UNIVERSITI PUTRA MALAYSIA

PRODUCTIVITY AND PROXIMATE ANALYSES OF SPIRULINA (ARTHROSPIRA PLATENSIS) IN DIFFERENT FLOATING WATER-BASED PHOTOBIOREACTOR DESIGNS

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By

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Chairman : Hishamuddin Omar, PhD
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Various concepts of photobioreactor (PBR) have been discovered scientists around the world. Though, only definite photobioreactor designs would be suitable for growing microalgae in a certain geographical climate outdoor condition. This study focused on developing simple floating water-based photobioreactor (PBR) without any computerized controlled systems. The aim of this study was to identify the chronology in developing best design overall for a photobioreactor. Major part of the design involved the aspect of the structure materials, shape and rigidness, whereas minor parts of the photobioreactors design includes the aeration placement, agitation or mixing, coloration corresponding to the high light intensity, temperature control, the size of the openings for the free exchange of gas and support for stable float on the water. Experiments performed would indicate these simple floating photobioreactors whether there are either significant or insignificant effects on the productivity of *Arthrospira platensis* (*Spirulina*) compared to the simple land based PBR especially under tropical climate.

In outdoor condition in Malaysia, the weather patterns are extremely unexpected. Weather patterns that usually occurs can be categorized into three namely, first, humid or wet weather that have a high frequency of rain; mixed weather which has average frequency of rain occurrence, overcast with thick cloud layer and also relatively have high light intensity; finally, dry weather having less rain, often exposed to high temperatures and bright sunlight.

Floating photobioreactor (PBR) has been designed in two distinct rigid shapes (Octagonal and Cylindrical) built using recycled water bottles, Polyethylene terephthalate (PET).While another form of simple floating PBR was designed more flexible as floating enclosure and was custom made from

High Density Polyethylene (HDPE) materials. Simple land based PBR was prepared with High-density Polyethylene (HDPE) plastic bag, (25cm x 50cm). For every minor modification, 10 days of *A. platensis* cultivation inside all PBRs were conducted with daily monitoring of growth parameters. The expected outcome from this experiment shall anticipate that floating PBRs would gives higher yield in term of biomass dry weight and specific growth rate of cultured *A. platensis* in comparison of land based PBR. In any case, developing and third world countries could use simple floating PBRs for commercial applications instead of investing on impractical and complicated photobioreactor designs.

For proximate analyses, the average total protein content (%) of *A. platensis* cultured in Cylindrical and Octagonal PBR under dry weather condition was higher significantly ($p < 0.05$) at 61.18±0.45 and 60.58±0.62 than other PBRs respectively. Total carbohydrate content (%) of *A. platensis* cultured in Cylindrical PBR (dry weather condition) was significantly ($p < 0.05$) higher at 26.71±1.43. While the lowest lipid content recorded in Flexible PBR, 0.66 ± 0.579% respectively. Highest scored by Cylindrical PBR, 7.883±0.28 under mix weather condition.

The study indicated simple floating photobioreactor system for practical commercial cultivation system. Water based cultivation system has been seen promising compared to land based cultivation system. Several advantages were determined as more dry biomass and productivity of Spirulina were achieved compared to common practice of land based cultivation system. Moreover, this simple enclosed floating photobioreactor system may be an economical starter approach for modern farmers in order to maintain high quality, cleanliness and purity of Spirulina.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PRODUKTIVITI DAN ANALISIS PROKSIMA SPIRULINA (*ARTHROSPIRA PLATENSIS*) YANG DIKULTUR DALAM PELBAGAI REKA BENTUK FOTOBIOREAKTOR YANG TERAPUNG BERASASKAN AIR

