UNIVERSITI PUTRA MALAYSIA

INVESTIGATION ON THE USE OF RED AND YELLOW LIGHT-EMITTING DIODES (LEDS) TO PROMOTE GROWTH, PROXIMATE COMPOSITION AND CELL MORPHOLOGY OF MARINE MICROALGA *TETRASELMIS* SP.

NUUR FATIN BINTI KAMARUL ZAMAN

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NUUR FATIN BINTI KAMARUL ZAMAN

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Investigation on the use of red and yellow light-emitting diodes (LEDs) to promote growth, proximate composition and cell morphology of marine microalga *Tetraselmis* sp.", by Nuur Fatin Binti Kamarul Zaman and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Project

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti PerubatanVeterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek

Pengkajian penggunaan diod pemancar cahaya merah dan kuning dalam mengalakkan pembesaran, komposisi proksimat dan sel morfologi di dalam mikroalga marin *Tetraselmis*sp.

Oleh

Nuur Fatin Binti Kamarul Zaman

2016

Penyelia: Profesor DatoDr. Mohamed Shariff Mohamed Din

Penyelia bersama: Dr Sanjoy Banerjee

Jarak gelombang cahaya yang berbeza mempengaruhi pembesaran dan komposis proksimat mikroalga marin *Tetraselmis sp.* dengan menggunakan diod pemancar cahaya merah dan kuning , lampu pendarfluor sebagai kawalan. Tiada perbezaan ketara ($p < 0.05$) di antara diod pemancar cahaya merah , kuning dan lampu pendarfluor sepanjang 11 hari kecuali lampu pendaflour lebih tinggi

berbanding LED merah pada hari ke 12 & 13 pengkulturan dari sudut densiti sel , optikal sel dan spesifik kadar pertumbuhan spesifik. Kandungan lipid dan protein lebih tinggi di bawah diod pemancar cahaya merah , manakala komposisi karbohidrat tinggi di bawah lampu pendarflour. Dari sudut morfologi sel menggunakan *scanning electron microscope* (SEM), tiada perbezaan dari sudut morfologi sel diantara tiga lampu. Di dalam kajian ini menunjukkan fasa permulaan mempunyai bentuk eliptikal manakala eliptikal lebar pada fasa akhir. Tambahan lagi, kehadiran proses pembagian sel dan flagella dapat dijumpai. Keputusan kajian ini menunjukkant *Tetraselmis* sp. mempunyai persamaan dari sudut kadar pembesaran and dan morfologi sel di bawah lampu merah, kuning dan pendarfluor**.**

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 – Project

Investigation on the use of red and yellow light-emitting diodes (LEDs) to promote growth, proximate composition and cell morphology of marine microalga *Tetraselmis* sp.

By

Nuur Fatin Binti Kamarul Zaman

2016

Supervisor: Professor Dato Dr. Mohamed Shariff Mohamed Din

Co-supervisors: Dr Sanjoy Banerjee

The growth and proximate composition of marine microalga, *Tetraselmis* sp.using red and yellow color LEDs and fluorescent as a control were compared under different wavelengths. There were no significant differences ($p > 0.05$) between the red, yellow and fluorescent lights during the 11 days of culture except fluorescent light had higher cell density and optical density compared to red on day 12 & 13. However,specific growth rates showed similar values. Lipid and protein content was significantly higher under red LEDs whereas, carbohydrate

composition show significant higher under floursescent light. In terms of cell morphology observe under scanning electron microscope (SEM), the sizes of the cells among the treatments did not reveal significant difference. The cells were broad elliptical in initial stage and elliptical in final stagein in all three lights. There were also dividing cells and flagella observed on the cells. Final stage showss the cell is more folded especially under the yellow LED and significant difference between initial and final in term of size. The results showed that *Tetraselmis* sp. had similar growth rate, and and cell morphology under the red, yellow LEDs and fluorescent light wherease high carbohydrate in florescent light and high protein and lipid under red LED.

Key words: Growth, proximate composition, *Tetraselmis* sp. light wavelength, morphology

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1. INTRODUCTION

Microalgae are unicellular plants that are able to manufacture their own food material by photosynthesis. It is important in aquaculture development because it is one of the primary food sources for a large number of aquatic organisms. Microalgae need light as their main energy source thus it known as photoautotrophic organism. The effects of light intensity, salinity, media nutrients, and temperature, on the growth and proximate composition of microalgae have been widely explored (Brown et al., 1997). Furthermore, light conditions affect directly the growth and photosynthesis of microalgae.

In tropical countries, microalgae are used for larval feeding. It is produced in indoor hatcheries to avoid bio-contamination. Thus, indoor hatcheries are believed to improve the quality and reliability of microalgae production. According to Thompson et al., (1992) culturing microalgae outdoors under ambient conditions is less costly as compared to indoor. However outdoor production will exposed to high seasonal unpredictability and cause the cells exposed to rapid fluctuations to light and temperature. In addition, the climate where the microalgae are grown is often suboptimal as an example there is a lack of sufficient light during the rainy season due to cloud cover. Thus, all these parameters will influence the proximate composition and growth of the microalgae.

Recently, wavelength of light has been found to influence the growth of marine microalgae (Loong et al., 2014). Colored LEDs have a fixed wavelength and are considered to be more efficient than fluorescent light. Thus, it can improve

the quality and quantity of microalgae biomass (Martins, 2014). Adjusting the environmental conditions is one of the effective ways to optimize the growth rates of microalgae (Mata et al. 2010). Since LEDs have relatively narrow wavelengths bands, it allows them to produce specific wavelengths (color). Besides that it is easily obtained and in small size in various forms and is easy to use. LEDs also have an advantage in that it consumes low energy, and is easy to install that make LEDs more environmentally friendly than other sources of light. In addition, the LEDs do not produce as much heat as other types of lights. Thus it can prevent overheating of the growth medium surrounding the microalgae cells (Koc et al., 2013).

Hence, this study was undertaken to fulfil the following objectives:

i. To investigate the effects of red and yellow LEDs on the growth rate of *Tetraselmis* sp.

ii. To examine the effects of red and yellow LEDs on nutritional profile: protein, lipid and carbohydrate, content of *Tetraselmis* sp.

iii. To examine the morphology of *Tetraselmis* sp. cultured under the red and yellow LEDs

For this research, the following hypotheses were proposed:

1. Ho: There are no effects on growth and nutritional profile enhancement and morphology of microalgae culture using red and yellow LEDs.

2. Ha: There are effects on growth and nutritional profile enhancement and morphology of microalgae culture using red and yellow LEDs.

2. LITERATURE REVIEW

2.1 *Tetraselmis* sp.

Tetraselmis sp. is a green marine microalga. This type of microalga is one of the motile phytoplankton, which has four flagella. *Tetraselmis* sp. is very important as the main supplier of energy and organic substances in aquatic ecosystems, an important food and fatty acids for sea animals. *Tetraselmis* sp. contains high concentration of natural lipid and also produces amino acids for aquaculture (Muller, 2003).

