EFFECT OF DIFFERENT PHOTOPERIOD ON GROWTH RATE OF *Spirulina platensis*

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DECLARATION

I declare that this dissertation is the result of my own independent work except where otherwise state.

Date: 18 April, 2007

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ABSTRAK

Cahaya merupakan factor penghad dalam pengkulturan *Spirulina platensis* secara besar-besaran. Kajian ini telah dijalankan untuk menentukan tempoh pencahayaan yang memberikan kesan terbaik terhadap pertumbuhan *Spirulina platensis. Spirulina platensis* telah diberikan empat rawatan tempoh pencahayaan yang berbeza, iaitu 12L:12D, 9L:15D, 6L:18D, dan 3L:21D. Walaupun keputusan analisis ANOVA menunjukkan tiada kesan signifikan terhadap pertumbuhan *Spirulina platensis*, jika dilihat berdasarkan keputusan pertumbuhan dan kadar pertumbuhan, terdapat sedikit perbezaan pertumbuhan di antara rawatan pada akhir eksperimen. *Spirulina platensis* menunjukkan pertumbuhan dan kadar pertumbuhan yang lebih baik di bawah rawatan 12L:12D berbanding dengan rawatan yang lain. Terdapat pertambahan berat kering sebanyak 125.89% pada *Spirulina* dengan kadar pertumbuhan sebanyak 0.0905 d^{-1}. Berdasarkan kepada keputusan ini, kesimpulan yang dapat dibuat ialah rawatan 12L:12D memberikan kesan pertumbuhan yang terbaik jika dibandingkan dengan rawatan yang lain.

ABSTRACT

Light has been the main limiting factor in mass culturing of *Spirulina platensis*. Therefore this study has been conducted to determine photoperiod that promotes the fastest growth. *Spirulina platensis* was cultured in four type of photoperiod; 12L:12D, 9L:15D, 6L:18D, and 3L:21D. Although the result of the ANOVA analysis shows that there was no significant effect on the growth rate of the *Spirulina platensis*, based on the growth and growth rate result, there was a slight indication of difference in growth can be seen at the end of the culture period between the treatments used. *Spirulina platensis* shows much better growth and specific growth rate under 12L:12D treatment compare to other treatments. *Spirulina platensis* obtained a 125.89% increased in dry weight and 0.0905 d^{-1} growth rate. Based on these results, a conclusion that can be drawn is that 12L:12D treatment promotes the best growth rate compare to other treatment that was used in this study.

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°C	celcius
%	percent
g	gram
L	liter
ppt	liter parts per thousand
K'	growth rate
h	hour
ANOVA	analisis of variance
p	signicant value
L-D	light dark cycle

CHAPTER 1

INTRODUCTION

1.1 Background of Study

It is not easy to define microalgae because they represent a taxonomically diverse group of organisms. One definition that might be suitable for microalgae is "photosynthetic single-celled or colonial microorganisms", although most of these minute organisms will continue on growing even without the present of radiance if dissolved sugars are provided in the culturing medium (Wilkfors, 2000). The interest in these phototrophic organisms lies in their ability to produce biomass for food, feed and fine chemicals, using solar energy. The range of habitats for microalgae is astonishing, they are found all over the world. Most microalgae are found in the water, but they are also known to inhabit on the surface of all type of soils (Tomaselli, 2004). Microalgae are also found in fresh, saline and hypersaline waters, in polar ice, attached to plants, and even in symbiotic relationships with fungi animals (Wilkfors, 2000).

Microalgae are referred to as autotrophs, a word that when it is translated literally means self feeding. This term is no doubt appropriate because of the photosynthesis process, in which light energy is converted to chemical energy to support the heterotrophic processes of the rest of the cell; creating sugars and oxygen from carbon dioxide and water. In cyanobacteria, the photosynthesis process occurs in cellular structures, called lamellae, which have no membrane (Wilkfors, 2000).

