UNIVERSITI PUTRA MALAYSIA

PRODUCTIVITY ENHANCEMENT OF SPIRULINA (Arthrospira platensis) USING FLEXIBLE FLOATING-BED PHOTOBIOREACTOR, MIXING METHODS AND NITROGEN SOURCES

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By

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Nowadays, microalgae are becoming the focal point to solve malnutrition or malnourishment, especially in developing countries. Malnutrition significantly causes thousands of deaths especially among children. In Malaysia, mass cultivation of Spirulina has not been well explored. This is due to the lack of information and scientific data pertaining to Spirulina cultivation under Malaysian climate.

Therefore, the aim of this study was to develop a new culture system for Spirulina mass cultivation. The study was conducted under outdoor condition settings. *Arthrospira platensis* was cultured inside flexible floating-bed photobioreactor whereas land based photobioreactor was established as control. Half concentration of standard Kosaric medium from the original amount was used in this study. Both physical and growth parameters were determined daily while the nutritional content was determined at day-8 for cycles I, II and III. The pH variations recorded normal ascending patterns throughout the cultivation period. No significant difference ($p > 0.05$) of dissolved oxygen concentrations was found, however, land based photobioreactor showed slightly higher reading on average. A significantly higher ($p < 0.05$) culture temperature recorded inside land based photobioreactor was 32.97 ± 0.50 ºC, while the culture temperature inside flexible floating-bed was 28.97 ± 1.40 ºC. No significant difference ($p > 0.05$) of total chlorophyll and biomass variations were observed. Both flexible floating-bed and land based photobioreactors showed no significant difference in terms of protein content ($p > 0.05$) but recorded higher protein content of more than 45% of 1 g cell dry weight. Both photobioreactors recorded lower lipid content of not more than 1.5% of 1 g cell dry weight.

Subsequently, in outdoor environment and half strength of media concentration, *A. platensis* was cultured inside flexible floating-bed photobioreactor with different mixing methods namely aeration and agitation. Aeration mixing recorded

significantly lower ($p < 0.05$) dissolved oxygen concentrations. Lower values of temperature were also recorded ($p < 0.05$) between flexible floating-bed photobioreactor with aeration and land based photobioreactor. *Arthrospira platensis* with aeration mixing contained significantly more ($p < 0.05$) total chlorophyll content. Biomass concentration also revealed higher values ($p < 0.05$) for aeration mixing compared to agitation mixing. Major differences were observed for many parameters as higher dissolved oxygen concentration inhibits cell growth significantly. On average, protein content was higher ($p < 0.05$) in aeration mixing up to 46.57 ± 1.53%, compared to agitation at 22.47 ± 2.17%. Vice versa, agitation mixing recorded significantly higher ($p < 0.05$) carbohydrate content compared to aeration mixing. No significant difference ($p > 0.05$) was recorded for lipid content. Specific growth rate recorded for aeration was 0.223 1/day while for agitation was 0.121 1/day.

Both $NaNO_3$ and CH_4N_2O displayed promising growth performance of *A. platensis* for outdoor cultivation inside flexible floating-bed photobioreactor. No significant difference ($p > 0.05$) of optical density, total chlorophyll content and biomass concentration was recorded between the two different nitrogen sources for outdoor cultivation. However, optical density and total chlorophyll recorded slightly higher readings for $NaNO_3$ due to the nitrogen atom being readily able to be used by the cell in culture media compared to CH_4N_2O. Cultivation with $NaNO_3$ as nitrogen source revealed significantly higher ($p < 0.05$) protein content up to 46.57 ± 1.53%. Meanwhile, CH_4N_2O recorded lower protein content but the synthesis of carbohydrate content was significantly higher, up to 48.08 ± 1.27%. The specific growth rate of $NaNO_3$ was 0.223 1/day better than CH_4N_2O.

The study indicated higher growth in flexible floating-bed with aeration. $NaNO_3$ was better than CH_4N_2O. Bio-economic study has determined that the compound, CH_4N_2O is more cost effective as it is cheaper and readily available in the market.

