EFFECTS OF BG11, SKM AND MKM CULTURE MEDIA ON OPTICAL DENSITY OF *Spirulina platentis* CULTIVATION

by

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THESIS DECLARATION

I declare that this thesis entitle 'Effects of BG11, SKM and MKM Culture Media on Optical Density of Spirulina platentis Cultivation' is the result of my own research except as cited in the references for any degree and is not concurrently submitted in candidature of any degree.

Signature student:

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I certify that the Report of this final year project entitled Effects of BG11, SKM and MKM Culture Media on Optical Density of *Spirulina platentis* Cultivation by Nurul Ainaa' Binti Md Zuki, matric number E17A0052 has been examined and all correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Sustainable Science) with Honors Faculty of Earth Science, University Malaysia of Kelantan.

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Effects of BG11, SKM and MKM Culture Media on Optical Density of *Spirulina platentis* Cultivation

ABSTRACT

Spirulina platensis is blue-green microalgae and well known due to its unique nutritional quality. Spirulina also blue green algae that have attract a great deal of popularity in health products. In this research study, the culture medium are using BG11 medium, SKM and MKM and to analyse their effect on *S. platensis* by using growth parameter and also to determine the best culture medium for large cultivation. Thus, the optical density was recorded due to measure the growth performance on *S. platensis*. The optical density (OD) is one of the most important parameters in Spirulina cultivation. In this research, the cultivation system was conducted in 1 L of three conical flasks for every mediums. The 10 ml of culture were diluted with 10 ml of every different culture mediums for every conical flasks. The production of Spirulina in different culture mediums were observed for ten days of cultivation. Besides that, the initial pH also was recorded and also aerated by electric aerator. The data analysis was growth estimation by optical density measurement had been determined in a spectrophotometer at 620 nm. The results had shown, MKM the most suitable culture medium for cultivation of *S. platensis* due the growth performance on optical density. The results also shown, the alternative low-cost culture medium which was MKM had been successfully formulated for the cultivation of *S. platensis*.

Kesan Kultur Media BG11, SKM dan MKM terhadap Ketumpatan Optik dalam Pertumbuhan *Spirulina platentis*

ABSTRAK

Spirulina platensis adalah mikroalga biru-hijau dan terkenal kerana kualiti pemakanannya yang unik. Spirulina juga alga hijau biru yang telah menarik banyak popularitи dalam produk kesihatan. Dalam kajian penyelidikan ini, medium pertumbuhan menggunakan medium BG11, SKM dan MKM dan untuk menganalisis kesannya terhadap *S. platensis* dengan menggunakan parameter pertumbuhan dan juga untuk menentukan kultur medium pertumbuhan terbaik untuk penanaman besar. Oleh itu, ketumpatan optik direkodkan kerana mengukur prestasi pertumbuhan pada S. platensis. Ketumpatan optik adalah salah satu parameter terpenting dalam penanaman Spirulina. Mengukur ketumpatan optik pertumbuhan sel berguna untuk mengukur kepekatan biomas. Dalam penyelidikan ini, sistem penanaman dilakukan dalam 1 L dari tiga kelalang kon untuk setiap medium. 10 ml kultur dicairkan dengan 10 ml setiap medium yang berbeza untuk setiap kelalang kon. Penghasilan Spirulina dalam kultur medium yang berbeza diperhatikan selama sepuluh hari penanaman. Selain itu, pH awal juga dicatat dan juga disalurkan oleh aerator elektrik. Analisis data adalah estimasi pertumbuhan dengan pengukuran ketumpatan optik telah ditentukan dalam spektrofotometer pada 620 nm. Kajian ini menunjukkan, MKM sebagai kultur medium yang paling sesuai untuk penanaman S. platensis kerana komponennya berpatutan dan mudah. Hasilnya telah ditunjukkan, media pertumbuhan kos rendah alternatif yang merupakan MKM telah berjaya dirumuskan untuk penanaman *S. platensis*.

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LIST OF ABBREVIATIONS

BG 11 medium	Blue green 11 medium
BNM	Bristol's Modified medium
Ca	Calcium
$CaCl_2.2H_2O$	Calcium carbonate dihydrate
$CO(NH_2)_2$	Urea
$CuSO_4.5H_2O$	Copper sulphate pentahydrate
$Co(NO_3)2.6H_2O$	Cobalt(II) Nitrate Hexahydrate
$EDTANa_2$	Ethylene Diamine Tetraacetic Acid DiSodium
Fe	Iron
Fe EDTA	Ferric EDTA
$FeSO_4.2H_2O$	Iron(II) sulfate
H_3BO_3	Boric acid
K	Potassium
K_2HPO_4	Dipotassium phosphate
K_2SO4	Potassium sulfate
$MgSO_4.7H_2O$	Magnesium sulphate heptahydrate
MKM	modified Kosaric medium
Mn	Magnesium
$MnCl_2.4H_2O$	Manganese(II) chloride tetrahydrate
Na	Sodium
NaCl	Sodium Chloride
NASA	National Aeronautics and Space Administration
$NaCO_3$	Sodium carbonate
$NaHCO_3$	Sodium Bicarbonate
$NaNO_3$	Sodium nitrate
$NaMoO_4.2H_2O$	Sodium molybdate dihydrate
Na_2MoO_3-	Sodium molybdate
NaOH	sodium hydroxide

NH_4VO_3	Ammonium metavanadate
$NiSO_4.7H_2O$	Nickel(II) sulfate heptahydrate
OD	Optical Density
P	Phosphorus
PX	Cell produtivity
RO	Reverse osmosis
SKM	standard Kosaric medium
SM	Standard medium
SPSS	Statistical package for the social sciences
TC	Culture period
$Ti_2(SO_4).6H_2O$	Titanium(III) sulfate
UMK	Universiti Malaysia Kelantan
USA	United State America
UTM	Universiti Teknologi Malaysia
WHO	World Health Organization
$ZnSO_4.7H_2O$	Zinc Sulfate Heptahydrate
ZM	Zarrouk medium

LIST OF SYMBOLS

%	Percentage
L/min	Liter per minute
<	Less than
~	Approximately
Mg/g	Milligram per gram
^{O}C	Temperature
g/500ml	Gram per millilitre
g/ L	Gram per litre
nm	Nanometre
µm	Micrometre

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Algae are one of photosynthetic organisms that converts sun energy into chemical energy through the process of photosynthesis. Algae have a simple structure of reproductive mechanisms and also contain their own different compounds structure and function. Next, algae can be categorized into three types which are macroalgae, microalgae and cyanobacteria with their own characteristics (Becker, 2007). However, cyanobacteria is sometimes can be categorized as microalgae. After that, microalgae and cyanobacteria are photosynthetic organisms that can be divided into multicellular and unicellular. One of the characteristics of microalgae and cyanobacteria is both of them can grow in selective media and do not contaminate with other microorganisms when cultivating in open system (Borowitzka, 1999). The cyanobacteria are also known as 'blue-green algae' in a group of prokaryotic because they contain photosynthetic pigments such as chlorophyll and phycocyanin. In general, cyanobacteria are known as Spirulina from species of *Arthrospira* (Soni et al., 2017).

Spirulina are filamentous cyanobacterium of planktonic photosynthesis with the higher appearance in the water bodies that have excessive carbonate and bicarbonate with a pH value is 9.5 (Soni et al., 2017). Spirulina are the oldest living plant live since 36 billion years ago on the earth. In 1915, a Spanish scientist named Hernando Cortez had found out Spirulina as food resources at Lake Texcoco, Mexico (Vonshak, 2014). Then, Pierre Dangeard had observed about the advantages of Spirulina on health due to the flamingos that eat Spirulina are still alive.

