

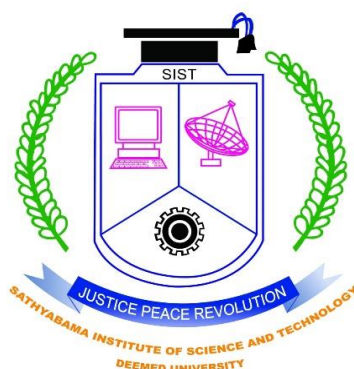
Effects of different wavelengths and intensities of light on growth of *Chlorella vulgaris*

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

by

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DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC | 12B Status by UGC | Approved by AICTE
JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

April - 2021



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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Rackshaya.R.C. (37230065), Vaishali.K. (37230092) who carried out the project entitled "Effects of different wavelengths and intensities of light on growth of *Chlorella vulgaris*" under our supervision from January, 2021 to March, 2021.

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We, Rackshaya.R.C. (37230065) and Vaishali.K. (37230092), hereby declare that the Project Report entitled “Effects of different wavelengths and intensities of light on growth of *Chlorella vulgaris*” done by us under the guidance of Dr.V.Ramesh Kumar (Internal) and Mr.G.Narendra Kumar (External) at Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

The wavelength of the light, the intensity of light and the duration with which the microalgae are subjected to specific wavelengths of the light are the three important factors that determine the quantity and quality of biomass production. LEDs are known to stimulate the growth of microalgae. *Chlorella vulgaris* was subjected to different colored light environments, different intensities of those lights and three different media to analyze their difference in biomass production for specific wavelength of light, intensity of light and growth media. 100mL of the microalgal strain was taken in different conical flasks. 50mL BG-11 media was added to each flask. The flasks were subjected to four different LED lights (red, green, blue and yellow) of three different light intensities ($247 \mu\text{mol m}^{-2}\text{s}^{-1}$, $299 \mu\text{mol m}^{-2}\text{s}^{-1}$ and $345 \mu\text{mol m}^{-2}\text{s}^{-1}$) of each colour. The algae growing under the sun was considered as control. The light/dark regime given was 12/12 hours. They were cultivated for 15 days at 28°C culture temperature. The growth analysis revealed that the microalgae that are grown in F medium and green LED showed the highest growth in all the three light intensities and media. Green LED in F medium with the light intensity $299 \mu\text{mol m}^{-2}\text{s}^{-1}$ produced the highest protein content of about 50.6mg/mL, highest lipid content of about 4.62mg/mL and highest carbohydrate content of about 4.2mg/mL followed by sun, red LED, blue LED and yellow LED respectively. Hence, we are recommending these conditions for designing a bioreactor for growing microalgae to obtain higher quantity and quality of biomass.

Keywords: *Chlorella vulgaris*, microalgae, LED, light, growth, wavelength, red, green, blue, yellow, biomass, protein, lipid and carbohydrate.

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LIST OF SYMBOLS AND ABBREVIATIONS

β	Beta
$^{\circ}\text{C}$	Degree Celsius
μ	Micro
BSA	Bovine Serum Albumin
BOD	Biochemical Oxygen Demand
CO_2	Carbon dioxide
COD	Chemical Oxygen Demand
CUSO_4	Sodium Potassium Tartrate
cm	Centimeter
d	Deci
dcw	Dry Cell Weight
dgat	Diacylglycerol O-acyltransferase
E	Electromotive force
EDTA	Ethylene di amine tetra acetic acid
EPA	Eicosapentenoic acid
FAA	Free Amino Acid
FAS	Specific Fatty Acid
GC/MS	Gas Chromatography/Mass Spectroscopy
g	Gram
H_2O	Water
H_2SO_4	Sulphuric Acid
h	Planck's constant
K_2HPO_4	Potassium Hydrogen Phosphate
K_s	Specific Growth Rate Constant
L	Liter
LED	Light Emitting Diode

L/D	Light/ Dark
MgSO ₄	Magnesium Sulphate
m	Meter
mg	Milligram
mL	Milliliter
NH ₄ Cl	Ammonium chloride
NPQ	Non-Photochemical Quenching
nm	nanometer
OD	Optical Density
PBR	Photo Bioreactor
PCR	Polymerase Chain Reaction
PN junction	P type and N type junction
pH	Power of Hydrogen
psi	Pascal
RBS	Ribosome Binding Site
R ²	Coefficient Correlation
TFA	Total Fatty Acid
TDS	Total Dissolved Solid
UV	Ultraviolet
w/w	weight/weight

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CHAPTER 1

INTRODUCTION

1.1 ALGAE

Informal terms for a large and diverse group of photosynthetic eukaryotic organisms are "Algae". It is a polyphyletic grouping that includes species from multiple distinct evolutionary descendants of a common ancestor. Algae lack a sterile covering of cells around their reproductive cells and have chlorophyll as their primary photosynthetic pigment. Since algae contain chlorophyll pigments, light is essential for photosynthesis. Algae show a wide range of reproductive actions from simple asexual cell division to complex forms of sexual reproduction. One of the important examples of algae that have primary chloroplasts derived from the endosymbiotic cyanobacteria is Chlorophyta. Like the plants, the green algae contain two forms of chlorophyll, which they use to capture light energy for the production of sugars, but unlike plants, they are primarily aquatic.

1.2 MICROALGAE

Microalgae are regarded as a promising feedstock for bioenergy production due to their fast growth rate, high productivity, and low nutritional requirements (Levina et al., 2020). Some of the advantages of microalgae over plants are

- (1) They have a higher growth rate
- (2) They don't need nutrient-rich water to grow
- (3) High CO₂ fixation rate
- (4) No requirement of arable land
- (5) They can grow autotrophically.

Most green microalgae essentially contain proteins, carbohydrates, fats, and nucleic acids in different proportions. It is found that there are some types of microalgae that contain up to 70% of their mass by unsaturated fats (Yap et al., 2016).

1.3 LIGHT SOURCE

Both artificial and natural light sources are used for the production of photoautotrophic microalgae (Blanken et al., 2013). A light-emitting diode (LED) is a semiconductor

appliance that discharges light when electric current is passed through it. Light is produced when all the electrons that are carried in the semiconductor materials combine. LED is similar to a PN junction diode that illuminates light when activated. LEDs have become more efficient and are used almost everywhere because it consumes less energy, it is cost-efficient, it produces exceptional color range and generates low radiated heat. For the past few years, Light Emitting Diodes (LEDs) have become the most preferred artificial light source due to their durability and lack of toxic elements such as mercury and more economical than other fluorescent lamps. The advantage of using LEDs is that they produce fast responding diodes and these diodes emit mono or multi-chromatic light at the desired wavelength, thus making it ideal for the studies on the light requirements of microalgae.

1.4 MICROALGAE - *Chlorella vulgaris*

Chlorella vulgaris is a unicellular eukaryotic green freshwater marine microalgae belonging to the family Chlorellaceae. It is a rich source of protein and lipid. It contains 10% minerals and vitamins, 5% fiber, 20% carbohydrates, 20% fat, and 45% protein (w/w, dry basis) (Belasco et al., 2019). It is known for its rapid growth. They are highly distributed in Japan.

1.5 CLASSIFICATION OF *Chlorella vulgaris*

Class	Trebouxiophyceae
Order	Chlorellales
Family	Oocystaceae
Genus	Chlorella
Species	<i>Chlorella vulgaris</i>

1.6 APPLICATIONS OF *Chlorella vulgaris*

They find their application in the food, pharmacy, and cosmetic industries. They are determined as a plant growth enhancer since they have many growth promoters and have played a vital role in building and sustaining soil fertility, thereby raising the growth and yield of several crops (Deepa et al., 2010). It is reported that *Chlorella vulgaris* on mixotrophic cultivation yielded higher lipid content and higher lipid productivity

irrespective of culture medium, which made it a potential source for the consideration of biodiesel production.

CHAPTER 2

LITERATURE REVIEW

Chlorella vulgaris (*C. vulgaris*) was promising microalgae to simultaneously achieve biomass production, carbon dioxide (CO₂) fixation, nutrients removal and proteins production especially under different conditions of CO₂ gas and wastewaters. Results presented that maximal specific growth rate of *C. vulgaris* was 0.21–0.35 d⁻¹ and 0.33–0.43 d⁻¹ at 0.038% and 10% CO₂ respectively, and corresponding maximal CO₂ fixation rate was attended with 4.51–14.26 and 56.26–85.72 mg CO₂·L⁻¹·d⁻¹. *C. vulgaris* showed good wastewater removal efficiency of nitrogen and phosphorus at 10% CO₂ with 96.12%–99.61% removal rates. Nitrogen fixation amount achieved 41.86 mg L⁻¹ when the initial NH₄Cl concentration was set at 60 mg L⁻¹ at 10% CO₂. Improved total protein (25.01–365.49 mg) and amino acids (24.56–196.44 mg) contents of *C. vulgaris* biomass was observed with the increasing of added CO₂ and ammonium concentrations. Moreover, the developed kinetic function of *C. vulgaris* growth depends on both phosphorus quota and nitrogen quota with correlation coefficient (R^2) ranging from 0.68 to 0.97. Computed maximal consumed nutrients concentrations ($\Delta C_{\max}$) based on Logistic function are positively related to initial NH₄⁺-N concentrations, which indicated that adding ammonium could stimulate the utilization of both phosphorus and nitrogen (Wenwen et al., 2021).

Microalgae are regarded as promising feedstock for bioenergy production due to their fast growth rate, high productivity and low nutritional requirements. However, large-scale biomass production for bioenergy currently is not economically viable due to low productivity and high cultivation costs of microalgal biomass. Optimal growing conditions for specific strains must be set up to increase the microalgae productivity. Therefore, in present study we focus on finding the optimal light intensity conditions for three microalgae cultivation intended for large-scale biomass production in outdoor raceway ponds supplemented with artificial LED illumination. We tested light intensity in a range of 30 to 200 $\mu\text{mol m}^{-2}\text{s}^{-1}$. The impact of light intensity on the growth and biomass production of three microalgae strains was assessed by measurements of optical density and dry cell weight. Our study shows that light intensity of 30 and 50

$\mu\text{mol m}^{-2}\text{s}^{-1}$ is too low to maintain the high growth rate of microalgae strains studied. The results suggest that *C. vulgaris* has lower light requirements compared to *C. sorokiniana* and *C. reinhardtii*. The highest biomass yield was achieved at light intensity of $150 \mu\text{mol m}^{-2}\text{s}^{-1}$ for *C. vulgaris* and at $200 \mu\text{mol m}^{-2}\text{s}^{-1}$ for *C. sorokiniana* and *C. reinhardtii* (Levina et al., 2020).

The effects of wavelengths of light-emitting diode (LED), nitrate concentration, and salt concentration were evaluated for the two-phase culture of the microalgal species *Phaeodactylum tricornutum*, *Dunaliella tertiolecta*, and *Isochrysis galbana* on cell growth and lipid production. Blue LEDs produced the highest biomass of *P. tricornutum* at a nitrate concentration of 8 mg/L, reaching 0.97 g dcw/L with a specific growth rate (μ) of 0.047 h^{-1} , followed by *I. galbana* with 0.79 g dcw/L and $\mu = 0.040 \text{ h}^{-1}$ and *D. tertiolecta* with 0.55 g dcw/L and $\mu = 0.028 \text{ h}^{-1}$. Of the three microalgae, *P. tricornutum* had the highest specific growth rate of $\mu_{\text{max}} = 0.070 \text{ h}^{-1}$ and lowest saturation constant of $K_s = 4.18 \text{ mg/L}$, resulting in fast cell growth. The highest lipid production was obtained under green LED wavelength stress on day 14, reaching 60.6% (w/w) of the dry cell weight among the three microalgae. The main fatty acids produced by the three microalgae were myristic acid (C14:0), palmitic acid (C16:0), oleic acid (C18:1), and arachidic acid (C20:0), which comprised 72.68%–84.16% (w/w) of the total fatty acids content under three stresses (Jung et al., 2018).

There are 150,000 microalgae species. Scientists identified and classified 30,000 species. Only 10–15 species are utilized and studied for wastewater treatment, biogas upgrading, biomass production, and lipid content. LED can raise microalgae performance to the highest. Therefore, numerous empirical investigations have discussed for years about using various circumstances like light intensity, dark: light ratio, and wavelength. Though, studies have not discovered the ultimate LED for achieving maximum growth. Thus, this mini-review article shows the recent conducted studies on various microalgae species under LED impact to discover a range of optimal conditions. Besides, future perspective presents the next stage of research and development on using LED for microalgae growth (Al-Amshawee et al., 2020).

Microalgae are viable sources of biological compounds for biodiesel production. In this study, effects of various types of nitrogen sources and nutrients concentrations in the growth medium and different LED light wavelengths and intensities on biomass production of green algae *Chlorella vulgaris* were investigated. Warm white light with 80 $\mu\text{mol}/\text{m}^2/\text{sec}$ light intensity was determined as the optimal light for biomass production. The results indicated that microalgae growth with urea as a nitrogen source was higher than that of other nitrogen sources such as sodium nitrate, ammonium carbonate and ammonium chloride. Maximum biomass concentration (1.37 g/L) was obtained under the following media compositions: urea 0.25 g/L, K_2HPO_4 0.04 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.06 g/L, and ammonium ferric citrate 0.01 g/L. Microalgae growth data under the different light wavelengths and intensities were fitted with a mathematical model (Khalili et al., 2015).

Spanish consumers' knowledge and attitudes towards microalgae as food were assessed using a specific questionnaire - a total of 3084 Spanish consumers over 18 years old were considered in the current study. Overall, the questionnaire revealed that there is a huge lack of knowledge on basic aspects of microalgae, especially within the youngest respondents (18–24 years old) and for those aged over 65 years old. Approximately 85% of all respondents scored the statement "*There is a big lack of information about microalgae*" with either 4 or 5 (using a 5-point hedonic scale), demonstrating that microalgae and their potential applications are unknown to the majority of the Spanish population. Spanish consumers consider microalgae as: (i) sustainable and environmentally friendly, (ii) nutritious and healthy, and (iii) safe, which is of key importance for food products. The main reasons that were given for not consuming microalgae were lack of information available on the product and lack of consuming habit. The current study also demonstrated that increasing consumer's knowledge on microalgae has the potential to increase consumer choice and market shares of microalgae-enriched products. Results reported herein could be extrapolated to other European countries, although perception and attitudes towards food can vary depending on cultural considerations (Tomas et al., 2021).