Abstrak tesis yang dikemukakan kepadaSenatUniversiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENAMBAHBAIKAN PRODUKTIVITI SPIRULINA (*Arthrospira platensis)* DENGAN PENGGUNAAN FOTOBIOREAKTOR FLEKSIBEL TERAPUNG, KAEDAH PENCAMPURAN DAN SUMBER NITROGEN

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Pada masa kini, mikroalga mejadi perhatian utama untuk mengatasi masalah kekurangan zat makanan terutamanya di negara membangun. Kekurangan makanan mengakibatkan ribuan kematian. Di Malaysia khususnya, pengkulturan skala besar masih kurang dipraktikan. Ini kerana kurangnya ilmu dan kajian saintifik berkenaan pengkulturan Spirulina di bawah cuaca Malaysia.

Oleh itu, objektif kajian ini adalah untuk membangunkan sistem kultur baru untuk pengkulturan Spirulina berskala besar. Kajian dijalankan di kawasan terbuka. *Arthrospira platensis* dikultur di dalam fotobioreaktor fleksibel terapung dan fotobioreaktor atas tanah adalah sebagai kawalan. Kepekatan yang digunakan adalah separuh daripada kepekatan asal media Kosaric. Kedua-dua parameter fizikal dan pertumbuhan diukur setiap hari sementara itu kandungan nutrisi ditentukan pada hari ke-8 untuk kitaran I, II dan III. Bacaan pH menunjukan bacaan meningkat sepanjang tempoh pengkulturan. Tiada perbezaan yang signifikan ($p > 0.05$) direkodkan untuk oxygen terlarut tetapi fotobioreaktor atas tanah menunjukan bacaan sedikit tinggi secara puratanya. Bacaan yang lebih tinggi ($p < 0.05$) direkodkan oleh suhu kultur di dalam fotobioreaktor atas tanah iaitu 32.97 ± 0.50 ºC, manakala fotobioreaktor atas tanah ialah 28.97 ± 1.40 ºC. Tiada perbezaan signifikan ($p > 0.05$) jumlah klorofil dan biojisim yang direkodkan. Kedua-dua fotobioreaktor tidak menunjukan perbezaan yang signifikan ($p > 0.05$) tetapi merekodkan bacan protein melebihi 45% bagi setiap 1 g berat kering sel. Kedua-dua fotobioreaktor merekodkan bacaan lipid tidak lebih daripada 1.5 % bagi setiap 1 g berat kering sel.

Seterusnya, dalam keadaan pengkulturan di kawasan terbuka dan separuh kepekatan media, *A. platensis* dikultur dengan dua kaedah pencampuran di dalam fotobioreaktor fleksibel terapung iaitu pengudaraan dan pencampuran mekanikal.

Kaedah pencampuran pengudaraan mencatatkan bacaan oksigen terlarut yang rendah ($p <0.05$). Suhu yang direkodkan juga mencatatkan bacan yang lebih rendah ($p < 0.05$) di dalam fotobioreaktor fleksibel terapung dengan pengudaraan berbanding fotobioreaktor atas tanah. *Arthrospira platensis* dengan campuran melalui pengudaraan merekodkan bacaan jumlah klorofil lebih baik ($p < 0.05$). Biojisim juga menunjukan bacan lebih baik ($p < 0.05$) berbanding kaedah pencampuran mekanikal. Perbezaan signifikan direkodkan dalam banyak parameter disebabkan oleh kepekatan oksigen terlarut yang tinggi di dalam pencampuran mekanikal yang mana merencat pertumbuhan. Secara purata, kandungan protein lebih tinggi ($p < 0.05$) direkodkan oleh pencampuran pengudaraan iaitu 46.57 ± 1.53%, manakala, pencampuran mekanikal adalah 22.47 ± 2.17%. Sebaliknya, kandungan karbohidrat lebih tinggi ($p < 0.05$) direkodkan pencampuran mekanikal berbanding pengudaraan. Kadar pertumbuhan untuk pengudaraan dan mekanikal adalah 0.223 1/day dan 0.121 1/day.