Furthermore, Spirulina are consists of cell wall that are soft and complex can cause the protein and sugar are easily to dissolve better than other algae (Merali, 2020). Spirulina also consists up to 74 % protein in dry weight and high in minerals, vitamins, fatty acids and pigments (Barrocal et al., 2010). Then, Spirulina are been called 'future food' because they have more than fifty essential nutrients include vitamins, minerals and amino acids (Jung et al., 2019). Therefore, the United Nation World Food Conference has already appointed Spirulina as a future food in 1974 (UNWFC, 1974). Additionally, Spirulina have been listed by World Health Organization (WHO) as one of the best superfoods in the world and NASA also states Spirulina as portable space travel food due to high amount of nutrients but in small quantities (Khan et al., 2005).

Besides that, the cultivation of microalgae and cyanobacteria have been commercialized by the industry sector in Japan in 1960s, while in Mexico, China and USA in 1970s (Science & Milledge, 2014). Nowadays, Spirulina have become high demand for industrialization in the world. For example, Spirulina have obtained huge popularity in the industry of food health and raising in aquaculture diet of vitamin and protein supplements. The productions of Spirulina have become one of the health products, especially in protein and diet supplements. It is in high demand for a solution to counter the malnutrition issues, especially in poor countries.

According to WHO, malnutrition is defined as lack of nutrition or underweight and it has become a serious public health problem due to the increased risks in health illness and morbidity. In Sustainable Development Goals (SDG), the second goal is to achieve zero hunger. This goal is about to end hunger and malnutrition problems especially for children in developing countries (Goal 2: Zero hunger UNDP, 2020). According to the United Nation's website, it had stated there are 45% of children under five years old die due to lack of nutrition in the region of Africa or Sub-Saharan Africa (Goal 2: Zero Hunger, 2020). Moreover, the global population reached 7.67 billion on 1st January 2019. The population will increase by about 80 million people per year for the duration from 2015 to 2020, so that the United Nations predicts about 9.7 billion people in 2050 (Jung et al., 2019). Therefore, Spirulina are the best opportunity as a food supplement for malnutrition issues due to many nutrients and proteins.

After that, the growth of Spirulina includes three main phases, namely planting, harvesting and processing. The production system of Spirulina have two types which are open system and closed system (Zhang, 2018). The cultivation of Spirulina in an open system has been widely discovered for higher production in recent years (Boussiba et al., 1988). The open system has been classed into some of natural waters such as lagoons, lakes, container and artificial pond (Singh & Sharma, 2012). Meanwhile, photobioreactor system is the closed system known due to closed equipment which is does not have the process of exchange gasses and being contaminated to the environment. It is probably one of the main measures that should be taken to ensure the effective mass cultivation of algae (Ugwu et al., 2008). This is because the photobioreactor has many advantages over than open system and is commonly used in operation to produce high biomass productivity due it can control some cultural environments.

1.2 Problem Statement

Spirulina platensis is blue-green microalgae and well known due to its unique nutritional quality. *Spirulina platensis* also consist high amount of nutrients and easy to grow in the water. Nutrition condition is one of the essential factors in the assessment of microalgae productivity and its growth conditions (Markou et al., 2014). When contemplating large-scale cultivation of *Arthrospira*, the culture medium is the second main factor influencing production costs, accounting for 25% of overall costs (Vonshak, 2014). Another way to minimize the cost of nutrients is to reduce the concentration of the widely used normal medium without impacting algal growth.

Furthermore, the introduction of commercial fertilizers and industrial grade chemicals into the culture medium is a significant cost-saving component in the large-scale cultivation of *Arthrospira*. There are many types of culture medium had been used such as Conway medium, BG11 medium, Seawater medium and F/2 medium. Besides that, Zarrouk's medium has become an effective standard medium (SM) for Spirulina culture for centuries. However, in this research study the effect of medium culture using BG11 medium, SKM, and MKM were analysed based on growth parameter which is optical density.

1.3 Objectives

The objectives of this study are:

1. To prepare and cultivate *S. platensis* in BG11, SKM and MKM media.
2. To analyse the effects of BG11, SKM and MKM culture media on optical density in the cultivation of *S. platensis*.

1.4 Scope of the Study

This study is to analyze the effect of medium growth on *Spirulina platensis* by growth parameter. A growth parameter is an optical density at 620 nm. The optical density (OD) is one of the most important parameters in Spirulina cultivation. Measuring the OD of cell growth is useful to measure the biomass concentration. Growth estimation by optical density measurement is generally determined in a spectrophotometer (Jagannathan & Sathish, 2018).

Then, the mediums for cultivation of *S. platensis* are Blue Green 11 medium also known as BG11 medium, standard Kosaric medium (SKM) and modified Kosaric medium (MKM). After that, the stock solution of BG11 medium, SKM, MKM were prepared in a 1.0 L of the conical flask by taking the correct amount of solution from each stock and a volume of up to one litre marked with distilled water was made (Sharkerl et al., 2007).

Besides, the cultivation of *S. platensis* are conducting in conical flasks with an electric aerator at the laboratory Faculty of Earth Science, Universiti Malaysia Kelantan (UMK). The initial pH also be recorded and the cultivation was conducting for 10 days.

1.5 Significance of the Study

Many elements have to be provided for the growth of Spirulina, such as carbon (C), oxygen (O), hydrogen (H), nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), sulfur (S), phosphorus (P) and trace elements with the major nutrients being carbon, oxygen, hydrogen, nitrogen, phosphorus, and potassium. The first three are obtained from water and air while the latter three have to be absorbed from the

culture medium (Xin et al., 2010). Nitrogen is one of the essential elements for the growth, development, reproduction, and other physiological activities of Spirulina. The nitrogen source and concentration also affect the accumulation of lipid in Spirulina. Usually, ammonium salts, nitrates, and urea are used as nitrogen sources, but their absorption rates and utilization are different (Xin et al., 2010).

In this research study, the culture mediums are using BG11 medium, SKM and MKM and to analyze their effect on *S. platensis* by using growth parameter. Spirulina required high nutrient inputs and salt concentrations compared to *Scenedesmus* and *Chlorella*. This might be the reason for Spirulina was naturally grown in salt lakes exclusively (Saranraj, 2014). The difficult culture medium currently in use in various centers of production were small alternations of the medium first developed by Zarrouk's for Spirulina culture. Spirulina required a medium of high alkalinity and a steady supply of bicarbonates ions.

Besides that, there are many growth parameters such as optical density, dry weight biomass and chlorophyll content. However, this study is using optical density to measure the growth performance of *S. platensis*. Optical density (OD) is one of the most important parameters in Spirulina cultivation. Measuring the OD of cell growth is useful to measure the biomass concentration.

In addition, to maintain a healthy culture, monitoring the growth is very essential. Spirulina would grow optimally when the nutrients and light sources are sufficient. The Spirulina will die after the stationary phase and the debris will accumulate in the culture medium or environment.

CHAPTER 2

LITERATURE REVIEW

2.1 Background history of Spirulina

According to Jung et al., (2019), there are first photosynthetic life forms on earth during 3.6 billion years ago and also provide earth planet's first oxygen which is known as ‘blue green algae’. Cyanobacteria are the blue-green algae which has the biological connection between algae and green plants (Koru, 2012). The Spanish explorers attacked Mexico in the 17th century, it found that the Aztecs living in the Valley of Mexico in the capital Tenochtitlan were harvesting from the lake a "healthy food." Spanish chroniclers defined fishermen with good net harvesting from the lagoons this blue-colored "techuitlatl" and making it a blue-green cake (U, 2014). Spirulina are multicellular and filamentous blue green algae that have attract a great deal of popularity in health products. It have historically been used as a food substitute by people living around alkaline lakes where it is naturally available. For example, the community living in the Kanem’s area adjacent to Chad Lake that have very low rate of malnutrition (U, 2014).