Oleh

MOHAMED AMAR NAQQIUDDIN BIN ABDUL KADER

Julai 2016

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Pelbagai konsep fotobioreaktor (PBR) telah ditemui ahli-ahli sains di seluruh dunia. Akan tetapi, reka bentuk fotobioreaktor yang sesuai sahaja dapat meningkatkan pertumbuhan mikroalga dalam keadaan iklim geografi tertentu. Kertas kerja ini memberi tumpuan kepada pembangunan fotobioreaktor (PBR) terapung berasaskan air yang mudah tanpa memerlukan sistem berkomputer yang rumit untuk mengawalnya. Tujuan kajian ini adalah untuk mengenalpasti susur galur reka bentuk yang paling ringkas dan terbaik secara keseluruhan bagi sesebuah fotobioreaktor. Reka bentuk sesebuah fotobioreaktor majoritinya melibatkan aspek struktur, bentuk fizikal dan ketegarannya, manakala aspek-aspek yang lain yang perlu ditekankan termasuk peredaran kultur yang konsisten, warna yang sesuai untuk reflex cahaya pada tahap yang tinggi, kawalan suhu kultur, saiz bukaan bagi pertukaran bebas gas dan sokongan bagi pengapungan stabil atas air. Ujian yang telah dilaksanakan akan member sedikit sebanyak pendedahan bahawa fotobioreaktor terapung dengan ringkas berasaskan air ini sama ada memberi impak yang jelas atau kurang kepada produktiviti *Arthrospira platensis* (Spirulina) berbanding dengan sistem pengkulturan mudah berasaskan darat terutamanya yang diuji sepenuhnya di bawah iklim tropika.

Corak cuaca kawasan lapangan di Malaysia pada kebiasaannya tidak dapat dijangkakan. Corak cuaca yang kebiasaannya berlaku boleh dikategorikan kepada tiga iaitu pertamanya, cuaca yang lembap yang mempunyai kekerapan yang tinggi turunnya hujan; keduanya cuaca yang bercampur yang mempunyai kekerapan kejadian hujan, mendung dengan lapisan awan yang tebal dan juga mempunyai limpahan cahaya matahari yang agak tinggi dan; akhirnya, cuaca kering yang kurang turunnya hujan, menghadapi kenaikan suhu yang tinggi dan mempunyai keamatan cahaya matahari yang terang.

Fotobioreaktor terapung (PBR) yang ringkas telah direka dalam dua bentuk tegar yang berbeza (PBR yang bersegi dan yang mempunyai bentuk silinder) dibina menggunakan bekas botol air, Polyethylene terephthalate (PET). Manakala satu lagi bentuk PBR terapung yang ringkas direka lebih fleksibel seperti bentuknya sampulan plastik terapung yang mana bahannya diperbuat dari Polyethylene Berketumpatan Tinggi (HDPE). Pengkulturan berasaskan darat pula menggunakan PBR yang telah direka ringkas dengan hanya menggunakan beg plastik Polyethylene yang berkepadatan tinggi (HDPE), (25cm x 50cm). Untuk setiap eksperimen yang dijalankan, *A. platensis* melalui pengkulturan selama 10 hari dalam semua fotobioreaktor dengan pemantauan parameter pertumbuhan harian secara teliti. Hasil yang diharapkan dari eksperimen ini akan memberi ilmu pengetahuan yang lebih mendalam sama ada system fotobioreaktor terapung yang ringkas ini dapat membuahkan hasil yang lebih atau kurang dalam jangkaan berat kering dan bagaimanakah jangkaan seterusnya pula pada kadar pertumbuhan bagi *A. platensis* jika dibandingkan dengan sistem pengkulturan berasaskan darat. Sehubungan dengan itu, negara-negara membangun dan negara dunia ketiga perlu mengambil peluang yang sedia ada menggunakan sistem fotobioreaktor terapung yang ringkas ini untuk aplikasi komersial dan juga perlu berhenti melaburkan masa dan wang untuk mereka bentuk fotobioreaktor yang tidak praktikal dan terlalu rumit.

Untuk analisis proksima , purata jumlah kandungan protein (%) daripada *A. platensis* yang dikulturkan dalam PBR berbentuk silinder dan oktagon di bawah keadaan cuaca kering adalah lebih tinggi dengan ketara ($p < 0.05$) masing-masing pada 61.18 ± 0.45 dan 60,58 ± 0,62 daripada PBR yang lain. Jumlah kandungan karbohidrat (%) daripada *A. platensis* yang dikulturkan dalam PBR yang berbentuk silinder (keadaan cuaca kering) adalah ketara (p < 0.05) lebih tinggi pada 26.71 ± 1.43. Manakala kandungan lipid (%) yang paling rendah telah dicatatkan melalui PBR fleksibel iaitu 0.66 ± 0,579. PBR berbentuk silinder pula telah merekodkan bacaan lipid yang tertinggi, 7,883 ± 0.28 di bawah keadaan cuaca yang bercampur.

