knowledge of microalgal kinetics and growth dynamics become the short point of it. The combination of both systems is also possible for cost effective microalga production (Schenk *et al.*, 2008).

Although previous generation of biofuels, mainly produced from food crops, oil seeds, animal fat, and waste cooking oil have been implemented and commercialized for a long time, their production systems still have some economic and environmental boundaries in their feasibility to meet market demand, especially in this era of modernization, climate change, mitigation and economic growth can affect their productivity (Mata *et al.*, 2009). As production capacity increases, the competition with agricultural lands allocated for food production will also be increased. In details, biofuel supplied by previous generation of feedstock can only afford approximately 0.3% (~12 million tons in year 2007) of the current global oil demand, indirectly lead to unquenchable future energy fuel demand. Based on research, 8% of plant-based oil production is used as biodiesel (Schenk *et al.*, 2008) and this has led to an increase of the oil crops' price from years to years.

It is well-known that surface of the Earth exposed to abundant solar energy in the form of sunlight. Consequently, this makes the light energy required for lipid synthesis in green organisms highly available. However, there is an issue that even all the available lands are planted with oil-producing crops, the supply would only be able to satisfy less than half of our energy consumption today under the estimated yield and assumptions. Thus, biofuel production is not proportional to the global fuel requirements. Besides that, the vigorous exploitation of land has resulted in non-sustainable practices serious environmental problems. For example, the deforestation of rainforest regions in Brazil and South East Asia for soybean and oil palm plantations as crops for biofuel production (Schenk *et al.*, 2008).

This research is aimed to mainly characterize and sustainably produce microalga as the feedstock for biofuel production using aquaponics system. Aquaponics system is used to retain the productivity at satisfactory level without causing major contamination from environmental pollution, wastage of energy, and resources. Moreover, it is environmental friendly and high economic value. It is able to solve problems faced

potentially solved. However, integration of microalga culture with aquaponics system, received very little research conducted, and less literature available.

The main challenges faced throughout this research are that algae harvesting in the right timing is required to achieve maximal harvest at the peak period for desired end-products (Halfhide *et al.*, 2014). Freshwater used in aquaponics system is generally obtained from tap water and distilled water, which were heavily contaminated with metals and it is too toxic for microalgae growth (Andersen, 2005). Therefore, expansion of aquaponics system with additional chambers to accommodate aquaculture, hydroponic and microalga ecosystem is necessary. The need of light exposure to fulfill photosynthetic requirement of microalgae may evaporate water and increase the temperature within the system, all these should be taken into consideration to observe then make adjustment as the prevention from system failure.

1.3 OBJECTIVES OF THE RESEARCH

Generally, this research aims to sustainably produce and characterize microalga as the resource of biofuel production using aquaponics system. The detailed objectives are as below:

1. To characterize and sustainably produce microalga species using aquaponics system.
2. To investigate water quality parameters for the production of microalgae.
3. To characterize production of Fatty Acid Methyl Ester (FAME) as biofuel from extracted microalgae lipid.

1.4 SCOPE OF STUDY

To define my research, the explanation for the title is necessary. Firstly, "sustainable production", is the capability to tolerate and retain, it is how a systems keep active and productive continuously, with the ability to be maintained at certain rate or level. The sustainable production is the process of synthesis or generation without causing contamination, waste of energy and resources, friendly to environment, with high economic value.

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