The community of Kanembu live along the shores of Lake Chad gathers wet algae in clay containers, remove the water from cloth sacks, and spread the algae on

the sandy shores of the lake to dry the heat. After the wet algae been cooled, these algae cakes are taken by women for sale in the local market (Abdulqader et al., 2000). Therefore, in 1967 Spirulina have been described in the International Association of Applied Microbiology as a "wonderful potential food supply" (Koru, 2012). In commercial usage, the generic name, Spirulina, refers to the dried biomass of cyanobacterium known as *Arthrospira platensis* which is a whole substance of biological origin and *A. Platensis* and *A. maxima* are generally used as milk, nutritional supplements and feed supplements. The reintroduction of Spirulina as a safe food for human use in the late 1970s and early 1980s was associated with a variety of unfounded arguments that relate Spirulina to a 'super-agent' that could do almost everything, from cancer therapy to antibiotics and antiviral purpose. The cultivation of microalgae and cynobacteria have been started for commercialize by the industry sector in Japan on 1960s and in Mexico, China and USA is on 1970s (Science & Milledge, 2014). Nowadays, Spirulina have become high demand for industrialization in the world. For example, Spirulina have obtained a huge popularity in the health food industry and a raising popularity for aquaculture diet of vitamin and protein supplements.

2.2 The morphology and characteristics of Spirulina

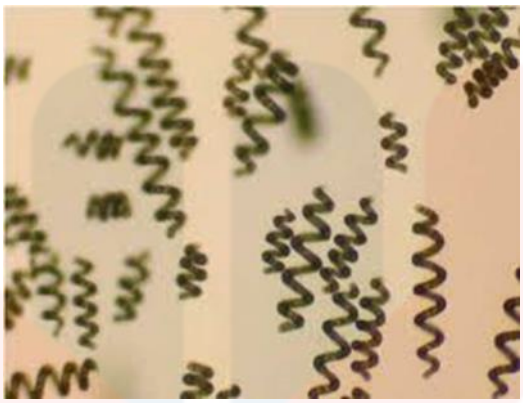

Figure 2.2 The microscopic image of the Spirulina microalgae (Koru, 2012)

Spirulina are filamentous cynobacteria due to the alignment multicellular cylindrical trichomes in entire length of open left hand helix (Vonshak, 2014). Phylum belongs to Cyanophyta and family of Oscillatoriacea. Spirulina is an invisible organism living in a range of habitats including ground, sand, marshes, brackish water, seawater, and fresh water. Spirulina identified as blue green filaments made up of cylindrical cells organized under a microscopic examination in unbranched, helicoidal trichomes. The filaments are elastic, gliding around their axis and lack of heterocysts (Cifferi Orio, 1985). Then, the smaller species cytoplasm is homogenous and septa hardly visible. However, the major organisms including *S.Platensis* and *S.Maxima* have a granular cytoplasm comprising gas vacuoles and septa that is clearly noticeable. The inter- thylakoid space is reduced by the existence of electronically transparent protein gas vesicles, with the cylindrical form allowing ability of floating (Cifferi Orio, 1985).

P.J. Turpin had extracted Spirulina from a freshwater sample in 1827 and Wittrock and Nordstedt also had been documented helical, septal and green blue algae which is called Spirulina jenneri *f.platensis* (Habib & M. Ahsan, 2008). However, the first taxonomic paper had been published by Stizenberger did not appeared until 1852.

Stinzenberger had been provided the name *Arthrospira* to new genus due to presence of septa, helical shape and multicellular structure (Cifferi Orio, 1985). In 1982, Gomont was assigned to the genus Spirulina, and the septal type to the genus *Arthrospira* (Cifferi Orio, 1985). In addition, the morphologies of *Arthrospira* are significantly classified by helix form, pores distributed in the cell wall, septum visibility under light microscopy, diameter and trichome level fragmentation (filaments) (Vonshak, 2014). Besides that, the capsule of Spirulina have fibrillar structure which is cover any filament that surrounding it. The unusual presence of the capsule along the filaments in the *S.platensis* are differentiating morphological feature that be applied to the *S.maxima* (Cifferi Orio, 1985). After that, the species of *Arthrospira* (Spirulina) exhibit great plasticity in morphology. It is because to natural conditions such as temperature and other physical and chemical influences, and likely even to genetic changes (Koru, 2012).

2.3 Nutritional content of Spirulina

The Spirulina have higher content of macronutrient and micronutrient. There are many chemical compositions in the dry weight of Spirulina such as proteins, carbohydrates, vitamins and minerals. The protein content of the drymass is approximately 55-70%, however compared to other often used protein sources such as milk, beef, eggs the proteins produce fewer lysine and sulfur amino acids (methionine, cysteine), but also much more than any other food. Phycobiliproteins constitute major portion of proteins, the most important being phycocyanin with 7-13 % of the drymass. The carbohydrate content is 10-20 %, with lipids acconting for 9-14 % of that. Spirulina is rich in minerals including K, P, Na, Ca, Mn and Fe representing 6-9 % of

the drymass. Vitamin A, B and C are also present, with an average B-carotene content of 1,4 mg /g-1 of drymass, translating to 0,25 mg of vitamin A (Ali & Saleh, 2012).

Table 2.2 The nutrient composition of spirulina

Nutrient	Percent of composition	Description
Carbohydrate	13.5 – 15	Glucose, mannose, rhamnose, galactose, xylose, 2-O-methyl – L-rhamnose and 3-O-methyl-L-rhamnose
Protein	60 – 65	Essential of amino acids such as methionine, lysine, leucine, isoleucine, threonine and valine. Nonessential of amino acids such as glycine, cystine, arginine, aspartic acid, serine, alanine and glutamic acid
Minerals	~ 7	Iron, calcium, zinc, potassium, magnesium, sodium, copper, phosphorus chromium
Fats (lipids)	5 – 6	gamma-linolenic acid or GLA, linoleic acid
Pigments	< 1	Chlorophyll, phycocyanin, xanthophylls, alpha and beta carotenes
Moisture	6 – 13.5	

(Source: Koru, 2012)

Table 2.3 The vitamins content of Spirulina in powder

Vitamin	mg 100/g
Beta carotene	140
Vitamin B_6	0.8
Vitamin B_{12}	0.32

Table 2.3 (Continued)

Vitamin K	2.2
Folic acid	0.01
Thiamin B_1	3.5
Niacin B_3	14.0

(Source: Koru, 2012)

2.4 The cultivation systems of Spirulina

The growth of Spirulina includes three main phases which are namely planting, harvesting and processing. The selected strains have being used in specially built ponds for cultivating algae. One of the essential criteria in Spirulina cultivation is continuous agitation of the water. Algal culture agitation is required to keep nutrients equally distributed and to open up all the cells to sunlight. The washes of biomass have been drying and grinds because want to achieve the requirement of particle size.

Figure 2.4 The open pond of Spirulina (Meristem Journeys, 2018)

There are two types of cultivation system of Spirulina which are open system and closed system (Zhang, 2018). The cultivation of Spirulina in open systems have been widely discovered for higher production in the recent years (Boussiba et al., 1988). The open systems have been classed into some of natural waters such as lagoons, lakes, container and artificial ponds. The open systems like tank, circular

pond and big shallow of pond have been used frequently because they are low in cost of the operation and production of Spirulina (Ugwu et al., 2008). However, the open system has some disadvantages which are, due to lack of light intensity and low evaporation, it needs a large space for the building and the process of diffusion carbon dioxide to atmosphere. The open systems also have low aeration system and can effect in biomass productivity. Moreover, contaminate by other organisms and fast growing heterotrophs have been limited the growth of algae in open systems to certain species that can thrive under intense conditions (Soni et al., 2017).