Kajian menunjukkan sistem fotobioreaktor terapung ringkas ini menepati ciri-ciri praktikal untuk digunakan sebagai sistem pengkulturan moden yang komersial. Sistem pengkulturan berasaskan air dilihat lebih berpotensi berbanding dengan sistem pengkulturan yang berasaskan darat. Beberapa kelebihan telah dapat dikenalpasti daripada analisis berat kering dan produktiviti Spirulina tersebut yang dilihat telah meningkat pencapaiannya berbanding dengan sistem biasa pengkulturan berasaskan darat. Selain itu, sistem tertutup fotobioreaktor terapung yang ringkas ini adalah satu-satunya permulaan sebagai satu pendekatan ekonomi yang baru bagi petani moden supaya dapat mengekalkan kualiti dan kebersihan Spirulina yang tinggi.

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I certify that a Thesis Examination Committee has met on 26 July 2016 to conduct the final examination of Mohamed Amar Naqqiuddin bin Abdul Kader on his thesis entitled "Productivity and Proximate Analyses of Spirulina (*Arthrospira platensis*) in Different Floating Water-Based Photobioreactor Designs" 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 Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor Philosophy.

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%	Percentage
°C	Degree
µg	Microgram
µg day^{-1}	Microgram per day
µg g^{-1}	Microgram per gram
µl	Microlitre
µm	Micrometre
µmol L^{-1}	Micromolar per litre
µmol m^{-2} s^{-1}	Micromolar per second metre square
ANOVA	Analysis of variance
Ca	Calcium
$CHCl_3$	Chloroform
cm	Centimetre
CO_2	Carbon dioxide
Cr	Chromium
Cr (III)	Trivalent chromium
Cr (VI)	Hexavalent chromium
CrO_4^{2-}	Chromate
CrO_7^{2+}	Dichromate
Cu	Copper
DW	Dry weight
H_2O_2	Hydrogen peroxide
g L^{-1}	gram per litre

KCl	Potassium chloride
L	Litre
M	Molar
MeOH	Methanol
Mg	Magnesium
mg L^{-1}	Milligram per litre
mg mL^{-1}	Milligram per mililitre
mL	Millilitre
mm	Milimetre
mM	Milimolar
N	Nitrogen
Na_2CO_3	Sodium carbonate
nm	Nanometre
P	Phosphate
PBR	photobioreactor
ppm	Part per million
rpm	Round per minute
SD	Standard deviation
SE	Standard error
Se	Selenium
SPSS	Statistical Package for Science Social
UV	Ultraviolet
v/v	volume/volume

CHAPTER 1

INTRODUCTION

1.1 Introduction

Numerous studies have shown that microalgae benefit the environment by sequestering CO_2 and producing more O_2 while utilizing less water than normal crops (Kargupta *et al.,* 2015). Microalgae have higher productivity compared to terrestrial crop per size of production in long term. Due to great diversity and simplicity of its form, the evolution and performance of microalgae is beyond prospect than the same process from terrestrial crops which may take some years (Hannon *et al*., 2010). From thousands of microalgae species existed, few hundred have been successfully cultured in laboratories and less than 100 species have been commercialized. The most common microalgae commercially produced are *Chlorella vulgaris*, *Dunaliella salina* and Spirulina (Spolaore *et al*., 2006). Of all the species cultured, Spirulina (*A. platensis*) is widely cultured in many geographical regions of the world because it lives in alkaline medium which make it less susceptible to contamination. Most Spirulina producing regions lies in semi arid area where climate are stable, less cloud cover, ample sunshine and receiving less rain (Vonshak *et al.,* 1982). Malaysia on the other hand lies in the equator where it is believed to be less suitable for microalgae cultivation due to indistinct weather, frequent cloud covers and high precipitation which dilute growth medium.

Scientists have suggested cultivating microalgae as one of proficient ways to encounter escalating issues such as global warming and climate change which are among the most debated issues in the world presently. The magnitudes of these occurrences have profound effect to the global flora and fauna diversity whereby it could cause hundreds or thousands of species extinction. Global warming can alter atmospheric air and water circulation patterns which influence the weather patterns of air exchanges, rainfall distributions, wind speed, wind directions, precipitation, cloud covers and other variables (Barry and Chorley, 2009). Industrialization, forest destruction and other human activities are the major producer CO_2 and CH_4 which led to the formation of greenhouses gases causing global warming. The consequences of global warming are climate change producing phenomena like extreme weathers: tornadoes, huge typhoons, thunderstorms, flash floods, drought, acid rain, raising sea levels and more. Mitigation of global warming requires concerted efforts from major producer of greenhouse gas nation (Cop21paris, 2015). Although many nations have pledge to reduce greenhouse production, this will take time. Meanwhile scientific communities worldwide have provided numerous evidences pertaining to global warming including declining results caused by the atmosphere disturbance towards productivity of agriculture (Smith *et al.,* 2015).

