UNIVERSITI PUTRA MALAYSIA

MODIFICATION OF KOSARIC'S MEDIUM WITH NITROGEN AND PHOSPHORUS FOR Spirulina (Arthrospira platensis) IN SHELTERED OPEN CULTURE SYSTEM

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By

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

MODIFICATION OF KOSARIC'S MEDIUM WITH COMMERCIAL NITROGEN AND PHOSPHORUS FOR SPIRULINA *(Arthrospira platensis)* IN SHELTERED OPEN CULTURE SYSTEM

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August 2015

Chairman: Hishamuddin Bin Omar, PhD
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Presently the world is experiencing serious environmental crisis such as global warming that effect the source of human nutrition hence, increasing starvation, and malnutrition. Demand for microalgae as supplemental food had increased steadily in recent years. However, in Malaysia, cultivation of microalgae is scanty due to lack of knowledge in microalgae production in variable weather conditions. One of the fundamental problems is lack of cheap and effective fertilizer.

The aim of this research was to develop economical and inexpensive fertilizer for a commercial production and give higher productivity of *A. platensis*. The objective was implemented by substituting all the nitrogen and phosphorus sources present in Kosaric Medium (SM) with locally available commercial fertilizers. The *A. platensis* was treated with 19 treatments include SM as control with three different nitrogen sources (ammonium nitrate, ammonium sulphate, and urea) and three different phosphorus sources (phosphoric acids, triple superphosphate (TSP), and diammonium phosphate (DAP)). Two grades of nitrogen concentration representing 50% (high concentration) and 25% (low concentration) of SM nitrogen concentration were compared in this study. 30L *A. platensis* was grown in each polyethylene (PE) bag in outdoor condition for seven days in first cultivation, ten days in second cultivation and 8 days in third cultivation. The growth parameters (optical density, biomass and chlorophyll *a* concentration) and biochemical content (proteins, carbohydrate and lipid) were recorded and compared with *A. platensis* cultivated in SM.

Significant differences of the growth parameters and biochemical composition were recorded for the different nitrogen and phosphorus sources and concentration. The result of this study revealed that *A. platensis* cultivation during dry season had better performance and *A. platensis* could utilize ammonium nitrate (ammonium nitrate + triple superphosphate in low concentration) most efficiently and gave the highest productivity on day 8 with

biomass, chlorophyll, and protein yield of 1.24 ± 0.004g/L, 11.41 ± 0.21mg/l, and 62.5%, respectively with average of pH, 10.12 ± 0.08 which was significantly different ($p<0.05$) with other treatments. This was better than that of SM (0.73± 0.01g/L, 8.64 ± 0.13mg/L, and 52%, respectively). Further increased in nitrogen concentration will limit growth.

This study indicated that the utilization of ammonium nitrate and TSP as nitrogen and phosphorus has the potential to replace the expensive Kosaric media. The modified medium can be used cost effectively for large scale mass production of protein rich *A. platensis* and yields similar performances to Kosaric medium.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENGUBAHSUAIAN MEDIA KOSARIC DENGAN NITROGEN DAN PHOSPHORUS UNTUK SPIRULINA (*Arthrospira platensis*) DALAM SISTEM PENGKULTURAN DI LUAR MAKMAL YANG TERLINDUNG

Oleh

NURSHAZREEN BINTI MASHOR

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Perubahan iklim yang ekstrim juga dikenali pemanasan global semakin kritikal pada masa ini memberi kesan ke atas sumber makanan dan nutrisi manusia terjejas menyebabkan kebuluran dan kekurangan zat makanan. Permintaan alga sebagai makanan tambahan semakin meningkat pada abad ini. Walau bagaimanapun, pengkulturan mikroalga di Malaysia tidak dilakukan secara besar-besaran dan masih dijalankan di dalam makmal disebabkan kekurangan kajian dan pengetahuan dalam pengkulturan mikroalga yang dijalankan dalam keadaan cuaca yang berubah-ubah.

Oleh itu, tujuan kajian ini dijalankan adalah untuk menghasilkan media kultur yang efektif dengan kos media yang berpatutan untuk pengkulturan *A. platensis* berskala besar serta memberikan produktiviti yang lebih tinggi. Objektif kajian telah dilaksanakan dengan menggantikan sumber nitrogen dan phosphorus dalam media Kosaric (SM) dengan baja nitrogen dan phosphorus komersial tempatan yang sedia ada. Pertumbuhan *A. platensis* diuji dengan 19 rawatan termasuk standard media (SM) sebagai tanda aras. Tiga jenis sumber nitrogen komersial (ammonium nitrat, ammonium sulfat, dan urea) dan phosphorus (asid phosphoric, triple superphosphate (TSP), and diammonium phosphate (DAP)) diuji dengan kepekatan tinggi (50%) dan rendah (25%) daripada kepekatan asal sumber nitrogen dan phosphorus dalam SM.