Successful microalgae culture is generally dependent upon nutrients, light, pH, turbulence, temperature and salinity. Light energy has been known to be a limiting factor controlling the distribution of the microalgae. Seasonal variations in illumination with latitude result in different seasonal production patterns in tropical, temperate, boreal and Polar Regions (Kennish, 1994).

The microalgae that is proposed in this study is *Spirulina platensis,* which is also known as *Arthrospira platensis.* It is a photosynthetic, filamentous, spiral-shaped and multicellular blue-green microalgae. They inhabit a wide variety of habitats, particularly alkaline, brackish and saline waters (Qiang, 2004*b*). Cyanobacteria contain chlorophyll a and their traditional name of blue-green was derived from their color which is due to the presence of phycocyanin and phycoerythrin, which usually mask the chlorophyll pigmentation (Tomaselli, 2004). Cyanobacteria or blue-green algae are prokaryotes, that is, cells that have no membrane-bound organelles, including chloroplasts (Sheath and Wehr, 2003).

The chemical composition of microalgae is dependent on the environmental parameters such as temperature, illumination, pH value, and mineral content of the medium, carbon dioxide supply and mixing velocity. The desired biochemical constituents of the *Spirulina* can be obtained by altering its culture conditions (Becker, 2004a) *Spirulina Platensis* is rich with protein, beta-carotene, vitamin-B12 and γ-Linolenic compare to other source of food (Dang, 1990). *Spirulina platensis* contains all the essential amino acid which makes it a complete source of protein.

Microalgae faces frequent changes in irradiance in the natural environment, microalgae have developed several acclimation mechanisms in order to cope with such changes. In light-limiting conditions, microalgae tend to increase their pigmentation by increasing the number of their photosynthetic units. Inversely, under supra-optimal irradiance, microalgae reduce their pigmentation (Masojidek *et al*., 2004). The timescale according to which the cells have to respond to those changes varies from seconds to days (Vonshak and Torzillo, 2004)

Spirulina have been consumed by human for hundreds of years and there are various ways of consuming it. *Spirulina* contains nutrients that are found in small quantity in other food source. The nutrient content of *Spirulina* makes it a desired food source and it can be taken as health supplement. Today, *Spirulina* are sold as dietary food in health food stores worldwide (Qiang, 2004*b*).

In aquaculture, microalgae are use as live feed for aquatic larvae such fish and shrimp. Microalgae have the attributes that are suitable to be utilized as live feed, their size which range from 1 μm to 15 μm makes them easy for larvae to consume (Brown and Miller, 1992). *Spirulina platensis* is used as feed for penaid shrimp larvae, bivalve mollusk, brine shrimp and marine rotifers. Among all the algae utilized in commercial aquaculture, *Spirulina platensis* probably has the broadest range of applications. Many studies on using *Spirulina platensis* as live feed have shown positive result, this proves the statement of the potential of *Spirulina platensis* in aquaculture (Becker, 2004*b*).

1.2 Justification of Study

The potential of *Spirulina platensis* in aquaculture and for human consumption has been recognized throughout the world. Many research on mass production of *Spirulina platensis* have been done by other country such as Taiwan, Japan, China and Vietnam (Dang , 1990). The effort of these countries is showing a positive result and success. In Malaysia, research on microalgae especially on *Spirulina platensis* is limited. Lack of knowledge makes it impossible for mass production of *Spirulina platensis* in Malaysia. *Spirulina* have a wide range of environment tolerance, therefore it is not impossible for *Spirulina platensis* to be cultured massively in Malaysia.