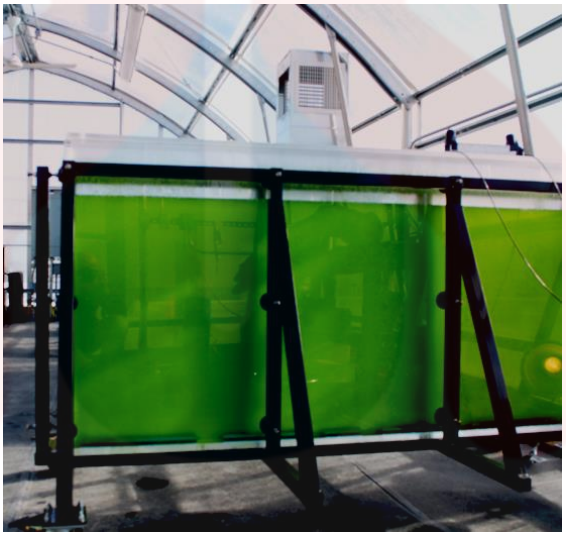

Figure 2.5 The closed system of Spirulina known as flat plate photobioreactor (Lam et al., 2017)

Besides that, another cultivation system of Spirulina is closed system. This closed system also known as photobioreactor due to closed equipment. The closed equipment do not have the process of exchange gasses and being contaminated to environment. There are some examples of photobioreactors which are flat plate photobioreactor, tubular photobioreactor, internally illuminated photobioreactor and vertical column photobioreactor (Soni et al., 2017). In order to resolve issues with open ponds, a great deal of attention is now being given to the creation of appropriate closed structures such as flat plate, tubular, vertical column and internally illuminated

photobioreactor. The photobioreactors also able to control some cultural environments, for examples to reduce the loss of carbon dioxide, the supply of water, optimum temperature, light intensity, density of culture, gas change, level of pH and aeration system. The photobioreactors have many advantages than open system and is commonly used in operation to produce high biomass productivity. The cultural system of Spirulina might be using natural or artificial light for illuminated. In addition, some cultural systems like open pond and flat plate are using natural light for illuminated (Soni et al., 2017). In general, the photobioreactors are using artificial light internally or externally for illuminated. For examples, florescent lamp or another light that are being provided. In fact, certain photobioreactors can be quickly controlled. Tempering may simply be done by placing the photobioreactor in a constant temperature area. This technique is limited to lightweight photobioreactors but large-scale outdoor devices, such as tubular photobioreactors, cannot be conveniently controlled without strong technological commitment.

2.5 Environmental factors of Spirulina

Microalgae production and chemical composition are influenced primarily by specific environmental factors such as temperature level, light intensity, accessible carbon dioxide, pH level and nutrients.

2.5.1 Effect of Spirulina on various nutrients

Spirulina are need macro and micronutrients such as carbon, nitrogen, phosphorus, potassium, arsenic, chlorine, calcium, iron, copper, nickel and cobalt for process productivity.

Different culture media are used to start new cultures according to the water source. The water used should be clean or filtered to avoid growth of other algae. Water often contains enough calcium, but if it is too hard it will cause muds. Portable water is convenient whereas RO treated water is the best to grow Spirulina (Soni et al., 2017). In addition, carbon also is an important resource for cyanobacteria production.

Besides that, the synthesis of amino acids which are make up proteins and other cellular components that needs nitrogen (Vaittinen, 2018). Nitrogen (N) is the most important nutrient which contributes to biomass production. The nitrogen content of the microalgae biomass may range from 1% to more than 10% and can depend across various classes (e.g. low in diatoms) and organisms based on their source and availability (Sukumaran, Nulit, et al., 2018). For examples, urea seems to be more desirable due to affordable and produces two nitrogen atoms in the one molecule of 46% nitrogen. According to Stanca and Popovici (1996), demonstrated that the utilization of urea as a nitrogen source in *Spirulina platensis* cultivation leads to an increase in both the total biomass and the biomass chlorophyll content. Urea is easily assimilated by *Spirulina platensis*, probably due to its spontaneous hydrolysis to ammonia under alkaline cultivation (Devanathan & Ramanathan, 2013).

2.5.2 Effect of Spirulina on temperature level

The influence of temperature exerts on biochemical processes and influences algae on biochemical composition, it is causes temperature become one of the most significant environmental variables. Spirulina with their maximum development temperature decreases between 35 ºC and 38 °C (Cifferi Orio, 1985). The microalga Spirulina will be developed at higher temperatures with high light intensity resulting

in minimal protein content and enhanced carbohydrate content (Sukumaran, et al., 2018).

Then, the effect of temperature on growth rate of Spirulina, the composition of biomass and pigment production has been studied. The crop growth kinetics also show a wide variety of temperature tolerances from 20 °C to 40 °C and the maximum growth rate, cell production with maximum accumulation chlorophyll and phycobilliprotein have been found at 35 °C (Kumar et al., 2011). The Spirulina is at least or almost zero during the night.

2.5.3 Effect of Spirulina on pH level

Vincent and Silvester (1979), stated the pH level is directly affected the physiological properties of algae and nutrient availability. The solubility of carbon and minerals is determined by pH level in the culture through directly or indirect (Zealand, 1979). Next, for mass production the bicarbonate and sodium ions consumption and also the optimum pH of the Spirulina nutrient media has been changed from 8.4 to 9.5. The Spirulina produce well at pH values from 9 to 11. It also help that other strains are stop from contaminating the tank because cannot survive in the alkaline atmosphere (Soni et al., 2017). Additionally, another function of high pH level in Spirulina cultivation is to prevent another algae grow in the system. Therefore, to keep the high level of pH or change, a large number of sodium bicarbonate must present in cultural medium (Soni et al., 2019)

According to Pandey et al. (2010), the influence of pH on development of *Spirulina platensis*, protein and the amount of chlorophyll was examined and the dry

weight of *Spirulina platensis* was 0.91g/500ml and the quality of protein and chlorophyll was 64.3% and 13.2 mg/gm at pH 9.

2.5.4 Effect of Spirulina on light intensity

Most photoautotrophic organisms, including photosynthetic bacteria, cyanobacteria and higher plants, transform light energy into chemical energy through process photosynthesis. It also report light quality, intensity and duration are important factors in the production of algal (Soni et al., 2017). One of the major challenges in microalgal biotechnology is the efficient use of energy, particularly when an improvement in the yield of biomass is required (Vaittinen, 2018).

Spirulina is growing in the existence of light (photosynthesis), maximum sunlight illumination might not be necessary (30 % of full sunlight is adequate, except that more might be needed to heat up the morning culture quickly). The need for light intensity for growth varies from organism to organism and often demands a specific range of intensity for production. Then, the chemical reactions, including protein synthesis and respiration, occur within Spirulina during dark times (Vaittinen, 2018).

Watanabe and Hall (1996), investigated the photosynthetic productivity of the filamentous cyanobacaterium Spirulina platensis in a coneshaped helical tubular photobioreactor. It was constructed with 0.255 m^2 basal area and conical shape. The inner surface of the photostage was illuminated with compact fluorescent cool white lamps and the input radiation energy was 1249 kj d^{-1}. The maximum photosynthetic efficiency obtained was 6.83 per cent which is corresponded to a production rate of 15.9 g dry biomass m^{-2} d^{-1}.

