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Evaluation of Relationship between Light Intensity (Lux) and Growth of *Chaetoceros muelleri*

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Abstract

Microalgae have the most significant contribution towards the global environment, oxygen production and carbon cycle consequently understanding marine microalgae is vital. Hence the factors that affect the growth should be considered and studied in advance before the culturing of these algae. Photosynthesis is most important in plant growth. Thus this study tries to find out the optimum light intensity (Lux) required for achieving maximum yield of *Chaetoceros muelleri* in a laboratory culture.

The inoculation of the algae was done under controlled environment where the cells were counted and assumed to be the same for all. The pure strand of *C. muelleri* with f2 media (8 sets of 3 flasks) were placed in front of the light source at lux values of 0 lux, 500 lux, 1000 lux, 1500 lux, 2000 lux and 2500 lux. The cell count was the basic result that was collected. At every 24 hours cells were counted and readings were recorded.

The experiment lasted 7 days. The result showed that there was significant growth that is the number of cells increased exponentially as the Light intensity (lux) increased till the culture was exposed to a 1000 lux where maximum cells were counted during the culture period (168 hours). From previous studies it was evident that the optimum light intensity for flask culture in laboratory was 1000 lux. However, results from the experiment show that the cell growth with 1500 lux is very close and similar to 1000 lux. Some recommendations after the experiment are; that there is very little literature on the above topic and that this area requires a lot more attention as Fiji's Aquaculture Industries are blooming thus there is a need for further research and study around the same area.

Keywords: Microalgae; Lux; *Chaetoceros muelleri*

Introduction

Microalgae are defined as minute photosynthetic plants and can be found in both seawater and freshwater [1]. These have similar properties as land based plants but they have to be submerged in an aqueous environment where they have efficient access to water, carbon dioxide and other nutrients.

Nowadays, there are several marketable applications of microalgae; some of which include (i) they are used to enhance the nourishing value of food and animal feed owing to their chemical composition, (ii) they play a crucial role in aquaculture and (iii) they can be assimilated into cosmetics. Moreover, they are cultivated as a source of highly valuable molecules [2]. Microalgae are also used in the production of pharmaceuticals, diet supplements, pigments and biofuel [3] and represent the largest, yet one of the most poorly understood groups of microorganisms on earth [4]. A handful of microalgae species out of the many that have been found are mainly cultured for live aquaculture feed and *Chaetoceros muelleri* [5] is one of them.

The hypothesis for this experiment is to find whether light is required to culture *Chaetoceros muelleri*. Secondly to examine the amount of light required to give the maximum yield of the product. Also show the relationship between the amount of light required and the growth rate of *Chaetoceros muelleri*.

Microalgal culture has to be conducted under specialised conditions as explained by Coutteau [6]; the most important parameters regulating algal growth are *nutrient quantity and quality, light, pH, turbulence, salinity and temperature*. Concentrations of cells in phytoplankton cultures are generally higher than those found in nature. Algal cultures must therefore be enriched with nutrients to make up for the deficiencies in the seawater. Macronutrients include nitrate, phosphate and silicate. Here the paper describes the general

set for culturing Microalgae: Temperature 16-27°C, Salinity 12-40g/l and Light Intensity (lux) 1,000-10,000 lux depending on the volume of culture.

As with all plants, microalgae photosynthesize, i.e., they assimilate inorganic carbon for transformation into carbon-based matter. Light is the source of energy which drives this reaction and in this regard intensity, spectral quality and photoperiod need to be considered. Light intensity plays a vital role, but the necessities fluctuate critically with the culture penetration and the density of the algal culture: at higher depths and cell concentrations the light intensity must be increased to penetrate through the culture 1,000 lux is suitable for Erlenmeyer flasks, 5,000-10,000 lux is required for larger volumes [6]. Light may be natural or supplied by fluorescent tubes. Too high light intensity (e.g. direct sun light, small container close to artificial light) may result in photo-inhibition. Also, overheating due to both natural and artificial illumination should be avoided. The duration of artificial illumination should be minimum 18h of light per day, although cultivated phytoplankton develops normally under constant illumination [7].

Background

Chaetoceros is probably the largest genus of marine planktonic

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diatoms with approximately 400 species described. Although a large number of these descriptions are no longer valid. It is often very difficult to distinguish between different *Chaetoceros* species. Several attempts have been made to restructure this large genus into subgenera and this work is still in progress. However, most of the effort to describe species have been focused in boreal areas, and the genus is cosmopolitan [7], so there are probably a large number of tropical species still undescribed.

