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BOLU ABANT İZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
Department of Biology

EFFECTS OF GREEN LIGHT SPECTRUM SUPPLEMENTATION ON GROWTH OF GREEN MICROALGAE SCENEDESMUS QUADRICAUDA

MASTER OF SCİENCE

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BOLU, MAYIS - 2023

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Effects of green light spectrum supplementation on growth of green microalgae Scenedesmus quadricauda submitted by **Asmaa DABBAS** and defended before the Examining Committee Members listed below in partial fulfillment of the requirements for the degree of **Master of Science** in **Department of Biology, Institute of Graduate Studies of Bolu Abant Izzet Baysal University in 2.05.2023** by

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ABSTRACT

EFFECTS OF GREEN LIGHT SPECTRUM SUPPLEMENTATION ON GROWTH OF GREEN MICROALGAE SCENEDESMUS QUADRICAUDA
MSC THESIS
ASMAA DABBAS
BOLU ABANT IZZET BAYSAL UNIVERSITY
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Microalgae has a significant role with high-value product, lighting condition is a potential source to manage and increase algae growth. LED light has shown promising results in the growth rate of microalgae and the activity of secondary metabolites.

S. quadricauda was grown under monochromatic LED light wavelengths, i.e., white, red, blue, green and also dichromatic red/ blue LED in ratios of 1/3, 3/1 at the total light intensity of 50 µmol photons m^{-2} s^{-1}, to measure the dry weight, optical density, and chlorophyll concentration. The results indicated that the red and blue light combination was the optimum light wavelength for high optical density and chlorophyll concentration in *S. quadricauda.* The order of growth rate R3/B1> R1/B3> Blue> Red> White> Green.

Green light yielded the best chlorophyll production in *S. quadricauda,* red light was the lowest chlorophyll results. Furthermore, the green light was supplemented gradually into the combination of red: blue (1: 3) at the light intensity of 2, 4, 6, 8, and 10 µmol m^{-2} s^{-1} as an expense of red and blue light.

Treatment with 6 µmol/ m^{-2} s^{-1} green light supplemented with red/blue combinations was the highest growth rate result and the lowest is with 2 µmol/ m^{-2} s^{-1}. No statistical differences between any of the treatments. Supplementation of green light showed that there is no negative or positive effect on the growth of *S. quadricauda* species. Seems that supplementation of green light is compensated for red and blue light expenses although green light alone has a suppressive effect on the growth of cultures of *S. quadricauda*.

KEYWORDS: *Scenedesmus qaudricuada,* LED, Red Light, Blue Light, Dichromatic Combination, Green Light

ÖZET

YEŞİL İŞIK DALGA BOYU KATKISININ SCENEDESMUS QUADRİCAUDA MİKROALG TÜRÜNÜN BÜYÜMESİNE OLAN ETKİLERİ
YÜKSEK LİSANS TEZİ
ASMAA DABBAS
BOLU ABANT İZZET BAYSAL ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ
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(TEZ DANIŞMANI: ASSIST. PROF DR. MURAT TELLİ)
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Mikroalgler, yüksek değerli ürünlerle önemli bir role sahiptir, Aydınlatma koşulları, alg büyümesini yönetmek ve artırmak için potansiyel bir kaynaktır. LED ışığı, mikroalglerin büyüme hızında ve ikincil metabolitlerin aktivitesinde umut verici sonuçlar göstermiştir.

Kuru ağırlık, optik yoğunluk ve klorofil konsantrasyonunu ölçmek için, 1/3, 3/1 oranlarında, 50 µmol foton m^{-2} s^{-1} toplam ışık şiddetinde, *S. quadricauda*, monokromatik LED ışık, yani beyaz, kırmızı, mavi, yeşil ve ayrıca dikromatik kırmızı/mavi dalga boyları altında büyütüldü. Sonuçlar, *S. quadricauda*'da, yüksek optik yoğunluk ve klorofil konsantrasyonu için kırmızı ve mavi ışık kombinasyonunun optimum ışık dalga boyu olduğunu gösterdi. Büyüme oranı sırası R3/B1> R1/B3> Mavi> Kırmızı> Beyaz> Yeşil.

S. quadricauda'da yeşil ışık en iyi klorofil üretimini sağlarken, kırmızı ışık en düşük klorofil üretimini sağlamıştır. Ayrıca yeşil ışık, 2, 4, 6, 8 ve 10 µmol m^{-2} s^{-1} ışık yoğunluğunda, kırmızı ve mavi ışık gideri olarak, kademeli olarak kırmızı: mavi (1:3) kombinasyonuna eklendi.

Kırmızı/mavi kombinasyonları ile eklenen 6 µmol /m^{-2} s^{-1} yeşil ışıkla muamele en yüksek büyüme oranı sonucuydu ve 2 µmol /m^{-2} s^{-1} ile en düşük büyüme oranı sonucuydu. Muamelelerin hiçbiri arasında istatistiksel fark yoktur. Yeşil ışığın eklenmesi, *S. quadricauda* türlerinin büyümesi üzerinde olumsuz veya olumlu bir etkisinin olmadığını göstermiştir. Yeşil ışığın tek başına *S. quadricauda* kültürlerinin büyümesi üzerinde baskılayıcı bir etkisi olmasına rağmen, yeşil ışığın eklenmesi, kırmızı ve mavi ışık giderlerini telafi ettiği görülmüştür.

ANAHTAR KELİMELER: *Scenedesmus qaudricauda*, LED, Kırmızı Işık, Mavi Işık, Dikromatik Kombinasyon, Yeşil Işık.

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LIST OF ABBREVIATIONS AND SYMBOLS

S. quadricauda : *Scenedesmus quadricauda*

CO_2 : Carbon dioxide

ATP : Adenosine triphosphate

NADPH : Nicotinamide adenine dinucleotide phosphate

PSI : Photosystem I

PSII : Photosystem II

Cytb6f : Cytochrome b6f complex

P680 : Primary electron donor in PSII

P700 : Primary electron donor in PSI

FD : ferredoxin

FNR : ferredoxin-NADP reductase

PQ : plastoquinone

PC : plastocyanin

LHPC : Light Harvesting Protein-Chlorophyll system

LHC : Light Harvesting Complex

Rubisco : Ribulose biphosphate carboxylase/oxygenase

RuBP : Ribulose Bis-Phosphate

G3P : Glyceraldehyde-3-Phosphate

Chl : Chlorophyll

HID : high-intensity discharge

LED : Led Emitting Diodes

DNA : Deoxyribonucleic acid

G phase : Growth

M phase : Nuclear division

CP : Commitment point

C : Cytokinesis

S phase : DNA replication

NPQ : Non-photosynthetic quenching

ROS : Reactive oxygen species

BG-11 : Blue-green (culture medium) No.11

R/B : Red/ Blue light

R : Red light

G : Green light

B : Blue light

W : White light

Tr : Treatment

ANOVA : Analysis of variance

nm : Nanometers

SPSS : Statistical Package for Social Sciences

OD : Optical Density

SD : Standard deviation

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1. INTRODUCTION

Microalgae are unicellular organisms responsible for photosynthesis; light energy is converted into chemical energy (1). Microalgae (eukaryotic and prokaryotic cyanobacteria) are a huge class of photoautotrophic organisms, with organisms able of heterotrophic, mixotrophic, or photoheterotrophic existence (2). They can grow rapidly, especially under favorable environmental conditions (3). The majority of algae are aquatic in either fresh or marine waters, however, certain kinds of algae may be found growing in a variety of environments.

Microalgae are divided into two prokaryotic divisions (Cyanophyta and Prochlorophyta) and nine eukaryotic divisions (Glaucophyta, Rhodophyta, Heterokontophyta, Haptophyta, Cryptophyta, Dinophyta, Euglenophyta, Chlorarachniophyta, and Chlorophyta). The three most significant groups are green algae (Chlorophyta), red algae (Rhodophyta), and diatoms. Pigmentation, life cycle, and basic cellular structure are critical factors for classification. (4) A detailed classification of microalgae is provided by Richmond (5).

1.1 Ecology and Biodiversity of Microalgae

Microalgae are crucial for preserving ecological balance and energy transfer in the food web as a primary producer having huge genetic and phenotypic diversity in sizes.

Using sunlight, algae are photosynthetic organisms concerting carbon dioxide and H_2O photosynthesis to generate organic molecules, carbohydrates, fatty acids, proteins, and secondary metabolites. Ecologically, algae have a fundamental role in the food chain because it is responsible for the survival of almost all aquatic species (6).

The nutrient cycle is a process of nutrient movement through the ecosystem between organisms, the nutrient will be transformed into other chemical forms to be utilized by different organisms and recycled again, the microalgae play an important role in the nutrient cycling in several mechanisms (7). Like carbon dioxide fixation that is used by algae through photosynthesis (8), and also in nitrogen fixation since the algae could be used as soil fertilizers (9).

The worldwide distribution of microalgae in the ecosystem makes their genetic and phenotypic variety noticeable. The blue-green algae (cyanobacteria) are prokaryotes related to many common bacteria. These algae are also thought to be the ancestors of some higher algae and plants' chloroplasts (figure 1.1) (10). Dinoflagellates and euglenoids are mesokaryotes, which may have certain traits between prokaryotes and eukaryotes. Red algae resemble mesokaryotes, while green algae are closely related to higher plants as eukaryotes (11).

Ecological evaluations of aquatic environments need taxonomic-level identification of algae to determine the environmental conditions supporting the algae's growth and thus the composition will vary according to their taxonomic group (12).

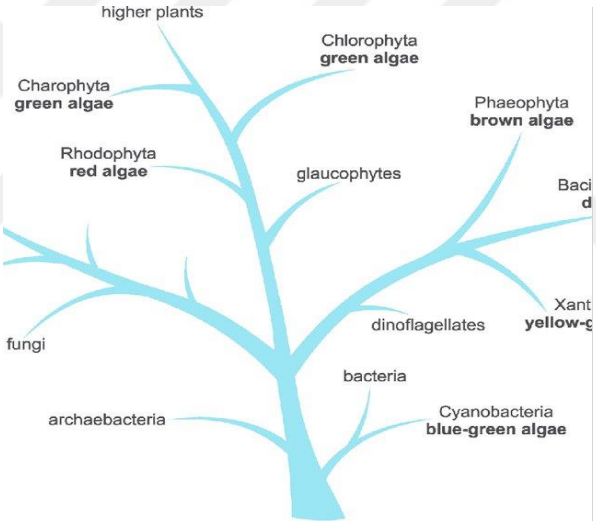


Figure 1.1. Simplified phylogenetic tree (12).

1.2 Photosynthesis in Algae

Photosynthesis is the process by which photosynthetic organisms attract sunlight and convert it into organic molecules and biomass (13). Photosynthesis has two phases: light reactions, in which the solar energy is converted into chemical energy phase; adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) and dark phases. In the dark reactions, ATP and NADPH (activated by the presence of photons) are the force of the Calvin-Benson-Bassham cycle to generate carbohydrates (14). Both stages occur inside the chloroplast, and the thylakoid membrane contains chlorophyll-containing pigment molecules.