Kedua-dua $NaNO_3$ and CH_4N_2O menjanjikan pertumbuhan yang bagus untuk pengkulturan di kawasan terbuka dengan fotobioreaktor fleksibel terapung mengandungi *A. platensis*. Tiada perbezaan yang signifikan direkodkan oleh ketumpatan optik, kandungan jumlah klorofil dan biojisim untuk kedua-dua sumber nitrogen. Walau bagaimanapun, ketumpatan optik dan kandungan jumlah klorofil menunjukan bacaan lebih tinggi kerana atom nitrogen daripada $NaNO_3$ lebih tersedia digunakan oleh sel di dalam media berbanding CH_4N_2O. Pengkulturan menggunakan $NaNO_3$ menunjukan bacaan protein lebih tinggi ($p < 0.05$) iaitu 46.57 ± 1.53%. Manakala, CH_4N_2O menunjukan bacaan karbohidrat lebih tinggi ($p < 0.05$) iaitu 48.08 ± 1.27%. Kadar pertumbuhan bagi $NaNO_3$ adalah 0.223 1/day yang mana lebih baik daripada CH_4N_2O.

Kajian menunjukan pertumbuhan lebih tinggi di dalam fotobioreaktor fleksibel terapung dengan kaedah pengudaraan. $NaNO_3$ adalah lebih baik daripada CH_4N_2O. Kajian bio-ekonomi menunjukan penggunaan CH_4N_2O lebih menjimatkan untuk pengkulturan berskala besar kerana harga pasaran yang lebih murah dan mudah diperolehi.

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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 Master of Science. The members of the Supervisory Committee were as follows:

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This is to confirm that:

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%	Percentage
DO	Dissolved oxygen
CO_2	Carbon Dioxide
$\mu molm^{-2}s^{-1}$	Micromoles per square meter per second
CH_4N_2O	Urea
cm	Centimetre
mm	Millimetre
USA	United States of America
DNA	Deoxyribonucleic acid
g	Gram
g/l	Gram per litre
NO_2	Nitrogen dioxide
HCL	Hydrochloric
m^2	Square meter
nm	Nanometre
mg/l	Milligram per litre
O_2	Oxygen
ml	Millilitre
NaCl	Sodium chloride
$NaNo_3$	Sodium Nitrate
NaOH	Sodium hydroxide
NH_3	Ammonia
NH_4	Ammonium

mg	Milligram
PE	Polyethylene
rpm	Rotation per minute
kg	Kilogram
UPM	Universiti Putra Malaysia
UV	Ultraviolet
µm	Micrometer
RO	Reverse osmosis
ºC	Centigrade Celsius
kg/m	Kilogram per meter
hp	Horse power
PVC	Polyvinylchloride
RCBD	Randomized Complete Block Design
PET	Polyethylene terephthalate
rpm	Round per minute
AOAC	Association of Analytical Communities
NaOH	Sodium hydroxide
SPSS	Statistical Package for the Social Sciences
OH	Hydroxide
UVR	Ultraviolet radiation
KNO_3	Potassium nitrate
W	Watts
cm/s	Centimeter per second

CHAPTER 1

INTRODUCTION

1.1 Problem Statement

Nowadays, microalgae are becoming the focal point to solve malnutrition or malnourishment, especially in developing countries. Malnutrition significantly causes thousands of deaths especially among children. Malnutrition also leads to many diseases such as scurvy; due to lack of vitamin C, kwashiorkor; due to lack of carbohydrates and physical retardation; due to lack of essential nutrients. Befitting its high productivity and nutritional values (Jimènez, 2003), Spirulina could become most instrumental in solving food scarcity. Some Africa countries such as Somalia, which is situated in the Horn of Africa has suitable weather and location for microalgae cultivation. The mass cultivation of Spirulina in this region has not been well explored due to the lack of knowledge about its mass cultivation system.

Presently, Spirulina is grown commercially in countries such as the United States of America (USA), Taiwan, China, Japan, Israel, India, Thailand and a host of other countries (Spolaore *et al.*, 2006). However, there has been no report of Spirulina grown commercially in Malaysia because of the lack of scientific studies conducted. In distinct dry and wet seasons and in semi-arid areas, Spirulina can be grown in open pond (Richmond *et al.*, 1990), but under indistinct weather like that of Malaysia's, rain water can dilute growth medium (Radman, 2007) and cause other microalgae contamination (Madkour *et al.*, 2012).