2.5.5 Effect of Spirulina on aeration system

Aeration is very important to achieve high production and better yields of Spirulina. This can be accomplished by rotators, which through gentle agitation of rising cells hold the cells in suspension. Dubey (2006), had stated aeration which could be achieved by rotators, and which provides agitation of growing cells to maintain the cells in suspension, has been described as very necessary in getting good quality and better yields of Spirulina species

The species of Spirulina generates large yield of biomass at aeration of the growth medium (bubbling with air). Hence, aeration system can enhances the chlorophyll content of the species Spirulina. The aeration also gives the Spirulina filaments a homogenous distribution throughout the growth system for adequate exposure to illumination. It also helps to diffuse the concentration of carbon dioxide uniformly and removes inhibitory substances as oxygen (Vonshak, 2014). However, if aeration is not adequate, the energy utilization efficiency and the production of biomass will be low (Soni et al., 2017). Therefore, aeration system is a basic necessity for the Spirulina cultivation.

2.6 Growth phases of algae cultures

The growth phases is shown in 5 stages. The aim is to sustain the exponential increasing process in continuous cultures to increase production. In addition to reduced biomass production, the nutritional value of algae cultivate under growth-limiting conditions is inferior to algae cultivated in optimal conditions.

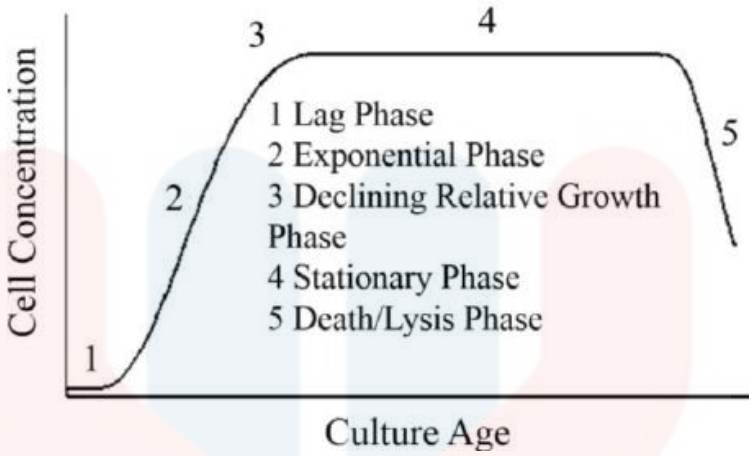

Figure 2.6 The five growth phases of algae (Price & Farag, 2014)

The figure above shows the five different phases of growth. As the culture is being transformed into the new growing medium, physiological adaptation usually occurs. This process is called stage of induction or lag (1). Lavens and Sorgeloos (1996), had stated a little change in cell density is excepting during this relatively long phase. The cells continue dividing exponentially after the lag step. Then, exponential phase (2) is relatively short, because as the density increases the cells begin to shade each other. Laven and Sorgeloos (1996), also stated in the declining growth phase (3), some physical or chemical factors, such as nutrient availability, light or pH, begin to limit growth, the cell division from the exponential phase will slow down.

After that, a stationary period (4) follows the exponential process, where the culture is in a state of controlled development and relatively constant density of cells. The phase of the crash or death (5) may be triggered for multiple reasons such as water quality degradation, nutrient depletion, overheating, excessive pH or pollution may push the culture into a phase characterized by a rapid decline in cell density (Laven & Sorgeloos, 1996).

2.7 Growth medium

According to history, there several techniques and simple cultural media were developed in the late 1800s and early 1900s (Thakare et al., 2018). Due to their high potential for morphological and physiological adaptation to different conditions, both algae and cyanobacteria often serve as pioneer microorganisms in biodiversity. Algae were first researched in culture by Beijerinck more than a century ago on 1890, who was the first human to deal with axenic cultures (Thakare et al., 2018). However, Beijerinck, Knop and Bristol have made several improvements to the culture media that have helped to enhance the growth of algae in the artificial culture media. The composition of Bristol's Modified medium (BMM) are $CaCl_2.2H_2O$ 1 g, Pringsheim's soil-water 40 mL, Iron(III) chloride solution (1%) < 1 mL, NaCl 1 g, $MgSO_4.7H_2O$ 3 g, $NaNO_3$ 10 g, KH_2PO_4 3 g, K_2HPO_4 3 g. In previous study had been reported, the nitrogen source, $NaNO_3$, which is contained in a higher percentage of Bristol Modified Medium than the BG11 medium (Idenyi et al., 2016). Besides that, Idenyi et al., (2016) also stated that ferric iron solution in BMM was the source of iron in the medium resulting in higher biomass production.

Spirulina required high concentrations of nutrients and salts compare to *Scenedesmus* and *Chlorella*. This may be the factor that Spirulina was naturally grown mostly in salt lakes (Ogawa et al,. 1970). Microalgae require both macronutrients and micronutrients for their growth and metabolic function. Types of algal medium are determine by the chemical composition of these nutrients. Several culture mediums abound for the growth of algae, even it is important to know which medium is best for optimum growth of the organisms. There are different media preparations according to the local growing conditions.

The different culture mediums have been tried for cultivation of spirulina such as Zarrouk's media (Zarrouk, C. 1966), Rao's media (Singh, S. 2006), CFTIR media (Venkataraman et al., 1995), OFERR media (Singh, S. 2006), Revised media (RM6) (Raoof et al., 2006) and Bangladesh medium (Khatum et al., 1994). The chemical compositions of different growth media are compared in Table 2.7.

Table 2.7 The comparison chemical composition of growth media

Ingredients	Zarrouk's media (gms/L)	Rao's media (gms/L)	CFTRI media (gms/L)	OFERR media (gms/L)	George's media (gms/L)	Reduced cost media (gms/L)
$NaHCO_3$	16.80	15	4.5	8.0	-	16.8
K_2HPO_4	0.50	0.50	0.5	-	00.02	0.235
K_2SO_4	2.50	2.50	1.5	-	-	-
NaCl	1.00	0.60	1.0	0.5	-	0.353
$MgSO_4.7H_2O$	0.20	0.20	1.0	5.0	0.02	0.471
EDTA	0.08	0.04	1.2	0.16	-	-
$CaCl_2.2H_2O$	0.04	-	-	-	-	0.353
$FeSO_4.2H_2O$	0.01	0.008	0.04	-	-	0.176
H_3BO_4	2.86	-	0.01	0.05	-	0.265
$MnCl.4H_2O$	1.180	-	-	0.052	-	2.86
$ZnSO_4.7H_2O$	0.222	-	-	-	-	1.81
Na_2MoO_3-	0.015	-	-	-	-	0.222
$CuSO_4.5H_2O$	0.074	-	-	-	-	0.0177
NH_4VO_3	22.9	-	-	-	-	0.079
$NiSO_4.7H_2O$	47.8	-	-	-	-	-
$NaWO_2$	17.9	-	-	-	-	-
$Ti_2(SO_4).6H_2O$	4.4	-	-	-	-	-

Table 2.7 (Continued)

$Co(NO_3)_2.6H_2O$	4.4	-	-	-	-	0.118
$CO(NH_2)_2$	-	-	-	0.2	-	0.088
Fe EDTA	-	0.20	-	-	-	-
Ferric citrate	-	-	-	-	0.035	-
Peptone	-	-	-		1.00	-

Source : (Soni et al., 2017)

Nowadays, Zarrouk's medium has been an effective standard medium (SM) for Spirulina culture for many years (Zarrouk, 1996). From the previous study, the nitrogen content of ZM will potentially contribute to a maximum biomass concentration of 4.6 g/L for Spirulina (Delrue et al., 2017). However, the reduced cost media can be as efficient as ZM in terms of final concentration of biomass, chlorophyll and protein content.

CHAPTER 3

MATERIALS AND METHODS

3.1 Preparation for growth medium

In this research study, a cyanobacteria was used is *Spirulina platensis*. The mother culture of *Spirulina platensis* had been obtained from Algae Laboratory at Universiti Putra Malaysia (UPM). The culture mediums for *Spirulina platensis* are Blue Green 11 medium known as BG11 medium Table 3.1, standard Kosaric medium (SKM) Table 3.2 and modified Kosaric medium (MKM) Table 3.3.