The benefits of *Spirulina platensis* as health supplement had gain recognition from the world community and this included the people of Malaysia. So far Malaysia had to import many of these *Spirulina platensis* health products from other countries to meet the increasing demand. This can be avoid or if not, mitigate if mass production of *Spirulina platensis* is done in Malaysia and producing our own *Spirulina platensis* product. But to do so, intensive study is needed to obtain more knowledge on the *Spirulina platensis.*

Manipulation of irradiance for outdoor mass production of *Spirulina platensis* can be done in economical way (Wong and Chang, 1999). These problems had inspired me to conduct this study, which is to determine the effects of different photoperiod on growth rate and biochemical constituents of *Spirulina platensis.*

1.3 Objectives of Study

The objectives of this study are:

i. To determine the effects of different photoperiod on the growth rate of *Spirulina platensis.*
ii. To identify suitable photoperiod that would promote optimum growth rate of *Spirulina platensis*.

1.4 Hypothesis of Study

The hypothesis of this study would be that the different photoperiod will affect the growth rate of the *Spirulina platensis.*

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to Blue-green Algae (*Spirulina platensis*)

Algae are considered to be a loose group of organisms that have all or most of the following characteristics: aquatic, photosynthetic, simple vegetative structures without a vascular system, and reproductive bodies that lack a sterile layer of protecting cells. (Sheath and Wehr, 2003). Microalgae can be classified into five main classes and they are the blue-green algae (Cyanophyceae), brown algae (Phaeophyceae), green algae (Chlorophyceae), red algae (Rhodophyceae), and diatoms (Bacillariophyceae) (Stottrup and McEvoy, 2003). The *Spirulina platensis,* which is proposed in this study, belongs to the class Cyanophyceae.

Cyanobacteria are a large and morphologically diverse group of phototrophic prokaryotes with such wide ecological tolerance that they occur in almost every habitat on earth. All cynobacteria are photoautotrophs, deriving the ATP and NADPH required for CO_2 fixation from electrons released from water by photolysis. Many can also grow photoheterotrophically, obtaining energy from light and getting carbon from organic compounds, although such growth is usually much slower than in the light (Adams, 2000).

Spirulina platensis is a multicellular, filamentous cyanobacterium, consisting of blue-green filaments of cylindrical cells in unbranched helicoidal trichomes. The mature trichomes of *Spirulina platensis* are few millimeters long and consist of cylindrical cells of 3-12 μm in diameter (Ciferri, 1983). *Spirulina platensis* are photoautotrophs, therefore, any factor that affects photosynthesis will influence the growth of *Spirulina platensis.* The major factors that are known to affect the growth of *Spirulina platensis* are light intensisty, temperature, salinity, and alkalinity (Qiang, 2004*b*).

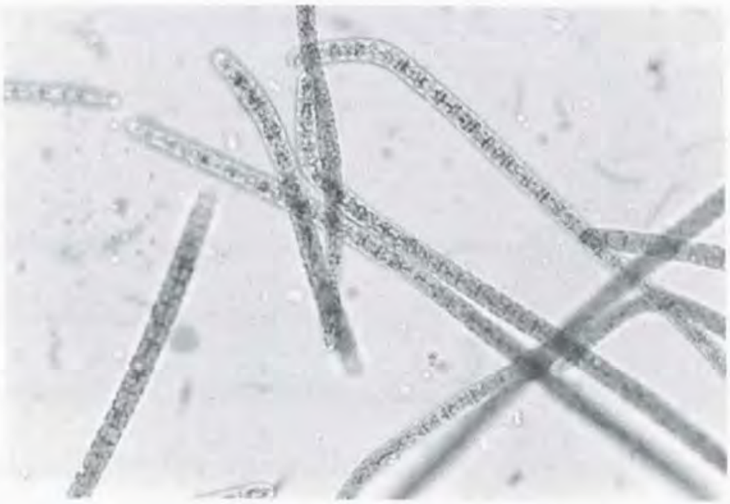

Photo 1.1 *Spirulina platensis* under light microscope
(Source: Kennedy A. A, University Malaysia Sabah)

The maximal growth rate for *Spirulina platensis* was obtained at pH 9.5-9.8. The reason why *Spirulina platensis* has been successfully culture at large-scale is because the fact that it thrives in the high pH environment. The type of environment in which the

Spirulina platensis is cultured makes the life of other microorganisms rather difficult (Qiang, 2004*b*). *Spirulina platensis* is more known as dietary supplement rather than as aquaculture feed because of it extractable chemical (Wilkfors, 2000).