Photosystem II (PS II), Cytochrome (Cyt) b6f, Photosystem I (PS I), and the ATP synthase are the four protein supercomplexes that make up the photosynthetic machinery in the thylakoid membrane. Only the reaction-center chlorophylls of PS II (P680) and PS I (P700) transform light energy into chemical energy, with all other reactions taking place. PSI and PSII both collect sunlight and permit two energetically uphill steps. PSII photochemically breaks down H_2O into O_2 and protons and reduces plastoquinone, then PSI oxidizes plastoquinol via Cyt b6f and creates the reductant NADPH, which converts CO_2 to carbohydrates (via the Calvin-Benson cycle) by using ATP from ATP synthase. (15).

In figure 1.2, one of the chlorophyll's electrons is activated by sunlight if it absorbs a photon with a specific energy. With numerous other chlorophylls, this chlorophyll molecule is situated inside the photosystem (16). This excitement is transferred from one molecule to another to tighten the reaction center complex (which also contains two molecules of chlorophylls) Plastocyanin, plastoquinone and ferredoxin facilitate the transfer of electron between photosystems.

The layout of Photosystem II (PSII), cytochrome b6f, plastoquinone (PQ), Photosystem I (PSI) connected with ferredoxin (Fd) and ferredoxin-NADP reductase (FNR), and adenosine triphosphate (ATP) synthase complexes, plastoquinone (PQ), plastocyanin (PC), light-dependent splitting water reaction into oxygen and hydrogen molecule (16).

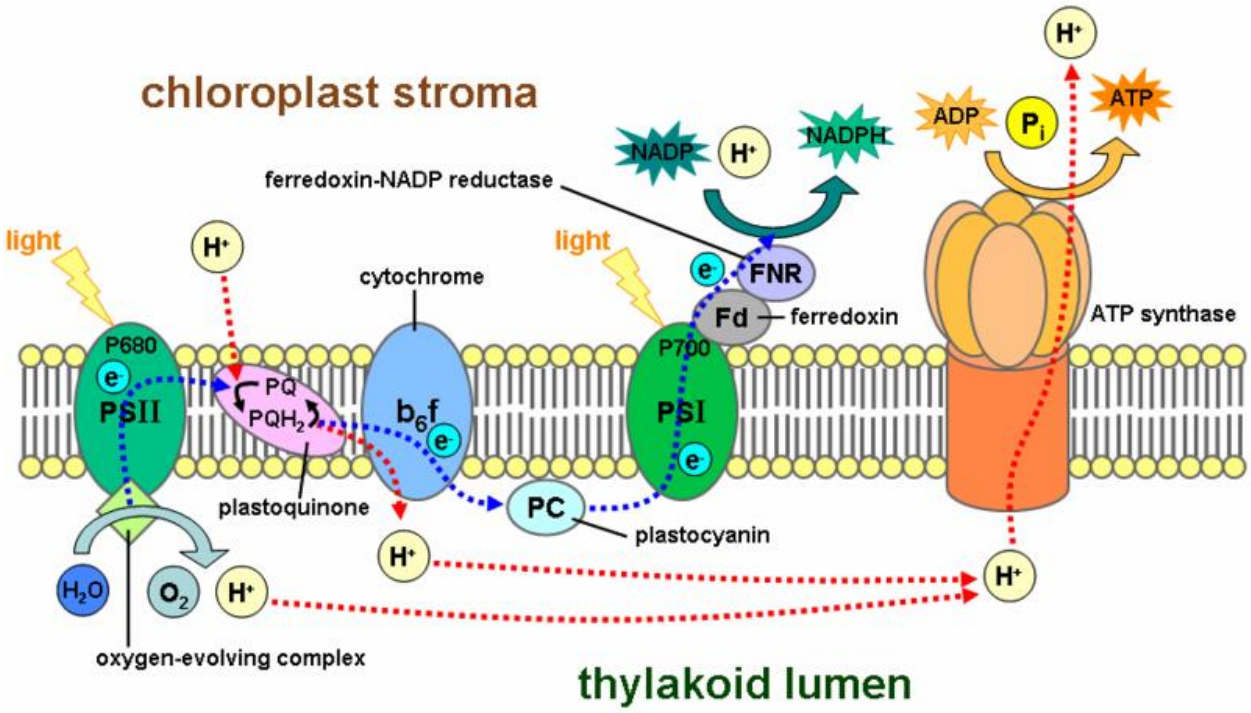


Figure 1.2. Graphic illustration of photosynthetic electron transport.

1.2.1 Light Harvesting System

A set of photo pigment proteins that absorb light energy, these pigments work together with other compounds catching the light into the cell to create a very complex Light Harvesting Protein-Chlorophyll system (LHPC), it also termed an antenna, that is implanted in thylakoid membranes (17). It provides exciting neighbors passed on between molecules until it reaches the reaction center to drive oxygenated photosynthesis.

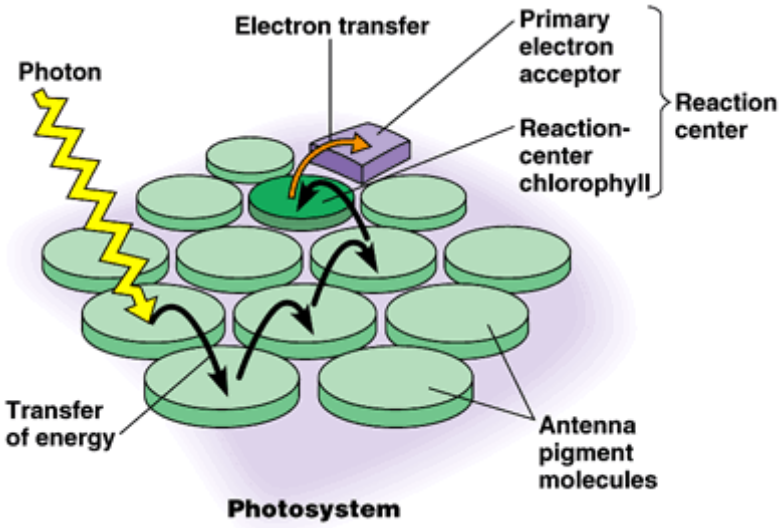


Figure 1.3. Energy flow through an antenna complex (19).

Higher plants and green algae have light-harvesting complexes (LHCs), including carotenoids, chlorophyll a, and chlorophyll b. Both photosystem basic structures consist of a central complex and a light-harvesting complex, designated LHC I and LHC II for PSI and PSII, respectively (18).

As we mentioned before, light must be captured by absorbing compounds in the initial step of the process of photosynthesis. Since chlorophyll a and other pigments have different absorption spectra, the combination present in each microalgal cell enables absorption in a much broader spectral range than if photosynthetic systems consisted of just one such pigment.

Photosystem I have nearly pure chlorophyll-a while photosystem II has chlorophyll-b in a significant level, these processes consume energy from wavelengths with somewhat different frequencies (680 and 700 nm) (15).

Phycobiliproteins (phycocyanin, phycoerythrin), which are light-harvesting accessory pigments, also carotenoids are correspondingly crucial components in the system (19).

Table 1.1. The absorption wavelength of the most common light harvesting pigments (23).

Pigment	Color	Absorption band range (nm)
Chlorophylls	Green	450–475 nm 630–675 nm
Carotenoids	Yellow-orange	400–550 nm
Phycobilins	Blue-red	500–650 nm

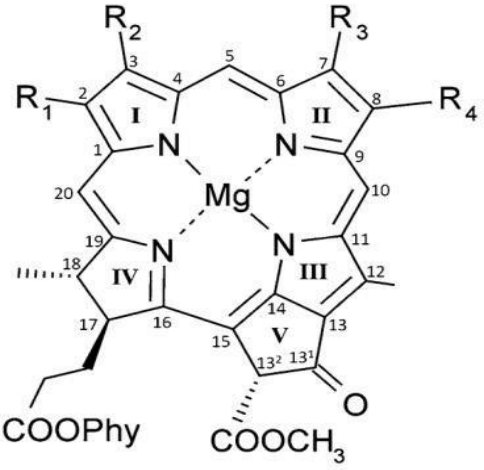
1.2.1.1 Chlorophyll

It is a group of pigments found in plants, algae, and other organisms. It produces energy and gives green color by absorbing visible light above blue and red wavelengths, which will only reflect green.

Chlorophyll is vital for photosynthesis; there are many different forms of chlorophyll, such as chlorophyll a and b, which differ only in one functional group inside the porphyrin ring (figure 1.5) to absorb photons in a certain way. Although chlorophyll d (Chl-d) may now be regarded as a significant pigment, the photosynthetic systems of cyanobacteria, photosynthetic protists (algae), and land plants are all structured around chlorophyll a (Chl-a) (20).

Chlorophyll c and chlorophyll d have been discovered in many types of algae and cyanobacterium with an important function in photosynthesis (21). Besides there is chlorophyll f (22).

Chl-a, which plays the photochemical role in both Photosystem I (PSI) and Photosystem II, has long been regarded as the most important of all these Chls. Chl-a is an excellent photosynthetic pigment for visible light and is likely to predominate in most oxygenic photosynthetic situations; however, it requires augmentation in the blue, green, orange and near-infrared regions of the spectrum, whereas Chl-d most likely plays a very limited role where mainly near-infrared (NIR) light (23).



Name	R_1	R_2	R_3	R_4
Chlorophyll *a*	CH_3	$CH{=}CH_2$	CH_3	CH_2-CH_3
Chlorophyll *b*	CH_3	$CH{=}CH_2$	CHO	CH_2-CH_3
Chlorophyll *d*	CH_3	CHO	CH_3	CH_2-CH_3
Chlorophyll *f*	CHO	$CH{=}CH_2$	CH_3	CH_2-CH_3

Figure 1.4. Structure of different types of chlorophyll (24).

1.2.2 Dark Light Reaction

Calvin cycle is a biological process found in the stroma of the chloroplast that uses ATP and NADPH to introduce energy. There are three stages to this: 1) An enzyme called RuBisCo captures CO_2 from the atmosphere and attaches it to 5-carbon sugar called Ribulose Bis-Phosphate RuBP to make 6 molecules of 3-phosphoglycerate (3-PGA), which is the rate-limiting step; 2) Using 6 molecules of ATP and 6 molecules of NADPH via reduction Glyceraldehyde-3-P is produced by converting 6 molecules of 3-PGA into 6 molecules (G3P) 3) Phosphorylation RuBP is regenerated, more ATP is used in phase 3 to return a portion of the pool of glyceraldehyde 3-phosphate to RuBP (25,26).

1.2.2.1 Carbon fixation

CO_2 is "fixed" from an inorganic form into organic molecules. Rubisco attaches the CO_2 to RuBP. The product of the reaction is a six-carbon intermediate that splits in half to form two molecules of 3-phosphoglycerate (26).

