EFFECTS OF DIFFERENT LIGHT INTENSITY ON *Nannochloropsis* sp. GROWTH IN OUTDOOR CULTURE

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I would like to declare that this dissertation is my original writing, except the data and facts that is stated with its sources and origins.

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ABSTRACT

Nannochloropsis sp. is one type of phytoplankton which play an important role for larval fish and rotifer production. This study have been done to determine the growth of *Nannochloropsis* sp. in different light intensity. This research divided into three treatments which are 2.5%, 25% and 100% of light intensity. The results showed the high mean of growth on *Nannochloropsis* sp. obtained from 100% light intensity treatment with the maximum value 23.33 million cells per mL. The treatment also showed less phosphate and nitrite content in medium culture which were 1.25 mg/L and 2.50 mg/L. However, there was no significance difference ($P< 0.05$) for growth and nutrient concentration in medium culture on 100% and 25% light intensity treatment. When the 100% light intensity exposed to the heavy rain, the number of cells decline dramatically and become unstable condition. Therefore, this research recommended that *Nannochloropsis* sp. culture should be done under 25% light intensity for outdoor culture.

ABSTRACT

Nannochloropsis sp. is one type of phytoplankton which play an important role for larval fish and rotifer production. This study have been done to determine the growth of *Nannochloropsis* sp. in different light intensity. This research divided into three treatments which are 2.5%, 25% and 100% of light intensity. The results showed the high mean of growth on *Nannochloropsis* sp. obtained from 100% light intensity treatment with the maximum value 23.33 million cells per mL. The treatment also showed less phosphate and nitrite content in medium culture which were 1.25 mg/L and 2.50 mg/L. However, there was no significance difference ($P< 0.05$) for growth and nutrient concentration in medium culture on 100% and 25% light intensity treatment. When the 100% light intensity exposed to the heavy rain, the number of cells decline dramatically and become unstable condition. Therefore, this research recommended that *Nannochloropsis* sp. culture should be done under 25% light intensity for outdoor culture.

ABSTRAK

Nannochloropsis sp. adalah sejenis fitoplankton yang memainkan peranan penting bagi penghasilan larva ikan dan rotifer. Kajian ini dijalankan bagi menentukan tahap pertumbuhan *Nannochloropsis* sp. pada pancaran cahaya yang berlainan. Kajian ini dibahagikan kepada tiga keadaan dimana pengkulturan *Nannochloropsis* sp. dilakukan dibawah 2.5% , 25% dan 100% pancaran cahaya. Keputusan menunjukkan min pertumbuhan fitoplankton tertinggi pada sampel 100% pancaran cahaya dengan nilai maksimum 23.33 juta sel per mL. Sampel ini juga menunjukkan kandungan fosfat dan nitrit yang rendah dalam medium kultur iaitu sebanyak 1.25 mg/L dan 2.50 mg/L. Namun demikian, tiada perbezaan bererti ($P< 0.05$) bagi sampel 100% dan 25% pancaran cahaya bagi pertumbuhan sel dan kepekatan nutrien pada medium kultur. Apabila sampel 100% pancaran cahaya terdedah pada hujan lebat, bilangan sel semakin berkurangan dan berada pada keadaan yang tidak stabil. Oleh yang demikian, kajian ini mencadangkan agar pengkulturan *Nannochloropsis* sp. secara komersial dijalankan dengan faktor pancaran cahaya matahari sebanyak 25%.

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°C	degree centigrade
%	percentage
ppt	part per thousand
mL	milliliter
L	liter
g	gram
mg	milligram
sp.	species
Sig.	significance
M	million
µm	micrometer
PUFA	polyunsaturated fatty acids
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
UMS	Universiti Malaysia Sabah
SPSS	Statistical Package of Social Science

CHAPTER 1

INTRODUCTION

1.1 Aquaculture in Malaysia

Referred to T.V.R Pillay in Aquaculture Principles and Practice, aquaculture described as culturing any type of aquatic animals and plants in fresh, brackish and marine environment. It is also known as aquatic crops which use to increase fishery production especially for high commercial value species and fulfill the market (Lokman, 1992). As we know, in Malaysia, fisheries sector still plays the role in providing the fish as protein sources. However, the production getting decline from year to year due to overfishing. Aquaculture is a potential activity to overcome the problem of limited fish sources. This activity began since 3000 years before in Asia and still lack of knowledge in Malaysia. Therefore, many research should be done to improve the techniques and skills including of broodfish management, seeds production, health and disease, fish nutrition and even microalgae culture.