Light (wavelength, intensity, and light/dark cycle) have been considered as one of the most important parameters for microalgae cultivation. In this paper, the effect of medium

frequency intermittent light on *Auxenochlorella pyrenoidosa* (formerly *Chlorella pyrenoidosa*) cultivation was investigated. Three parameters of intermittent light, light intensity, light/dark ratio, and light/dark cycle were employed and the influence of these parameters on the productivity of *Auxenochlorella pyrenoidosa* was studied. The biomass yield and growth rates were mainly affected by the light fraction and cycle time. Light with $220 \mu\text{E m}^{-2}\text{s}^{-1}$ light intensity was determined as the optimal light intensity for biomass production. At the light intensity of $420 \mu\text{E m}^{-2}\text{s}^{-1}$, the results indicated that the intermittent light improved the biomass production with larger light/dark ratio compared with the continuous light. At a lower mean light intensity over time, the intermittent light should be more suitable for biomass growth and the decrease in the light/dark ratio (L/D) will lead to higher biomass productivity. The light/dark cycle time has little influence on the biomass yield (Chen et al., 2020).

The purpose of this study was to establish a chloroplast transformation system in *Chlorella vulgaris* for expressing recombinant proteins. The 16S-*trnI/trnA*-23S in the inverted repeat region of *C. vulgaris* chloroplast genome was used as the flanking fragments in the homologous recombination vector. Meanwhile, the selectable marker *aadA* gene was promoted by the endogenous 16S promoter, and two codon-optimized antimicrobial peptide NZ2114 and Piscidin-4 genes linked by endogenous ribosome binding site (RBS) in a polycistron were regulated by the *rbcL* promoter of *C. vulgaris*. The plasmid was transformed into the chloroplast of *C. vulgaris* via micro particle bombardment and the transformants were screened using spectinomycin. After multiple rounds' selection, the homoplastic transformants were identified using polymerase chain reaction (PCR), Southern blotting, and Western blotting. This research provided an efficient chloroplast genetic system for *C. vulgaris*, and will be beneficial for the algal further research and application (Kang et al., 2021).

LEDs have specific wavelengths that can positively influence the production of microalgal biomass and biomolecules of interest. Filling the gaps in the literature, this study evaluated the effect of different LED wavelengths and photoperiods on protein productivities and free amino acid (FAA) profile of *Spirulina* sp. LEB 18 cultures. The best protein productivity results were obtained in red and green LED cultures using

integral and partial photoperiods, respectively. In these experiments, protein productivities increased 2 and 1.6 times, respectively, compared to the control culture using fluorescent light. Green LEDs in partial photoperiod provided also the highest concentrations of essential and non-essential FAA, about 1.8 and 2.3 times higher, respectively, than control cultures. LEDs showed to be a promising sustainable light source for increasing protein productivity and FAA concentration in *Spirulina* sp. LEB 18 cultures (Denise et al., 2020).

The spatial and spectral distribution of light was essential for *H.pluvialis* growth and astaxanthin yield. In this work, experiments and numerical simulation with red and blue light in different ratios have been carried out on *H.pluvialis*. Although the light transmittance along the incident direction decreased significantly with increasing blue light ratio, the growth of *H.pluvialis* did not show linear relation with the absorption of blue light. Due to the red/blue light ratio of 2:1 matching the chlorophyll a/b ratio well, it produced the highest biomass and photosynthetic pigments, which were 19% and 62% higher than that under red light, respectively. The trend of maximal photochemical yield (F_v/F_m) and non-photochemical quenching (NPQ) change were similar to the content of chlorophyll and carotenoids separately. The highest carotenoids content and NPQ has been observed under red/blue light in the ratio of 2:1, which might bring *H. pluvialis* strong self-protection from high light and high astaxanthin yield (Li et al., 2020).

This study aims to characterize the potential of three strains of microalgal species (*Chlorella sorokiniana* KNUA114 and KNUA122; *C.vulgaris* KNUA104) for use as feedstock, based on their fatty acid compositions. Each strain was molecularly identified using four marker genes (ITS, SSU, *rbcl*, and *tufA*) and phylogenetically characterized. *C.sorokiniana* and *C.vulgaris* collected from Ulleung Island, South Korea, were homologous with other known species groups. Samples' fatty acid components were measured using GC/MS analysis in growth temperatures of 10°C, 25°C, and 35°C. The growth rate of *C.sorokiniana* strains was higher than that of *C.vulgaris* under high temperature conditions, confirming the potential industrial applicability of the former as feedstock material. Additionally, saturated fatty acid contents and productivities increased as biological resources of the *C. sorokiniana* strains were higher than those

of *C.vulgaris* under high light intensity and temperature conditions. These results suggest that the fatty acid components of *C.sorokiniana* strains may potentially be used as biological resources (e.g., feedstock) (Yun et al., 2020).

Biogas is a promising renewable energy which has to be upgraded to meet the efficient combustion standard. The microalgae biogas upgrading system used in this study could effectively upgrade biogas and simultaneously reduce biogas slurry nutrients. Red light was the optimum light wavelength for microalgae growth, biogas upgrading, and biogas slurry nutrient reduction. Only moderate light intensities (i.e., 400, 600, 800, and 1000 $\mu\text{mol m}^{-2}\text{s}^{-1}$) were suitable for microalgae growth. The optimal lighting strategy should be an incremental light intensity strategy since it could avoid photo inhibition at the initial culture phase and insufficient light intensity at the latter culture phase. Under this lighting strategy, the microalgae dry weight was $446.98 \pm 25.32 \text{ mg l}^{-1}$; the methane concentration in the upgraded biogas was $92.87 \pm 4.10\%$; the chemical oxygen demand, total nitrogen, and total phosphorus removal efficiency was $92.67 \pm 5.14\%$, $80.87 \pm 6.25\%$, and $79.33 \pm 6.18\%$, respectively (Cheng et al., 2016).

This study evaluated light emitting diodes (LEDs) as a light source in *Spirulina* sp. LEB 18 cultures in terms of growth parameters and biomass composition. Different photoperiods (partial and integral) and colors (blue, green, red and white) were assessed. Blue, green, red and white LEDs increased biomass productivity and maximum specific growth rate of such cultivations. The maximum biomass concentration ($1.77 \pm 0.02 \text{ g L}^{-1}$) was obtained when red LEDs in integral light photoperiod were applied to cultivations. The biomass composition showed around 12.8% carbohydrates (ww^{-1}), 57.4% proteins (ww^{-1}) and 12.7% lipids (ww^{-1}). The major fatty acids produced during cultivations were palmitic, linoleic and γ -linolenic. Green LEDs in partial light photoperiods promoted a higher concentration of phycocyanin ($126.39 \text{ mg g biomass}^{-1}$). The potential of LEDs as an energy source in *Spirulina* sp. LEB 18 cultures was demonstrated by the biomass and bioproducts photo stimulation (Prates et al., 2018).

Haematococcus pluvialis accumulates large amounts of astaxanthin during its

haematocyst stage. Cyst germination is a biological pretreatment method for improved astaxanthin extraction with potential to replace energy-intensive homogenizer-based mechanical cell cracking methods. The present study demonstrates effects of different LED light wavelengths on haematocyst germination and on its astaxanthin biosynthesis pathway. Blue light enhanced the germination efficiency and slowed the nitrogen consumption rate, resulting in significantly higher astaxanthin content and improved extractability compared with white and red lights, that also had lower germination rates. After 5 days, the total astaxanthin extractability under blue light was 4.0 and 6.7 times higher than for white and red lights, respectively. The blue light receptor gene *photo* was significantly induced which upregulated the biosynthesis pathway genes *psy* and *pds*, as well as *dgat1* and *dgat2d*. Hence, blue light triggers germination and astaxanthin biosynthesis, providing a strategy for improved extraction while modulating higher biosynthesis during germination (Ruijuan Ma et al., 2018).

Microalgae are affected by the amount of light received. This parameter can be controlled by changing the light source and altering the reactor used for their growth. In this study, the effect of different colors of light was analyzed in the growth of *Dunaliella tertiolecta*, observing that blue lighting systems reached a biomass 10 times superior to the one generated by orange lightning systems. This growth effect was seen in a novel tubular internally illuminated photobioreactor. In this photobioreactor, the blue reactor produced 1.7 times the biomass of the red reactor, with the particularity that the latter showed an oscillating behavior in its growth. From irradiance models, the light dispersion coefficient is higher than the absorption coefficient when using red light. In contrast, with blue light, the value of the scattering coefficient is almost null (Rebolledo et al., 2019).

Outdoor cultivations of microalgae for commercial scale production usually rely on natural sunlight following the 'Sine' regime, but most lab scale studies adopt 'Block' or 'Continuous' regimes, leading to poor prediction of outdoor cultivation results. To fill this gap on β -carotene production by *Dunaliella salina*, the effects of light regimes ('Block', 'Sine', 'Continuous') on cell growth and β -carotene accumulation in *D. salina* were investigated in this study. The results showed significant differences between these

regimes. When receiving the same amount of illumination, 'Sine' mode inferred 10–55% higher biomass yield and 31–58% higher β -carotene yield than those under the 'Block' mode. The biomass concentration, as well as protein and total fatty acid (TFA) content, has no significant difference between different light regimes, but the starch content in the 'Continuous' light regime was higher than that of 'Sine' and 'Block' regimes. β -carotene accumulation under 'Continuous in summer' and 'Simulated diurnal irradiance in summer' regimes were correlated with increase (C18:1, C18:2) and decrease (C16:4, C18:3) of specific fatty acids (FAs) contents, rather than TFA content. Meanwhile, it was found that β -carotene accumulation was correlated with periodical Fv/Fm fluctuations under different light regimes, and indicated that exposure to periodical strong light is more favorable for β -carotene accumulation, rather than continuous light. These results indicated the significance of using the 'Sine' light regime since it not only provided better predictions on outdoor production, but also helped to increase biomass and β -carotene productivities (Xi et al., 2020).

The light spectrum is an important parameter that affects microalgae growth, but to date has not been thoroughly studied in microalgae biofilm-based systems. In this study, the effect of light spectra on biofilms of two microalgae (*Chlorella* sp. and *Nannochloris oculata*) was explored by investigating their growth, photosynthetic property, and main organic composition cultured under white, blue, green, and red light. The results indicated that blue light and red light were more efficient in enhancing cells growth than white light. Microalgae cultured under blue light had higher photosynthetic and photoprotective capability. The highest lipid accumulation was achieved for microalgae cultured under blue light, while the highest carbohydrate accumulation was obtained under red light. Protein accumulations in cells cultured under these four light conditions were almost the same. The results of this study may have important implications in selecting suitable light spectra for microalgae biofilm-based cultivation systems (Yuan et al., 2020).

Extraction of multiple high-value products is recommended for sustainability of the microalgal production platform. This study proposes a process for selective extraction of carotenoids and chlorophylls with ethanol, followed by alkaline pH extraction of proteins

from wet, freeze-thawed *Chlorella vulgaris* biomass. A biomass-to solvent ratio of 1:5 and 3 extraction stages were required to achieve maximum extraction yield of chlorophylls and carotenoids. The main compounds in the ethanol extract were identified as lutein, chlorophyll a and chlorophyll b. The lutein and total chlorophyll yield in the extracts were 5.4 mg/g and 15.4 mg/g dry biomass respectively. Effective protein release from freeze-thawed biomass was contact-time dependent and > 76% of total protein could be extracted in 15 min via bead-milling and in 6 min by high-pressure homogenization at 15000 psi. Ethanol extraction of pigments affected protein solubility, and an alkaline pH was required to release the same total protein. Concentration and fractionation of proteins was carried out using a two-stage membrane filtration process and 78–80% of proteins remained in the 300 kDa retentate. Ethanol treatment and higher pH conditions did not negatively impact membrane filtration, nutritive value, or the emulsification properties of protein concentrates (Kulkarni & Nikolov, 2018).

This work evaluated the potential of microalgae of *Chlorella* sp., *SL7A*, *Chlorococcum* sp., *SL7B* and *Neochloris* sp., *SK57* cultivated in river water contaminated with pharmaceutical effluent for biomass and lipid production. It has been observed that fast growing algae in this medium is *Neochloris* sp. *SK57*. Maximum biomass and lipid yield was obtained from *Neochloris* sp. *SK57* (0.52 g/l) and *Chlorococcum* sp. *SL7B* (0.129 g/l) along with dry cell weight of lipid was 28%. The increase in biomass and lipid in this media is due to assimilation of organic nutrients and stress due to other components present in the river water. Fatty acid profile of algal biomass showed that saturated fatty acids production is enhanced in oils of *Neochloris* sp. *SK57*, and its suitability in food and fuel applications. Water quality of the river water was monitored before and after algal cultivation. Results showed that quality of river water was improved after algal cultivation (Pattnaik et al., 2018).

Conditions of light-emitting diode (LED) wavelength, light intensity, nitrate concentration, and time of exposure to green LED light stress in a two-phase culture were optimized for lipid production with three species of microalgae. Among the three microalgae, *Nannochloropsis oculata* showed the highest specific growth rate (μ_{max}) of 0.037 h⁻¹ and the lowest saturation constant (*K_s*) of 1.32 mg/L. The highest lipid contents of the

three microalgae in the second phase under green LED light stress were 52.0% (w/w) for *Nannochloropsis salina* at 2 days, 53.0% (w/w) for *Nannochloropsis oceanica* at 2 days, and 56.0% for *N. oculata* at 2 days. Fatty acid analysis of the microalgae showed that 85–87% (w/w) of total fatty acids from *N. salina*, *N. oceanica*, and *N. oculata* consisted of palmitic acid (C16:0) and oleic acid (C18:1).n (Anamika et al., 2020).