These pathways are centered on the catalytic activity of Rubisco (ribulose-1,5-bisphosphate carboxylase oxygenase) (Figure 1.4)

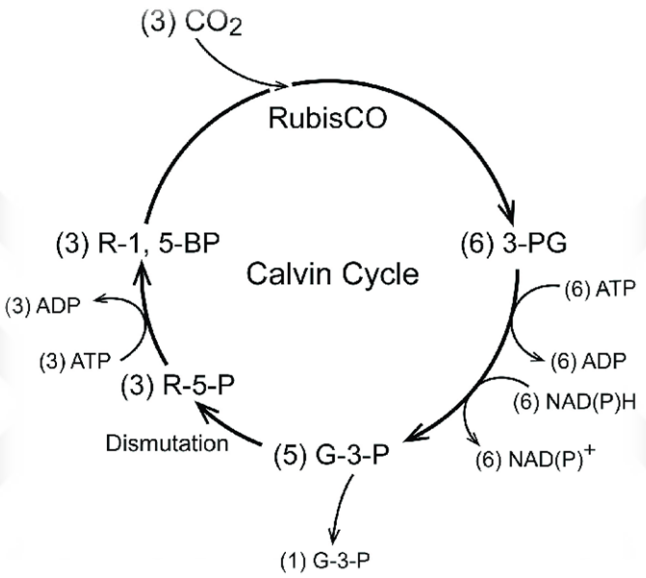


Figure 1.5. Graphic illustration of carbon fixation via Calvin Cycle (22).

1.3 Microalgae Biotechnological Potential

The microalgal biotechnological industry has an extensive consideration due to an exceptional source of bioactive compounds whereby huge biodiversity, e.g., lipids, proteins, carbohydrates, sterol, carotenoids, chlorophylls, nucleic acids, phenolic compounds, and nutraceuticals are produced in a limited period from the same harvested microalgal biomass (27). They are used for producing biomass with various compounds that can be utilized in the food, medication, cosmetic industries, and many other applications (28).

Due to a variety of species, the algae of the marine environment will offer a nutrition supply and valuable molecules (29). Protein, lipids, and carbohydrates are

the main components of algae. Protein is an alternative resource as the cells of algae can synthesize all amino acids; likewise, they can supply the essential ones to humans and animals (30). The average quality of many algae is identical to or even higher than that of other common plant proteins (31).

Microalgae also represent a vital supply of almost all essential vitamins (e.g., A, B1, B2, B6, B12, C, E, biotin, nicotinate, folic, and pantothenic acid). Moreover, pigments like chlorophyll, carotenoids, and phycobiliproteins are present (31). Then different algae species contain these bio components in different proportions (32).

Eukaryotic microalgae can be utilized in CO_2 emission reduction procedures and wastewater treatment; besides, they are the most potential feedstocks for producing large quantities of bio-based commodities, including food, feed, fuel, and high-value metabolites (13).

Algae are one of the main targets for sustainable biofuel generation and CO_2 consumption due to their photosynthetic capabilities. Furthermore, the algae provide us with food, fuel, and many other essential products for the past and potentially in the future (6).

Usage in bioenergy and pharmaceutical industries is considered a cost-effective source of energy. Biofuel applications, biodiesel being the leading interest as microalgal biomass is a unique input for biorefinery, by the composition of biomolecules, especially fatty acids, that are measured as a powerful alternative for biodiesel production (33).

Biomedical applications are obtained from the omega-3 fatty acids, carotenoids, and phycobiliproteins (34). Likewise as a source of active pharmaceutical substances that can be utilized to treat cancer, viruses like AIDS and Herpes Simplex, and drug-resistant bacterial strains (6). It can be used as antimicrobial treatment against bacteria and viruses (35). For other practical uses include cosmetics, agricultural herbicides, prebiotics, and nutraceutical applications (36).

Certain microalgae are considered the most significant therapeutic agents to remove or uptake various poisons. For instance, *S. quadricauda* contains substances like dimethomorph, pyrimethanil, and isoproturon; it can be used as a fungicide and herbicide (37).

The availability of high value products leads to increasing the market size production of microalgae. Global market size of microalgae is 11.4 Bn $ in 2022 and expected to be in 2028 to total 18.1 Bn $ (38).

1.4 The Mass Production of Microalgae in Biotechnological Application

Microalgae are capable to grow rapidly double their biomass and reach high yields to provide funding source of bioproduct (39). Without applying pesticides, herbicides, or fungicides (29,40).

In general, microalgae culturing system can be mostly classified into two categories, which are the open pond and photobioreactor. (Figure 1.6)

For the new development of photobioreactors, the light source has turn out to be one of the major to take into consideration to achieve an adequate irradiation, since microalgae are phototrophic organisms, which indicates that the culturing altered by the huge effect of light (41).

a)

b)

Picture 1.1. Microalgae culturing system: a) lab-scale open pond system (43). b) closed-system photobioreactor (44).

Table 1.2. Comparison between open pond cultivation system and photobioreactor.

Open pond	**Photobioreactor**
Suitable for large scale, low energy demand, simple and easy to scale up (42).	Closed system with efficient design, unsuitable for large scale (43).
Cost-efficient cultivation system (42).	High initial cost, applicable for specific species. (43).
Low biomass productivity (43).	Enhanced metabolic efficiency and improved biomass productivity (43).
High tendency for contamination by bacteria or protozoa which generates unsuitable and toxic products (44).	Highly controlled growth conditions for microalgal culture and reduce the chances of contamination (45).
Unable to monitor certain growth parameters such as temperature and light intensity which could alter the growth rate of microalgae (44).	Offer well management on culture conditions (45).

1.5 Environmental Impact of Microalgae

Cultivation of microalgae does not need only arable land; it can also be achieved by using wastewater which can support the bioremediation of wastewater (46).

In aquatic ecosystems, some microalgae may effectively remove the contamination from highly polluted wastewater overloaded with nutrients (nitrogen, phosphate) and toxic metals to avoid the growth of unfavorable phytoplankton blooms (47). Moreover, due to microalgae's CO_2 bio fixation capacity, it notably helps in decreasing the greenhouse effect (4).

The uptake of nutrients from wastewater to support microalgal growth, with using sunlight and CO_2, the microalgae biomass will be provided as it could be used for other applications, besides high value chemical will be produced (Figure 1.1) (48).

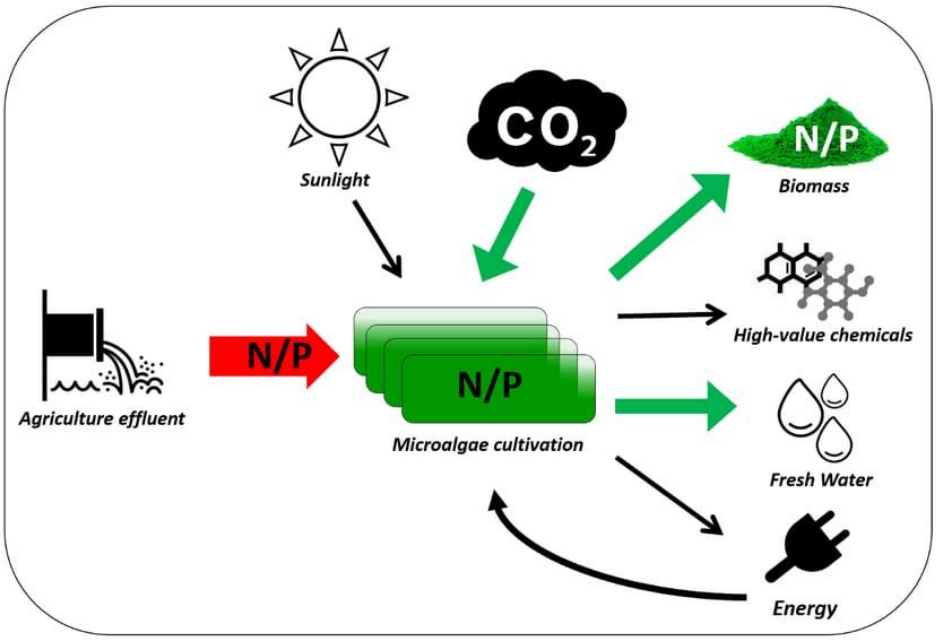


Figure 1.6. Microalgae recycling nutrient with wastewater treatment (8).

1.6 Light

In the cultivation system, microalgae needs light, nutrients, and carbon dioxide in order to produce organic compounds. Light demand is one of the most crucial factors, in order to improve the photoautotrophic growth of microalgae; light should be delivered at the proper intensity, duration, and wavelength (49). Sunlight and artificial lamp use are the two ways to generate light energy. The benefit of using sunlight as a light source is that it is free and available. On the other hand, day/night cycles, shifting weather patterns, besides seasonal fluctuation and unstable intensity are some of its drawbacks. Besides, each of these elements depends on the environment and the region (50).

The light must be regulated in photosynthesis, especially light intensities and wavelengths since their contribute to the formation of intracellular content and the production of biomass (49). Light intensity should be optimized according to the type of species although as we increase the light intensity, the growth will be increased. The growth will then be negatively impacted by too little or too much light intensity, which may result in photoinhibition or photo limitation (51).

Artificial lighting can prevent these irradiance level changes including fluorescent lamps (FLs) and light-emitting diodes (LEDs). Likewise, sustained lighting will result in enhanced productivity as biomass is not evaporated during the night (50). For autotrophic microalgae, which use the photosynthetic process to create organic molecules, visible light is their principal source of energy which is between 400-750 nm. (Figure 1.7)

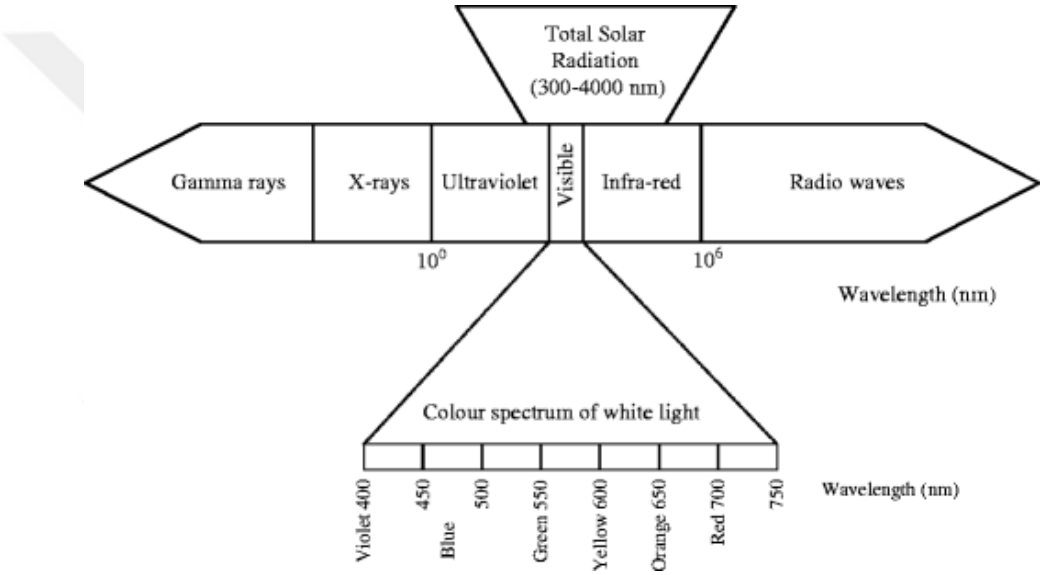


Figure 1.7. Visible light spectrum pattern in the electromagnetic spectrum (49).

1.6.1 LED Light

It is a solid-state semiconductor source that when forward voltage is applied, emit narrow-spectrum light. Efficiency and longevity are the two benefits of LEDs that are most frequently mentioned.