1.2 Microalgae

According to Tomaselli (2004), microalgae, refers to the microscopic algae *sensu stricto* and the oxygenic photosynthetic bacteria (Cyanophyceae). These two groups of phototropic organisms have potential in utilization in food, feed and fine chemicals. There is also another term for microalgae which is phytoplankton. Microalgae also described as unicellular eukaryotic algae, a dominant community populating in fresh and sea water ecosystem (Barnabé, 1994).

Microalgae are suspended in the water and carried passively with the water flow. However, there are some species that have flagella which allow them to move even in a short distance. Although the size of microalgae is small, sometimes it is necessary for the phycologist to divide into three categories depending on the sizes. It is convenient to identify the suitable method to collect the microalgae. The largest microalgae with the size range 20 – 200 μm known as microplankton. Nanoplankton is categorized for the microalgae with 2 – 20 μm size range while the smallest microalgae which the size are less than 2 μm are called picoplankton.

In the nature, microalgae have been the primary producers in food chain of the marine and freshwater ecosystem (Feuga, 2004). The microalgae will be eaten by the zooplanktons, before being consumed by the larger animals such as fishes. Because of the role in aquatic ecosystem, microalgae have been used for aquaculture sector for rearing the aquatic animals like fish, mollusks and crustacean.

In past years, many microalgae species were introduced such as *Chlorella* sp., *Nannochloropsis* sp., *Isochrysis* sp. and *Tetraselmis* sp. The main application of microalgae is related to nutritional content either uses in fresh or as food additives. The purpose of culturing the microalgae in this sector is used directly to feed farm animals and human. Other than that, it is used for feeding the larval forms of cultured species which can consume the microalgae directly in hatcheries (Moretti *et al.*, 1999).

The selections of microalgae species for applying in aquaculture sector depend on several criteria. The first criteria for microalgae to be selected are high nutritional content for both fish larvae and rotifers. As a result, the growth and survival rate of larvae can be increase during the rearing period (Feuga, 2004). Besides, it is absence of toxicity for their consumers. The microalgae also should have suitable dimensions and digestibility. Other than that, it is an advantage if the species has high rate of reproduction because it can increase the production in a short period and reduce the costs at the same time. The next criteria for microalgae is having good capacity to adapt standardized mass rearing condition while the last is the species has reliability and affordability for doing mass production (Moretti *et al.*, 1999).

Table 1.1 Characteristics and use of microalgae in Aquaculture (Source: Barnabé, 1994)

Species	Normal size	Size range	T ° C optimal	Salinity S ‰	Concentration Millions/mL	Species to which this is fed
Unicellular green algae						
Tetraselmis suesica	6-7	4-11	< 22	25	2-5	B-R-A-P
Dunaliella tertiolecta	4-5	3-11				C
Dunaliella salina	4-5					C
Chlorella	2-4		< 28		50	Low
Nannochloris	1-3		< 28	5-30	50	R
Unicellular brown algae						
Isochrysis galbana	3	2-7	16-20		5-15	B
Monochrysis lutheri	4	2-9	16-20		10-20	B
Pseudoisochrysis	5-6		16-20		Idem	C-P_e
Unicellular diatoms						
Phaeodactylum tricornutum	5	3-20	18-26			B-P_e
Diatom chains						
Skeletonema costatum	50	3-9				B-C
Chaetoceros calcitrans (rendered unicellular by ultrasound)	100+	3-5	16	20-30		B-H

A: Artemia, B: Bivalves (larvae + spat), C: Cupped oyster, H: All oyster species, P_e: Penaeid shrimps, R: Rotifers, P: Clams.

1.3 *Nannochloropsis* sp.

Nannochloropsis sp. is one of marine microalgae which is classified under Eustigmatophyceae class (Tomaselli, 2004). Previously, it is known as Chlorophyceae class because of similar characteristics. Some of phycologist also classified *Nannochloropsis* sp. under Xanthophyceae. However, after a few experiment and research conducted, the results shows clearly that eustigmatophycean differed greatly from the others. Eustigmatophycean is lack of chlorophyll, other than chlorophyll a content and have high violaxanthin content (Fietz, 2005). This eukaryotic unicellular alga is spherical in shape and categorized under picoplankton because of the small range of the sizes (Moretti *et al.*, 1999). *Nannochloropsis* sp. has high nutritional value especially in polyunsaturated fatty acid. Therefore, it is possible to do biomass production to fulfill the demand.