As the periodic emission of light pulses by light emitting diodes (LEDs) is known to stimulate growth or induce high value biocompounds in microalgae, this flashing light regime was tested on growth and biochemical composition of the microalgae *Nannochloropsis gaditana*, *Koliella antarctica* and *Tetraselmis chui*. At low flashing light frequencies (e.g., 5 and 50 Hz, Duty cycle = 0.05), a strain-dependent growth inhibition and an accumulation of protein, polyunsaturated fatty acids, chlorophyll or carotenoids (lutein, β -carotene, violaxanthin and neoxanthin) was observed. In addition, a 4-day application of low-frequency flashing light to concentrated cultures increased productivities of eicosapentaenoic acid (EPA) and specific carotenoids up to three-fold compared to continuous or high frequency flashing light (500 Hz, Duty cycle = 0.05). Therefore, applying low-frequency flashing light as a finishing step in industrial production can increase protein, polyunsaturated fatty acids or pigment contents in biomass, leading to high-value algal products (Serena Lima et al., 2021).

Biogas is a well-known, primary renewable energy source, but its utilizations are possible only after upgrading. The microalgae-based bag photo-bioreactor utilized in this research could effectively upgrade biogas and simultaneously reduce the nutrient content in digestate. Red light was determined as the optimal light wavelength for microalgae growth, biogas upgrading, and digestate nutrient reduction. In the range of moderate light intensities (i.e., 800, 1200, 1600, and 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$), higher light intensities achieved higher biogas upgrade and larger digestate nutrient reduction. Methane content attained the highest value of $92.74 \pm 3.56\%$ (v/v). The highest chemical oxygen demand, total nitrogen, and total phosphorus reduction efficiency of digestate were $85.35 \pm 1.04\%$, $77.98 \pm 1.84\%$, and $73.03 \pm 2.14\%$, respectively. Considering the reduction and economic efficiencies of the carbon dioxide content of biogas and digestate nutrient as well as the biogas upgrading standard, the optimal light

intensity range was determined to be from 1200 to 1600 $\mu\text{mol m}^{-2}\text{ s}^{-1}$ (Yongyun et al., 2013).

Light-emitting diodes (LEDs) will become one of the world's most important light sources and their integration in microalgal production systems (photobioreactors) needs to be considered. LEDs can improve the quality and quantity of microalgal biomass when applied during specific growth phases. However, microalgae need a balanced mix of wavelengths for normal growth, and respond to light differently according to the pigments acquired or lost during their evolutionary history. This review highlights recently published results on the effect of LEDs on microalgal physiology and biochemistry and how this knowledge can be applied in selecting different LEDs with specific technical properties for regulating biomass production by microalgae belonging to diverse taxonomic groups (Peter et al., 2014).

Chlorella pyrenoidosa is a fresh water microalgae used as substrate for production of value added products like biodiesel. In this investigation *C.pyrenoidosa* was grown for ten days in an internally illuminated bubble column photobioreactor at: 450 lux green light (495–570 nm), 480 lux red light (620–750 nm), 1663 lux blue light (450–495 nm), 1986 lux white light (380 to 750 nm). The results after a period of ten days of growth, showed that *C.pyrenoidosa* has grown better under blue light when compared to the other types of light. *C.pyrenoidosa* biomass produced 33.8% of lipid content when grown under blue light, indicating its potential as an alternative source of biodiesel (Asuthkar et al., 2016).

The increase in industries leads to high discharge of effluents, which in turn pollutes the environment critically. Algal technology is a very efficient method to treat this effluent by using a bioremediation process. Algae can be cultivated under certain conditions of temperature, light and sufficient nutrients to produce value added products. Many research works were undertaken to increase the efficiency of the production rate in both indoor and outdoor cultivation systems by designing special Photo-Bioreactors (PBR's) to increase the rate of photosynthesis. Duration and intensity of light have more impact on the growth and photosynthesis of microalgae. Micro algae have excellent potential

for industrial applications to treat effluent. Micro algae need a light/dark regime for productive photosynthesis. The LED (Light Emitting Diode) has the potential to stimulate the growth rate of algae. Experiments conducted in the current research work showed that, the growth rate of *Chlorella vulgaris* (algal species of our interest) is increased by 5.31% (RED LED), 3.72% (BLUE LED) and 6.31% (WHITE LED) at varying effluent concentrations respectively in laboratory conditions. Also the study clearly shows that the Total Dissolved Solids (TDS) is reduced significantly by 15%, COD by 23.7%, BOD by 93% and Total Suspended Solids (TSS) by 99.8% under Blue LED and under White LED, TDS is reduced by 20%, COD by 27.3%, BOD by 97%, and TSS by 99.99%. The result clearly proves that the biodegradation rate is significantly increased and physicochemical parameters varied for different wavelengths of Blue and White LED lights (Devaraj et al., 2017).

Algae are fast growing microorganisms and beneficial in many ways, especially concerning food supplement, alternative energy, and wastewater treatment. Naturally, they need a light: dark regime for productive photosynthesis. To have more insight into how light affects their growth, we investigate the effect of light sources (fluorescent and LED) on algae growth both experimentally and computationally via modeling. It was found that growth in the fluorescent light source is relatively better than in other sources. Computational data analyses and detailed discussions are presented. Lastly, a more generalized growth model of microalgae for incorporation of other factors and variables is necessary for more realistic modeling of real-world systems (Satthongs et al., 2019).

Integrations of two-phase culture for cell growth and lipid accumulation using mixed LED and green LED wavelengths were evaluated with the microalgae, *Phaeodactylum tricornutum*, *Isochrysis galbana*, *Nannochloropsis salina*, and *Nannochloropsis oceanica*. Among the single and mixed LED wavelengths, mixed LED produced higher biomass of the four microalgae, reaching 1.03 g DCW/L *I. galbana*, followed by 0.95 g DCW/L *P. tricornutum*, 0.85 g DCW/L *N. salina*, and 0.62 g DCW/L *N. oceanica* than single LED or fluorescent lights at day 10. Binary combinations of blue and red LEDs could produce the high biomass and photosynthetic pigments in the four microalgae. The highest lipid accumulation during the second phase with the exposure to green LED

wavelengths was 56.0% for *P. tricornutum*, 55.2% for *I. galbana*, 53.0% for *N. salina*, and 51.0% for *N. oceanica*. The major fatty acid in the four microalgae was palmitic acid (C16:0) accounting for 38.3–47.3% (w/w) of the total fatty acid content (Ra et al., 2018).

The biomasses of microalgae are rich in different types of polysaccharides like pigments, antioxidants, fatty acids, and anticancer chemicals. They are used as additives for the production in various food industries, pharmaceutical, cosmetics and nutraceuticals. These beneficial usages enforce the large-scale production of microalgae in photobioreactors. Photobioreactor provides a controlled environment of all the parameters helping the efficient growth within a small period of time. It uses different light sources such as sunlight, fluorescent lamp and incandescent lamp. Nowadays light emitting diodes (LEDs) are capturing more attention as one of the light sources in the photobioreactors due to their narrow bandwidth spectrum. The advantages, such as small size, lightweight, durable and efficient in terms of longer operating life, make the LEDs more compatible as the source of light in the photobioreactors. The amount of light given off is much higher compared to their power consumption. This review summarizes the knowledge on the use of different LEDs in photobioreactors for the microalgae growth (Wong et al., 2016).

Light wavelength is an essential parameter of microalgal growth and light spectrum quality affects the algal metabolism. Light-emitting diodes (LEDs) emit a very narrow wavelength which addresses fundamental as well as applied aspects of light color on biomass and value-added compound formation in microalgae. In this study, *Arthrospira platensis*, *Chlorella vulgaris* and *Scenedesmus obliquus* were cultivated in Bold's basal medium under five different LED illuminations (white, blue, green, yellow and red) to augment growth, pigment, protein, carbohydrates and lipid production. Dry weight of microalgae at the end of 10 days cultivation period revealed that higher content under the LED illumination was in the order of red>white>blue. Highest Chlorophyll a content of 8.4 mg g⁻¹ was recorded in *C. vulgaris* under red LED. Whereas green LED illumination has resulted in higher content of Chlorophyll b with 2.0, 1.98 and 1.68 mg g⁻¹ in *A. platensis*, *C. vulgaris* and *S. obliquus* respectively. Similarly the highest carotenoid content of 6.1 mg g⁻¹ in *A. platensis* was recorded in green LED. Total

carbohydrates of microalgae were increased under blue light and the maximum content of 52.1 mg g⁻¹ was found in *C. vulgaris*. Protein content in the range of 102.7-97.5 mg g⁻¹, 77.9-76.5 mg g⁻¹ and 51.9-49.4 mg g⁻¹ was observed in *A. platensis*, *C. vulgaris* and *S. obliquus* under blue and green LED. Lipid content of 5.68 % and 4.96 % was obtained in *C. vulgaris* and *S. obliquus* under blue LED. This was followed by 4.37% and 3.86% under red LED illumination for the same species. Based on the results, selection of blue and red LED illumination to enhance microalgal growth and cellular composition is possible in the selected microalgae (Raqiba et al., 2019).

Cultivation of microalgae in wastewater could significantly contribute to wastewater treatment, biodiesel production, and thus the transition to renewable energy. However, more information on effects of environmental factors, including light intensity, on their growth and composition (particularly fatty acid contents) is required. Therefore, we investigated the biomass and fatty acid production of four microalgal species, isolated in the Northern hemisphere and grown at three light intensities (50, 150 and 300 $\mu\text{Em}^{-2}\text{s}^{-1}$) (Nzayisenga et al., 2020).

Algae can serve as feedstock for many high value bioproducts and biofuels production. The key to economic algal biomass production is to optimize the growth conditions. This study presents the effect of light wavelengths and growth medium composition on the growth of *Chlorella vulgaris*. Different light wavelengths [blue, clear (white), green, and red] were used to test their effect on algal growth. Growth media formulations were varied to optimize the growth media composition for maximized algal biomass production. Experimental study was conducted in three phases to evaluate:

- (1) The effect of different light wavelengths;
- (2) The effect of the recommended growth medium at 25%, 50%, and 100% of suggested composition; and
- (3) The effect of nutrient concentrations (nitrogen and phosphorous).

The effect of these factors was evaluated through specific algal growth rates and volumetric biomass productivities during the entire growth period. In this study, blue light performed better (higher growth rate and biomass productivity) at longer growth periods

(10–14 days) compared to clear, red and green light wavelengths. The growth media and nutrient effect results indicate that the growth of *C. vulgaris* is higher at 50% suggested growth media composition compared to 100% growth media composition which results in approximately 50% potential reduction in chemical costs for large scale algal production (Matthew et al., 2014).

CHAPTER 3

AIM AND SCOPE

3.1 AIM

To analyze the growth and biomass production of *Chlorella vulgaris* by subjecting it to different wavelengths of light, intensities of light and different media.

3.2 SCOPE

- High biomass production helps in more yield of biofuel in bioprocess industries.
- *Chlorella vulgaris* biomass can be used as a feed for fishes in aquaculture.
- They are used as immunostimulants in medicine and as a growth enhancer.
- They are widely used as food supplements since they are rich in protein and lipid content.
- The pigments present in the algal biomass find their application in cosmetic industries.

CHAPTER 4

MATERIALS AND METHODS

4.1 EQUIPMENT AND GLASSWARES

Microscope, spectrophotometer/colorimeter, gravimeter (CG-5 model), sonicator, autoclave, incubator, vortex mixer, water bath, air pump, electronic balance, refrigerated centrifuge, LED lights (red, blue, green and yellow - $247 \mu\text{mol m}^{-2}\text{s}^{-1}$, 10 bulbs, 33 cm; red, blue, green and yellow - $299 \mu\text{mol m}^{-2}\text{s}^{-1}$, 20 bulbs, 66cm & red, blue, green and yellow- $345 \mu\text{mol m}^{-2}\text{s}^{-1}$, 30 bulbs, 1m), sunlight, cardboard boxes, aluminium foil, spatula, butter paper, test tube stand, slide, cover slip, eppendorf, 250ml conical flasks, measuring cylinder, beaker, test tubes, pipette, tips and cuvette.

4.2 REAGENTS AND CHEMICALS

- Alkaline copper reagent
50mL of Solution A (2% Na_2CO_3 in 0.1N NaOH) + 1mL of Solution B (0.1% CuSO_4 in sodium potassium tartrate).
- Folin's phenol reagent
Dilute the reagent with distilled water in 1:2 ratio.
- BSA (Bovine Serum Albumin)
Stock solution: 100mg BSA is made up to 100mL with distilled water.
Working solution: 10mL of stock BSA + 90mL distilled water.
- Anthrone reagent
2g Anthrone in 1L concentrated H_2SO_4 .
- Glucose and other carbohydrates stock solution
200g glucose per mL of distilled water (the same stock solution for other carbohydrates).

4.3 MICROALGAL STRAIN SELECTION AND COLLECTION

Considering the application and availability of the microalgae, *Chlorella vulgaris* was selected as a source and was isolated from the department of Biotechnology, Sathyabama Institute of Science and Technology (SIST). The strain was confirmed as *Chlorella vulgaris* by identifying them under compound microscope (Fig.4.1).