Compared to other artificial light sources, the most recent high-power LEDs are essentially equivalent to high-intensity discharge (HID) lamps and more efficient than incandescent and fluorescent lamps (52). As presented in the figure (1.8), the LED's design has a greater conversion efficiency, and has a longer expected life

Since LED lights typically emit little heat, placing the LED close to an algae specimen is safe. It can distribute light uniformly and sufficiently to penetrate through the culture in the photobioreactor. Additionally, it is portable, sturdy, and flexible to use with quick response, and mercury-free (54).

LEDs can last up to 50,000 hours longer (like fluorescent lamps and incandescent lamps) due to their high quality in temperature control than traditional lighting sources (52).

As it shown in the figure (1.10), LED spectrum could provide the wavelength with higher level of accuracy. Comparing sunlight to LED light, LED is close to mimic sunlight spectrum.

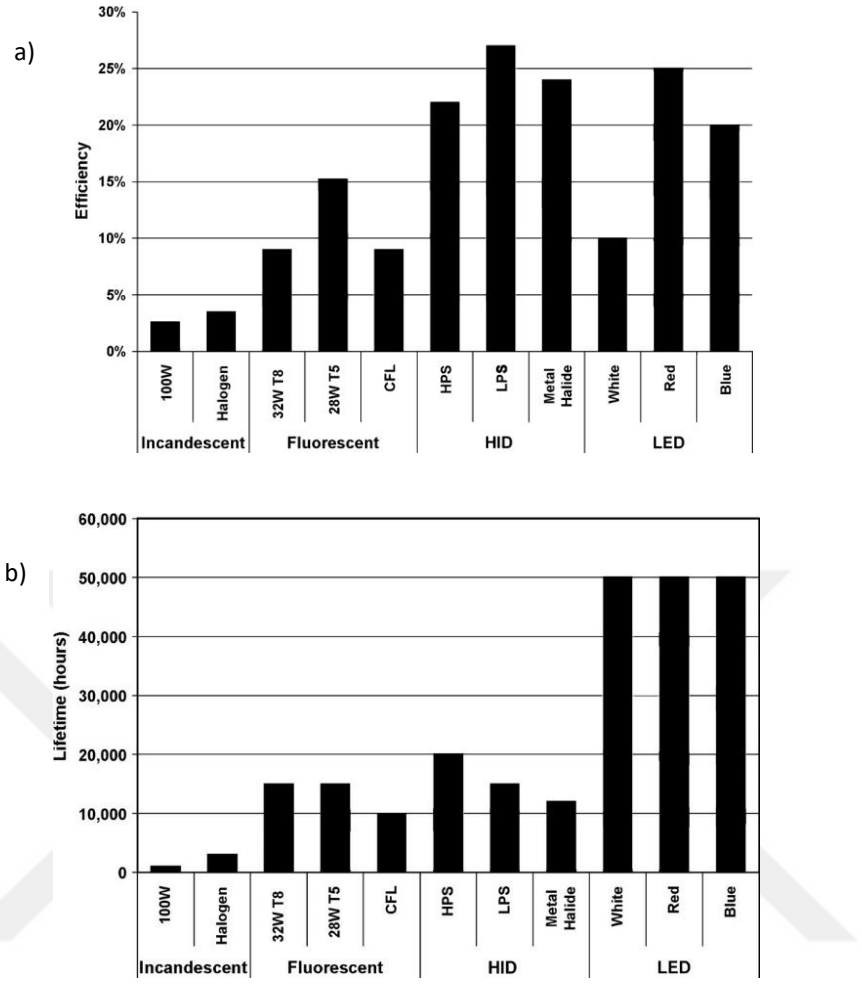


Figure 1.8. Comparing: of various electric light sources: a) the luminous efficiency. b) the lifetime.

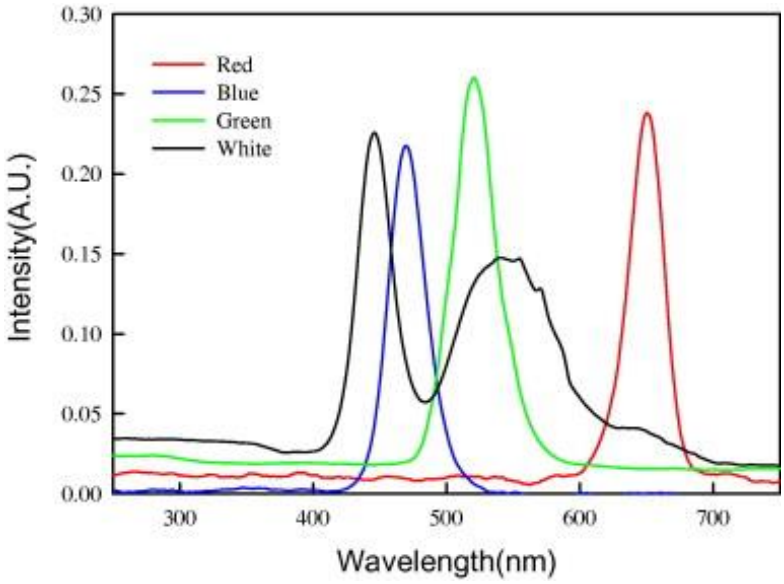


Figure 1.9. The emission spectrum of blue, red, green and white LED light (54).

a)

b)

c)

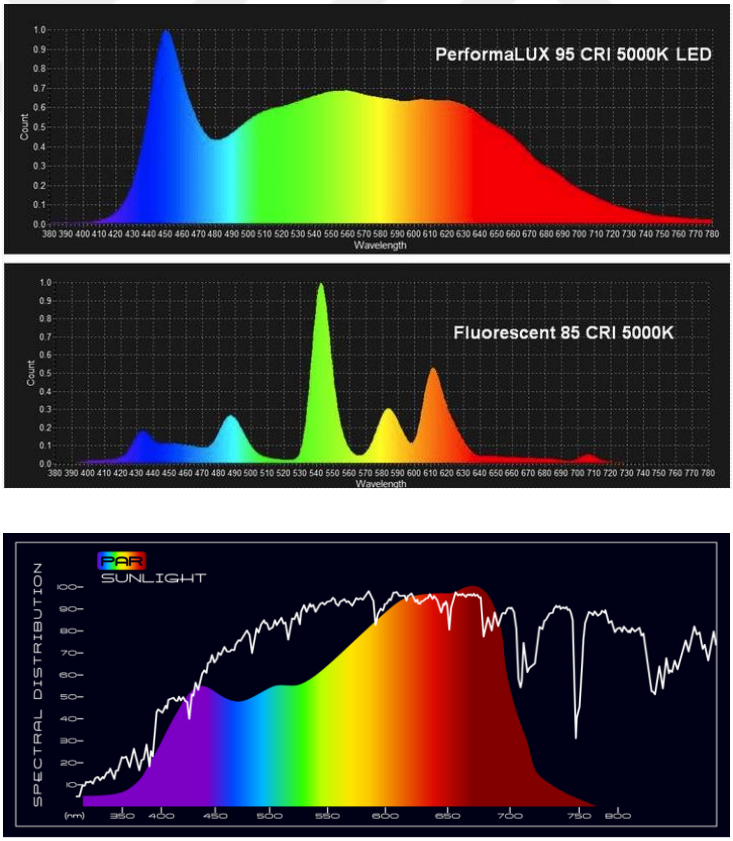


Figure 1.10. Comparison between a) LED, b) the flourescent light spectrum, and c) sunlight spectrum (51) (52).

1.7 *Scenedesmus quadricauda*

Scenedesmus was first described by Meyen in 1829, it is a genus of very diverse species of colonial green algae, they are nonmotile along with mostly consist of oblong cells joined together in a single row (53). *S. quadricauda* is a quick growing non-motile green alga consisting of 2-4-8 oblong cylindrical cells usually in a single row (53).

Systematic position of *Scenedesmus* is as follows: (53).

Empire: Eukaryota

Kingdom: Plantae

Phylum: Chlorophyta

Class: Chlorophyceae

Order: Sphaeropleales

Family: Scenedesmaceae

Subfamily: Scenedesmoidea

Genus: Scenedesmus Meyen 1829

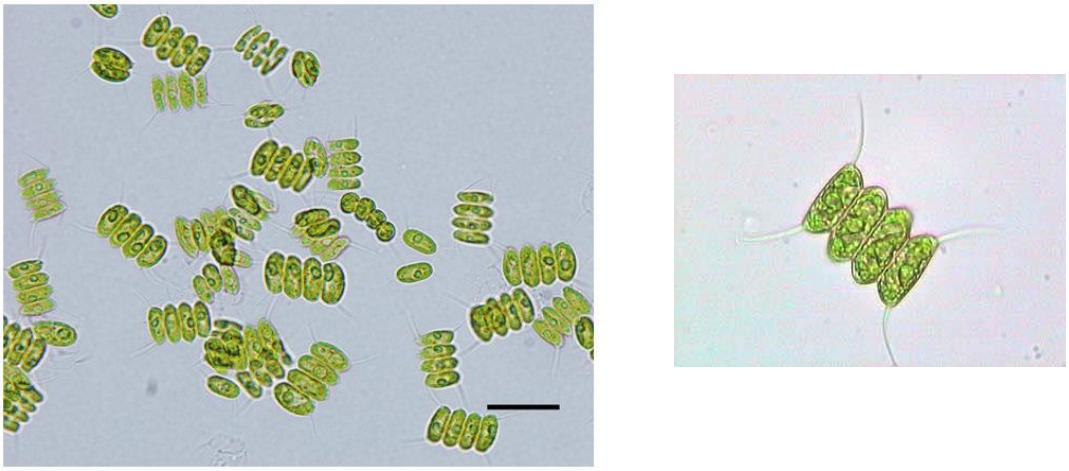


Figure 1.11. Illustration of the *Scenedesmus quadricauda* freshwater algae 4 or more cells are connected side by side, organized linearly or zigzag; the cell wall is often smooth (54,55)

1.7.1 Life Cycle of *S. quadricauda*

S. quadricauda's were previously described by Egan and Trainor (56), it was found that *Scenedesmus* most commonly replicates through asexual mode in both culture and nature. Moreover, autospores and autocolonies are produced. Each *Scenedesmus* has the ability to produce either a unique unicellular stage or a 4 celled colony through two subsequent, non-vegetative cell divisions.

1.7.2 Cell Cycle of *S. quadricauda*:

Binary fission is when the mother cell divided into two daughter cells, most of green algae divided by complex multiple fission; A common cell cycle while the mother cell can divide into more than two daughter cells. It consists of a growth phase, synthesis phase, second growth phase, and mitosis, followed closely by cell division (57).

The mother cell divides into 2n daughter cells, where ***n*** is an integer from 1 up to 15, the number of daughter cells is highly affected by external conditions, which depends on the species type and the growth rate. Hence, under very favorable condition the maximum number of daughter cells will be produced (i.e., 8 to 16 or ***n*** = 3–4) (57).