A number of systems have been developed to culture microalgae but all require intensive care due to the local climate and thus are costly. Spirulina cultivation has been carried out in many systems such as open pond, vertical or horizontal tubular reactors (Miron *et al.*, 2000; Barbosa *et al.*, 2004) and membrane photobioreactors (Kumars *et al.*, 2010b). Each system was developed to meet specific weather and location requirements in order to achieve higher productivity. Every system has its own set of advantages and disadvantages. For instance, open pond cultivation system is easily exposed to contamination which could pose enormous problems in large scale cultivation. Studies have stated that recent technology for microalgae cultivation using closed photobioreactor is much more expensive. Other than that, Kumar et al. (2010b) also have mentioned that uncontrolled supply of CO_2 in close photobioreactor systems could lead to greenhouse gas emissions. Pultz (2001) also explained that dissolved oxygen (DO) concentrated in close photobioreactor could become an inhibiting factor for microalgae growth. Although Malaysia has the potential to be Spirulina producer, lack of studies and subsequently expertise, on mass cultivation of Spirulina under Malaysian climate, makes it difficult to be realised. Frequent rain renders it impossible to culture in an open system. Therefore, it is necessary to design a culture system that is able to avoid growth media dilution while at the same time mitigate risks of contamination from other microorganisms and foreign compound pollutions.

1.2 Justification of Study

Arthrospira platensis or commonly known as Spirulina has been recognized as one of super foods, rich with proteins, vitamins, carbohydrates, and possessing various medical properties (Soundarapandian and Vasanthi, 2008). Many publications recorded the potential of Spirulina in health care. Spirulina is very productive (Rodrigues, 2010) and can survive in wide environmental conditions (Vonshak, 1997). Essentially, Spirulina contains about 62% amino acid content, vitamin B_{12} and complex sugar (Estrada *et al.*, 2001). Recent studies have also shown that Spirulina produces Phycocyanin, which is a very important substance that acts as antioxidants and has radical scavenging properties (Benedetti *et al.*, 2006). That is why Spirulina is produced commercially either, for human or animal consumption or manufacturing pharmaceuticals and also as nutritional supplements (Chisti, 2007; Kumar *et al.*, 2010a).

Recently, a local company has patented a culture system that is capable of culturing various microalgae in outdoor enclosed system which turns out to be also cheap and scalable. Floating-bed photobioreactor is a microalgae cultivation system that is suspended in large water body such as sea, lake and river. Malaysia is surrounded by sea which is an advantageous geographical factor for microalgae cultivation. A large volume of water has large heat capacity which acts as temperature regulator. The ability to control temperature is important in microalgae cultivation especially Spirulina. Ravelonandro et al. (2008) mentioned that optimum level temperature is crucial for Spirulina cultivation. Besides that, the waves produced near the shores will provide what is known as natural mixing to the culture. The cultivation system should be well mixed to enhance the removal of accumulated oxygen (Travesio *et al.*, 2001). Floating-bed photobioreactor has the potential to be applied as the foremost Spirulina culture system in Malaysia. There are many different floating-bed systems that can be developed. As for this study, a flexible floating-bed photobioreactor is developed to study *Arthrospira platensis*'s performance and quality under Malaysian climate.

Although cultivation of Spirulina in a floating-bed has been conducted, information on the performance of microalgae grown in such system is sparse. Thus, a general direction of this research is to study the effect on growth performance and quality parameters of Spirulina cultured in flexible floating-bed photobioreactor. The effects of different mixing methods and different nitrogen sources were also investigated.

As such, the objectives of this study are:

1. To study the effect of flexible floating-bed photobioreactor on growth performance and quality of *A. platensis* under Malaysian climate.
2. To determine the effect of different mixing methods on *A. platensis* cultured in flexible floating-bed photobioreactor.
3. To identify the effect of different nitrogen sources on *A. platensis* cultured in flexible floating-bed photobioreactor.

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LIST OF PUBLICATIONS

Mohd Shahmen Mohd Yazam, Nurshazreen Mashor, Hishamuddin Omar, Sharizim Zulkifly and Ahmad Ismail. 2015. Productivity and Quality of *Arthrospira platensis* Grown in Flexible Floating-bed Photobioreactor under Malaysian Climate. *Acta Biologica Malaysiana,* 4: 84-93.

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