30L *A. platensis* telah dikulturkan dalam beg polyethylene (PE) diluar makmal selama tujuh hari pada pengkulturan pertama, 10 hari pengkuturan kedua dan lapan hari pada pengkulturan ketiga. Parameter pertumbuhan (ketumpatan optik, berat bersih dan kepekatan chlorophyll *a*) dan kandungan biokimia (protein, carbohidrat dan lipid) telah direkodkan dan dibandingkan dengan kesemua rawatan.

Pertumbuhan *A. platensis* dan komposisi biokimia dalam T16 telah menunjukaan perbezaan yang ketara secara statistik antara kesemua rawatan yang diuji. Hasil kajian menunjukkan pertumbuhan *A. platensis* adalah tinggi pada pengkulturan pada musim panas dan menunjukkan bahawa *A. platensis*

dapat menggunakan ammonium nitrat (ammonium nitrat + triple superphosphat, kepekatan rendah) secara optimum dan menghasilkan produktiviti tertinggi pada hari ke lapan dengan berat bersih, klorofil, dan protein 1.24 ± 0.004g/L, 11.41 ± 0.21mg/l, and 62.5%, masing-masing dengan purata pH, 10.12 ± 0.08. Ini ada lebih tinggi berbanding *A. platensis* yang dikulturkan dalam SM dengan masing-masing (0.73 ± 0.01g/L, 8.64 ± 0.13mg/L, and 52%). Peningkatan kepekatan nitrogen telah menyebabkan pertumbuhan *A. platensis* menjadi terhad.

Kajian ini menunjukkan bahawa penggunaan ammonium nitrat dan tsp sebagai sumber nitrogen dan phosphorus mempunyai potensi untuk menggantikan media Kosaric yang mahal. Medium yang diubahsuai boleh digunakan untuk pengeluaran *A. platensis* yang kaya protein secara berskala besar dan menghasilkan pertumbuhan yang lebih tinggi dengan kos yang efektif dibandingkan dengan media Kosaric.

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I certify that a Thesis Examination Committee has met on 3 August 2015 to conduct the final examination of Nurshazreen binti Mashor on her thesis entitled "Modification of Kosaric's Medium with Nitrogen and Phosphorus for Spirulina (*Arthrospira platensis*) in Sheltered Open Culture System" 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 Master of Science.

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LIST OF ABBREVIATIONS

%	Percentage
µl	Microlitre
µm	Micrometer
$\mu molm^{-2}s^{-1}$	Micromoles per square meter per second
CH_4N_2O	Urea
cm	Centimetre
CO_2	Carbon Dioxide
DAP	Diammonium Phosphate
DNA	Deoxyribo Nucleic Acid
g	Gram
g/l	Gram per litre
H_3PO_4	Phosphoric Acid
HCL	Hydrocloric
HDPE	High Density Polyethylene
m	Meter
mg/l	Miligram per litre
$MgCl_2$	Magnesium Chloride
ml	Mililitre
NaCl	Sodium chloride
$NaNo_3$	Sodium Nitrate
NaOH	Sodium Hydroxide
NH_3	Ammonia
NH_4	Ammonium
NO_2	Nitrite
PE	Polyethylene
rpm	Rotation per minute
SM	Standard Media
TSP	Triple Super Phosphate
UV	Ultraviolet
α	Alpha
β	Beta
ºC	Centrugade Celcius

CHAPTER 1

INTRODUCTION

1.1 Problem statement

Presently the world is experiencing serious environmental crisis. The quest for affluent lifestyle and economic dominance has resulted in global warming (Liz, 2014; Xue *et al.,* 2011; Soletto *et al.,* 2008). Accumulation of methane and carbon dioxide (CO_2) in the atmosphere trap solar energy and heat generated from human activities in the earth atmosphere resulting in present global warming. Global warming not only causing high atmospheric temperature but the effect is more far ranging. Global warming caused climate change which results in climate change altering weather pattern: atmospheric air circulation, oceanic water circulation causing too much rain in some part of the world, too little rain in other part of the world, typhoon, hurricane, tornado, and acid rain (Peter *et al.,* 2011). The collective effects cause havoc to the environment, and well being of the earth population. Diseases, rising of the sea water level and crop failure is threatening the world population particularly the poorer countries. As a consequence, the source of human nutrition is reduced, increasing starvation and malnutrition.

So much has been debated about global warming at international forum but the action has been very slow. This is because developed countries is unwilling to make the unnecessary action to reduce carbon foot print but instead accusing poorer countries of cutting the rainforest. United Nation has identified that bulk of the carbon emission originate from North America, Europe and China (Liz, 2014). Scientist has recommended various measures to reduce green house gas such as adopting green technology, reducing fossil energy consumption, recycling and microalgae cultivation (Christopher, 2014; Raja *et al.,* 2008; Converti *et al.,* 2006; Binaghi *et al.,* 2003).