S. quadricauda have applied as model organisms, primarily for research of photosynthesis, and cell-cycle regulation (58). In special environment, the cells normally divide twice or three times during a single cell cycle then the two-celled coenobia of *S. quadricauda* will be produced which be able to yield four- or eight-celled. (Figure 1.11) Perhaps certain situations may additionally affect the cells in celled-coenobia. One of the studies showed that if we improve in the duration of the light interval, highly developed cells in eight-celled coenobia will be triggered (59).

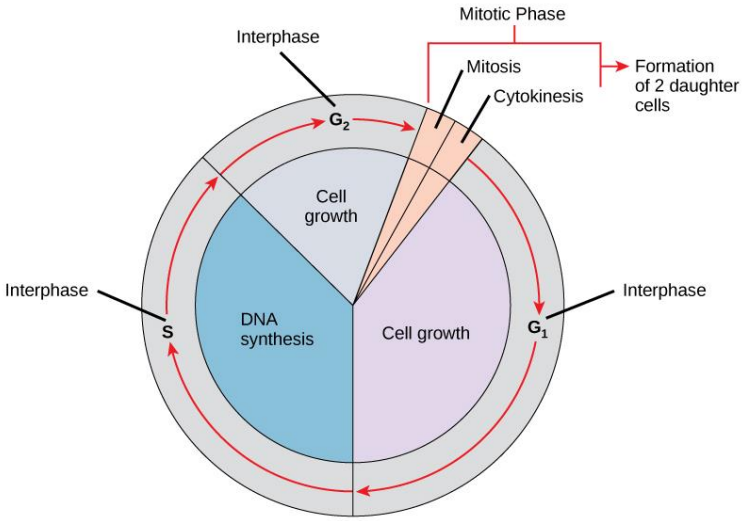


Figure 1.12. The cell cycle phases (60).

Growth, DNA replication, nuclear division, and cellular division, make up the typical reproductive sequence. It is depicted as a progression of the four stages G1, S, G2, (these three phases are called interphase, where the cell be prepared to the cell division) and M, which define the standard cell cycle through binary fission. In general, growth (G1 phase) is the stage where the necessary cell size is reached (61).

When the cell reaches a limiting value and composition of vital components, including energy reserves, at this point; such a cell is capable of moving through the reproductive cycle even in the absence of additional growth. This is called the commitment point or transition point or induction of division. The commitment point (CP) sly crucial for cell cycle development, since it could divide the algae cell cycle into pre / post CP stages (57).

The primary distinction between multiple fission cell cycles and binary fission cell cycles is that several commitment points are reached within a single cell cycle (57). Pre-replication stage, or pS, occurs between the CP and the start of DNA replication (57,61). The DNA replication–division sequence, is composed of DNA replication (S phase), G2 phase, nuclear division (M phase), and cytokinesis (C) (61).

In some algae, the transitional period between nuclear and cellular division; is thought to be extended referring to this phase as the G3 phase (57). It is simpler

to explain the mechanisms underlying the multiple fission cell cycle if we imagine the cell cycle as a sequence rather than a cycle.

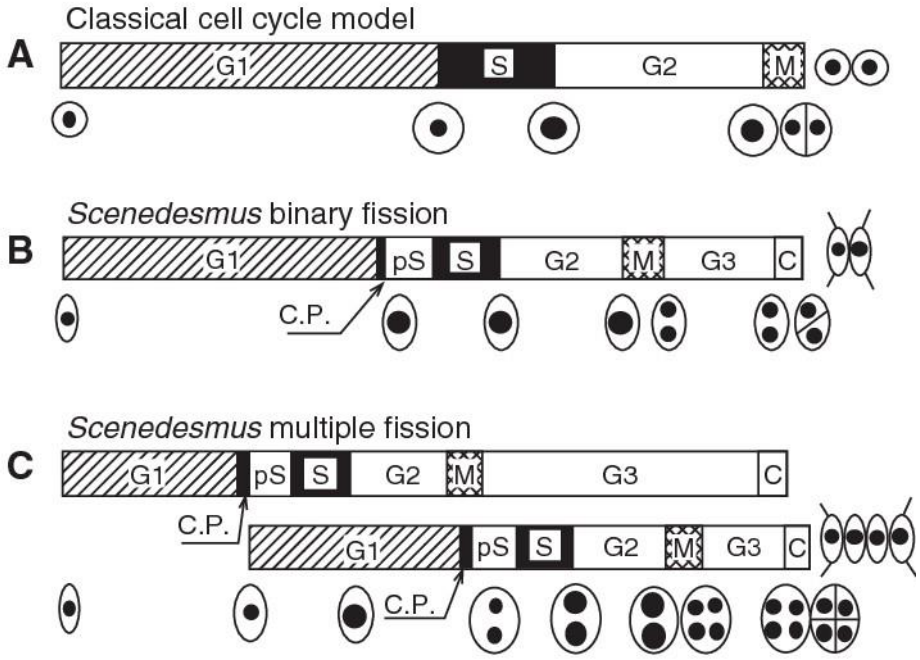


Figure 1.13. Diagram showing a comparison of classical and multiple fission cell cycles of *S. quadricauda.*

Diagram includes (A) classical cell cycle model for binary fission. (B) *Scenedesmus* divide into two daughter cells, *Scenedesmus* binary fission. (C) *Scenedesmus* that divides into four daughter cells, multiple fission two partially simultaneous overlapping cycles of growth and reproductive events appear in a single cycle in cells. The size and number of nuclei showed by the black marks inside, and large black spots show the development of the DNA (57).

The process started by the phase G1 by the size of the cell is achieved, and it is completed once the commitment point is reached (CP), which is the stage when the cell starts to trigger and terminate the sequence of the process, the processes is light and growth dependent before reaching CP whereas the remaining process of the cell cycle is light independent after CP. CPs for division into 2 (**B**), four (**C**) or eight. Varying on the length of illumination and light intensity. pS; is the replication phase, S; the DNA replication phase, G2; initiating mitosis then the nuclear division

M; and introducing the process leading to cell division at G3, the cell is cleaved at C phase. (Figure 1.12)

Multiple fission cell-cycle patterns basically divided into consecutive and clustered types; in *Scenedesmus* the pattern is consecutive (It also called *Scenedesmus*-type cell cycle). More than one CP, several rounds of DNA replication and nuclear divisions appear consecutively during the cell cycle, and cells develop into polynuclear (57).

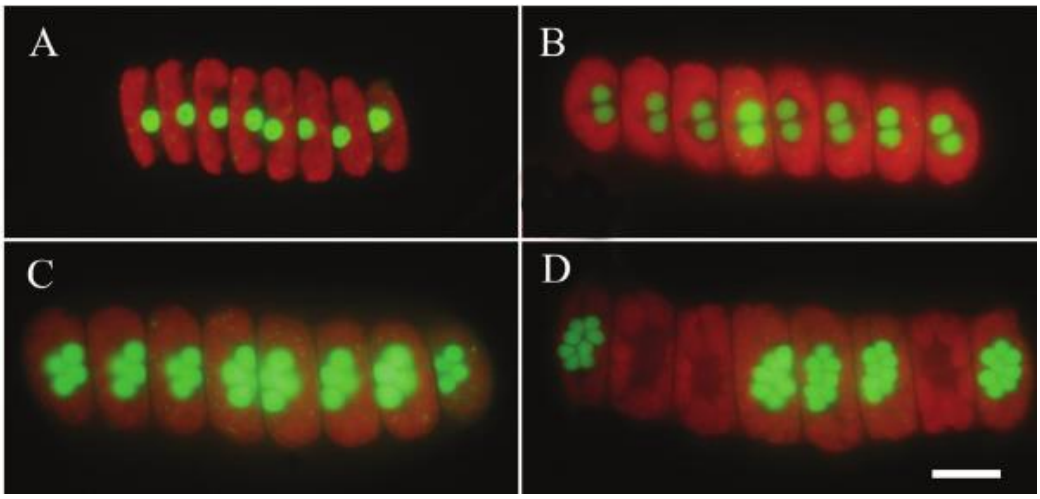


Figure 1.14. Fluorescence photomicrographs of consecutive pattern of *Scenedesmus quadricauda*; eight-celled coenobia of *Scenedesmus quadricauda* during the cell cycle. Nuclei showed as *yellow- green spots*. Chloroplasts as a *red color (*a) uninuclear daughter coenobium, (b) binuclear coenobium. (c) tetranuclear coenobium. (d) mother octanuclear coenobium. Dividing cells remained unstained (57).

1.7.3 The Effect of Different Light Wavelength on Algae

Water molecules has strong absorption of light with long or short wavelength; thus, the blue-green light will be entered deeply inside the water (62).

Different light wavelength will influence the growth of algae, as it is species-dependent due to the variation in pigments and photoreceptors thus different photosynthetic adaptation will be observed.

In general, the best response in most microalgae is under blue ($\lambda \approx$ 420-470 nm) or red light ($\lambda \approx$ 660 nm), this is related to the pigments in light harvesting complex which will absorb the suitable wavelength (63).

Chlorophyll a, chlorophyll b and the accessory pigments (phycobiliproteins) work together to absorb different light (red, yellow, green and blue). Chlorophyll a responsible for absorption of blue (430 nm) and deep red (630 nm), Chlorophyll-b has the peak of blue and the red light (460, 630 nm) (64). whereas phycobilin (accessory pigments) is related to the green wavelength (65).

Blue wavelength is used for gene transcription regulation and enzyme activation according to Ruyters (66). It will affect the photoreceptor such as cryptochromes, phytotropins and many other important receptors in plants and algae (67). Thus, blue light strongly boosts the formation of chlorophyll in many microalgal cultures.

Likewise, the blue light may involve in cell size regulation as in *Chlamydomonas reinhardtii* species, by shifting the CP of the cell cycle, larger cell size will be obtained (68).

Research of Atta et al. (69) was shown that the blue LED light has a short cultivation period, the blue LED is a more effective energy source than white fluorescent light for *Chlorella vulgaris*. Additionally, blue photons have a higher energy than what is needed for photosynthesis, they may trigger non photosynthetic quenching (NPQ), which produces reactive oxygen species (ROS) (70). Therefore, algae and plants produce photoprotective pigments to shield the photosynthetic system from ROS (e.g., xanthophylls) (70).

However, a study of Das et al. (71) showed that compared to exposure to green, red, or multichromatic white light LEDs, *Nannochloropsis* sp. produced more biomass when exposed to a monochromatic LED source of blue light.

Red wavelength has long wavelength with a crucial effect in growth in many algae, like in *Chlorella vulgaris* (72), It may promote the growth rate by accelerating the cell cycle and thus smaller cell size will be produced (73).
The growth of microalgae will be improved by using red or blue light, the combination of red and blue light is an important approach for optimal growth and biomass production in several species, since it will provide essential light quality to perform the photosynthesis properly. The ratio between red and blue is species-dependent as it is seen in recent studies (74,75).

White light has multichromatic wavelength, a study on *Scenedesmus* species by Ho et al. (76) showed that when compared to red, blue, and green monochromatic LEDs, white LEDs increased the amount of biomass produced and had a higher lutein production efficiency in S. obliquus FSP-3.