The stock solution of BG11 medium consists of (g/500ml) $NaNO_3$ 75.0, K_2HPO_4 2.0, $MgSO_4.7H_2O$ 3.75, $CaCl_2.2H_20$ 1.80, Citric Acid 0.30, Ammonium Ferric Citrate Green 0.30, $EDTANa_2$ 0.05, $NaCO_3$ 1.00 (R. Stanier et al., 1971).

Next, the stock solution of SKM consists of (g/L) $NaHCO_3$ 4.500 , NaCl 0.250, $CaCl_2$ 0.010, $MgSO_4.7H_2O$ 0.050, Epsom salt 0.050, $NaNO_3$ 0.625, K_2HPO_4 0.0250, Potassium Hydroxide 0.242, $FeSO_4.7H_2O$ 0.025 (Sukumaran, Nulit, et al., 2018).

Moreover, stock solution for MKM consists of (g/L) baking soda 4.500, Sea salt 0.250, $CaCl_2$ 0.010, Epsom salt 0.050, Phosphoric acid (10%) 0.070, Potassium Hydroxide 0.242, $FeSO_4.7H_2O$ 0.025 (Sukumaran, Nulit, et al., 2018).

All the culture medium in Table 3.1, 3.2, and 3.3 had been prepared followed the series, nutrient addition was performed and properly mixed before adding another to prevent precipitation and cloudiness in the growth medium from occurring. Therefore, all the stock solutions had been prepared in 1.0 L schott bottle with distilled water and put in the freezer. After that, the stock solution of BG11 medium, SKM, MKM were prepared in a 1.0 L of conical flask by taking the correct amount of solution from each stock and a volume of up to one litre marked with distilled water was made (Sharkerl et al., 2007).

Table 3.1 The medium composition of BG11

Medium composition of BG11		
No	Ingredients	Amount (g/500ml)
1.	$NaNO_3$	75.0
2.	K_2HPO_4	2.0
3.	$MgSO_4.7H_2O$	3.75
4.	$CaCl_2.2H_2O$	1.80
5.	Citric Acid	0.30
6.	Ammonium Ferric Citrate Green	0.30
7.	$EDTANa_2$	0.05
8.	$NaCO_3$	1.00

Table 3.2 The medium composition of SKM

Medium composition of SKM		
No	Ingredients	Amount (g/L)
1.	$NaHCO_3$	4.500
2.	NaCl	0.250
3.	$CaCl_2$	0.010
4.	$MgSO_4.7H_2O$	0.050
5.	Epsom salt	0.050
6.	$NaNO_3$	0.625
7.	K_2HPO_4	0.0250
8.	Potassium Hydroxide	0.242
9.	$FeSO_4.7H_2O$	0.025

Table 3.3 The medium composition of MKM

Medium composition of MKM		
No	Ingredients	Amount (g/L)
1.	$NaHCO_3$	4.500
2.	Sea salt	0.250
3.	$CaCl_2$	0.010
4.	Epsom salt	0.050
5.	$NaNO_3$	0.625
6.	Phosphoric acid (10%)	0.070
7.	Potassium Hydroxide	0.242
8.	$FeSO_4.7H_2O$	0.025

3.2 Cultivation system

In this study, the cultivation system had been conducted in 1 L of conical flasks for each mediums. Then, 10 ml spirulina were diluted together with 10 ml of mediums and a volume of up to one litre marked with distilled water. The production of Spirulina in different culture mediums were observed for ten days of cultivation (Naqqiuddin et al., 2014).

The environmental conditions for this study had been used 24 hours (12:12) of light intensity from fluorescent lamps in the laboratory. Besides that, the electric aerator (SOBO SB-988) in the Figure 3.2 was used to continuously aerate these cultured bottles (Sharkerl et al., 2007). The electric aerator was aerated with minimum and constant aeration.

Figure 3.2 SOBO SB-988

The pH also had been adjusted using sodium hydroxide (NaOH) to range 9 to 10. The average initial pH value of each conical flasks in BG11 medium, SKM, MKM was in the range of 9. When the pH value was about 9 in the *S. platensis* culture, the ammonia molecules reached the cells by diffusion and were directly absorbed (Zeng et al., 2012). According to Manmai et al. (2019), if the low pH of the medium, which was at acidic pH was not ideal for the growth of *S. platensis* and can cause that

microalgae to die (Manmai et al., 2019). This result is supported by that stated by De Oliveira et.al (1999) and Richmond et.al (1990), who hypothesized that the most acceptable pH for *S. platensis* cultivation is alkaline, often within the range of 8.5–9.5 (Zeng et al., 2012).

3.3 Measurement growth of Spirulina on different culture mediums

In this research study, cultivation of *S. platensis* in control and treatment had been measured using growth parameter which was optical density for evaluated the pattern of growth. Firstly, the optical culture density was measured which is defined as the absorption of visible radiation (absorption peak of chlorophyll) is at about 620 nm with a spectrophotometer (Hitachi U-1900) during ten days for each conical flasks (Sukumaran et al., 2014).

The blank that had been used for each culture was distilled water. Next, the growth of Spirulina had been observed and examined under a microscope to validate its purity.

3.4 Data Analysis

Microsoft Excel software was used for all tabulation, plotting and analysis (Kimathi, 2011). For each conical flask of three culture medium (BG11 medium, SKM, MKM), the reading of optical density at 620 nm during ten days were recorded, tabulated and plotted. Based on tabulated and plotted results, analysis of growth parameters of *Spirulina platensis* had been done by comparing the reading of optical density against days (time). The optical density was absorbance at 620 nm.

Thus, only one growth parameter was further discussed in Chapter 4 (result and discussion). The detailed results for the growth parameter were shown in Table 4.3.1, 4.3.2, 4.3.3 up to (a), (b), (c) respectively.

CHAPTER 4

RESULT AND DISCUSSION

The discussion explained mainly the results obtained from the study conducted as mention in Chapter 3 to achieve the objectives of this study. Its covers observation of *S. platensis*, growth parameter and effect of *S.platensis* growth on different culture mediums (BG11 medium, SKM, MKM)

4.1 Observation of *S.platensis*

The microscopic observation of *S. platensis* was observed under a microscope with magnification 100x (Figure 4.1) and 400x magnification at the laboratory of Faculty Earth Science (Figure 4.2).

Figure 4.1 The picture of *S. platensis* under microscope magnification number 10 x 10

Figure 4.2 The picture of *S. platensis* under microscope magnification number 40 x 40

The Spirulina helical shape and its transformation have also attracted scientific interest for decades. However, the microscopic and visual observation showed that the straight trichome had be seen.

N. B. Jeeji N. B. and Seshadri C. (1980) had to been stated straight trichomes can be found spontaneously occurring in the laboratory and outdoor cultures (Chaiyasitdhi et al., 2018). The ratio of helical to straight trichomes in mixed populations varies with culture conditions (N. B. Jeeji N. B. and Seshadri C., 1980).

Chaiyasitdhi et al. (2018) had been stated the lack of specialized cells, such as akinetes and heterocyst, found in other multicellular cyanobacteria, means that *Arthrospira* has to rely purely on the alteration of its non-specialized vegetative cells to respond to the fluctuating environment (Chaiyasitdhi et al. 2018). This can be seen through the overall-shape transformation of the trichome. Then, nutrient shortage also can lead to complete or nearly complete elimination of helical trichomes and domination of emerging straight trichomes in the population.