2.2 Effect of Growth Rate in Different Wavelength of Light

The growth rate and biomass production of marine microalgae are related to the wavelength (Shu et al., 2012). According Loong Teo et al., (2014) *Tetraselmis* sp. grows better under blue light compared to red light. This can be observed from the higher growth rate observed for *Tetraselmis* sp. under the blue LED condition resulting in higher biomass starting from the 2nd day when compared to red light condition. The biomass steadily increased starting from 1st day until day 16. However its remaining stationary due to nutrient depletion and wastes start to accumulate in the culture medium.

2.3 Proximate composition

According to Lourenço (2006), growth stage in microalgae cultures and the manipulation of the physical and chemical conditions of the cultures may result in differences in cell composition, as an example, variations in levels of lipid, protein, carbohydrate and other components of the cell. According to Brown et al. (1997), microalga composition varies by species in the proportion of protein(6 to 52%), lipid (7 to 23%) and carbohydrate (2 to 23%). Voskresenskaya

(1972) stated that the spectral composition of light can influence the physiological and biochemical changes in plants. Different light sources can vary the microalgae composition (Mercado et al. 2004).

2.4 Morphology

The taxonomy and morphology of the genus *Tetraselmis* have been relatively well studied. *Tetraselmis chuii* cell shape is compressed, elliptical to ovate, and size is (12–16) × (7–10) μm. The apical aperture hairs are abundant and single type (Hori et al. 1986). They have four flagella located in opposite pairs which are thick and of equal length, shorter than the length of the cell, covered with hairs and scales, and are inserted into an apical cell depression. Cells reproduce asexually by simple division within the mother cell commonly forming two daughter cells usually in an inverted position from each other however sexual reproduction is unknown (Graham et al, 2000). It is a motile cell, thick, bilaterally symmetrical, oval in shape when viewed from broad side, with a single apical furrow passing from one broad face to the other and cells elliptical when viewed from the narrow side. (Arora et al. 2013)

3. MATERIAL AND METHOD

3.1 Microalgae

The marine microalga strain *Tetraselmis* sp. was obtained from the Marine Science Centre (COMAS), University Putra Malaysia. The cultures were grown in filtered and autoclaved seawater (30 ppt, 7.5 pH) using Conway medium. The sterilized medium was kept for 2 days before inoculating the microalgae into the culture medium to allow sufficient time for CO_2 equilibration.

3.2 Inoculation of Microalgae to Culture Medium

Cultivation of *Tetraselmis* sp. having an initial cell density of 1×10^5 cells/ml was carried out in 2 L Erlenmeyer flasks using red and yellow LEDs corresponding to different wavelengths. Fluorescent light was used as a control. All the cultures were cultivated using Conway medium between 24-26˚C, 60 µmol/m^2/s light intensity and 30 ppt salinity. Duration of the experiment was 13 days. Each treatment and control had four replicates so as to ensure reproducibility of results.

The Conway medium consists of Solution A, Solution B and Solution C. 1mL of Solution A, 0.5ml of Solution B and 0.1ml of Solution C was added to 1000mL of filtered and sterilized (by autoclaving) seawater. Stated below are the compositions of chemicals in each solution:

Solution A – Stock solution

- KNO_3 = 100 g
- Na_3O_4P = 20 g
- Sodium EDTA =45 g
- Boric acid = 33.4 g
- $FeCl_3$ =1.3 g
- $MnCl_2$ = 0.36 g
- Distilled water = 1000 ml

Solution B – Trace metals solution

- $ZnCl_2$ = 4.2 g
- $CoCl_2$.= 4.0 g
- $CuSO_4$ = 4.0 g
- (NH_4) MoO_4 = 1.8 g
- Distilled water=1000 ml
- *Note: Acidify with HC1 to obtain a clear solution.

Solution C – Vitamins solution

- Vitamin B (Thiamin) = 200 mg
- Vitamin B12 (cyanocobalamine) =10 g

Figure 2.1 Inoculating of *Tetraselmis* sp. into the culture medium.