The exposure to accessory wavelength ($\lambda \approx$ 500-630 nm) is not enough to promote high growth in microalgae, as it will be observed under green light in most phototrophic algae (63). as in *Nannochloropsis* species study that performed a lowest growth rate is under the green light (77). Green wavelength may induce the pigmentation in microalgae (78). Also, several studies on plants have discovered that green light, especially when used as a monochromatic spectrum, suppresses plant species. (79,80)

Despite several studies have been investigated that the green wavelength is not essential considered to be least effective wavelength for photosynthesis for growth in plant and microalgae. But there is strong evidence supports the concept of green light is essential for photosynthesis and physiological reactions to the environment.

Studies on plants presented that chloroplasts will reflect only 10–50% of the green light that has a wavelength between 500 and 600 nm, the rest will be absorbed by pigments (81).

Phytochromes and cryptochromes are green light receptors but it is difficult to define the response of green light since these receptor are also responsible for red and blue light absorption spectrum. (79)

Green light may could penetrate deeper in the plant and dense microalgae, strong influence could be observed in many species (82). *S. quadricauda* studied by Niizawa et al. (83), dense microalgal cultures grown under green wavelength

illumination showed more effective biomass formation due to the appearance of "detour" and "sieve" effects.

Another study on *Spirulina platensis* conducted by Ravelonandro et al. (84) revealed that the green light was the key to achieving the highest levels of productivity. *Botryococcus braunii* indicated that mixed red-green-blue LEDs created the highest biomass, hence a novel strategy for combining green light with other LED light has been suggested (85).

2. AIM AND SCOPE OF THE STUDY

Aim of this study is focused on highlighting the best growth rate under illumination of a monochromatic and mixed dichromatic LED light spectrum of wavelengths blue, green and red LED light, in order to understand the interaction effects of light wavelength on the growth of the strain *S. quadricauda*. Thus, studying the impact of a gradual increase in the green LED spectrum on *S. quadricauda* development. Blue and red wavelengths have a significant role in photosynthesis among other light spectrum, but only a few studies have been conducted to examine the influence of employing green LED light spectrum.

The objective of this study is to investigate effects of monochromatic and combination of different blue and red-light spectrums together to find out optimum combinations for better growth rate of *S. quadricauda*. Then with gradually added green light proportions, we aim to test if there is an additive effect of green light in the growth rate of *S. quadricauda.*

3. MATERIALS AND METHODS

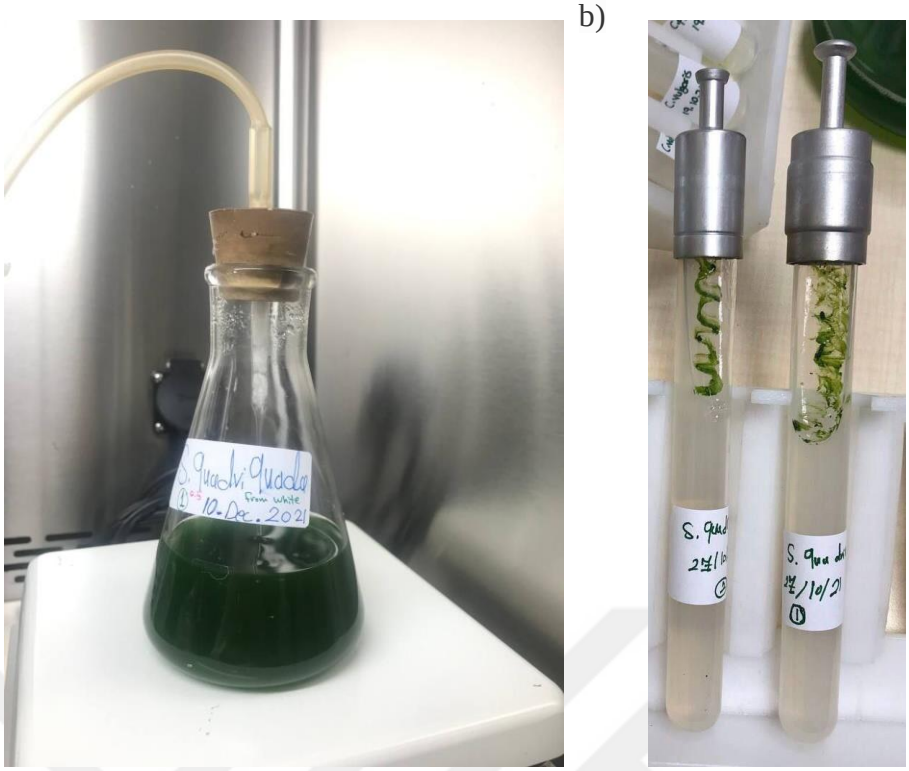
3.1 Microalgae Strain and Culture Condition

S. *quadricauda* microalgal stock was maintained in BG-11 medium (Table 3.1) under a LED light a 16:8 hrs light/dark photoperiod and "aerated" by a mixture of air and CO_2 (2%, v/v) at temperature 25°C ∓ 2°C with light intensity of 50 ∓ 2 µmol photon m^{-2} s^{-1} through inoculation of autoclaved flasks (250 ml capacity) every two weeks. Preparing the BG-11 mixture by using distilled water with the macro and microelement component to provide the appropriate medium for growing. The pH was adjusted in range of 7.2–7.4.

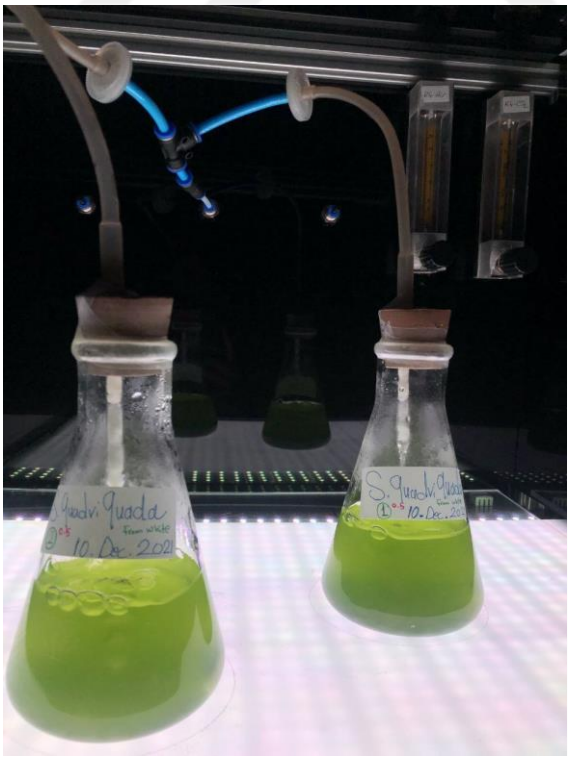
Table 3.1. Nutrient composition of BG-11 medium (83).

Macronutrient	**Concentration (mg/L)**
NaNO3	1500.00
K2HPO4 X 3H2O	40.00
MgSO4 X 7H2O	75.00
CaCl2 X 2H2O	36.00
Citricacid (C6H8O7)	6.00
Ferric Ammonium Citrate	6.00
Na2EDTA X 2HO	1.00
Na2CO3	20.00
Micronutrient	**Concentration (g/L)**
H3BO3	2.860
MnCl2 X 4H2O	1.810
ZnSO4 X 7H2O	0.222
NaMoO4 X 2H2O	0.390
CuSO4 X 5H2O	0.079
Co (NO3)2 X 6H2O	0.0494
Agar (solid medium)	15 g/L

a) b)



Picture 3.1. Algae culture in laboratory environment a) liquid medium b) solid medium (By adding agar after autoclaving)



Picture 3.2. LED light exposed effluent sample.

3.2 Experimental Culture and Light

Two experimental set up were used in the study. In the first experiment: 6 different light regimes with 3 replicates were applied as white, blue, red, green, R/B in the ratio of 1/3 and R/B in the ratio of 3/1 respectively in 250 mL of Erlenmeyer containing 200 ml BG-11 culture medium. In the second experiment: 6 different light regimes with 3 replicates were applied, green light was supplemented gradually into the combination of red: blue (1: 3) at the light intensity of 2, 4, 6, 8 and 10 µmol m^{-2} s^{-1} as an expense of red and blue light in 250 mL of Erlenmeyer containing 200 ml BG-11 culture medium, feed with aeration by a mixture of air and CO_2 enriched with (2%v/v) , under the light intensity of 50 µmol m^{-2} s^{-1} at 18/6 hours light/dark periods with constant temperature 25°C ∓ 2°C for 30 days of experimental duration.

The experimental light chambers were isolated with light proof Plexiglas to avoid interfering from external light sources. LED light intensities were adjusted using Ardinuo based software systems and measured using a portable light spectrometer (OHSP-350; Hopoocolor, China).

Experiment I: Light condition applied in the first experiment to find out the optimum light combinations of blue and red. Experiment II: R1/B3 group from the first experimental part used as control group in the second experiment, with supplementation of green LEDs light green with constant ratio (4%) as a fraction of red and blue light to explore the effect of green light on S. *quadricauda*.

Table 3.2. Different ratio of light intensity between R, G, B used at experiment I thus that they deliver the same number of photons 50 (μmol) per unit of time (s), per unit of algal suspension to investigate which one is the control one for the next experiment.

Culture	**Composition of different arrangements of LEDs (Total= 50 μmol m^{-2} s^{-1})**			**The percentage % of red: green: blue**
	Red	Green	Blue	
1. White (W)	20	16	13	40:32:26
2. Green (G)	0	50	0	0:100:0
3. Blue (B)	0	0	50	0:0:100
4. Red (R)	50	0	0	100:0:0
5. R1/B3	12.5	0	37.5	25:0:75
6. R3/B1	37.5	0	12.5	75:0:25

Table 3.3. Different ratio of light intensity between R, G, B used at experiment II by increasing green light in percentage of 4%.

Culture	**Composition of different arrangements of LEDs (Total= 50 μmol m^{-2} s^{-1})**			**The percentage % of red: green: blue**
	Red	Green	Blue	
1. Control	12.5	0	37.5	25:0:75
2. Treatment 1	12	2	36	24:4:72
3. Treatment 2	11.5	4	34.5	23:8:69
4. Treatment 3	11	6	33	22:12:66
5. Treatment 4	10.5	8	31.5	21:16:63
6. Treatment 5	10	10	30	20:20:60

The chromatic diagram for the second part of the experiment of all treatments using the LED light combination is shown in figure 3.1 below.