4.2 Growth measurement of *S.platensis*

Spirulina platensis was successfully cultivated for ten days in a selective culture medium such as BG11 medium, standard Kosaric medium (SKM) and modified Kosaric medium (MKM).

Spirulina platensis growth performance was measured by optical density. The optical density for every conical flask was determined in a spectrophotometer (Hitachi U-1900) at 620 nm daily. The absorbance values were shown as in Table 4.2., 4.3, 4.4 up to (a), (b), (c) respectively. After 10 days of cultivation, there were several differences of optical density measurement between 3 culture mediums.

a) BG11 medium

Table 4.2 Reading of optical density at 620 nm for ten days using BG11 medium

No of days	Optical density (nm)		
	Conical flask 1	Conical flask 2	Conical flask 3
1	0.011	0.008	0.010
2	0.014	0.015	0.012

Table 4.2 (Continued)

3	0.018	0.022	0.020
4	0.025	0.030	0.027
5	0.051	0.054	0.051
6	0.073	0.077	0.074
7	0.101	0.100	0.103
8	0.103	0.104	0.102
9	0.103	0.105	0.103
10	0.104	0.106	0.103

b) SKM

Table 4.3 Reading of optical density at 620 nm for ten days using SKM

No of days	Optical density (nm)		
	Conical flask 1	Conical flask 2	Conical flask 3
1	0.025	0.027	0.025
2	0.026	0.029	0.026
3	0.029	0.030	0.033
4	0.055	0.059	0.060
5	0.084	0.087	0.090
6	0.104	0.105	0.103
7	0.105	0.105	0.105
8	0.107	0.106	0.105
9	0.106	0.107	0.105
10	0.107	0.107	0.106

c) MKM

Table 4.4 Reading of optical density at 620 nm for ten days using MKM

No of days	Optical density (nm)		
	Conical flask 1	Conical flask 2	Conical flask 3
1	0.028	0.030	0.027
2	0.030	0.031	0.029
3	0.034	0.035	0.039
4	0.064	0.069	0.065
5	0.090	0.093	0.096
6	0.108	0.109	0.109
7	0.110	0.112	0.111
8	0.112	0.113	0.111
9	0.112	0.114	0.112
10	0.112	0.114	0.111

Table 4.5 The mean of optical density between three culture mediums at 620 nm

No of days	Optical density (nm)		
	BG 11	SKM	MKM
1	0.009	0.026	0.028
2	0.013	0.027	0.030
3	0.020	0.031	0.036
4	0.027	0.058	0.066
5	0.052	0.087	0.093
6	0.075	0.104	0.109
7	0.101	0.105	0.111

Table 4.5 (Continued)

8	0.104	0.106	0.112
9	0.104	0.106	0.113
10	0.104	0.107	0.112

Based on Table 4.5 mean of BG11 medium were shown, on day 1 until day 3 it was observed the mean value of optical density was 0.009, 0.013, 0.020. Meanwhile, for SKM showed the mean optical density on day 1 until day 3 was 0.026, 0.027, 0.031. Next, Table 4.5 for MKM presented 0.028, 0.030, 0.036 from day 1 until day 3. Manmai et al., (2019) reported the algae cells were adapted to the current environmental conditions at the initial stage from day 1 until day 3 of the culture although there were slight differences between the three different culture mediums (Manmai et al., 2019).

After day 3 Table 4.5, the mean value of absorbance was increased rapidly until day 7 which is from 0.027 to 0.101. Moreover, Table 4.5 for the next 4 days the optical density also increased which was 0.058, 0.087, 0.104, 0.105. Then, the optical density of MKM in Table 4.5 also had been increased rapidly from 0.066 to 0.111. Fatemeh & Mohsen, (2016) had reported during the second phase, cell density increases as a function of time t according to the logarithmic function: $C_t = C_0 e^{mt}$. C_t and C_0 are the cell concentrations at periods t and 0, respectively, and m= the real growth rate (Fatemeh & Mohsen, 2016).

Then, from day 8 until day 10 in Table 4.5 the absorbance value had been on stationary phase which was 0.104. Besides that, in Table 4.5 the mean value of optical density from day 8 until day 10 was 0.106 and 0.107. Meanwhile, in Table 4.5 was shown the mean value of optical density on day 8 until day 10 was 0.112 and 0.113.

In the stationary phase which was from day 7 until day 11, the microalgae cell density is constant since the limiting factors and the growth rate are stable (Carlsson et al., 2007).

4.3 *S. platensis* growth on different culture mediums

In 10 days, *S. platensis* was successfully cultivated in three different mediums which were BG11 medium, standard Kosaric medium (SKM) and modified Kosaric medium (MKM). The BG11 medium a small modification of the medium G-11 had been stated by Hughes, Gorham, and Zehnder (R. Y. Stanier et al., 1971). The composition had been shown in Table 3.1, 3.2 and 3.3. In several studies stated, BG11 medium was the successful medium for cyanobacteria. Then, the medium of Zarrouk is the first synthetic medium developed for the cultivation of *Arthrospira,* which has now been used as a typical medium in many research. (Zarrouk, C., 1966). Meanwhile, the Kosaric medium (KM) is a simplified and modified half-concentration from the medium of Zarrouk (Sharkerl et al., 2007). Based on Table 3.3 MKM, which was acted as the reference medium in the present inquiry, was approximately half of the original KM concentration and a quarter of the Zarrouk medium concentration.

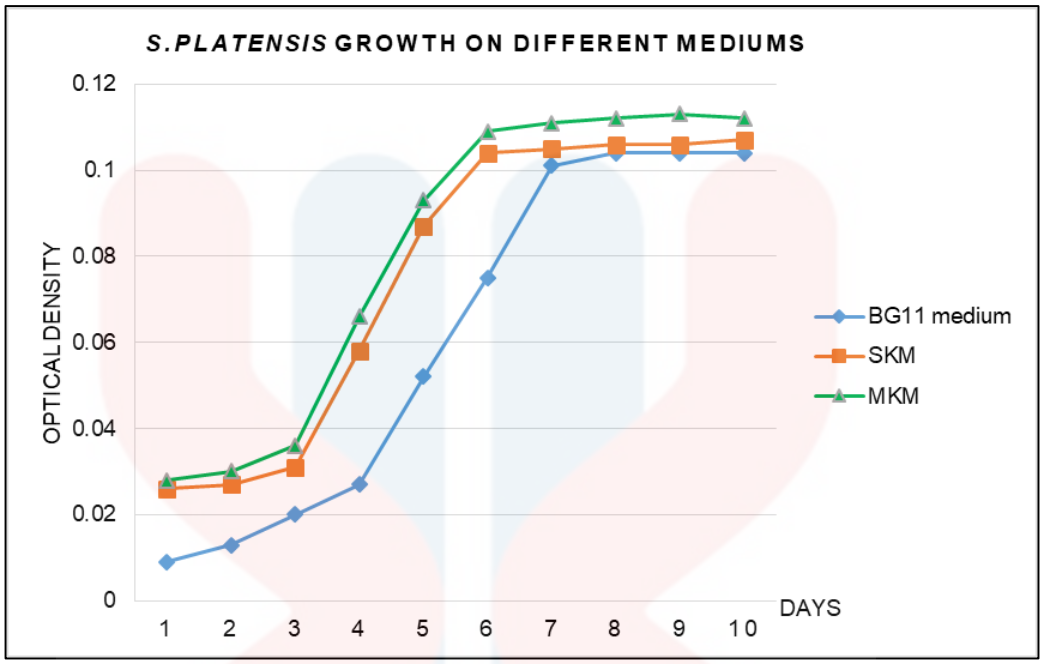

Figure 4.3 The graph of S.platensis growth between three mediums

S. platensis production plants for mass cultivation could be done in areas with suitable climatic conditions, particularly with the sunshine throughout the year. It is difficult to have an ideal growth due to different environmental factors like solar radiation, rain, wind, temperature fluctuation, etc. In this research, *S. platensis* was studied in laboratory culture.