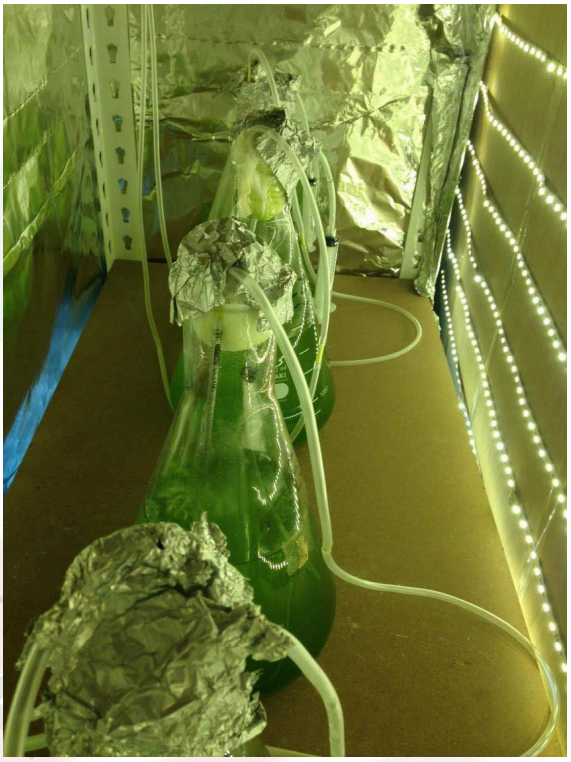


Figure 2.2*Tetraselmis* sp. culture under yellow LED

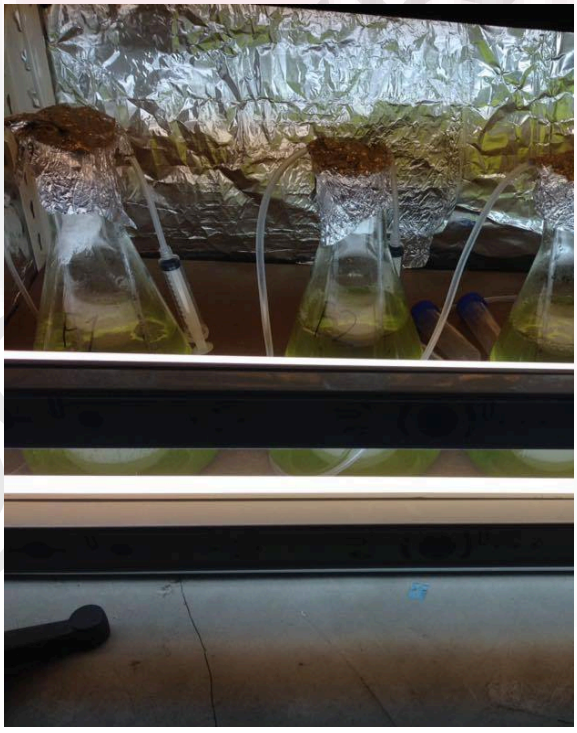


Figure 2.3*Tetraselmis* sp. culture under fluorescent light

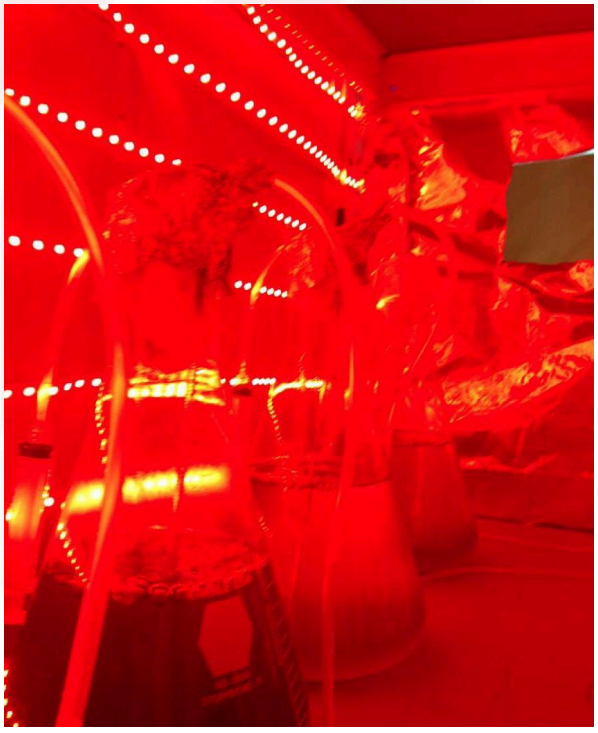


Figure 2.4 *Tetraselmis* sp. culture under red LED

3.3 Harvesting of *Tetraselmis* sp.

The culture was harvested by centrifuge at 9000 rpm for 10 min for 4 °C the supernatant was discarded. Autoclave of sea water was added and centrifuges again 9000 rpm 3 min at 4 °C.

3.4 Analysis of growth parameter

Microalgae growth was measured using cell density, and optical density (OD) method. Optical density reading was taken by spectrophotometer and Conway medium was used as blank. At every 24 h interval, the suspension of microalgae was stirred homogeneously and transferred in the quartz cuvette. The OD was read at 540nm. Cell counts of *Tetraselmis* sp. were measured using a haemocytometer. Cell numbers were determined daily by placing an aliquot of well-mixed culture suspension on a Neubauer haemocytometer. The cells were counted in five small squares in the centre block. The specific growth rate was calculated during the exponential growth period by the following equation;

$$\text{Specific growth rate} = \frac{\ln N_{t2} - \ln N_{t1}}{t_2 - t_1}$$

Where N_1 and N_2 are cell count on days t_1 and t_2, respectively

Microalgae cell morphology was observed under scanning electron microscope (SEM) to analyse cell structure changes. The samples of each treatment were taken on the 3rd day and at the end of the experiment (10thday). During sample preparation for SEM, the samples were centrifuged in suspension at 3000 RPM for 3 min and the supernatant were decanted. Then, the samples were fixed in 2.5% Glutaraldehyde for 4 h at 4 °C. After that, the samples were washed with 0.1M Sodium Cacodylate buffer for 3 times of 10 min each. After each change of the buffer, the samples were centrifuged and the supernatant was decanted. Next, the samples were post fixed in 1% Osmium Tetroxide for 2 h at 4 °C. After, post fixation, the samples were washed again with 0.1M Sodium Cacodylate buffer for 3 times at 10 min each. After each change of buffer the samples were centrifuged and the supernatant was decanted.

The samples were dehydrated using a series of acetone, which were 35%, 50%, 75%, 95% concentration every 10 min and 100% of acetone every 15 min for three times. Before changes of each concentration of acetone the samples were centrifuged first and the supernatant was decanted. After dehydration process cell suspension was pipette onto aluminium foil (1cm diameter) coated with albumin. The samples were transferred into specimen basket and put into the critical dryer for about ½ h. Then, mounting was done by sticking the sample onto the stub using double sided tape and coating with gold coat in sputter coater.

Figure 3.1: Mounting process was done by sticking the sample onto the stub using double sided tape and coating with gold coat in sputter coater.

Figure 3.2: Sample after coating with gold coat in sputter coater.

3.5 Proximate composition

3.5.1 Protein analysis

Protein was analysed according to the methods of Slocombe et al., (2013). Freezed dried microalgae sample (5mg) was weighed into test tubes. Water bath was set to 55°C. 200µl of 24 trichloracetic acid (TCA) was added to the samples to resuspend the dried microalgae samples. The samples were incubated at 95-100°C for 15min. The samples were later removed and allowed to cool. Once cooled, 600µL distilled water is added, diluting to the sample to 6% TCA. The sample was centrifuged at 13,000 rpm at 10°C for 20 min. Supernatant was discarded and pellet re-suspended in 0.5ml Lowry reagent D. Re-suspended samples were incubated in a water bath at 55°C for 1 h. Samples were allowed to

cool and centrifuged at 13,000rpm for 20 min at room temperature. Supernatant was transferred to a fresh tube.

Reagent for protein analysis:

A: 2% Na_2CO_3 in 0.1N NaOH

B: 1% NaK Tartrate in H_2O

C: 0.5% CuSO4.5H2O in H_2O

D: 48: 1: 1 of Reagent A: B: C

E: Phenol Reagent – 1 part Folin Ciocalteu-Phenol

The reagents A, B, and C can be stored indefinitely. However, reagents D and E have to be made up fresh every day (preferably just prior to use)

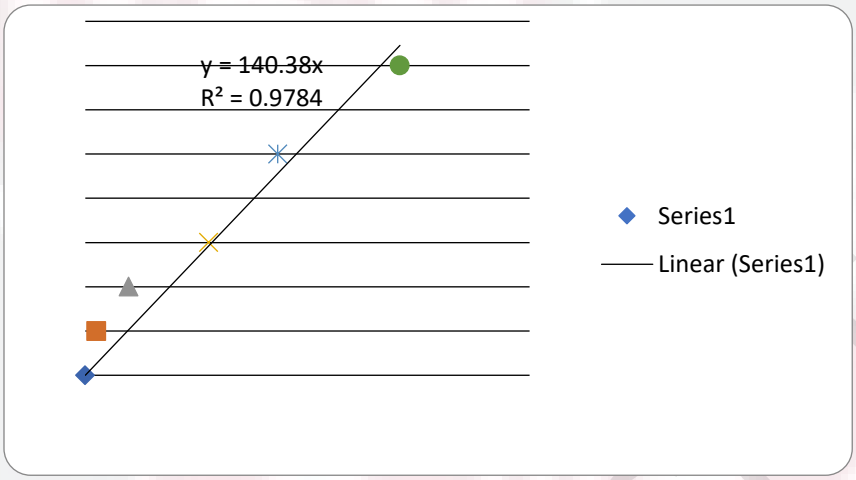
Standards solutions

- Make stock of 10mg/mL bovine serum albumin (BSA)
- Stock divided into 1-2mL aliquots of the following concentrations: 0.1, 0.5, 1, 2, 3, 4, and 5mg/mL