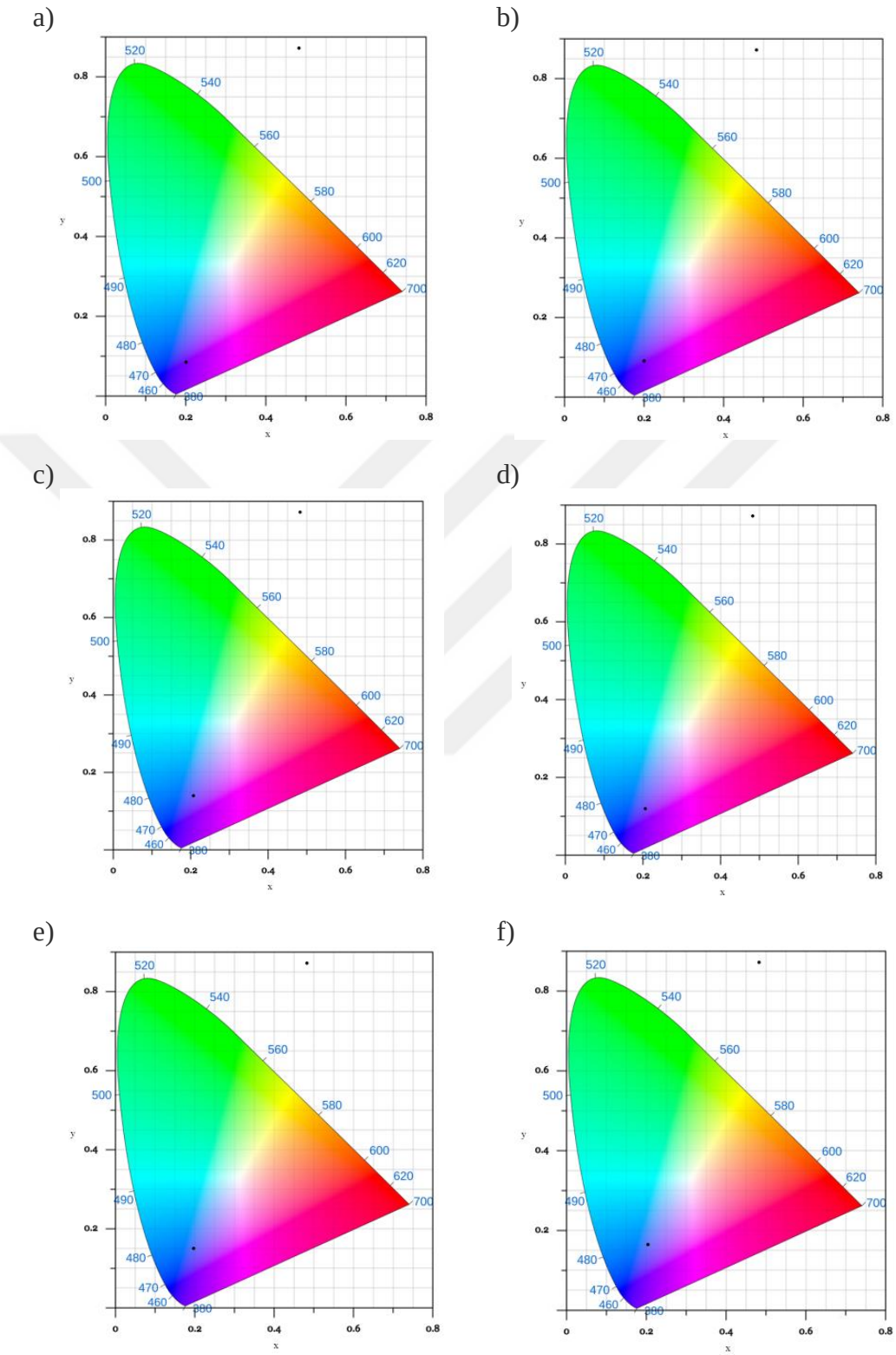


Figure 3.1. Chromatic diagram of experiment II; a) control group; b) treatment 1 (4% green light supplementation); c) treatment 3 (8% green light supplementation); d) treatment 3 (12% green light supplementation); e) treatment

4 (16% green light supplementation); f) treatment 5 (20% green light supplementation)

Picture 3.3. Working area under the laminar flow sterilized with ethanol in front of fire gas.

3.3 Measuring Optical Density of the Experimental Cultures

The growth pattern of the inoculated cultures was determined by measuring the Optical Density (OD) at 669 nm via UV VIS spectrophotometer every day by taking 2 ml of the growing sample under sterile condition.

Picture 3.4. The cultivation condition of *Scenedesmus quadricauda* under six light sources.

3.4 Chlorophyll Measurements

The chlorophyll of microalgae was estimated as follows, at every 48hr interval; Around 1 ml of the microalgae samples were transferred into 1.5 ml Eppendorf tube (Transferred into 15 ml Eppendorf tube later) and centrifugated at 5,000 rpm for 5 min. The supernatant was discharged, and 1 ml of methanol was added to the pellet by mixing with pipetting then vortex for 5 min, subsequently sonicated with the glass beds for 30 min at water bath at room temperature.

Samples were incubated overnight at 4°C then centrifugation again for 5 min at 15,000 g. After supernatant is removed, if the pellet is still green, additional 1 ml methanol was added, vortexed incubated for 2 additional hours, or 24hrs until the pellet becomes white. Repeat the procedure until the white pellet in the bottom will appear to make sure that the whole extraction of chlorophyll is performed.

Measuring the absorbance of total chlorophyll using spectrophotometer at different wavelength (645, 653, 663, 665, 666, 720).

Besides using the following equations of Arnon (1949) to calculate the chlorophyl content of each sample:

Chlorophyll a (µg/mL) = 12.7 (A663) − 2.69 (A645)

Chlorophyll b (µg/mL) = 22.9 (A645) − 4.68 (A663)

Total chlorophyll (µg/mL) = 20.2 (A645) + 8.02 (A663)

3.5 Dry Measurements

10 ml of the culture suspension of *S. quadricauda* were taken for dry weight estimation. Filtration by using a glass microfiber filter. The microalgae were entrapped in the filter. Drying in the oven 70 ºC overnight. The difference between the filter weights prior to and following filtration was used to determine dry weight.

3.6 Statistical Analysis

All experiments were performed in triplicates. Statistical analyses were carried out using the one-way ANOVA and Tukey's post-hoc test to use the SPSS software to determine the significance of the difference with a 95% confidence interval.

4. RESULTS

4.1 Monochromatic and Dichromatic LED Light

The growth rate of cultures was calculated under monochromatic white, blue, red, and green light exposure, as well as dichromatic red: blue in the ratios of 1:3 and 3:1.

The experiment was conducted under the light intensity of 50 µmol m^{-2} s^{-1} at 18/ 6 hours light/ dark periods “aerated” by a constant mixture of air and CO_2 (2%, v/v) at constant temperature 25°C ∓ 2°C.

4.1.1 Growth Rate

Growth rate of *S. quadricauda* measured as Optical Density (OD) at 669 nm was given in the figure 4.1. The highest OD value was recorded in the combination of R3:B1 at the end of the experiment.

The green and white lights have the lowest growth rates among all treatments. Table 4.1 show the comparative statistical significance level between the treatments based on Bonferroni P-value in Post-hoc Tukey HSD test. Almost all the growth rate values between the treatments were statistically significant and the order of growth rate is as follows: R3/B1> R1/B3> Blue> Red> White> Green. Seems that blue and red lights are more effective than white light on the growth rate of *S. quadricauda.* Similarly, blue and R1/B3 light setups displayed growth rates that were nearly identical (No statistical differences).

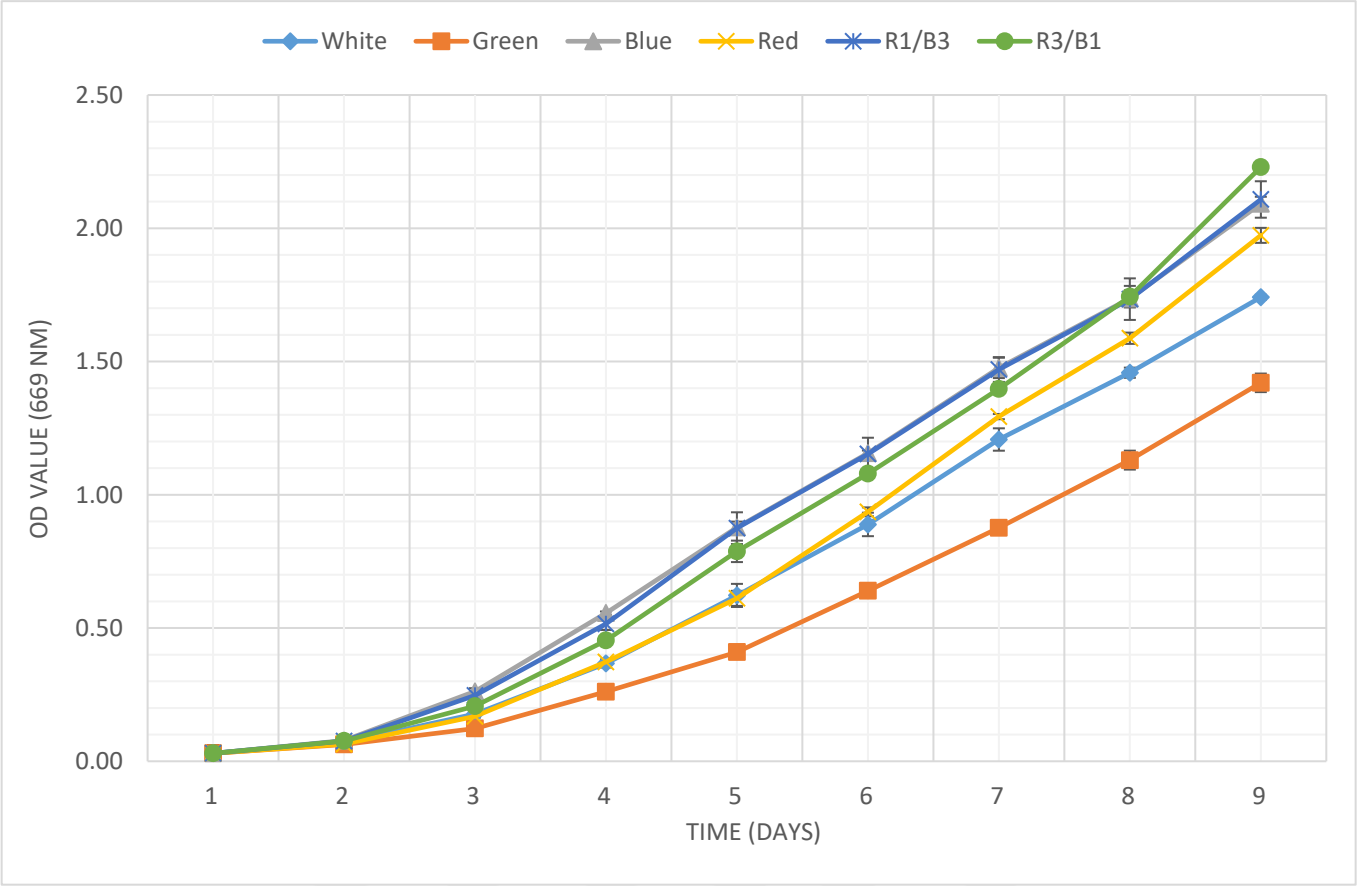


Figure 4.1. *S. quadricauda* growth curves under 6 different LED lights for 9 days experimental period by measuring the change in the optical density (Absorbance) at 669 nm with starting concentration at 0.03.

Table 4.1. Pairwise comparison of growth rate concentrations using Bonferroni P-value in Post-hoc Tukey HSD test with monochromatic and dichromatic LED light quality.

Treatments	***White***	***Green***	***Blue***	***Red***	***R1/B3***	***R3/B1***
White		0.001**	0.001**	0.001**	0.001**	0.001**
Green			0.001**	0.001**	0.001**	0.001**
Blue				0.01*	0.899	0.005**
Red					0.006**	0.001**
R1/B3						0.01*

Statistically significant values are $p<0.05$* and $p<0.01$**.