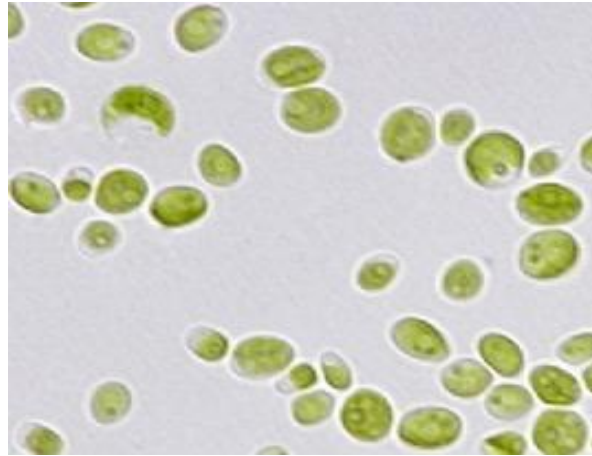


Fig: 4.1 Microscopic image of *Chlorella vulgaris*

4.4 GROWTH MEDIA PREPARATION

Three different media used for growing *Chlorella vulgaris* are BG-11 medium, F medium and Modified M-8 medium. They are selective media used for growing green and blue-green microalgae.

- BG-11 media composition

Ingredients	Grams / Liter
Sodium nitrate	1.500
Dipotassium hydrogen phosphate	0.0314
Magnesium sulphate	0.036
Calcium chloride dihydrate	0.0367
Sodium carbonate	0.020
Disodium magnesium EDTA	0.001
Citric acid	0.0056
Ferric ammonium citrate	0.006

Final pH after sterilization (at 25°C) is 7.1

- F medium composition

Ingredients	Moles
NaNO_3	5.3mM
$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	0.22mM
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	35 μM

Na ₂ EDTA.2H ₂ O	35 µM
CuSO ₄ .5H ₂ O	0.12 µM
Na ₂ MoO ₄ .2H ₂ O	0.078 µM
ZnSO ₄ .7H ₂ O	0.23 µM
CoCl ₂ .6H ₂ O	0.126 µM
MnCl ₂ .4H ₂ O	2.73 µM

Final pH after sterilization (at 25°C) is 7.2.

- Modified M-8 medium composition

Ingredients	Grams/ Liter
KNO ₃	3
KH ₂ PO ₄	0.74
Na ₂ HPO ₄ .2H ₂ O	0.26
CaCl ₂ .2H ₂ O	0.013
Fe EDTA	0.1816
MgSO ₄ .7H ₂ O	0.5153
MnCl ₂ .4H ₂ O	0.013
Al ₂ (SO ₄) ₃ .18H ₂ O	3.58
CuSO ₄ .5H ₂ O	1.83
ZnSO ₄ .7H ₂ O	3.2
H ₃ Bo ₃	0.01
Co(NO ₃) ₂ .6H ₂ O	1
Na ₂ MoO ₄ .2H ₂ O	1

Final pH after sterilization (at 25°C) is 6.5.

The media were prepared for 1000mL.

4.5 FABRICATION

Thirty six cardboard boxes of the same dimensions for each LED colour, for intensity of light and for different media respectively were taken and the inner surface was lined with aluminium foil. The LEDs were placed inside the box. To four of the boxes, 248 µmol m⁻² s⁻¹ light intensity LEDs were stuck inside; to another four of the boxes, 299 µmol m⁻² s⁻¹ light intensity LEDs were stuck and to the remaining four of the boxes, 345 µmol m⁻² s⁻¹ light intensity LEDs were stuck inside. The mouth of each conical flask was

sealed using aluminum foil. A hole was created and a tube to supply CO₂ was introduced. The tube was connected to a motor externally (air pump). The conical flasks were placed inside the box. The boxes were kept in a dark place devoid of sunlight. Fig.4.2 represents the cultivation of microalgae in different intensities of light.

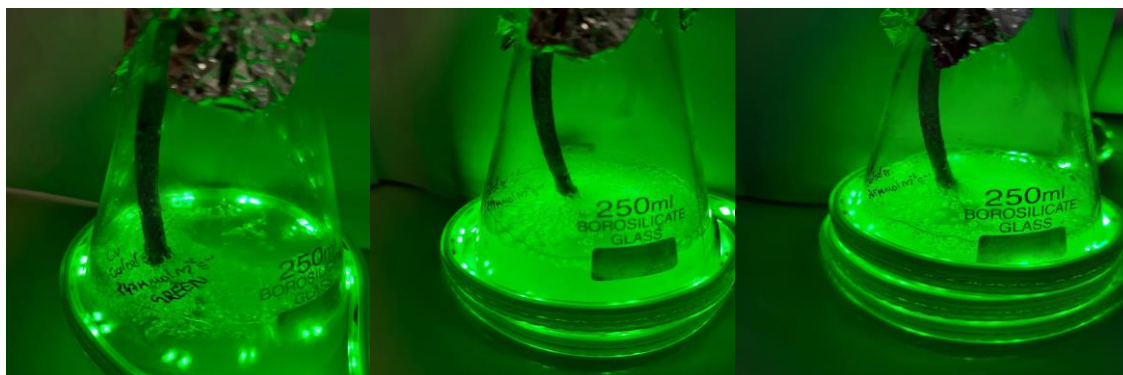


Fig: 4.2 Cultivation of microalgae in different intensities of light

4.6 CULTIVATION OF MICROALGAE

150mL of strain was taken in 250mL conical flasks. The flasks were placed inside the cardboard box containing a specific colour of LED light. Another 250mL conical flask was taken. 150mL of strain was added to it and kept under sunlight. This is used as a control. The strains were subjected to 12h/12h light/dark regime. They were cultivated for 15 days. The same procedure was carried out for all the three different intensities of light and three different media. Fig.4.3 shows the fabrication and the microalgal strain.



Fig: 4.3 Fabrication and microalgal strain

4.7 GROWTH ANALYSIS

For biomass production evaluation, all the tests were conducted in triplicate. Biomass yield was determined at the end of the batch cultivation for all cultures based on the dry cell weight. Dry weight was measured by the following procedure: 50 mL of homogeneous microalgal culture was collected from the culture flask and centrifuged at 10`000 rpm for 10 minutes at a room temperature in pre-weighted centrifugation tubes. Liquid part was discarded and tubes were dried at 80°C in the oven until constant weight, cooled to room temperature and weighted. Dry weight was calculated by subtracting initial tube mass from the mass of tubes with the dry biomass. Maximum biomass yield was calculated as grams of biomass per liter (g/L). Specific growth rate (μ) was calculated according to the equation

$$\mu = \frac{\ln(N_2 - N_1)}{t_2 - t_1}$$

Where N_2 and N_1 are culture optical densities at the time t_2 and t_1 , respectively.

4.8 ESTIMATION OF BIOCHEMICALS PRESENT IN *Chlorella vulgaris* BIOMASS

5ml of the microalgal strain of each sample was taken, sonicated and centrifuged. The supernatant was collected in a cuvette and was analyzed using a UV spectrophotometer.

4.9 PROTEIN ESTIMATION

The amount of protein present in *Chlorella vulgaris* biomass was calculated using Lowry's method. 11 test tubes were taken and named as B, S_1 , S_2 , S_3 , S_4 , S_5 , T_1 , T_2 , T_3 , T_4 and T_5 (Blank, Standard and Test). 0.5, 1, 1.5, 2.0 and 2.5ml of working standard solution of protein is pipetted out into a clean dry test tube marked as S_1 to S_5 respectively. 1 mL of microalgal sample was taken from blue LED, green LED, red LED, yellow LED and sun flask and was added to the test tubes T_1 , T_2 , T_3 , T_4 and T_5 respectively. The final volume was made to 2.5mL using distilled water. 2.5mL of distilled water serves as blank (B). 4.5mL of Alkaline copper sulphate solution and 0.5mL of Folin's phenol reagent were added to all the 11 test tubes. The contents were mixed well and kept at room temperature for 30 minutes. The intensity of the colour

developed was read colorimetrically at 620nm. A standard graph was drawn from the obtained values. From the graph, the amount of protein present in all the five samples was calculated.

4.10 LIPID ESTIMATION

The amount of lipid present in each sample was calculated using Gravimeter analysis. It is the most widely used method for determining lipid content of microalgae. It is also frequently used as a reference standard for validating other methods. The gravimetric method is a method of lipid extraction using solvents and the quantification of lipid is achieved by recording the weight of extracted lipids after evaporating extracting solvents. The extraction solvents used include the conventional ionic solvents, organic solvents, switchable solvent and CO₂-based solvent.

4.11 CARBOHYDRATE ESTIMATION

The amount of carbohydrate present in each sample was calculated by Anthrone method. Different concentrations (50, 100, 200, 300, 400, 500, 750 and 1000µg) of glucose stock solution (200µg/mL) were pipetted out into series of test tubes. They were then made up to 1ml with distilled water. Tube 1 is considered blank and the other eight were used for construction of the standard curve. Tubes 10 to 14 are for sun, blue LED, green LED, red LED and yellow LED. 5mL of Anthrone reagent was added to each tube and were mixed well by vortexing. The tubes were then cooled and incubated in a boiling water bath (100°C) for 10 minutes. The tubes were cooled at room temperature and the OD at 620nm was measured against blank. A standard absorbance (Y-axis) versus µg glucose (X-axis) curve was plotted to determine the amount of carbohydrate present in each sample.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 *Chlorella vulgaris* GROWTH IN DIFFERENT MEDIA AND INTENSITIES OF LIGHT

5.1.1 *Chlorella vulgaris* growth in BG-11 medium

Table 5.1 Cell growth in BG-11 medium with light intensity $247 \mu\text{mol m}^{-2}\text{s}^{-1}$

Day	Sunlight	Red	Blue	Green	Yellow
0	0	0	0	0	0
1	0.02	0.01	0.01	0.02	0.02
2	0.04	0.02	0.02	0.04	0.04
3	0.06	0.05	0.03	0.07	0.06
4	0.08	0.04	0.04	0.09	0.08
5	0.11	0.09	0.08	0.12	0.11
6	0.12	0.11	0.10	0.24	0.18
7	0.26	0.21	0.17	0.35	0.21
8	0.39	0.28	0.24	0.48	0.24
9	0.52	0.35	0.31	0.62	0.27
10	0.68	0.51	0.45	0.71	0.31
11	0.69	0.58	0.49	0.79	0.33
12	0.72	0.65	0.54	0.82	0.35
13	0.73	0.66	0.55	0.83	0.37
14	0.73	0.67	0.56	0.84	0.40
15	0.72	0.68	0.57	0.84	0.42

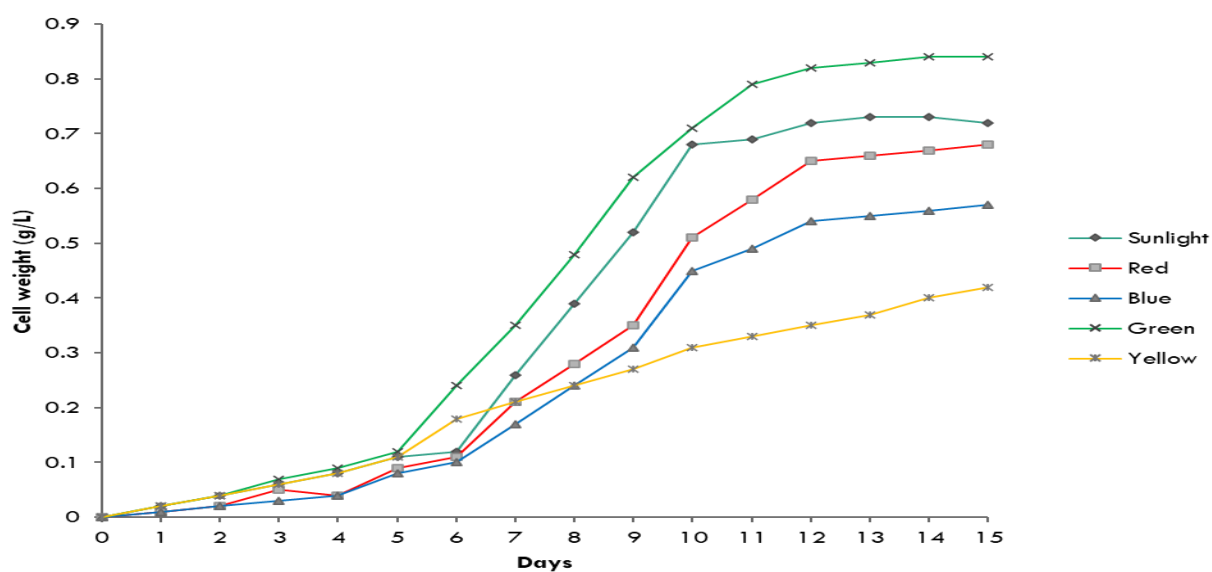


Fig: 5.1 Graphical representation of cell growth in BG-11 medium with light intensity $247 \mu\text{mol m}^{-2}\text{s}^{-1}$

Table 5.2 Cell growth in BG-11 medium with light intensity $299 \mu\text{mol m}^{-2}\text{s}^{-1}$

Day	Sunlight	Red	Blue	Green	Yellow
0	0	0	0	0	0
1	0.02	0.01	0.01	0.02	0.02
2	0.04	0.02	0.02	0.04	0.04
3	0.06	0.05	0.03	0.07	0.06
4	0.08	0.04	0.04	0.09	0.08
5	0.11	0.09	0.08	0.12	0.11
6	0.12	0.11	0.1	0.24	0.18
7	0.26	0.21	0.17	0.35	0.21
8	0.39	0.28	0.24	0.48	0.24
9	0.45	0.35	0.31	0.62	0.27
10	0.52	0.51	0.45	0.71	0.31
11	0.92	0.58	0.49	0.79	0.33
12	1.11	0.95	0.54	0.99	0.35
13	1.32	1.23	0.65	1.32	0.42
14	1.54	1.45	0.84	1.49	0.52
15	1.8	1.68	0.98	1.72	0.65