For protein quantification, 950µl of the sample protein was pipetteinto a new centrifuge tube. One ml of Lowry reagent D was added into the tubes and shaken and incubated for 10 min at room temperature. A 0.1ml of Folin- Phenol- water (Reagent E) was added to each individual sample after incubation and vortex immediately. The mixture was incubated for a further 30 min. Absorbance was measured using spectrophotometer at 600nm by using blank as mili-Q with Reagent E&D

Calculation % protein and % carbohydrate:

1. Calculate slope (m) and intercept (q) value
2. The R^2 value should be more than 0.90



Standard [carb] µg/L	ABS (600nm)
0	0
20	0.0369
40	0.1464
60	0.4164
100	0.6505
140	1.0623

DW of sample (mg)	Absorbance of samples (---- nm)	Conc. of protein/ carbohydrate in 25 ml	DW (mg/L)	protein / carbohydrate (%)
1	**2**	**3**	**4**	
		(Slope x abs of samples) + Intercept	(DW of sample / 25) x 1000 Start volume 25 mL	(3 / 4) x 100

3.5.2 Lipid analysis

Lipid was analysed according to the methods of Bligh modified lipid assay (1959) . Weigh of aluminium dishes was labelled and recorded and 15mg of the sample was weight and put into 15ml conical tube following which 3ml of methanol: chloroform was added and homogenised. The tube was centrifuged for 4 min at 6000rpm at 4 °C. Supernatant was then transferred into a clean tube and placed in ice and 3ml of methanol: chloroform was added into the tube containing sample residue and vortexed and homogenised.

The tube was centrifuged for 4 min at a medium speed of 4 °C until the two phases clearly separated. Upper phase was discarded (methanol and water) including the interfacial emulsion by a pipette suction. Upper phase was re-extracted by transferring the upper phase into clean test tube and 3 ml of chloroform was added and mixed by vortexing and centrifuged. The upper phase layer was again discarded and combined with the chloroform layer with the first extract. Then, the lower phase (chloroform) was transferred containing lipid into aluminium dish. The solvent was evaporated to dryness in 60°C at a dry oven.

Aluminium dish containing lipid residue was weighed to calculate the lipid concentration each sample.

1. Weight of aluminum dish : ① mg
2. Subsample weight : ②mg
3. After evaporation weight of aluminum dish : ③
4. Calculate the ratio of weight of aluminum dish contained with lipid

   Residualslipid concentration of subsample is ③-① = ④
5. So, original lipid concentration of subsample was

$$\frac{④}{②} X\ 100 = \%$$

Figure 3.4: Aluminium dish contained lipid residual

3.5.3 Carbohydrate analysis

Carbohydrate was analysed according to the methods of Dubois et al. (1956). Stock solution was prepared, using 20 g glucose in 20ml distilled water. (Concentration 1000 µg/L). Standard solution was prepared as below:

Standard solution of carbohydrate (µg/L)	
0 µg/L	Only distilled water 10ml
20 µg/L	0.2ml from stock solution + 9.8m distilled water
40 µg/L	0.4ml from stock solution + 9.8m distilled water
60 µg/L	0.6ml from stock solution + 9.8m distilled water
100 µg/L	1 ml from stock solution + 9.8m distilled water
140 µg/L	1.4 ml from stock solution + 9.8m distilled water

Solution A was 8g phenol in 2g distilled water heated at 100 °C. Five ml was taken from solution A and 100ml distilled water was added to make a 5 % phenol solution. Homogenised 5 mg of freeze dried sample with 5ml distilled water, then added in 20ml distilled water to make a total volume of 25ml. One ml homogenized sample and standard solution was taken in a test tube and 1 ml of Phenol 5% solution was added and allowed 30 seconds for the reaction period. Then 5ml concentration of H_2SO_4 was added and the reaction allowed for 30 seconds. Tubes were placed in cold water bath. After cooling the readings was take at 488nm by spectrophotometer.

Figure 3.3: Test tubes were placed in cold water bath

3.6 Statistical analysis

The collected data were analysed using one-way analysis of variance (ANOVA). Significant differences among the different treatments were determined using the Turkey multiple range test at 0.05 level of probability.

4. RESULTS AND DISCUSSION

4.1 Effect of the growth rate when exposed to different wavelengths

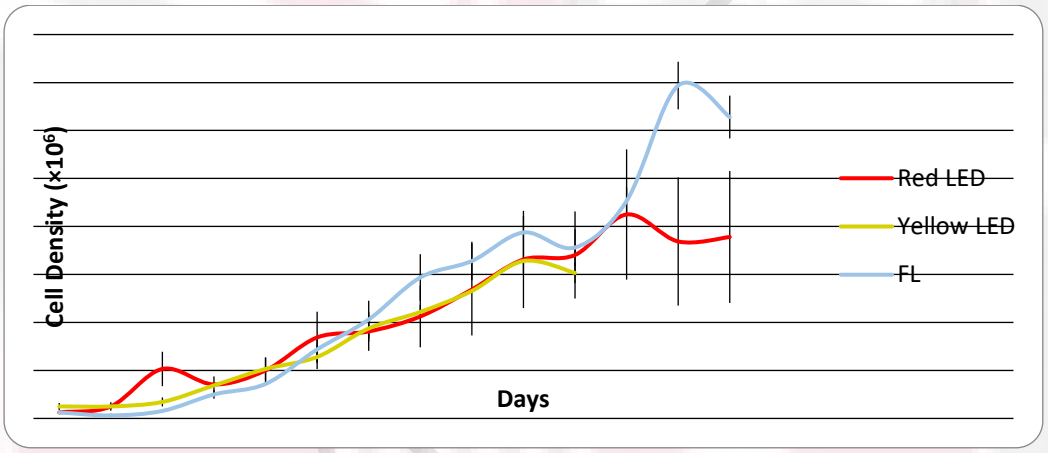


Figure 4.1: Cell density of *Tetraselmis* sp. when exposed to different wavelengths