There are three different methods in culturing *Nannochloropsis* sp. which are continuous, semi-continuous and batch culture (Moretti *et al.*, 1999). All of this methods can be applied in outdoor culture system or indoor culture system. Usually, in Mediterranean hatcheries, the microalgae produce by using batch culture. Batch culture is the most reliable but the least efficient because it takes a long period. However, it can be solved by identifying the major factor that can increase the production. Batch culture is suitable in doing large scale cultivation. For semi continuous and continuous culture, the techniques are quite efficient but it will cause waste and allow contamination of other organisms (Treece and Davis, 2000).

1.4 Effects of light intensity in *Nannochloropsis* sp.

In culturing *Nannochloropsis* sp., it is important for the culturist to maintain the growth condition in the optimal range in order to gain greatest yield. There are many factors influencing algal growth either in physical environment such as light, temperature, salinity and turbulence or in chemical environment like pH condition and nutrient. For large scale outdoor operations, light intensity is one of the environmental factors that can be manipulated. There are previous study in determination of the optimum light intensity in indoor culture of *Nannochloropsis* sp. The growth of this eustigmatophycean is best in the range of 1 000 to 2 000 lux (Moretti *et al.*, 1999).

Malaysia is a tropical country and it give advantage for culturists to carry out the mass production of *Nannochloropsis* sp. in outdoor culture. Eventhough light is the major factor in photosynthetic activity, high intensity may not guarantee the greatest yield. Therefore, the study on different light condition of *Nannochloropsis* sp. in outdoor culture conducted.

1.5 Objectives

The objectives of this study are:

1. To determine whether different light intensity affects the growth rate of *Nannochloropsis* sp.
2. To determine the optimum light intensity that promotes maximum growth on *Nannochloropsis* sp.
3. To study the difference of nutrient concentration on *Nannochloropsis* sp. medium cultured in different light intensity

CHAPTER 2

LITERATURE REVIEW

2.1 General Characteristics of *Nannochloropsis* sp.

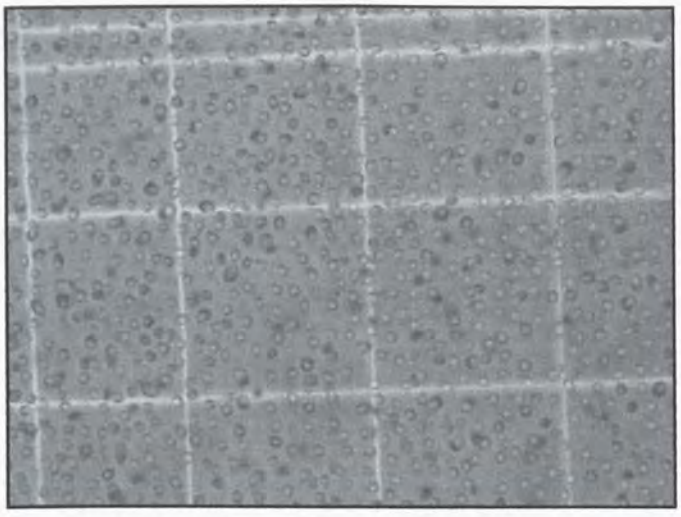

Photo 2.1 *Nannochloropsis* sp. cells

Nannochloropsis sp. is a picoplanktonic genus in marine environment and belonging to Eustigmatophyceae class (Tomasseli, 2004). Before, it was classified as a *Chlorella* sp. but then had been rejected because of no chlorophyll other than chlorophyll *a.* It is a eukaryotic unicellular algae and spherical in shape. However, some of them have

cylindrical to oval shape. This species is non-motile algae and move by the water flow. The size of *Nannochloropsis* sp. is in the range of 2-4 μm in diameter.

Nannochloropsis sp. has single chloroplast without pyrenoid and containing several bands of photosynthetic lamellae. The chloroplast endoplasmic reticulum is continuous with the nuclear envelope. This kind of microalgae is bounds with the polysaccharide cell walls and do not accumulate the starch (Tomaselli, 2004).

Referred to Moretti *et al.* (1999), the cells reproduce very fast through vegetative reproduction. Sometimes, it can reach 100% to 150% reproduction. The *Nannochloropsis* sp. growth exponentially or in other words it the population can be described under log-phase.

2.2 Biochemical Composition of *Nannochloropsis* sp.

Nannochloropsis sp. contain high nutritional compound such as sterol and fatty acids (Mohammady *et al.*, 2005). It has extremely high polyunsaturated fatty acids (PUFA) such as Eicosapentaenoic acid and Docosahexaenoic acid compare than other microalgae. Other than that, it also contains amino acids, vitamins and minerals. Furthermore, it has accumulated some secondary carotene, canthaxanthin and asthaxanthin. The major content of storage polysaccharide is β-glucan. The most important content are Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) which have major role in pharmaceutical industry and aquaculture sector.

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