Khin M. S. (2009) studied the growth of A. platensis on seawater-based Provasoli (PES) medium, seawater-urea medium, the seawater-based medium I and seawater-based medium II and seawater-based medium III in the laboratory. The optimal growth of A. platensis was found in seawater-based medium III which contained a low concentration of phosphate, a small amount of bicarbonate, nitrate, and FeEDTA. She reported that the maximum OD 0.79 with initial OD 0.2 was obtained on the 15th day of the experimental period.

In this present study, the growth of *S. platensis* was determined by three phases. The result in Figure 4.4 showed, the growth performance of *S. platensis* in

MKM which was the highest compared to SKM and BG11 medium during ten days. At the lag phase in Figure 4.4 (day 1 until day 3), the optical density of MKM was 0.028, 0.030, 0.036 which was slightly different than SKM but highly different compared to the BG11 medium. During the exponential phase (Figure 4.4) for MKM was highly increased from 0.066 to 0.111 respectively compared to SKM and BG11 medium. Next stationary phase (Figure 4.4), the pattern of the graph for MKM and SKM was similar compared to BG11 medium. This was because the behaviour of *S. platensis* in MKM was considered slightly different compared to the BG11 medium and SKM. Therefore, the result had been shown the ability of MKM, which mostly contains industrial grade chemicals, to provide optimum cultivation conditions for maximum algal growth.

MKM was successfully developed by substituting expensive laboratory grade chemicals for the main portion of SKM with cheap and easy commercial grade baking soda, sea salt, urea, phosphoric acid, potassium hydroxide and Epsom salt. In previous studies, Madkour et al., (2012) and Raoof et al., (2006) had been reported used both baking soda and sea salt in the revised medium (RM_6) and minimized cost-medium respectively (Sukumaran et al., 2018). The advantage of the use of sea salt was that the macro and micro minerals needed for the growth of *A. platensis* are partially supplied by this type of low-cost raw material (Madkour et al., 2012).

In addition, phosphoric acid has been used as an alternative source of phosphorus in Bangladesh medium (Khatun et al., 2006) compared to BG11 medium had used citric acid. However, the used of potassium hydroxide has never been proposed by any researcher as a component for growth media of Arthrospira. In this study, there was use of this alkaline potassium and had been increased the pH value of SKM was 9.13 and MKM at 9.54. Spirulina laboratory cultures have been reported to

show a wide range of optimum pH of 8–10. However, cells can deteriorate quickly by, for example, electromotive bursting or pH-induced agglomeration, as pH changes suddenly, and this can occur in growth systems that are not well buffered (Richmond et al., 1990)

Besides that, the development may have been related to medium BG-11 composition and moderate pH resulting in higher accumulation of biomass (Yadav et al., 2016). Medium BG-11 is poorly buffered and has a low phosphate content. A variety of other media used to grow blue-green algae are better buffered to have a greater supply of phosphate (R. Y. Stanier et al., 1971).

Based on Table 3.1, 3.2 and 3.3 had shown BG11 medium and SKM used $NaNO_3$ in composition of media compared to. The nitrate salts such as $NaNO_3$ and KNO_3 act as nitrogen sources that contribute to higher development of biomass, justifying their use in culture medium such as Paoletti, Zarrouk and Schlosser (Sassano et al., 2007). On the other hand, the use of NO_3 as a nitrogen source in SKM involves a high energy consuming enzyme catalyzed operation, with NO_3 reduced to NO_2 through nitrate reductase and followed by NO_2 reduced to ammonia by nitrite reductase, which contributes to energy loss and reduced algal yield.

Then, environmental conditions particularity of irradiance lux and temperature are important indicators of the production and general characterization of biomass. Generally, Anupama PR. (2000) had been reported in previous study, *S. platensis* growth is optimum from 30-35 ^{o}C, while high alkalinity is mandatory for spirulina growth (Dineshkumar et al., 2016). In this study, the temperature and irradiance lux were found to be better for growth as stated above.

Therefore, the used of MKM, which was composed of affordable and easily obtainable industrial chemicals, has lowered the overall growth cost by up to 97%. In comparison, the cost of MKM is also slightly lower relative to the previous studies.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this study, the cultivation of *S. platensis* in different culture medium which were BG11 medium, SKM and MKM was successfully. There was several differences of medium composition due to chemical components been used for culture of *S. platensis*. The BG11 medium a small modification of the medium G-11 had been state by Hughes, Gorham, and Zehnder (R. Y. Stanier et al., 1971). However, KM is a simplified and modified half-concentration from the medium of Zarrouk (Sharkerl et al., 2007). Thus, MKM was approximately half of the original KM concentration and a quarter of the Zarrouk medium concentration.

Based on the results, the optical density of MKM at 620 nm was recorded from 0.028 to 0.112 during ten days compared to SKM was from 0.0206 to 0.107 and its followed by BG11 medium which was had been the lowest from 0.009 to 0.104. Besides that, the pH values for every different culture mediums during cultivation was also recorded. The highest pH value had been recorded was 9.45 due to acceptable pH

for *S. platensis* cultivation is alkaline, often within the range of 8.5–9.5. Therefore, all the mediums suitable for cultivation of *S. platensis*.

In conclusion, MKM the most suitable culture medium for cultivation of *S. platensis* due the components were affordable and easy. The results have been shown, the alternative low-cost culture medium which was MKM had been successfully formulated for the cultivation of *S. platensis* by replacing the major chemical components of SKM with cheap and readily available industrial grade chemicals at a reduced level without affecting algal growth and yield. Therefore, the ability of MKM that can be used profitably for large scale cultivation of *Arthrospira* biomass in the modern environment due to a low-cost option that can be used profitably.

5.2 Recommendations

This study has reveal the ability of three different culture mediums used for cultivation of *S. platensis* with presence of CO_2 from aerator, system of cultivation. Hence, more future studies should be carried out to improve and enhance the effectiveness and efficiency of this approach.

This study shows some inadequacies and weakness in term of cultivation system design which is using 1 L of conical flask. The culturing Spirulina in the conical flask is limited to providing full information on the growth, production and processing of value-added chemicals, although it will include preliminary information on further demo or commercial level of cultivation (Gami et al., 2011).

In future, the cultivation of Spirulina can be cultivate in closed system also known as photobioreactor. The photobioreactors also able to control some cultural environments, for examples to reduce the loss of carbon dioxide, the supply of water,

optimum temperature, light intensity, density of culture, gas change, level of pH and aeration system. There are some examples of photobioreactors which are flat plate photobioreactor, tubular photobioreactor, internally illuminated photobioreactor and vertical column photobioreactor (Soni et al., 2017).

Next, the presence of CO_2 from electric aerator. The aeration need to be in minimum level and constant. The presence of both CO_2 and air increased the growth of Spirulina, as the carbon source gaseous bubbles formed the nutritional and microalgal metabolism equidistribution in the crop, and the concentration of CO_2 increased the exposure of carboxylase production in rubisco to fix CO_2 and produce biomass (Zeng et al., 2012).

After that, another recommendation is cultivation of Spirulina using urea as a nitrogen source via a feed batch process. The addition of urea was performed in four separate modes such as intermittent addition per 24 or 48 h, constant addition by exponential growth, added mass and continuous addition using a consistent mass rate (Costa, J. A., et al., 2003).

Lastly, another recommendation is measuring another growth parameters of spirulina such as biomass dry weight, specific growth rate and productivity. Cell productivity (PX) is measured as the ratio of cell concentration variance (X_m-X_i) to culture period (TC) (Danesi et al., 2011).

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