Recent studies have shown that microalgae are the potential candidate for renewable biofuel (Parmar *et al.,* 2011; Xin *et al.,* 2010; Chen *et al.,* 2009;). This is of no surprise because microalgae are one of the pioneer organisms living on earth and changing earth into habitable planet. Microalgae has the potential to reduce global warming because as stated by Garcia (2000), microalgae act as primary producer of oxygen where it uses CO_2 and produce oxygen (O_2), and functional in carbon and nitrogen fixation while at the same time produce valuable biomass high in protein and lipid. The advantage of microalgae cultivation is that it can be carried out on land or sea. Due to the limited land area to accommodate a growing population and agricultural activity, microalgae culture is very flexible and can be done at sea or freshwater areas. It does not require a large area for high productivity. Regarding to this, this biomass can be used for food, feed, and biofuel.

Unfortunately most of the researches are carried out in the laboratories focusing on growth studies where the environment and growth factors are being controlled (Anaga and Abu, 1996), finding the fast growing species (Day *et al.,* 2012) and high lipid content (Jiang *et al.,* 2011; Pruvost *et al.,* 2011). However very few of the studies is applicable in the real field conditions. This is because microalgae are very sensitive to the environment they are growing. Slight changes to the culture conditions will inhibit their growth. Therefore more effort should be devoted to microalgae culture in outdoor conditions. One of the microalgae adapted well in outdoor conditions is *Arthrospira* sp. commonly known as Spirulina.

A. platensis is a blue green algae which can be found in wide geographical distribution (Uday and Ahluwalia, 2013). It is very hardy species and can live where other microalgae facing difficulties to establish, therefore it is almost free from contaminating microalgae. *A. platensis* has been cultured in many parts of the world, good growth, and high in nutritional values where commercial cultivation of *A. platensis* are well established on a few countries including China, India, Bangladesh, America and Africa. *A. platensis* has been known as animal and human food in countries such as Asia and Africa. In addition, *A. platensis* has a high market demand and rich in pigments such as phycobiliproteins, beta carotene and luteina for pharmaceutical and nutriceutical industries (Raoof *et al.,* 2006).

1.2 Justification of study

As *A. platensis* is known as a potential solution to the problem of world protein source, various studies conducted and found that it has higher protein content than vegetables (Becker, 2007; FAO, 2001). *A. platensis* gives high productivity in a short time and easily adapted to a variety of climates.

Despite its potential and versatility, commercial *A. platensis* cultivation is almost nonexistent in Malaysia. This is because Malaysia lies in the tropic, with frequent rain and cloud covers and the condition is not conducive for outdoor cultivation. Most of the available references pertaining to *A. platensis* culture are from subtropical and arid region where the weather is fairly stable and clear sky (Isichei, 1990 and Hernandez *et al.,* 2009). Although Malaysia's weather is not conducive for outdoor cultivation, preliminary studies have shown that *A. platensis* can be grown in outdoor condition under a rain shelter (Puganeswary *et al.,* 2014). This study was conducted outside the laboratory to identify the potential of *A. platensis* growth and adaptation in the tropical rain forest climate with a variety of weather conditions.

In order for outdoor cultivation of *A. platensis* to be successful, the effect of weather conditions and nutrient requirement should be look into. Accordingly, management measures should be taken to ensure good microalgae cultivation is suitable for the environment in Malaysia in line with the development of the aquaculture industry. Since *A. platensis* growth is a function of environmental conditions such as light, temperature, and nutrient, it is necessary to study those interactions in Malaysia. In addition, to ensure the successful of *A. platensis* cultivation in large scale is dependent on effective development and economics culture. Study conducted by (Rodrigues *et al.,* 2010) found that over

25% of the total productivity of microalgae was related to the culture media that acts as a fertilizer to supply nutrients, particularly nitrogen and phosphorus.

Presently there is no cheap fertilizer available for commercial *A. platensis* cultivation in Malaysia. Therefore it is very important to develop cheap fertilizer from easily available chemicals to reduce production cost. There are several aspects that need to be improved in current fertilizer formula that will be used towards production of microalgae in a large scale in the future. Several factors have been studied throughout the study to compare the relationship between productivity and environmental factors change (Tomaselli *et al.,* 1997 and Prabuthas *et al.,* 2011). Hence the objectives of this study are:

1. To develop and improve the current fertilizer formula using cheap and effective nitrogen and phosphorus source for large scale production of microalgae especially in Malaysia
2. To determine the effect of different nitrogen and phosphorus source on *A. platensis* biochemical properties
3. To determine the effect of variable weather on *A. platensis* production and its biochemical composition.

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