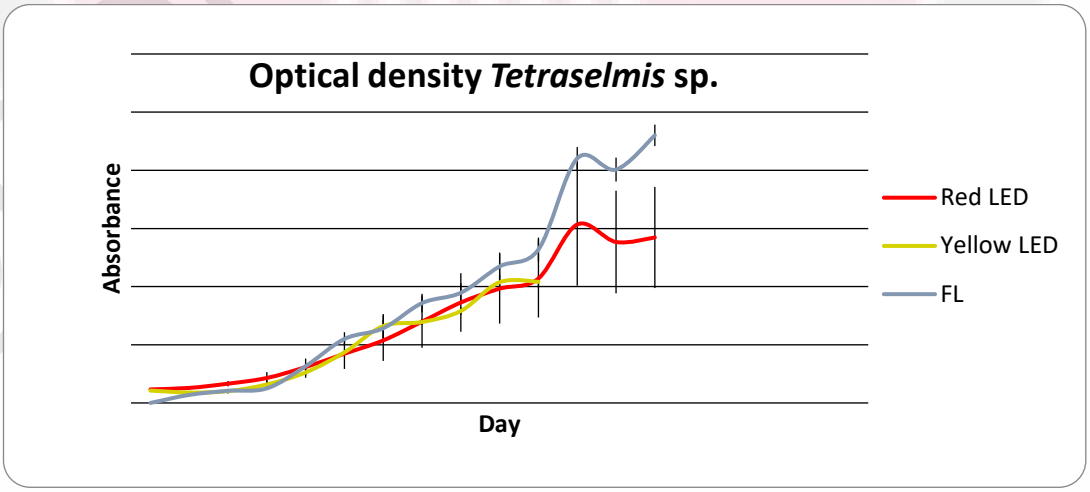


Figure 4.2: Optical density *Tetraselmis* sp. when exposed to different wavelengths

Table 4.1: Specific growth rate of *Tetraselmis* sp. when exposed to different wavelengths

Fluorescent light	0.208
Red LED	0.201
Yellow LED	0.18

Figure 9, shows there were no significant differences ($p > 0.05$) between the red, yellow LEDs and fluorescent light grown cultures during the 11 days in terms of cell density and OD. However it showed fluorescent light cell density is higher compared to red LED on day 12 and 13. Cultures grown in yellow LED reached the stationary phase earlier on day 9 and had shorter exponential phase therefore, were harvested 3 days earlier. Red LED and fluorescent light had exponential phase on day 4 while yellow LED on day 2. However red LEDs achieved stationary phase on day 11 followed by fluorescent light on day 12

Based on the cell count, specific growth rate was calculated using equation (1). As shown in Table1, the growth rates between three treatments showed similar specific growth rates.

According to Matthijs (1996), reproduction capacity of microalgae was dependent on the characteristic of light wavelength. Especially microalgae green pigment chlorophyll that can absorb the red light wavelength more efficiently compared to other wavelength. Photosynthesis needs light, absorption peaks of chlorophyll a and b which is at 662 nm and 642 nm, respectively while the red LED peak wavelength is at 659 nm fall between the chlorophyll a and b peaks. Thus, better light utilization will influence the biomass composition.

Since microalgae cells absorb red light through the green pigment chlorophyll, thus red LED would be likely to be more effective for photosynthesis and, in fact, the cell mass concentration of the microalgae grown in the red light was highest (Koc et al., 2012).

However in this study, fluorescent light and red LED show higher cell density during the 13 days of cultivation compared to yellow LED. The results are similar to the study of Abiusi et al., (2014) that showed growth that was much enhanced under red and fluorescent compared to blue and green lights.

In this study, the growth of microalga was monitored through cell count and OD because each technique has its own restrictions. The cell count technique has a disadvantage because the process is time consuming. Furthermore, incorrect counts during cell division can happen especially when similar-sized fragments are encountered that fail to separate or during cell division. Therefore, optical density also can be used as one of the method to calculate the cell density as it is very easy, practical and user friendly. (Banerjee et al, 2011).

4.2 Proximate analysis

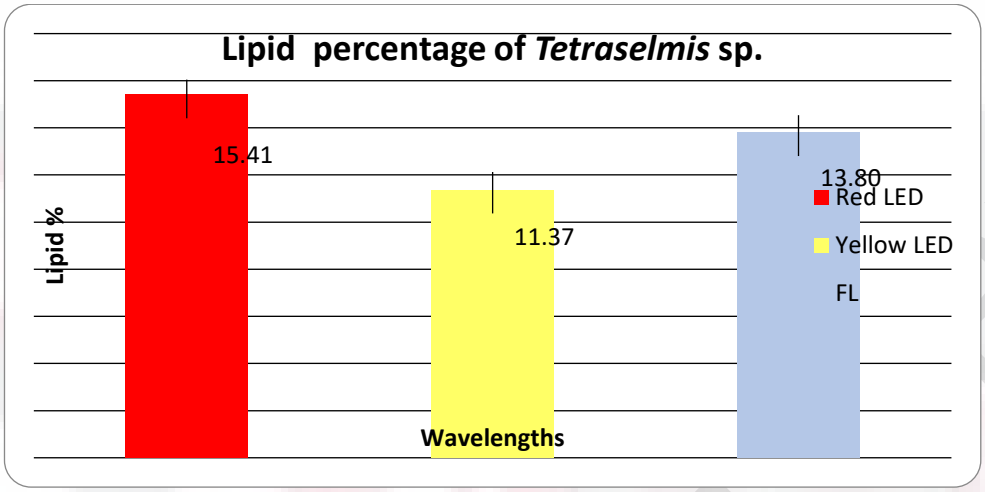


Figure 4.3 Lipid percentage *Tetraselmis* sp. when exposed to different wavelengths

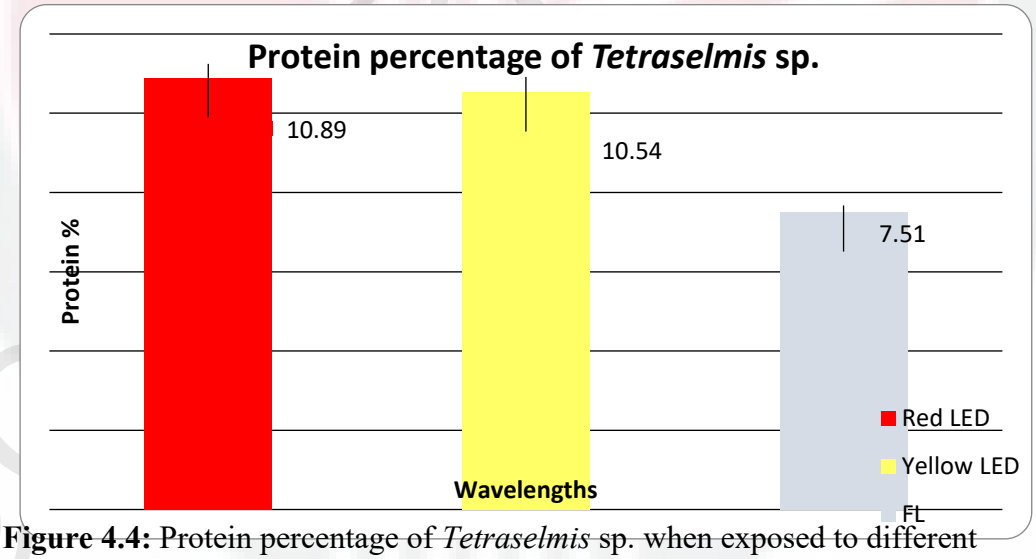


Figure 4.4: Protein percentage of *Tetraselmis* sp. when exposed to different wavelengths