2.2 The Importance of *Spirulina platensis*

The fact that by consuming *Spirulina platensis* promotes good health is no longer a myth, it has been proven by numerous of research and community around the world had accepted this fact. The demand for *Spirulina platensis* health products are increasing and researchers are trying to uncover the true potential of *Spirulina*. *Spirulina platensis* also have value in aquaculture due to its wide range of applications (Becker, 2004*b*). The annual production of *Spirulina platensis* biomass worldwide in 2000 was estimated to be about 2000 t (Qiang, 2004*b*).

Spirulina platensis has been consumed by human for at least 700 years, and maybe much longer, on the continents of America and Africa. For example, about 1300 AD, the Indians of Valley of Mexico harvested *Spirulina* from Lake Texcoco and made it into what they called *tecuitlatl*, *Spirulina* biomass which have been sun-dried and made into dry cake. And some where around that time, Africans in the area of Lake Chad also have about the same way of eating the *Spirulina* to that of the Mexican Indians, and they were called *dihe* (Qiang, 2004*b*)

Spirulina platensis has a number of health and therapeutic effects. For instance, it has the ability to reduce cholesterol and nephrotoxicity, inhibit the replication of several pathogenic viruses, enhance immune system and prevent the development of cancer. (Belay *et al*., 1993). *Spirulina platensis* is also a good source of fine chemicals. It contains excellent source of phycobiliproteins, a potent antitumor and anticancer drugs because of their ability to scavenge free-radical. *Spirulina* is also rich with Gamma-linolenic acid (GLA) which has the ability to prevent heart problem and reduce cholesterol (Qiang, 2004*b*)

Spirulina platensis could be the answer to the malnutrition that is suffered by developing countries. There was a study done in Vietnam, where *Spirulina platensis* powder was given as health supplement. The result was, children that were suffering from serious malnutrition recovered after they were treated with *Spirulina platensis* powder at the Thuanhai Hospital (Dang, 1990). There are also various experiments done by using *Spirulina platensis* as feed for aquaculture animals. Good growth rate was shown on the animals that were feed with *Spirulina*. Other animals, such as poultry and ruminant also show positive result when fed with *Spirulina* (Becker, 2004*b*).

2.3 The Growth and Life Cycle of *Spirulina platensis*

Growth is defined as an increase in living substance, usually the number of cells for unicellular microorganisms of total mass of cells for multicellular organisms. The most used parameter to measure change in cell number or cell mass per unit time is the growth rate (Tomaselli, 2004). *Spirulina platensis* has four growth phases.

a. Lag phase – The initial phase for the growth of *Spirulina platensis.* The growth lag could be the period of physiological adjustment due to changes in nutrient or culture conditions. It could also be due to the presence of non-viable cells or spores in the inoculum.
b. Exponential phase – At the late lag phase, *Spirulina platensis* have adjusted to the new environment and begins to grow and multiply. It will grow as long as mineral substrates and light energy are saturated.
c. Linear growth phase - *Spirulina platensis* growth is stable. This will continue as long as there is no change in the culture condition.
d. Death phase – The growth rate decrease. This maybe because of the decreasing mineral substrates or changes in light energy source (Lee and Shen, 2004).

2.4 Environmental Factors that affect the Growth and Biochemical Constituents of *Spirulina platensis*

Environmental factors, particularly light, temperature, nutrient status, and salinity, not only affect photosynthesis and productivity of cell biomass, but also influence the pattern, pathway and activity of cellular metabolism and thus dynamic cell composition. Some of these factors can be controlled while others are environment dependent (Qiang, 2004*a*).

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