Scientific Classification of *Chaetoceros muelleri*:

Empire	Eukaryota
Kingdom	Chromista
Subkingdom	Harosia
Infrakingdom	Heterokonta
Phylum	Ochrophyta
Subphylum	Khakista
Class	Bacillariophyceae
Subclass	Coscinodiscophycidae
Superorder	Chaetocerotanae
Order	Chaetocerotales
Family	Chaetocerotaceae
Genus	<i>Chaetoceros</i>

In the past there have been very few studies done on the relationship between the lux and the growth rate of *Chaetoceros muelleri*. As stated by Blinn [8] respiration and photosynthesis are the two significant processes in microalgal growth that occur simultaneously in the light. To know the rates of both processes, at least one of them has to be measured. To be able to measure the rate of light respiration of *Chlorella sorokiniana*, the measurement of oxygen uptake must be fast, preferably in the order of minutes. This experiment looks deep into the process of Respiration where the amount of Oxygen uptake *in situ*. The respiration rate is known to be related to the growth rate, and it is suggested that faster algal growth leads to a higher energy requirement, and as such, respiratory activity increases [8].

And as stated by Brown [9] equal inocula were added in the two bioreactors (1 and 3 cm light path); by the end of the second day, cell concentration was low in the 1-cm light path bioreactor, possibly indicating photo inhibition, since growth of the culture increased in the 3-cm light path bioreactor.. The optimal population density was obtained by a daily harvest of 5% of culture volume to be 2.39 gm⁻² day⁻¹ in 1 cm light path and 3.27 gm⁻² day⁻¹ in 3 cm light path. Light path length of 3 cm was found optimal in both with low and high initial cell densities [10].

As noted by the previous studies there has not been much research done on *Chaetoceros muelleri* in regards to the light and growth of the microalgae. Plus this research proposals will be one of the pioneer projects that will consider the relationship for the two variables in the labs as small experiment, from where more research can be carried out in order to use the investigate the intensity of light required for the maximum growth of *Chaetoceros muelleri*. Hence there is more attention required for the above pioneer project to be carried out successfully.

Materials and Methods

The experiment was carried out in the laboratory of the School of Marine Studies, University of the South Pacific (USP), Suva, Fiji.

Experimental organisms (*C. muelleri*)

A pure 20 ml sample of *C. muelleri* was purchased from Australian

National Algae Culture Collection (ANACC), CSIRO in Tasmania, Australia with the assistance of the USP Laboratory Technician.

Glassware preparation

The glassware used in this experiment was cleaned and washed with detergent and autoclaved on programme A (121°C and 20 psi) as provided on the autoclave for 15 minutes.

Water treatment

Seawater was strained using 1 µm (micro meter) cartridge filter and used for the preparation of the Media and Stock solution. After filtration the water was passed a Life Guard brand Ultra Violet Steriliser (Model no. - QL 40) that uses a 40 watt UV sterilizer bulb. All the above was done in the Aquaculture Laboratory (Wet Lab).

F/2 media preparation and modification

The procedures used to prepare the F/2 Media and also the Modification of F/2 media were followed from Guillard [11]. 75 g of NaNO₃ and 5g of NaH₂PO₄ H₂O were dissolved in 1 litre of filtered sea water and this was labelled as Solution A. Trace Metals was labelled Solution B, Silicates was labelled solution C. 1 ml of solution B and C was used for every litre of media. Vitamins were labelled Solution D and 0.5 ml of this solution was used for every litre of media.

Inoculation

A sample of pure *C. muelleri* is placed in a controlled environment for it to grow or reproduce. For this experiment the procedure used was adapted from Probert and Klaas [12] was followed. 100 ml of the f/2 media that was prepared earlier was filled into 250 ml Conical culture flasks which were covered using corks and aluminium foil. The prepared flasks were autoclaved under Programme A. After the Autoclave the flasks were removed and left overnight to cool in the algae room. All the flasks were inoculated with 10% of inoculum (*C. muelleri*) in the Laminar Flow cupboard. The cabinet was cleaned with 70% ethanol. The pipettes and micro pipettes were also autoclaved [13]. The Nitrile hand gloves used are to be sprayed with 70% ethanol and will be worn while inoculating. The mouth of the culture flask was sterilised by moving over a Bunsen burner flame.