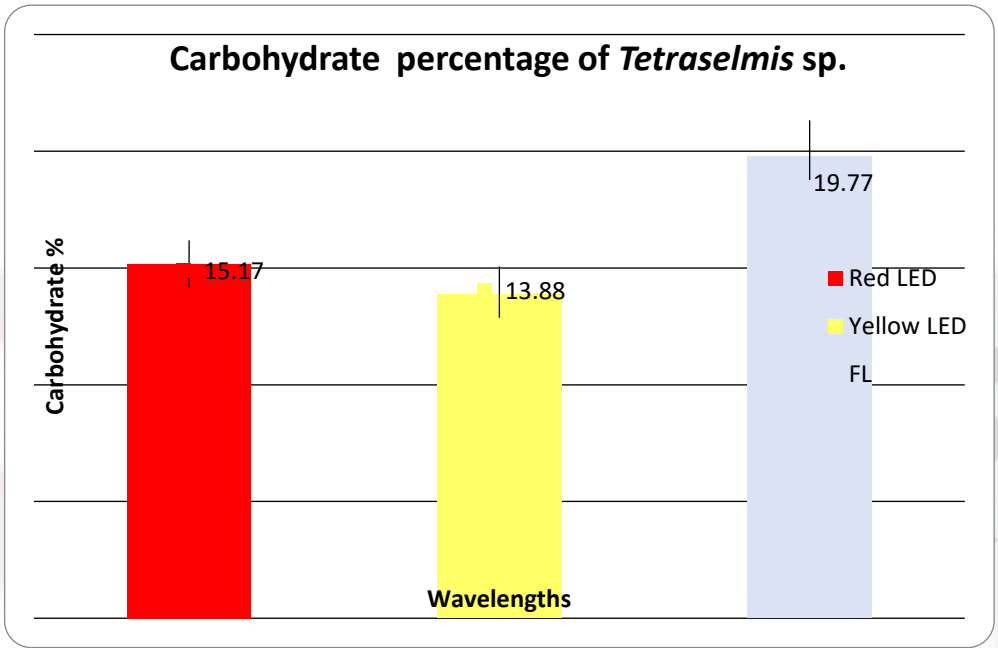


Figure 4.5: Carbohydrate percentage of *Tetraselmis* sp. when exposed to different wavelengths

Protein, carbohydrate and lipid composition of *Tetraselmis* sp. showed a significant difference ($p > 0.05$) between different wavelengths of light cultures.

Protein was significantly higher in red LED culture when compared with yellow LED and fluorescent light cultures. The red and yellow LEDs culture were not significantly different.. However, fluorescent had shown significant differences higher with red and yellow. Carbohydrate was significantly higher in fluorescent light culture followed by red and yellow LEDs culture. The red LED and yellow LEF cultures were not significantly different. However, fluorescent showed higher carbohydrate percentage compared to yellow LED culture.

. According to Senger (1987), favourable effects of blue light on protein synthesis metabolically or enzyme activation has been extensively investigated for unicellular green algae and higher plants, with red light favouring the accumulation of carbohydrates. However the current study showed higher

carbohydrate percentage in the fluorescent light compared to other treatments, and protein percentage was higher under red light when compared to fluorescent light. This finding is similar with the study by Abiusi et al., (2014) which also showed higher carbohydrate percentage under fluorescent light and protein under red LED. The lipid percentage was higher under red LED and is similar with studies done by Monika (2015) for different species, i.e., *Chorella* sp. Sánchez-Saavedra and Voltolina (2006) also stated that species specific differences in biomass composition with different light spectra. The author suggested the use of different light color change algal biomass composition. Besides that, Rivkin (1989) stated that light wavelength may manipulate cell composition relative to protein, polysaccharides, and lipids.

Studies by Brown et al. (1997) showed that microalgae composition vary by species in the proportion of protein (6 to 52%), lipid (7 to 23%) and carbohydrate (2 to 23%). In the current experiment, the protein, lipid and carbohydrate in *Tetraselmis* sp. cultured under different light conditions were within the ranges reported by Brown (1997).

4.3 Morphology

Table 4.2: Cell sizes of *Tetraselmis* sp. when exposed to different wavelengths

Treatment	Initial stage (µm²) Mean ± SD	Final stage (µm²) Mean ± SD
Red LED	26.06 ± 10.25	40.71 ± 6.23
Yellow LED	32.33 ± 7.51	37.81 ± 9.83
Flourescent light	31.10 ± 3.72	47.30 ± 2.50

When *Tetraselmis* sp. was examined under scanning electron microscope (SEM) there were no significant diffence in term of sizes between the treatments for initial and the final stages.

However,there was a significant size difference between initial and the final stage. However, the cells were similar in shape between three treatments , cells initial day exhibited broad eliptical and final stage is elliptical in shape. Besides that, on initial stage the number of cell is not as many compared to final stage which is in cluster indicated by observation under SEM. There were also present of dividing cells on initial stage and some of the cell with flagella.

At present there are no studies regarding the cell morphology under different wavelengths. However in this study, the cell show more folded on final stage compared to initial especially yellow light in term of observation under SEM. According to Oldenhof (2006) blue light can lead to larger cells. This is due to delayed during cell division and red light caused mother cells to divide at the smallest size. According to Abiusi et al., (2014) under red light, the daughter cells start to be released in a shorter time compared with white, blue and green lights, and the mother cells with green light were significantly smaller than with the other lights.

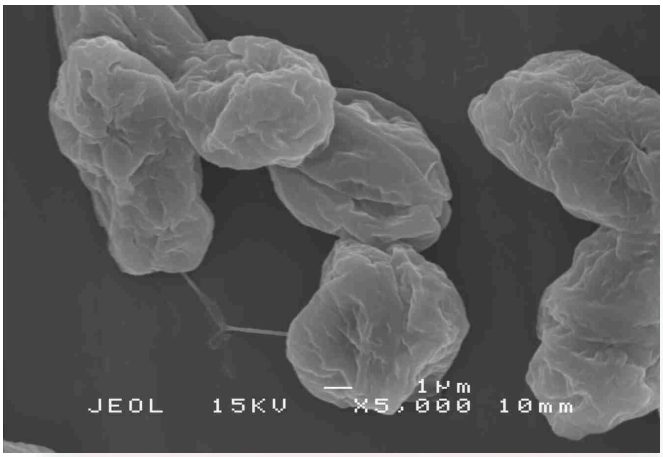


Figure 4.6: Fluorescent light on day 3- cell showed presence of flagella

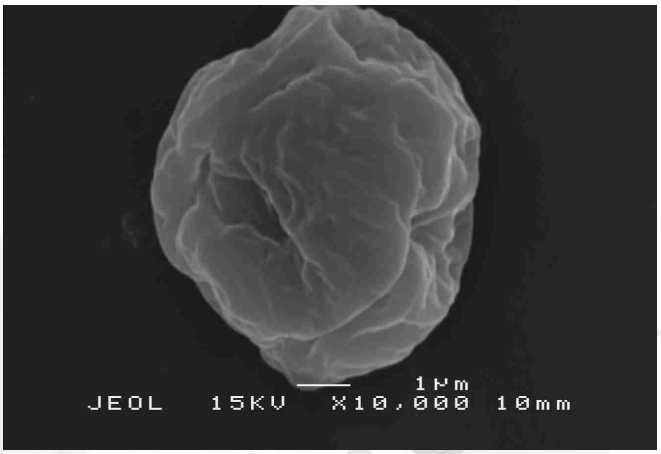


Figure 4.7: Fluorescent light on day 3 - individual cell length x width (6.8µm x 5.8 µm)