Published reports on cultivation in outdoor condition especially in Malaysia are scanty. Small scale culture studies of Spirulina in outdoor conditions in Malaysia have shown that Spirulina can be grown successfully despite of those limitations. *A. platensis* can be grown successfully in sheltered outdoor conditions achieving dry weight of 0.8 g L^{-1} in 8 days (Sukumaran *et al.,* 2014). With suitable rain shelter, production of microalgae can commence successfully without any contamination from the surrounding and frequent rainfalls. Using conventional mode of cultivation, a Malaysian company, Algae International Sdn. Bhd. have patented a culturing system of Spirulina floating bed method (FBM) photobioreactor. However, the information on its performance was not published.

Discovery of floating photobioreactor (PBR) offered better alternative in culturing microalgae rather than using land base culture system with rain sheltering. Most invented photobioreactor (PBR) for microalgae cultivation are designed to be land-based system. Land-based photobioreactor often designed to have bigger surface area for light exchange for photosynthetic process compared to water based. Scaling up land based photobioreactor would be very costly and have higher risk of contamination. The concept of floating photobioreactor in water area, such as big lakes, pools, ponds or reservoirs was later recommended alternatively for better production economically (Wiley *et al.,* 2013). Large water bodies have thermal stability as such that the range of water temperature for open pond or sheltered raceway pond may not fluctuate extremely. Vast volume of water could act as a regulator to maintain temperature. However for land-based photobioreactor, additional cooling systems are incorporated in the design of photobioreactor system. Radiated heat from land surface and radiant heat absorbed by the PBR structures during daylight increased the temperature inside the PBR, therefore it is necessary to have cooling system or shelters covering land based PBR to balance the temperature back to optimal condition (Mehlitz, 2009). Every cultivation system design has different aspects to be reviewed in terms of maintenance, culture managements and suitability of the growing microalgae species with the system. Thus, the growth of microalgae differs depends to the design of the cultivation system. Types of mixing or aeration, sunlight illumination intensity, temperature regulations, air exchanges, coloration, transparency, buoyancy and structure materials are important variables that require serious consideration. Culture conditions inside PBR are one of the important factors for the microalgae growth and intracellular substance accumulation (Yoon *et al.,* 2008). According to Teresa *et al.* (2010) these factors are divided into biotic, abiotic and operational factors. The examples of biotic factors are pathogens and competition by other microalgae. Meanwhile, abiotic factors are dissolved oxygen, CO_2, pH, salinity, light intensity, temperature, nutrient concentration and toxic chemical buildup by the microalgae itself. Next, the operational factors are described as energy to produce mixing, dilution rate, depth, harvest frequency and additions of fertilizer and bicarbonate. Enhancement of the basic requirements for *Spirulina* (*A. platensis*) such as nutrient assessment, salinity, and pH should be done to maximize the productivity.

Outdoor cultivation in foreign countries usually has high initial installation cost excluding the maintenance and management cost. The most familiar system, open channel raceway ponds were being used for microalgae cultivation in commercial scale. Although it is easy to construct and operate, open system have low productivity, require large space area and has high risk of contamination. Open channel raceway ponds are not practical in Malaysia because lands are limited, expensive plus frequent rains will cause dilution and contamination. Lack of capitals and technical expertise will make conventional PBR less viable preposition. However the discovery of simple floating photobioreactor/floating bed method is an attractive preposition because of its design simplicity, cheap to operate, scalable and can operate in any sheltered water bodies. Nevertheless information on most aspect of this simple floating photobioreactor in Malaysian perspectives is lacking.

1.2 Objectives

1.2.1 General Objective

This paper will focus on finding suitable design of floating photobioreactor system which is simple, practical, low maintenance cost, handy and scalable.

1.2.2 Specific Objectives

1. To explore different design of simple floating photobioreactor in term of durability: size, shape, coloration, rigidity and flexibility; buoyancy, long lasting and tough materials; mixing, aeration; and the opening sizes of gases exchange area.
2. To determine the growth performance of *Arthrospira platensis* in terms of cell density, biomass dry weight and chlorophyll *a* content in different floating photobioreactor designs.
3. To measure the productivity rate of *A. platensis* and the bioeconomic of different floating photobioreactor designs.
4. To analyze the proximate analyses composition of grown *A. platensis* in different floating photobioreactor designs.

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