Experimental setup

After the inoculation the rubber corks were bored to make holes and the glass tubing were inserted for the inlet (3-4 mm) and exhaust (2-3 mm) were placed to seal the mouth. The above setup was prepared in the laminar flow cabinet to avoid contamination. Once the inlet and exhaust lines are fixed the flasks were ready for setup in the algae room. Some of the parameters that were kept constant were; temperature at 18°C, preferred pH of 7-9 with reference to FAO, Instrumental Paper, 1999, salinity was between 20-35 ppt, Aeration was controlled by opening or closing the switch valves linked to the inlet line and the cell starter culture the cells were calculated in terms of cells/ml. the inoculation required 10% of the starter culture. During the preparation of all the twelve flasks efforts were made to ensure that approximately the same number of cells is transferred.

The variable

The variable in the experiment is the lux that is a standardized unit of measurement of the light intensity (which can also be called "illuminance" or "illumination") - as an example for reference purposes. One lux is equal to one lumen per square metre [14]:

$$1 \text{ lx} = 1 \text{ lm/m}^2 = 1 \text{ cd}\cdot\text{sr}\cdot\text{m}^{-2}$$

The flasks were placed at 500 lux, 1000 lux and at 1500 lux from the light source. The growth performance was measured in terms of cell density/ml.

Experimental design

Eighteen (18) trails were carried out that is six treatments with three replicates of each. The six treatments were based on the different lux; 0 lux, 500 lux, 1000 lux, 1500 lux, 2000 lux and 2500 lux. These were represented as treatment A, B, C, D, E and F respectively.

Sampling

Hemocytometer was used to count the cells [2]. From the time the inoculation was done the counting was done at every 24 hour time interval until the culture reached a stationary stage. From every flask a sample of 1 ml was taken and average of 3 counts were done for better result analysis. The experiment was repeated two more times to confirm and reconfirm results that were obtained in the first set.

Statistical analysis

The statistical analysis that was adapted by this experiment was SPSS. This showed the relationship between the amount of flux and the growth rate at different lux rates. The comparison of the differences of the treatments with the control was done by the Duncan's Test.

Results

Over the period of the experiment the results were collected and tabulated as Replicate 1, Replicate 2 and Replicate 3 (Table 1) from which an average data was calculated. Table 1 shows the average of all the three Replicates that was done. The values at the different lux (0 lux, 500 lux, 1000 lux, 1500 lux, 2000 lux and 2500 lux) were added and divided by 3.

$$\text{Average Cell Count at Different Lux} = \frac{\text{Cell count T1} + \text{Cell Count T2} + \text{Cell Count T3}}{3 \text{ Treatments}}$$

More over Figure 1 was constructed using Table 1, in order to see the relationship between the Cell counts at different lux with the time of each count.

According to the average growth rate of the *C. muelleri*, the figure states that the highest cell count is at 1000 lux. From the 0 hour at stated lux the cell count increased gradually. The maximum growth is shown at 1000 lux around 96 hours. As indicated by the figure that a maximum growth is reached than the slows and starts to decrease. This figure indicates the phases exponential phase (growth phase), stationary phase (Maximum point reached) than the death phase (no

more growth). At all the different lux (0 lux, 500 lux, 1000 lux, 1500 lux, 2000 lux and 2500 lux) there is a similar trend seen. The control (0 lux) showed little growth initially, than the cell count decreased to zero.

Moreover from the SPSS Statistics that was carried out indicated that there was a significant (df5, F298.54, p 0.00) difference between 6 treatments (0 lux, 500 lux, 1500 lux, 2000 lux and 2500 lux). 1000 lux was significantly higher than the control (p 0.00), 2000 lux (p 0.00) AND 2500 lux (p 0.00), however it was not significantly different from 500 lux (p 0.899) and 1500 lux (p 0.221). Thus it can be noticed that 1000 lux is more similar to 1500 lux. Figure 1 shows that at 1000 lux there is highest growth.

Discussion

The purpose if this experiment was to better understand the importance of light intensity in the growth of the *C. muelleri* as the time and the intensity was increased. The experiment was modelled in such a way that at different lux from a minimum to a maximum lux just to compare the cell count to observe the cell growth, the control was also set up to witness if there will be any growth at zero light intensity. The purpose of having the different lux setup for the experiment was to find out the best light intensity required for the Flask culture of *C. muelleri* in a controlled laboratory.

The main result of the experiment showed that growth rate varied at different Light intensity (Lux). *C. muelleri* can be cultured in a laboratory at different light intensity but according to the results the best amount of light for the maximum yield is 1000 lux, 500 lux and also 1500 lux are also similar but lower growth rate seen than 1000 lux.