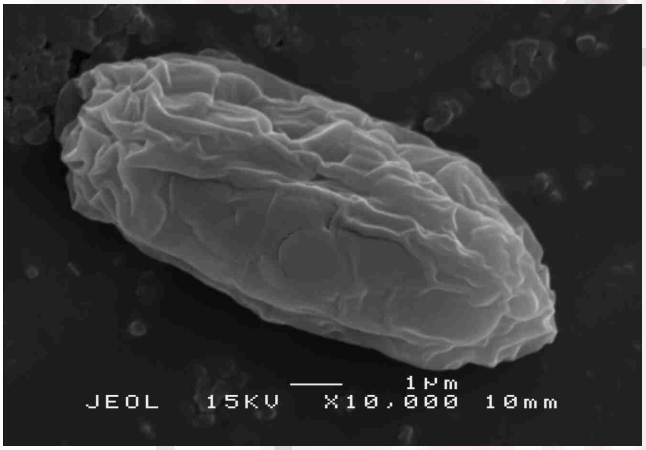


Figure 4.8: Fluorescent light on day 13 - clump of cells with average size of length x width (9-10 µm x 4.5-5.5 µm)

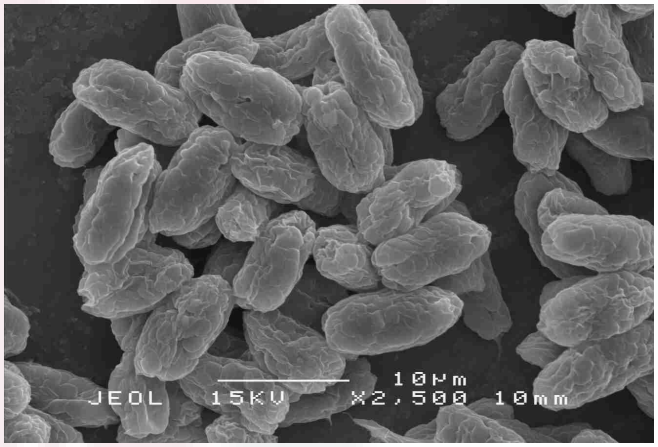


Figure 4.9: Florescent light on day 13, individual cells length x width (10.4 µm x 4.4 µm)

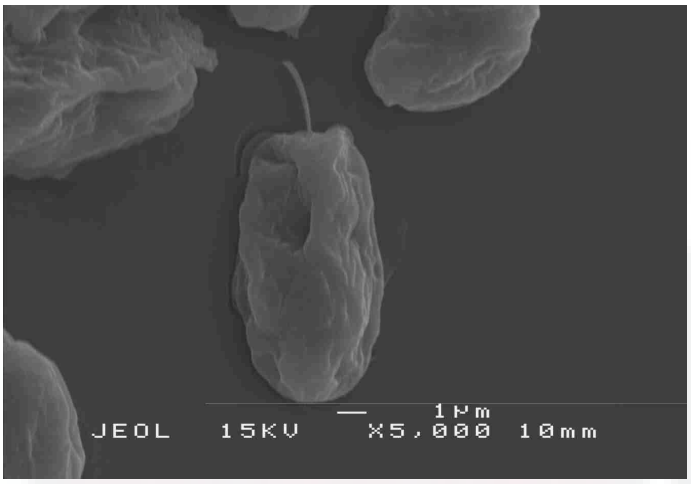


Figure4.10: Red LED on day 3- individual cells length x width (6.9μm x 4.3 μm)

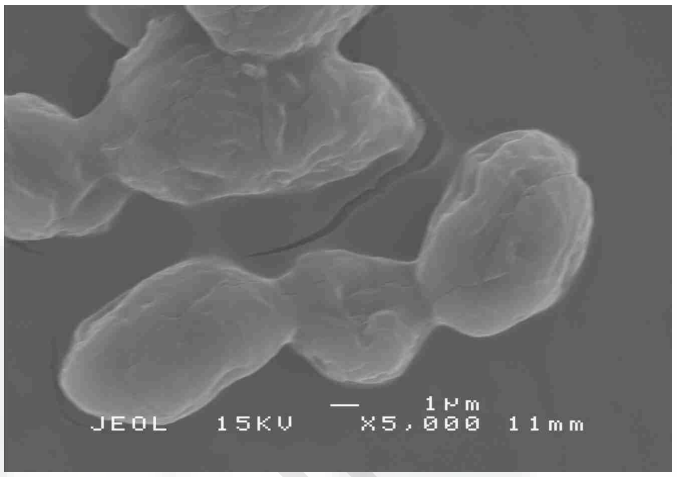


Figure 4.11: Red LED on day 3 – dividing cells were observed

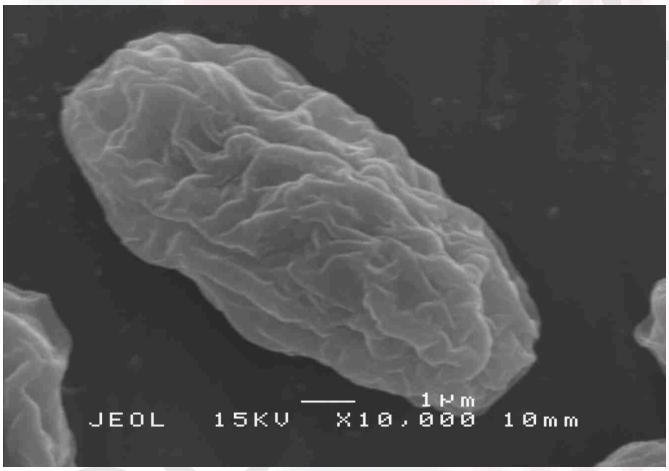


Figure 4.12: Red LED on day 13– individual cells length x width (9.1μm x 4.2 μm)