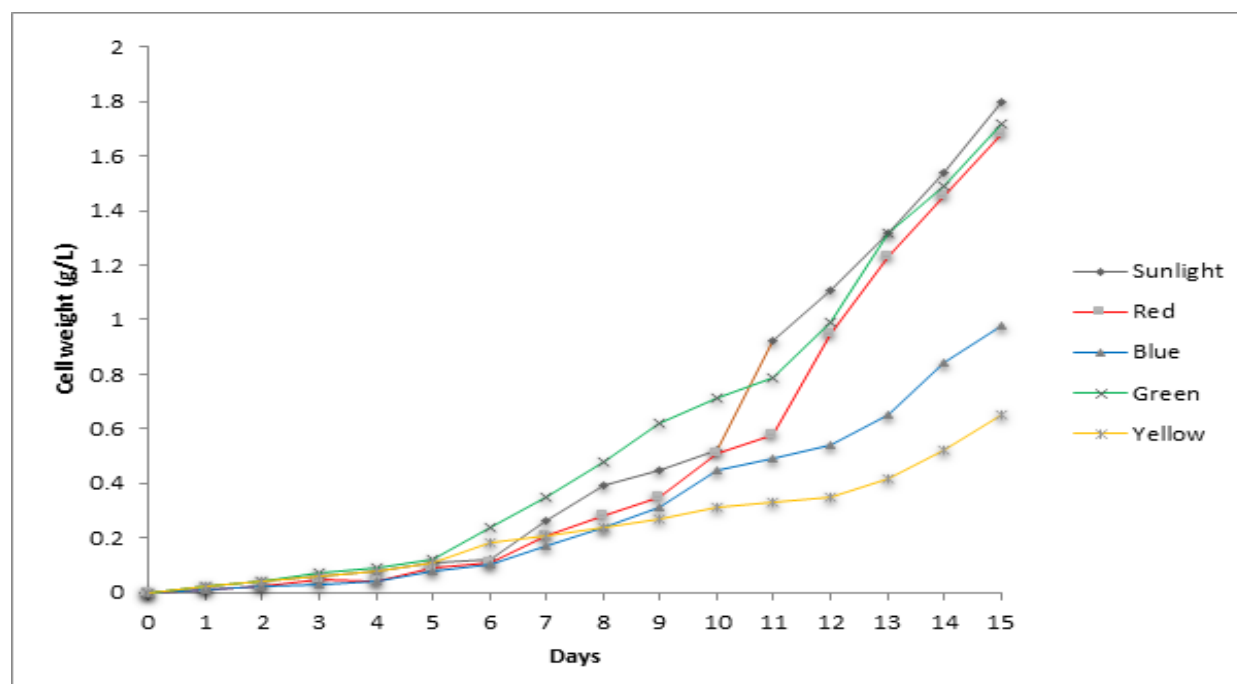


Fig: 5.2 Graphical representation of cell growth in BG-11 medium with light intensity $299 \mu\text{mol m}^{-2}\text{s}^{-1}$

Table 5.3 Cell growth in BG-11 medium with light intensity $345 \mu\text{mol m}^{-2}\text{s}^{-1}$

Day	Sunlight	Red	Blue	Green	Yellow
0	0	0	0	0	0
1	0.02	0.01	0.01	0.02	0.02
2	0.04	0.02	0.02	0.04	0.04
3	0.06	0.05	0.03	0.07	0.06
4	0.08	0.04	0.04	0.09	0.08
5	0.11	0.09	0.08	0.12	0.11
6	0.12	0.11	0.1	0.24	0.18
7	0.26	0.21	0.17	0.35	0.21
8	0.39	0.28	0.24	0.48	0.24
9	0.45	0.35	0.31	0.62	0.27
10	0.52	0.51	0.45	0.71	0.31
11	0.92	0.58	0.49	0.79	0.33
12	1.11	0.95	0.54	0.99	0.35
13	1.32	1.23	0.65	1.32	0.42
14	1.54	1.45	0.84	1.49	0.52
15	1.8	1.68	0.98	1.72	0.65

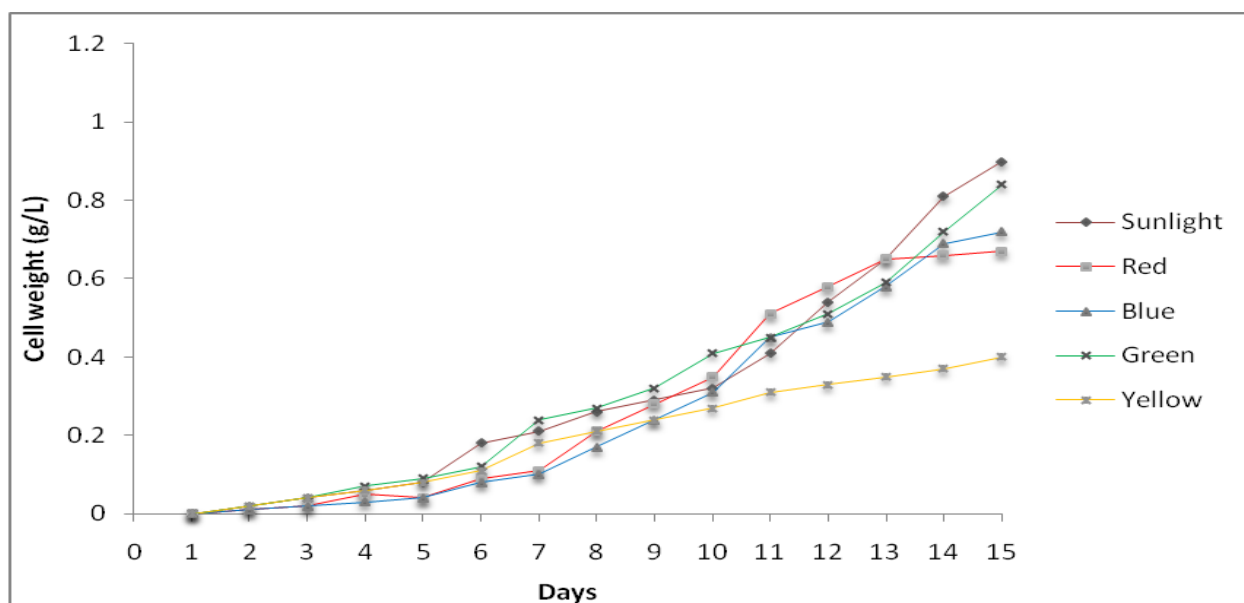


Fig: 5.3 Graphical representation of cell growth in BG-11 medium with light intensity $345 \mu\text{mol m}^{-2}\text{s}^{-1}$

The graphs above (Fig 5.1, 5.2 and 5.3) represent the amount of biomass of *C.vulgaris* produced in each intensity of light for each wavelength of light in BG-11 growth media. They infer that the highest biomass was produced by the microalgae *C.vulgaris* in green

LED amongst the 4 colours of LED. Among the three intensities of light, 299 $\mu\text{mol m}^{-2}\text{s}^{-1}$ light intensity produced the highest biomass of about 1.72g/L cell weight.

5.1.2 *Chlorella vulgaris* growth in modified M-8 medium

Table 5.4 Cell growth in modified M-8 medium with light intensity 247 $\mu\text{mol m}^{-2}\text{s}^{-1}$

Day	Sunlight	Red	Blue	Green	Yellow
0	0	0	0	0	0
1	0.26	0.23	0.21	0.29	0.11
2	0.41	0.36	0.31	0.47	0.21
3	0.50	0.42	0.40	0.54	0.37
4	0.57	0.49	0.48	0.59	0.42
5	0.61	0.58	0.53	0.71	0.51
6	0.89	0.67	0.66	0.89	0.62
7	0.94	0.73	0.71	0.96	0.70
8	1.02	0.99	0.87	1.11	0.84
9	1.12	1.09	1.04	1.17	0.96
10	1.20	1.17	1.13	1.24	1.16
11	1.35	1.24	1.21	1.34	1.22
12	1.44	1.39	1.36	1.50	1.29
13	1.49	1.45	1.41	1.57	1.37
14	1.56	1.51	1.49	1.64	1.41
15	1.62	1.58	1.53	1.81	1.46

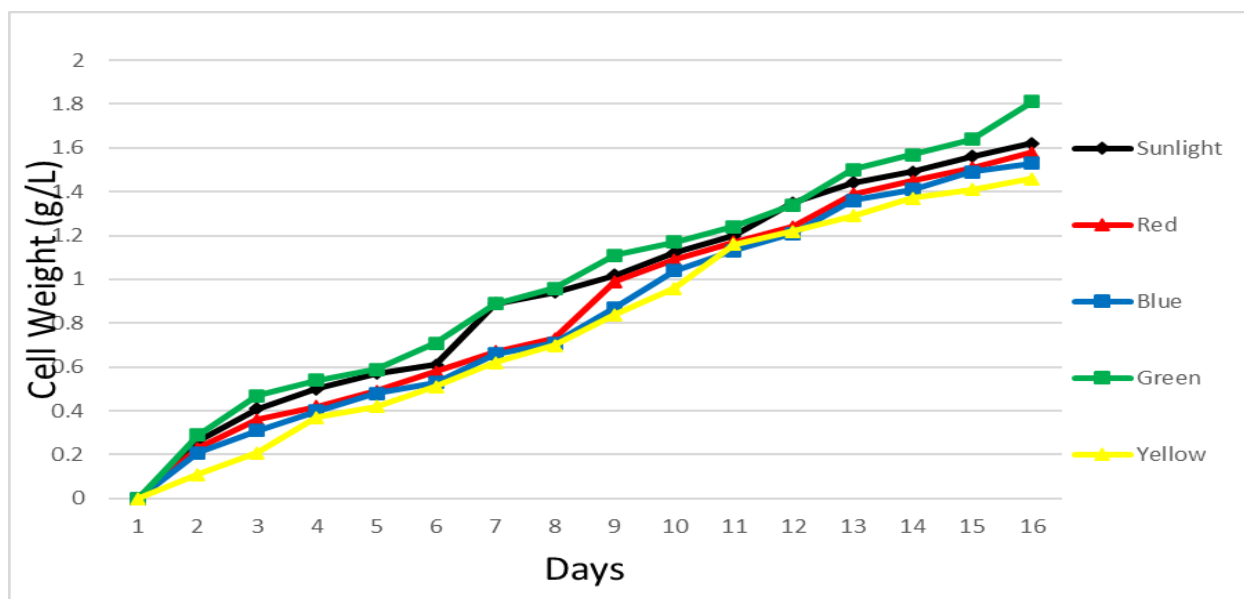


Fig: 5.4 Graphical representation of cell growth in modified M-8 medium with light intensity 247 $\mu\text{mol m}^{-2}\text{s}^{-1}$

Table 5.5 Cell growth in modified M-8 medium with light intensity $299 \mu\text{mol m}^{-2}\text{s}^{-1}$

Day	Sunlight	Red	Blue	Green	Yellow
0	0	0	0	0	0
1	0.07	0.08	0.07	0.09	0.08
2	0.15	0.14	0.11	0.19	0.32
3	0.24	0.24	0.20	0.33	0.64
4	0.62	0.60	0.58	0.90	0.96
5	1.36	1.29	1.18	1.42	1.09
6	1.39	1.39	1.32	1.44	1.12
7	1.42	1.40	1.34	1.55	1.19
8	1.46	1.41	1.38	1.57	1.22
9	1.53	1.49	1.47	1.61	1.34
10	1.72	1.68	1.61	1.73	1.39
11	1.94	1.77	1.72	1.97	1.45
12	2.11	2.10	1.98	2.19	1.51
13	2.53	2.46	2.23	2.63	1.59
14	2.87	2.68	2.41	2.94	1.73
15	3.13	2.92	2.52	3.55	1.98

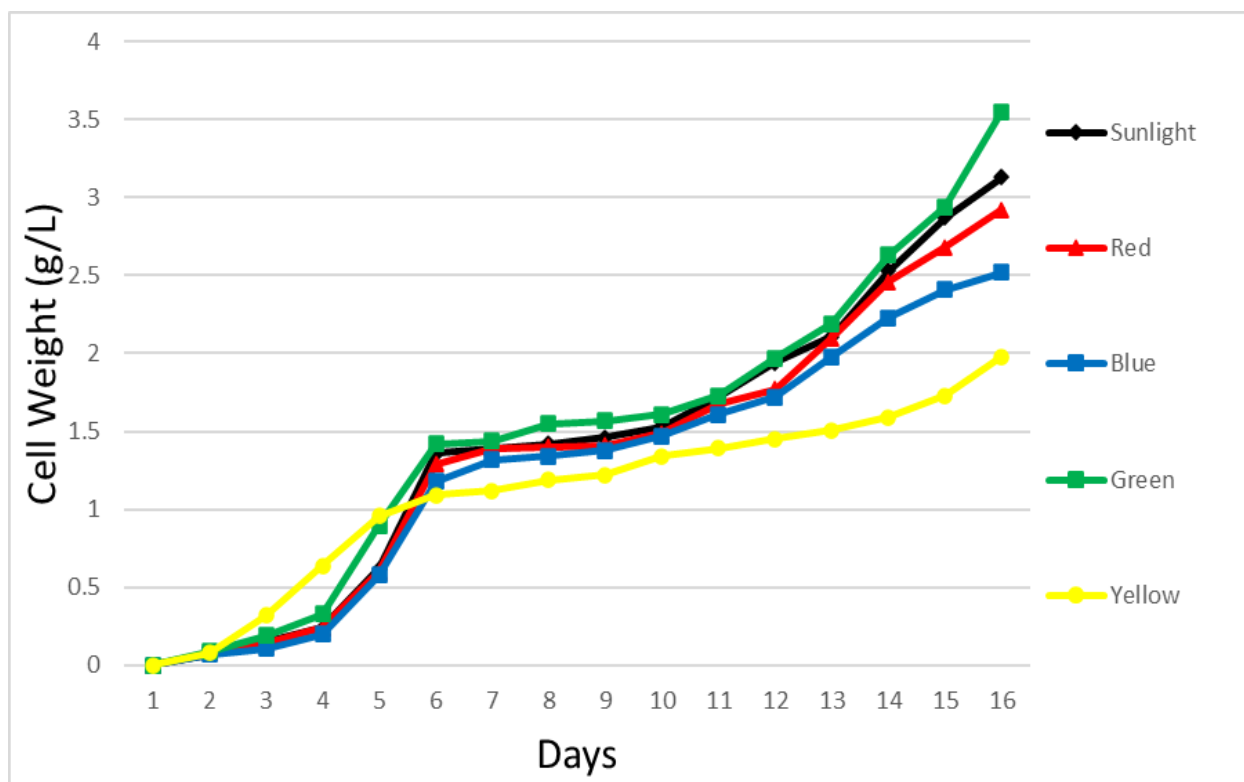


Fig: 5.5 Graphical representation of cell growth in modified M-8 medium with light intensity $299 \mu\text{mol m}^{-2}\text{s}^{-1}$