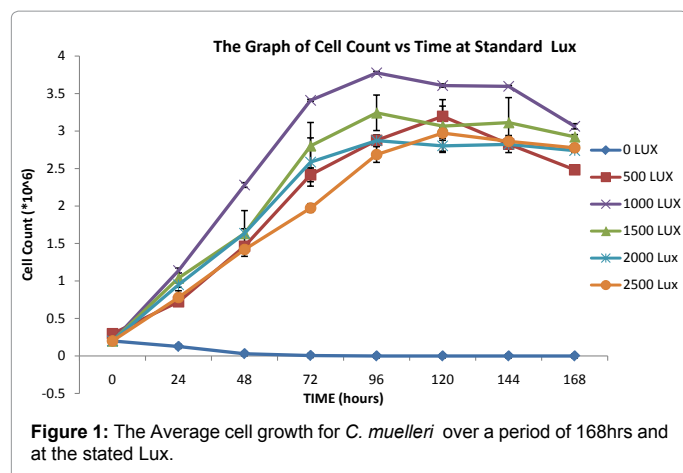
The experiment results is supported by Coutteau [6] where he stated that Light intensity played an important role, but the requirements varied greatly with the culture depth and the density of the algal culture: at higher depths and cell concentrations the light intensity must be increased to penetrate through the culture (e.g. 1,000 lux is suitable for Erlenmeyer flasks, 5,000-10,000 is required for larger volumes). Too high light intensity (e.g. direct sun light, small container close to artificial light) may result in photo-inhibition. This may cause overheating due to both natural and artificial illumination [15]. Thus in this experiment states that the higher the lux the slower the growth rate at around 2000 lux and 2500 lux the growth fast initially that it slows; there might be few factors affecting this one could be the light intensity too high for the cells to tolerate, or the high population increase might decrease the nutrients thus slow growth rate. Hence this indicates that too much light is not suitable for the growth of *C. muelleri*. Comparing this to 500 lux; this also has slow growth rate; thus only conclusion that can be drawn is less light intensity induces slow growth rate, as the flask has enough nutrients, light was the only factor that was low.

Naturally not only Light is required for the microalgae growth, there are other factors that affect the growth rate of *C. muelleri* which include right salinity, right pH, right amount of nutrients and vitamins [16]. The term nutrient "limitation" has been widely used in the literature in a variety of contexts [17], often interchangeably with the terms "deficiency" and "starvation." There are two distinctively different types of limitation: one referring to limitation of the maximum population density achieved (Liebig limitation) and one referring to limitation of growth rates

From the earlier research's carried out for the laboratory culture of *C. muelleri*, the light intensity required should be 1000 lux and the results for this experiment showed a similar outcome along with

Time (Hrs)	Average Cell Count					
	0 Lux	500 Lux	1000 Lux	1500 Lux	2000 Lux	2500 Lux
0	0.2	0.296667	0.2	0.2	0.2	0.2
24	0.126667	0.723333	1.14	1.04	0.95	0.78
48	0.03	1.463333	2.28	1.6333	1.636667	1.42
72	0.005667	2.416667	3.41	2.8033	2.586667	1.973333
96	0	2.876667	3.776667	3.2433	2.873333	2.686667
120	0	3.196667	3.606667	3.0667	2.803333	2.973333
144	0	2.826667	3.596667	3.1133	2.823333	2.863333
168	0	2.483333	3.063333	2.9233	2.74	2.776667

Table 1: The average cell count for the three treatments.



the other necessary factors that were kept constant. This experiment showed the effect of light on the growth of *C. muelleri* which was only one factor. There are further studies required in the similar areas. Once there is a better understanding of the culture of microalgae at a small scale, this could be used in the mass culture and used in the developing aquaculture industry in Fiji and the Pacific Islands.

Conclusion

This study examined the amount of light intensity required to give the best yield of *C. muelleri* in a laboratory culture. From the experiment carried out and the data analysis it may be concluded that the 1000 Lux is the optimum lux to produce the best yield. This is supported by the literature. It was noticed that as light intensity increased the growth of *C. muelleri* increased significantly to a limit at 500 lux, 2000 lux and 2500 lux where the growth rate slowed. This could be due to the either the decrease in the nutrient level in the flask or due to too less or over heat of the flask.

The relationship between light intensity required and the growth rate of *Chaetoceros muelleri* was also studied (Figure 1). Light is required for the growth of microalgae on the other hand too much light and too little light can slow the growth rate [17,18]. Thus the experiment indicated that 1000 lux to 1500 lux is the best light intensity in order to produce maximum yield.

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