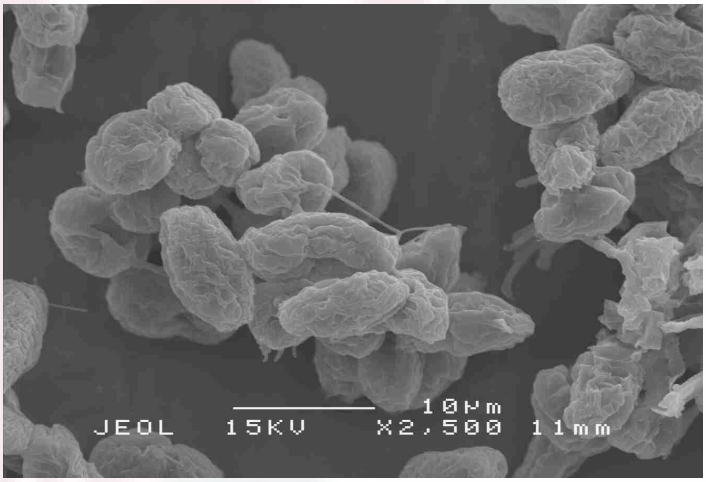


Figure 4.13 Red LED on day 13 - the cells were observed to be clumping and elliptical in shape

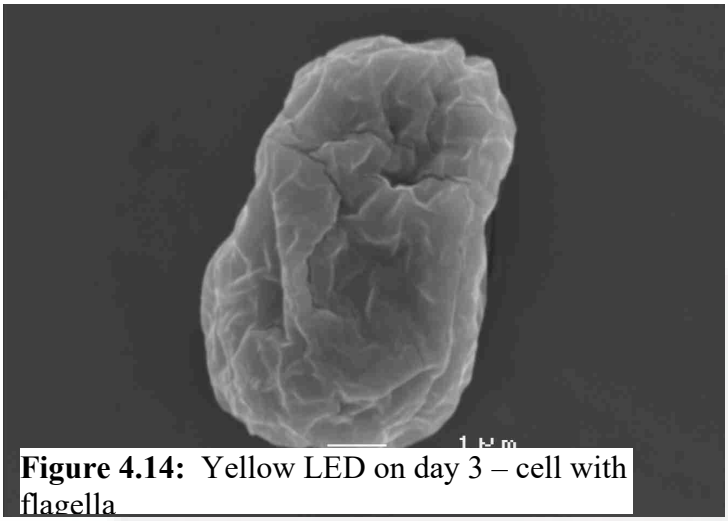


Figure 4.14: Yellow LED on day 3 – cell with flagella

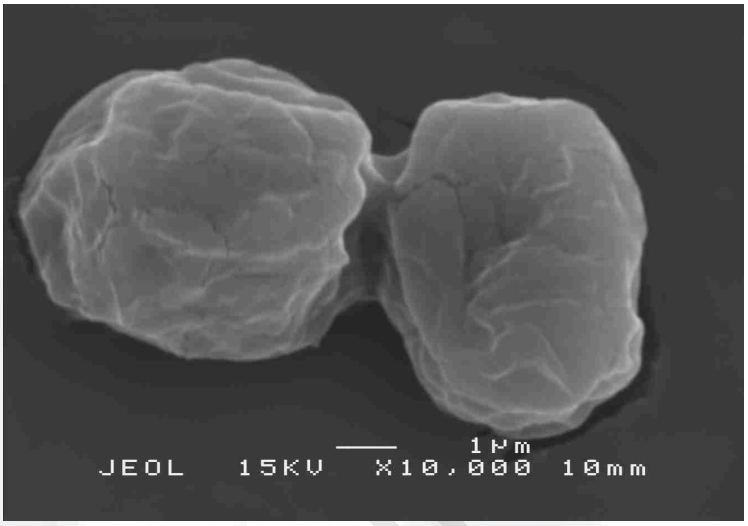


Figure 4.15: Yellow LED on day 3- dividing cells that are still enclosed by the parent cell wall.

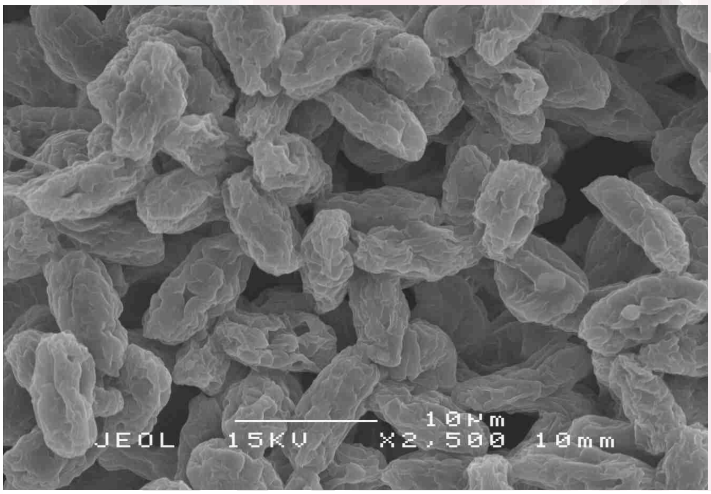


Figure 4.16 Yellow LED on day 10 – large number of cells in cluster a were observed

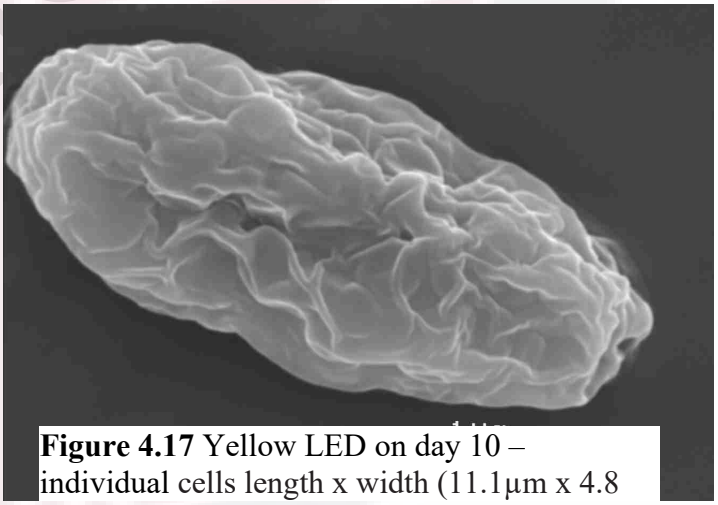


Figure 4.17 Yellow LED on day 10 – individual cells length x width (11.1µm x 4.8

CONCLUSION

Tetraselmis sp .was successfully cultivated for 13 days. According to the initial results, fluorescent, red and yellow lights showed no significant difference in terms of cell count, OD, cell morphology and specific growth rate. However, red light showed higher lipid and protein composition compared to other lights while carbohydrate was higher under fluorescent light.

RECOMMENDATION

Further studies are necessary to investigate whether similar results that were obtained in small volume culture of 2 litres could be obtained when the cells are cultured in larger volumes on a commercial scale.

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