Table 5.6 Cell growth in modified M-8 medium with light intensity $345 \mu\text{mol m}^{-2}\text{s}^{-1}$

Day	Sunlight	Red	Blue	Green	Yellow
0	0	0	0	0	0
1	0.01	0.01	0.01	0.02	0.01
2	0.04	0.02	0.03	0.04	0.02
3	0.44	0.19	0.44	0.45	0.18
4	0.52	0.26	0.51	0.53	1.27
5	0.59	0.31	0.59	0.59	0.35
6	0.66	0.37	0.65	0.67	0.38
7	0.97	0.52	0.96	0.95	0.54
8	1.0	0.94	1.0	1.0	0.94
9	1.18	1.0	1.17	1.19	1.0
10	1.26	1.12	1.23	1.27	1.11
11	1.38	1.21	1.38	1.41	1.20
12	1.56	1.33	1.55	1.57	1.32
13	1.84	1.39	1.83	1.84	1.38
14	1.93	1.46	1.90	1.95	1.46
15	1.96	1.53	1.94	2.11	1.51

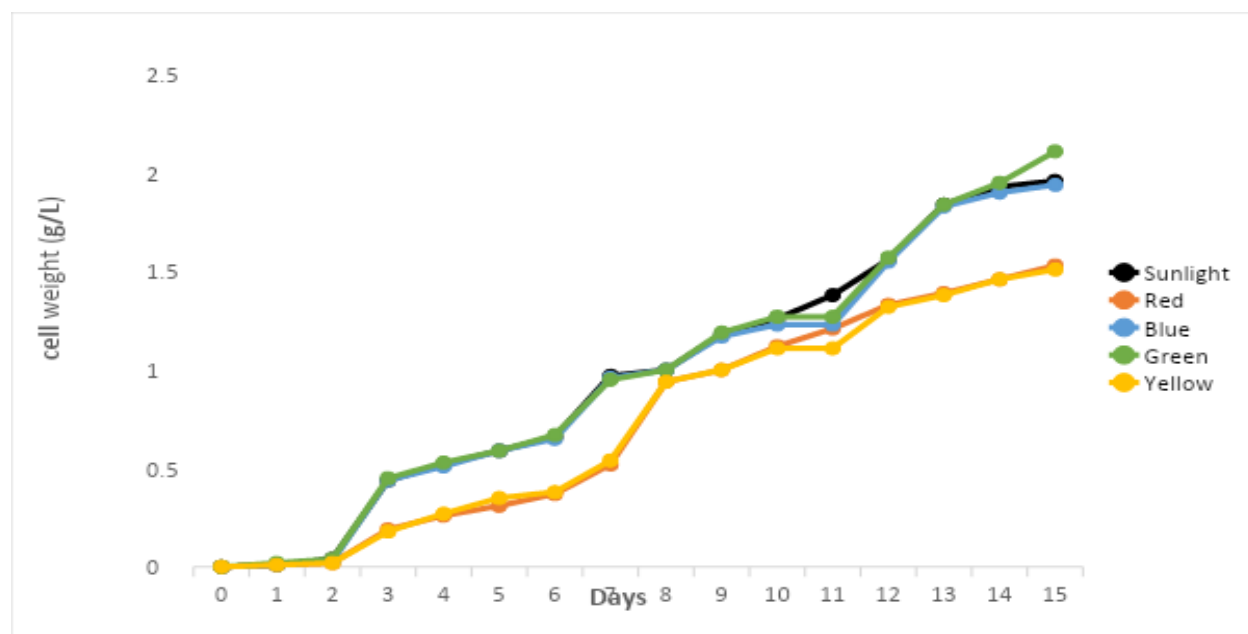


Fig: 5.6 Graphical representation of cell growth in modified M-8 medium with light intensity $345 \mu\text{mol m}^{-2}\text{s}^{-1}$

The graphs above (Fig 5.4, 5.5 and 5.6) represent the amount of biomass of *C.vulgaris* produced in each intensity of light for each wavelength of light in modified M-8 growth

media. They infer that the highest biomass was produced by the microalgae *C.vulgaris* in green LED amongst the 4 colours of LED. Among the three intensities of light, 299 $\mu\text{mol m}^{-2}\text{s}^{-1}$ light intensity produced the highest biomass of about 3.55g/L cell weight.

5.1.3 *Chlorella vulgaris* growth in F medium

Table 5.7 Cell growth in F medium with light intensity 247 $\mu\text{mol m}^{-2}\text{s}^{-1}$

Day	Sunlight	Red	Blue	Green	Yellow
0	0	0	0	0	0
1	0.02	0.12	0.01	0.15	0
2	0.15	0.15	0.02	0.23	0.01
3	0.18	0.28	0.03	0.42	0.02
4	0.23	0.65	0.04	0.84	0.02
5	0.35	1.54	0.12	1.92	0.03
6	0.68	2.05	0.15	2.36	0.03
7	0.87	2.46	0.18	2.72	0.04
8	2.04	2.53	0.24	2.74	1.13
9	2.55	2.97	0.46	3.01	0.14
10	2.75	3.08	1.28	3.24	0.16
11	2.94	3.45	1.87	3.76	0.18
12	3.02	3.56	2.19	3.98	0.19
13	3.52	3.64	2.32	4.08	0.22
14	3.93	3.67	2.64	4.10	0.25
15	4.11	4.24	2.93	4.92	0.28

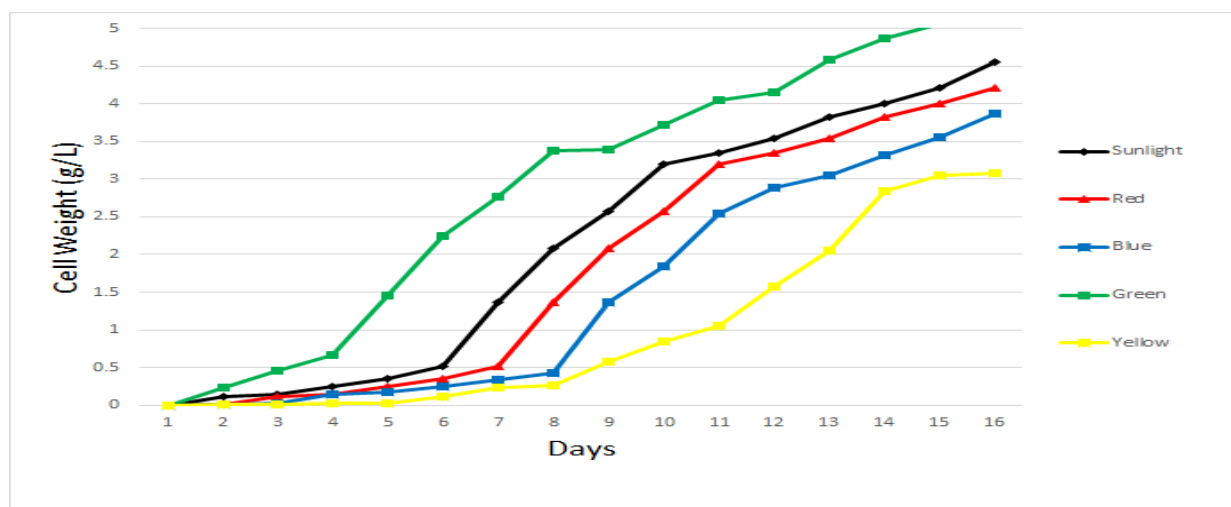


Fig: 5.7 Graphical representation of cell growth in F medium with light intensity 247 $\mu\text{mol m}^{-2}\text{s}^{-1}$

Table 5.8 Cell growth in F medium with light intensity $299 \mu\text{mol m}^{-2}\text{s}^{-1}$

Day	Sunlight	Red	Blue	Green	Yellow
0	0	0	0	0	0
1	0.12	0.01	0.01	0.24	0.01
2	0.14	0.12	0.02	0.45	0.01
3	0.25	0.14	0.15	0.66	0.02
4	0.35	0.25	0.18	1.45	0.03
5	0.52	0.35	0.25	2.24	0.12
6	1.36	0.52	0.34	2.77	0.24
7	2.08	1.36	0.43	3.38	0.26
8	2.57	2.08	1.36	3.40	0.58
9	3.20	2.57	1.84	3.72	0.85
10	3.35	3.20	2.55	4.05	1.05
11	3.54	3.35	2.88	4.15	1.58
12	3.82	3.54	3.05	4.58	2.05
13	4.01	3.82	3.32	4.87	2.84
14	4.22	4.01	3.55	5.06	3.05
15	4.55	4.22	3.87	5.25	3.08

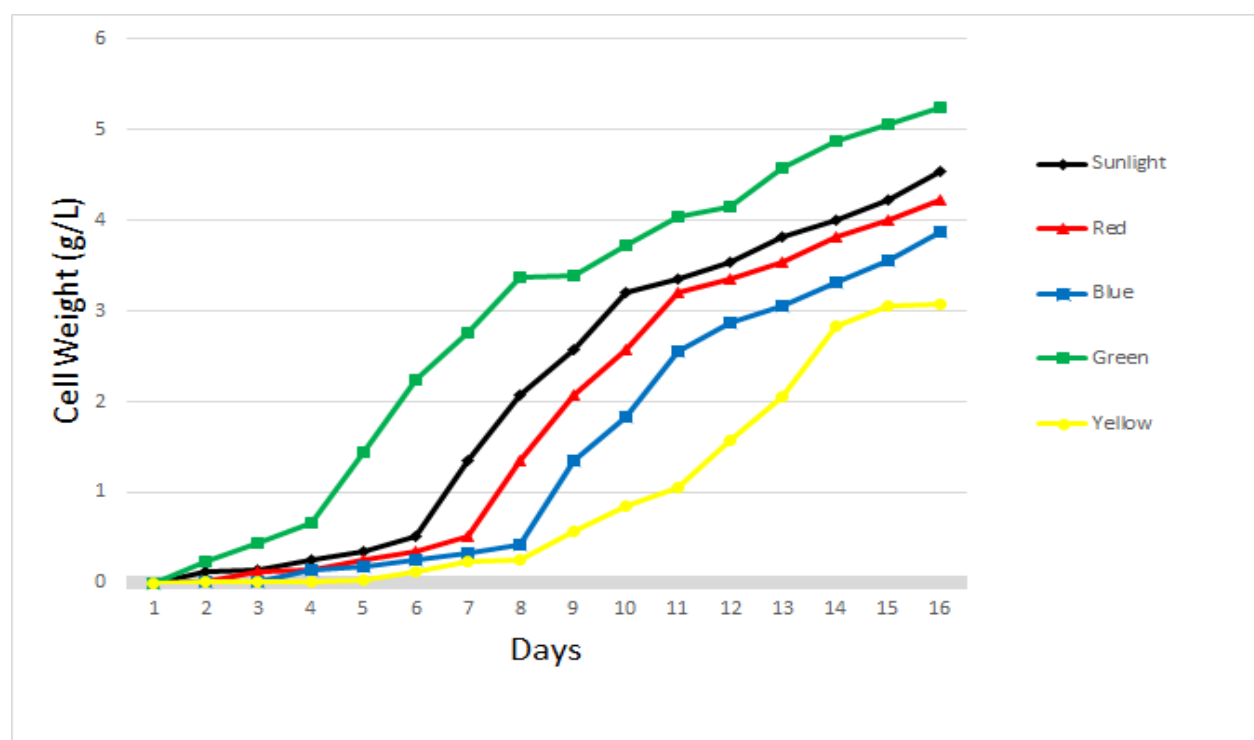


Fig: 5.8 Graphical representation of cell growth in F medium with light intensity $299 \mu\text{mol m}^{-2}\text{s}^{-1}$

Table 5.9 Cell growth in F medium with light intensity $345 \mu\text{mol m}^{-2}\text{s}^{-1}$

Day	Sunlight	Red	Blue	Green	Yellow
0	0	0	0	0	0
1	0.09	0.12	0.11	0.15	0.01
2	0.15	0.34	0.15	0.36	0.03
3	0.32	0.52	0.17	0.46	0.04
4	0.36	1.07	0.19	1.05	0.07
5	0.84	1.79	0.35	2.05	0.11
6	1.89	2.23	0.49	2.57	0.12
7	2.15	2.66	0.54	3.08	0.17
8	2.81	2.72	0.78	3.13	0.18
9	2.97	2.96	0.87	3.27	0.21
10	3.0	3.18	1.03	3.56	0.24
11	3.23	3.42	1.17	3.94	0.29
12	3.57	3.66	1.31	4.09	0.32
13	3.82	3.76	1.93	4.13	0.36
14	3.99	3.93	2.55	4.38	0.55
15	4.36	4.01	3.17	4.53	0.95

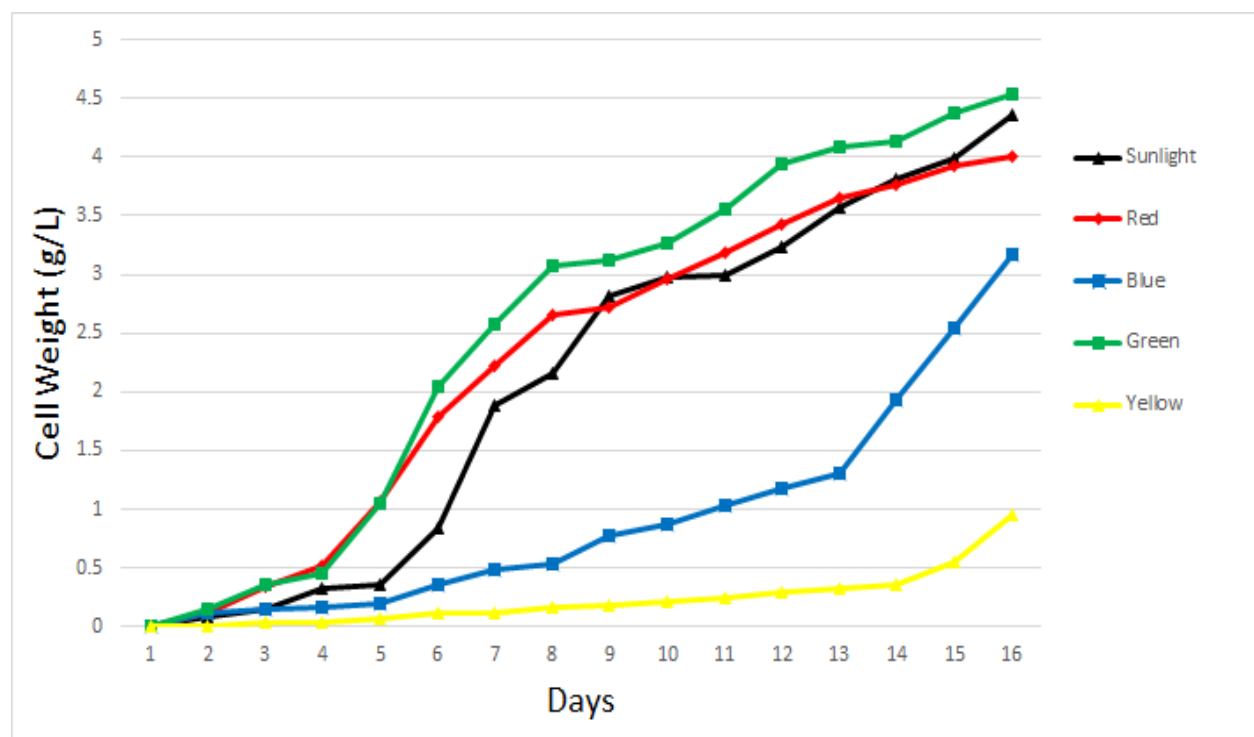


Fig: 5.9 Graphical representation of cell growth in F medium with light intensity $345 \mu\text{mol m}^{-2}\text{s}^{-1}$

The graphs above (Fig 5.7, 5.8 and 5.9) represent the amount of biomass of *C.vulgaris* produced in each intensity of light for each wavelength of light in F growth media. They infer that the highest biomass was produced by the microalgae *C.vulgaris* in green LED amongst the 4 colours of LED. Among the three intensities of light, 299 $\mu\text{mol m}^{-2}\text{s}^{-1}$ light intensity produced the highest biomass of about 5.25g/L cell weight.

5.2 ABSORBANCE PEAKS

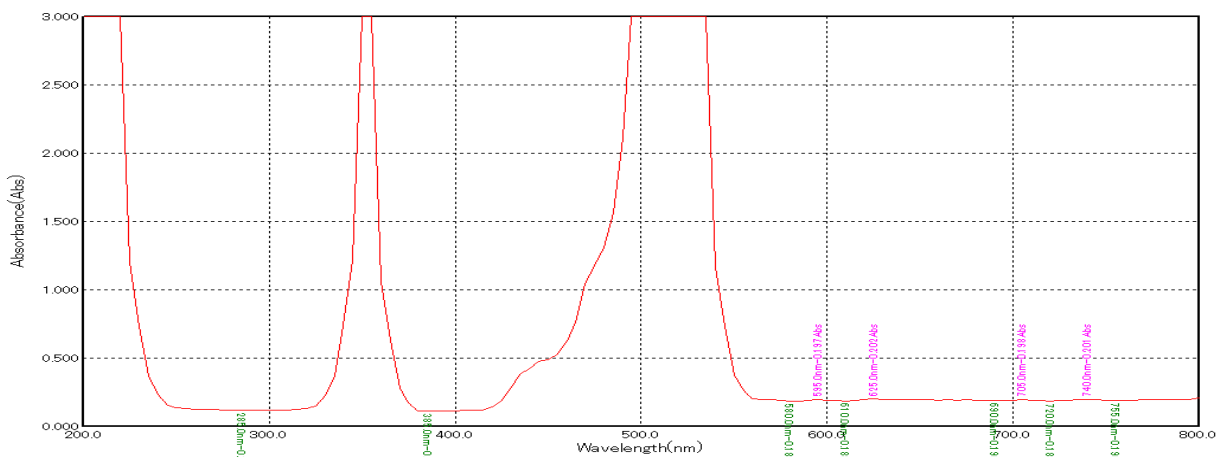


Fig: 5.10 UV absorbance peak of *C.vulgaris* grown in Sunlight

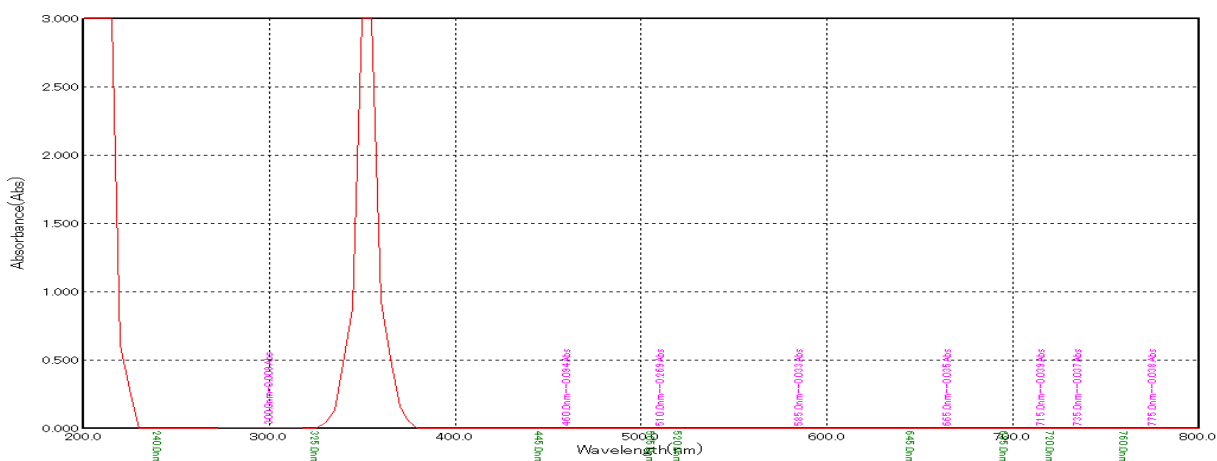


Fig: 5.11 UV absorbance peak of *C.vulgaris* grown in blue LED

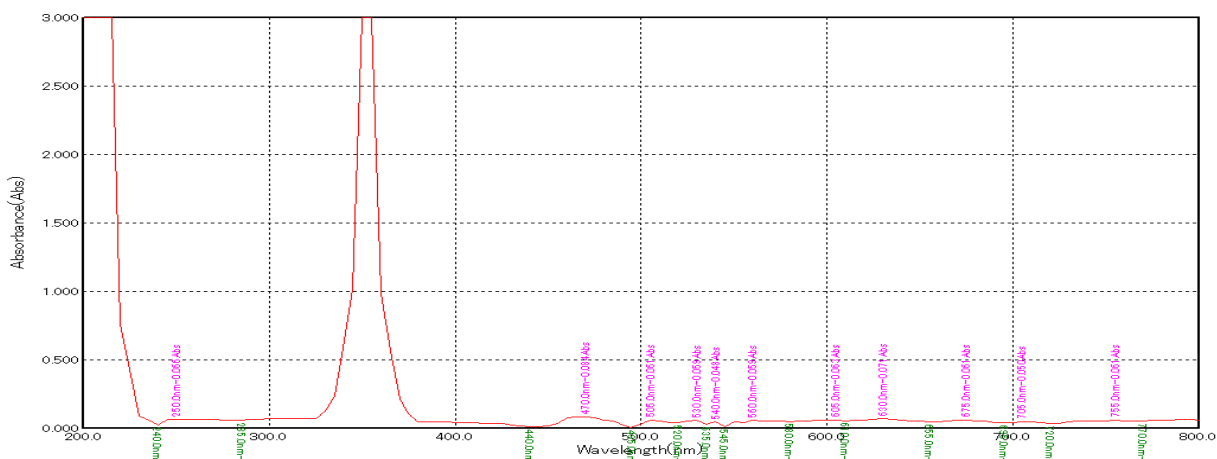


Fig: 5.12 UV absorbance peak of *C.vulgaris* grown in green LED

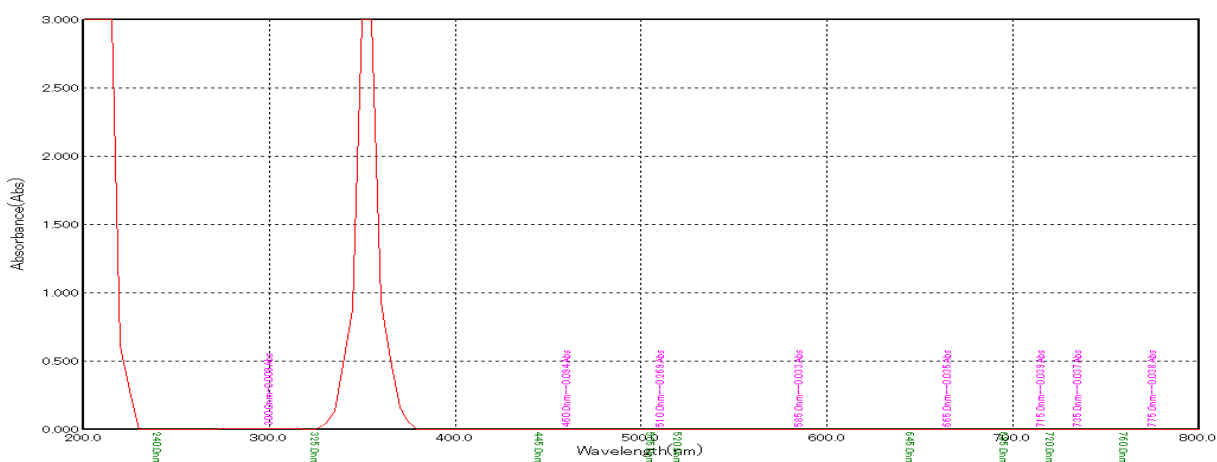


Fig: 5.13 UV absorbance peak of *C.vulgaris* grown in red LED

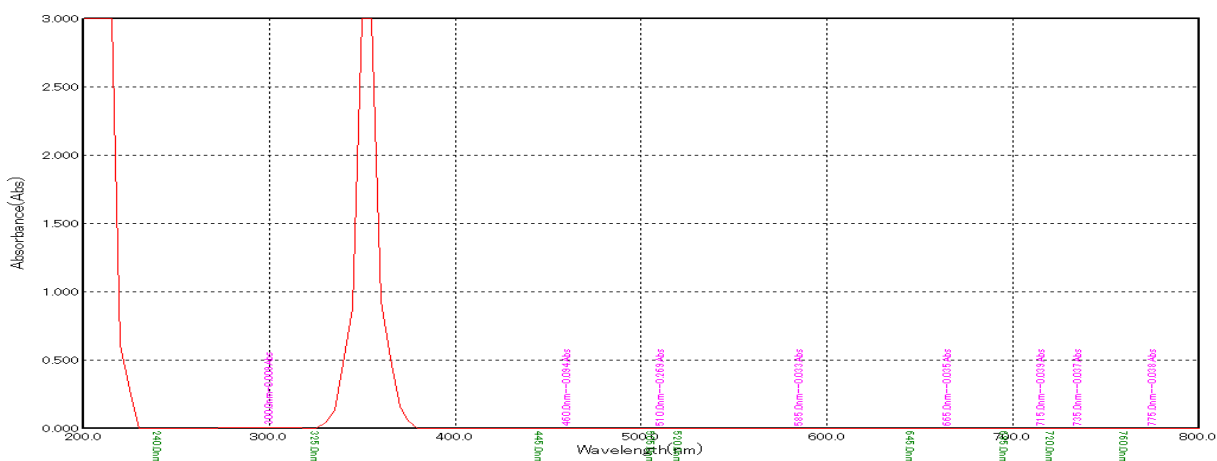


Fig: 5.14 UV absorbance peak of *C.vulgaris* grown in yellow LED

The graphs mentioned above (Fig 5.10, 5.11, 5.12, 5.13 and 5.14) show the different absorption peaks of each sample of *C.vulgaris* grown in different wavelengths of light. The wavelength maxima (peaks) help us to identify the different compounds present in the sample. These were obtained by using UV spectrophotometer.

5.3 Protein estimation by Lowry's method

Table 5.10 Protein estimation by Lowry's method

Day	Sunlight	Red	Blue	Green	Yellow
0	0.0	0.0	0.0	0.0	0.0
1	1.2	0.6	0.6	1.2	1.2
2	2.4	1.2	1.2	4.2	2.4
3	3.6	3.0	1.8	6.2	3.6
4	4.8	2.4	2.4	10.5	4.8
5	6.6	5.4	4.8	12.5	6.6
6	7.2	6.6	6.0	22.8	10.8
7	15.7	12.7	10.2	29.6	12.7
8	23.5	16.9	14.5	35.2	14.5
9	31.3	21.1	18.7	40.9	16.3
10	41.0	30.7	27.1	45.2	18.7
11	41.6	34.9	29.5	46.9	19.9
12	43.4	39.2	32.5	47.2	21.1
13	44.0	39.8	33.1	48.7	22.3
14	44.0	40.4	33.7	49.2	24.1
15	43.4	41.0	34.3	50.6	25.3

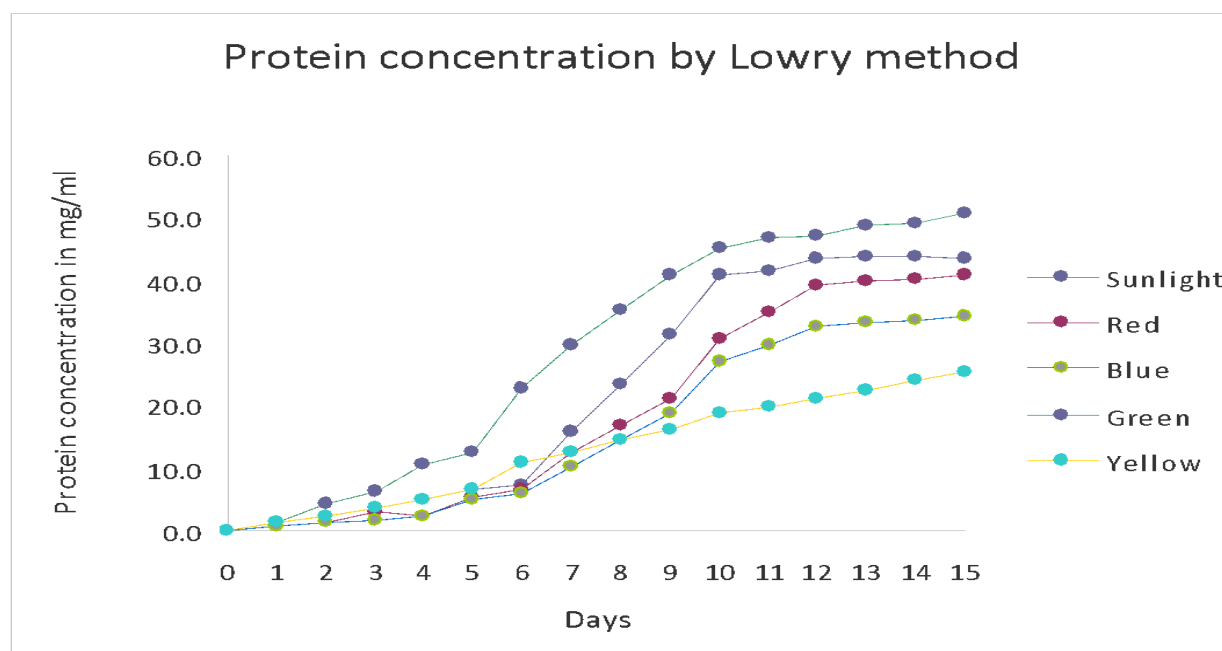


Fig: 5.15 Graphical representation of protein concentration in each biomass

5.4 Lipid estimation by Gravimetric analysis

Table 5.11 Lipid estimation by Gravimetric analysis

Day	Sunlight	Red	Blue	Green	Yellow
0	0	0	0	0	0
1	0.11	0.055	0.055	0.11	0.11
2	0.22	0.11	0.11	0.22	0.22
3	0.33	0.275	0.165	0.385	0.33
4	0.44	0.22	0.22	0.495	0.44
5	0.605	0.495	0.44	0.66	0.605
6	0.66	0.605	0.55	1.32	0.99
7	1.43	1.155	0.935	1.925	1.155
8	2.145	1.54	1.32	2.64	1.32
9	2.86	1.925	1.705	3.41	1.485
10	3.74	2.805	2.475	3.905	1.705
11	3.795	3.19	2.695	4.345	1.815
12	3.96	3.575	2.97	4.51	1.925
13	4.015	3.63	3.025	4.565	2.035
14	4.015	3.685	3.08	4.62	2.2
15	3.96	3.74	3.135	4.62	2.31

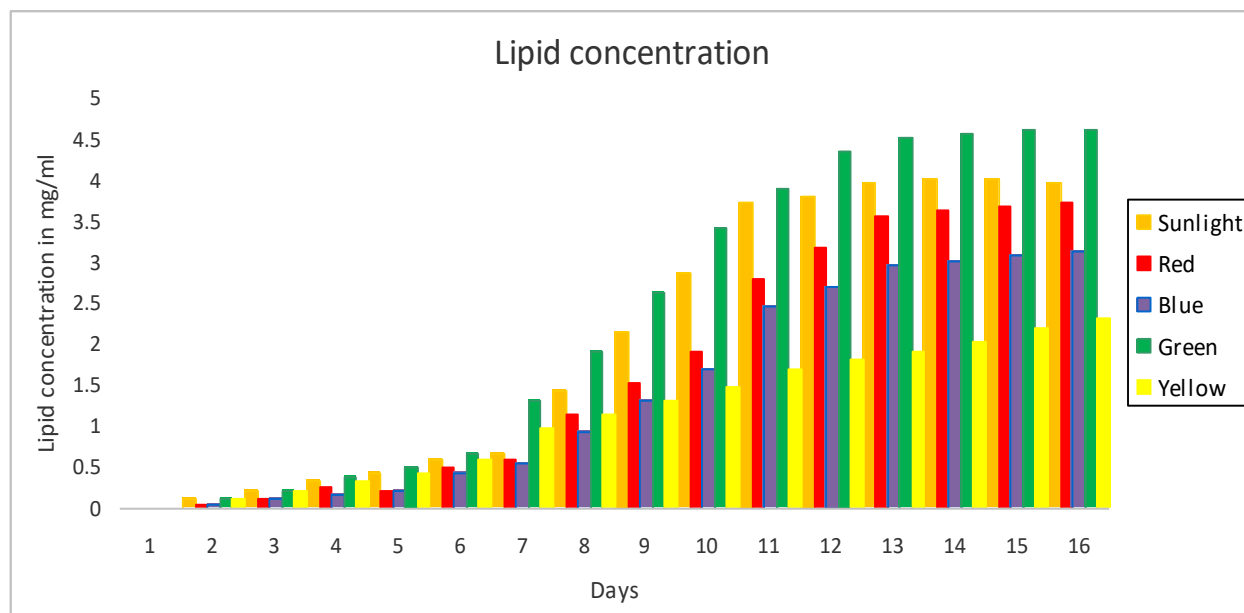


Fig: 5.16 Graphical representation of lipid concentration in each biomass

5.5 Carbohydrate estimation by Anthrone method

Table 5.12 Carbohydrate estimation by Anthrone method

Day	Sunlight	Red	Blue	Green	Yellow
0	0	0	0	0	0
1	0.1	0.05	0.05	0.1	0.1
2	0.2	0.1	0.1	0.2	0.2
3	0.3	0.25	0.15	0.35	0.3
4	0.4	0.2	0.2	0.45	0.4
5	0.55	0.45	0.4	0.6	0.55
6	0.6	0.55	0.5	1.2	0.9
7	1.3	1.05	0.85	1.75	1.05
8	1.95	1.4	1.2	2.4	1.2
9	2.6	1.75	1.55	3.1	1.35
10	3.4	2.55	2.25	3.55	1.55
11	3.45	2.9	2.45	3.95	1.65
12	3.6	3.25	2.7	4.1	1.75
13	3.65	3.3	2.75	4.15	1.85
14	3.65	3.35	2.8	4.2	2
15	3.6	3.4	2.85	4.2	2.1

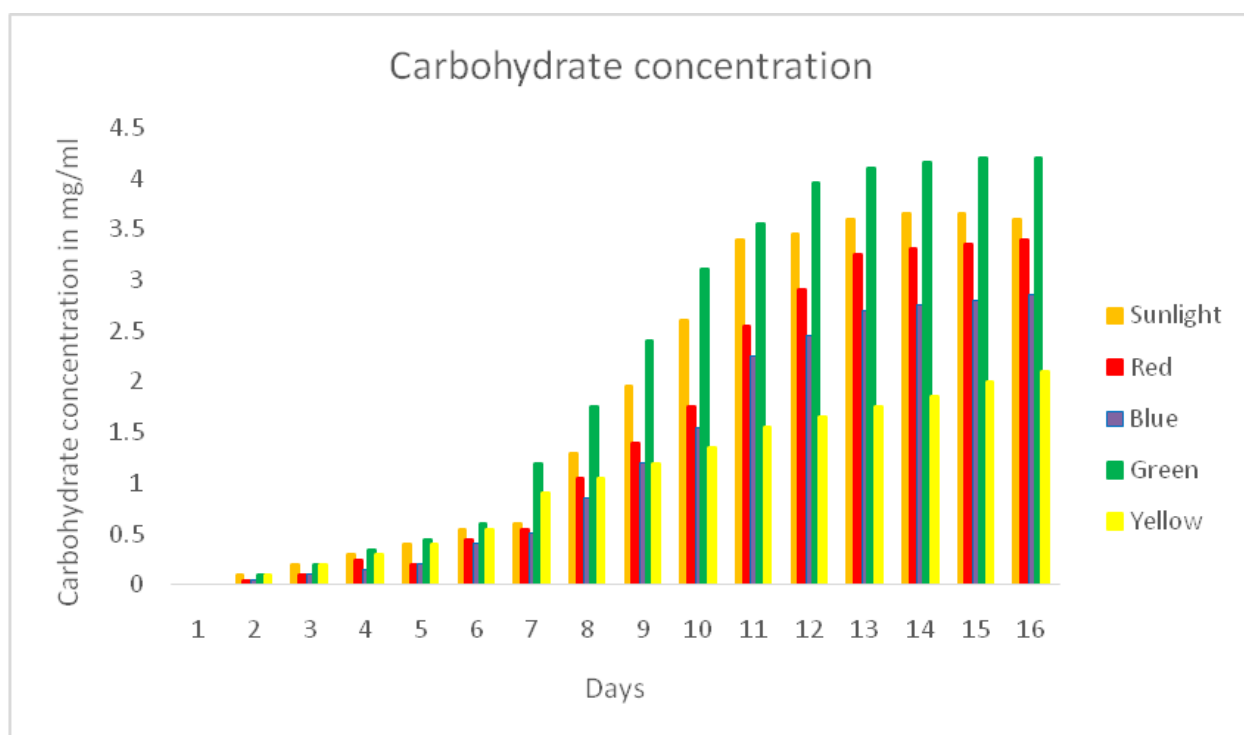


Fig: 5.17 Graphical representation of carbohydrate concentration in each biomass

Table 5.10 and Fig. 5.15 depict the amount of protein present in each sample grown in F medium in different wavelengths of light in the light intensity of $299 \mu\text{mol m}^{-2}\text{s}^{-1}$ estimated by Lowry's method. Table 5.11 and Fig. 5.16 represent the amount of lipid present in each sample estimated by using Gravimetric analysis. Table 5.12 and Fig. 5.17 show the amount of carbohydrate present in each sample calculated by Anthrone method each day. Green LED produced highest protein, lipid and carbohydrate in the microalgal sample followed by sunlight, red LED, blue LED and yellow LED.

CHAPTER 6

SUMMARY AND CONCLUSION

6.1 SUMMARY

Green marine microalgae *Chlorella vulgaris* was cultivated in three different media (BG-11 medium, F medium and modified M-8 medium) in three different light intensities ($247 \mu\text{mol m}^{-2}\text{s}^{-1}$, $299 \mu\text{mol m}^{-2}\text{s}^{-1}$ and $345 \mu\text{mol m}^{-2}\text{s}^{-1}$) of four different wavelengths of light (red, blue, green and yellow) and sunlight (control). 50mL of media was added to 100mL of microalgal strain (1:3 ratio) in 250mL conical flask. Air pump was fixed to each conical flask for aeration. All the flasks were kept in a dark place. The temperature was maintained as 28°C . The microalgae were cultivated for 15 days. The absorbance peak for each sample was observed by analyzing them in UV spectrophotometer. The optimal media, light colour and light intensity required to produce the highest biomass content of *Chlorella vulgaris* were determined. The amount of biomass, protein, lipid and carbohydrate accumulated were also calculated for each sample for all the three intensities of light and all the three media everyday. The biomass accumulated was calculated by weighing the dry weight of the samples everyday. Protein estimation was done by Lowry's method, lipid estimation was done by Gravimetric analysis and amount of carbohydrate present in each biomass was estimated by Anthrone method. Graph was plotted for each test to differentiate the efficiency of each wavelength of LED light and for easy comparison.

6.2 CONCLUSION

The growth medium that produced highest biomass among the three is F medium. It produced 4 times more biomass than BG-11 medium and 3 times more biomass than modified M-8 medium. Green LED produced the highest growth parameters in all the three intensities of light. The maximum biomass, protein, lipid and carbohydrate production was observed in green LED of light intensity $299 \mu\text{mol m}^{-2}\text{s}^{-1}$ than $247 \mu\text{mol m}^{-2}\text{s}^{-1}$ and $345 \mu\text{mol m}^{-2}\text{s}^{-1}$ in all the three growth media with highest cell weight in F medium as 5.25g/L, amount of protein as 50.6mg/mL, amount of lipid as 4.62mg/mL and amount of carbohydrate as 4.2mg/mL followed by sun, red LED, blue LED and yellow LED. In F medium, *Chlorella vulgaris* biomass accumulated up to 5.25g/L cell

growth in Green LED of light intensity $299 \mu\text{mol m}^{-2}\text{s}^{-1}$ which was higher than the other colours and sunlight. In F medium, when the green LED intensity was increased from $247 \mu\text{mol m}^{-2}\text{s}^{-1}$ to $299 \mu\text{mol m}^{-2}\text{s}^{-1}$, the biomass accumulated 1.15 times more and when the green LED intensity was increased from $299 \mu\text{mol m}^{-2}\text{s}^{-1}$ to $345 \mu\text{mol m}^{-2}\text{s}^{-1}$, the biomass accumulation depleted. In addition to being an environmentally friendly correct energy source, green LEDs have shown potential in the photostimulation of microalgae biomass and their biochemicals. The best medium to be used to achieve highest biomass of *Chlorella vulgaris* is F medium, the best intensity is $299 \mu\text{mol m}^{-2}\text{s}^{-1}$ and the best LED colour is found to be green. Hence, these conditions are recommended to design bioreactor in order to achieve the highest yield of biomass.

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