4.1.2 Chlorophyll Measurements

Table 4.2 shows the statistical significands of pairwise comparisons between treatments. Figure 4.2 shows OD values as measure of growth rate of experimental treatments. Although there is no clear statistical significance in pairwise comparisons of chl-a and chl-b values between treatments, green light seems to have the highest effects on chlorophyll concentration.

Interestingly, the chlorophyll content did not increase under the green light wavelengths for the first five days, where it increased dramatically to higher than the other LED light wavelength on the last day.

The order of chlorophyll a and b are arranged as the following: Green> R3/B1> Blue> R1/B3> White> Red

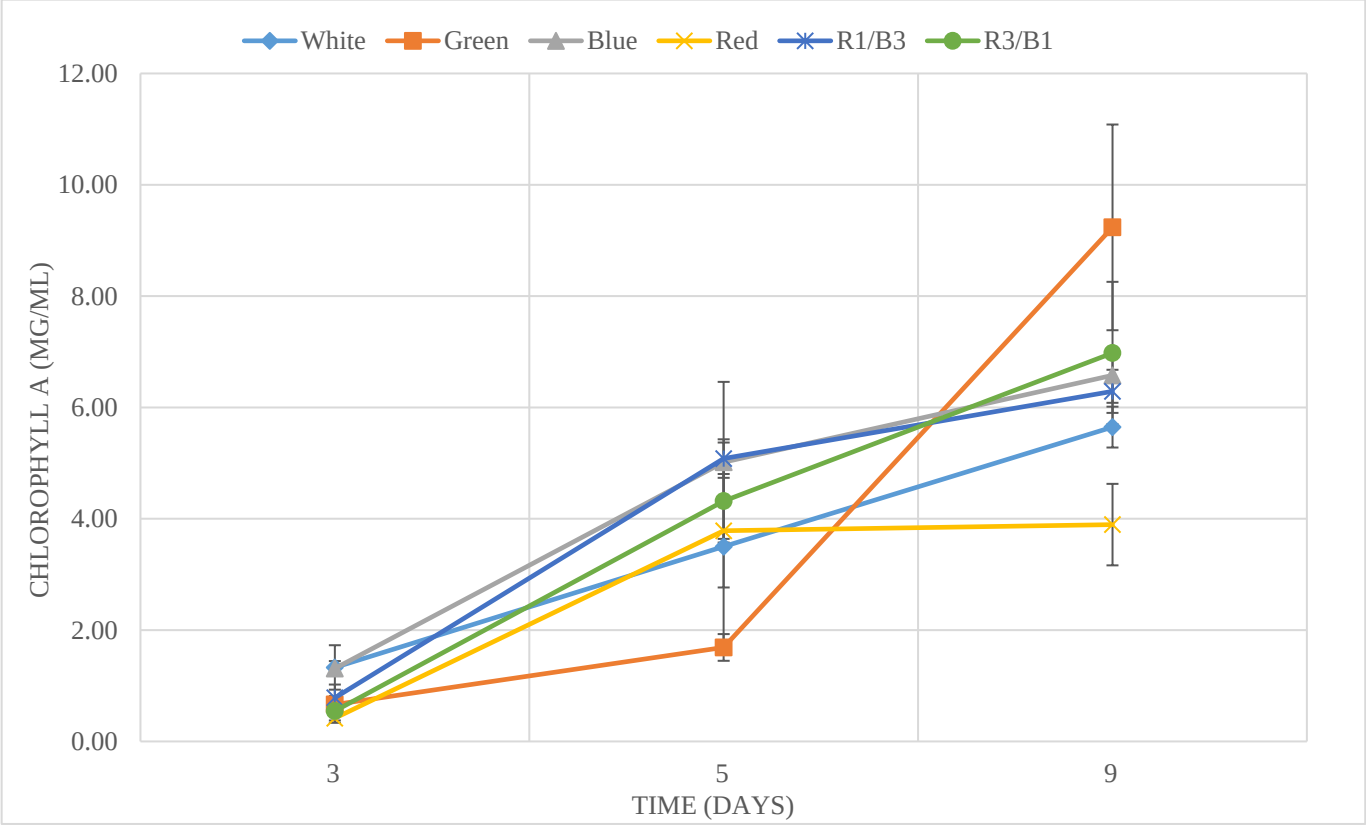


Figure 4.2. a) Chlorophyll a concentration (µg/mL) of *S. quadricauda* under 6 different experimental treatments.

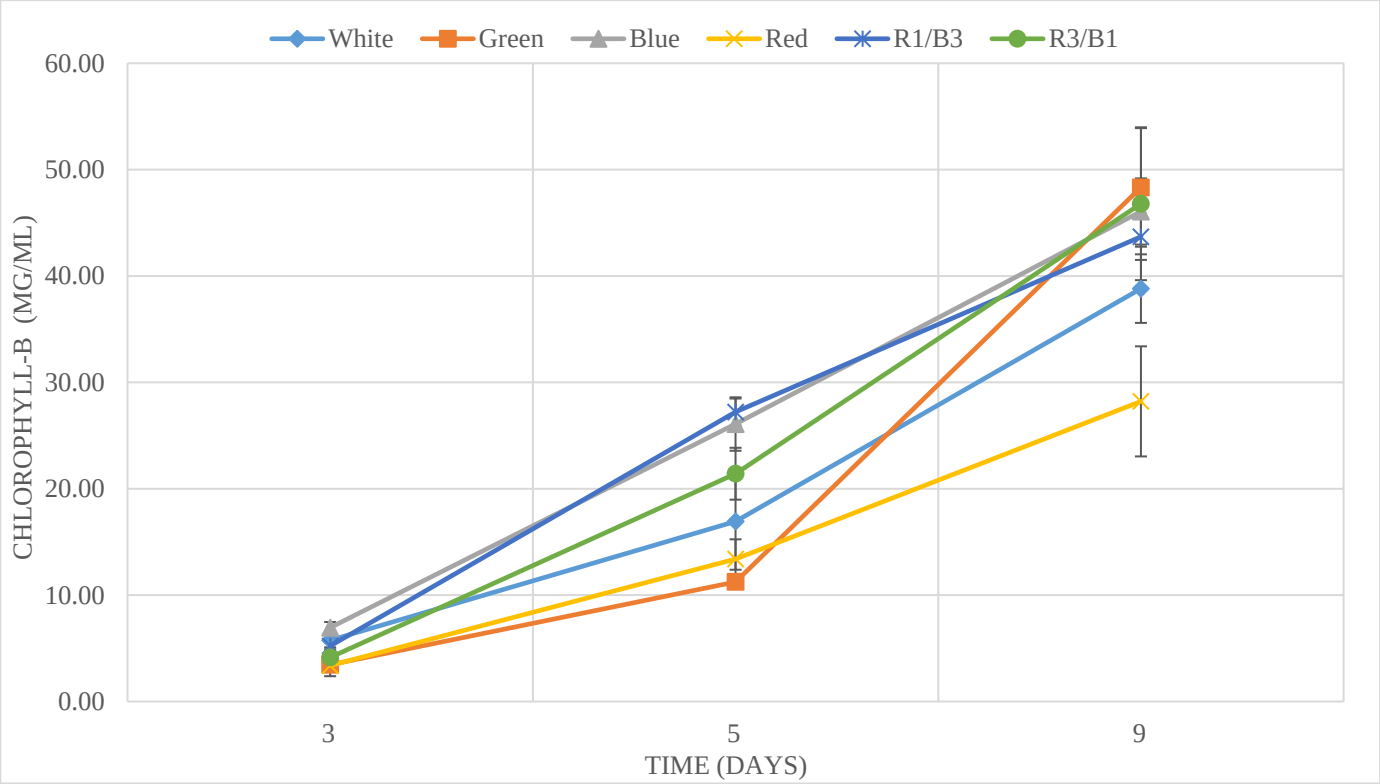


Figure 4.2. b) Chlorophyll b concentration (µg/mL) of *S. quadricauda* under 6 different experimental treatments.

Table 4.2. Pairwise comparison of chlorophyll-a concentration using Bonferroni P-value in Post-hoc Tukey HSD test with monochromatic and dichromatic LED light quality.

Treatments	***White***	***Green***	***Blue***	***Red***	***R1/B3***	***R3/B1***
White		0.009**	0.846	0.337	0.899	0.595
Green			0.063	0.001**	0.03*	0.137
Blue				0.060	0.899	0.899
Red					0.106	0.026*
R1/B3						0.899

Statistically significant values are p<0.05* and p<0.01**.

4.1.3 Biomass Productivity

Dry weight values show 2 statistical district clusters that white and green light has lower than blue, red, and dichromatic combinations. Although blue light has the highest value, there are no statistically significant differences between blue, red, and dichromatic treatments. (Figure 4.3, table 4.3) The order of dry weight is as follow: Blue> R3/B1> R1/B3> Red> White> Green

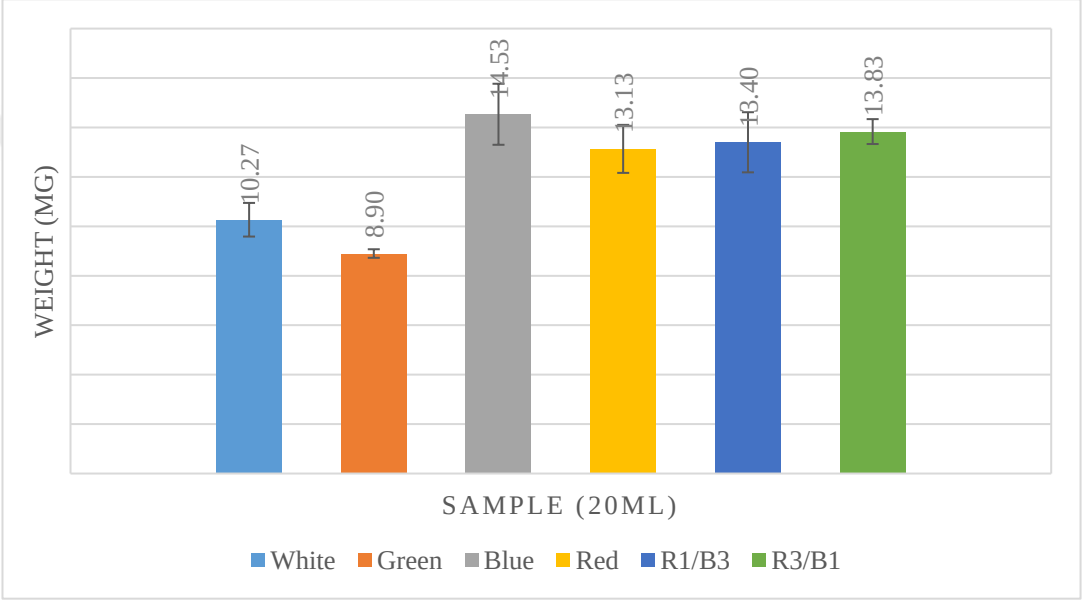


Figure 4.3. Dry weight measurements (mg) of *S. quadricauda* with standard deviation under monochromatic and dichromatic LED light on the last day (Day 9) of the experiment by using 20 ml of the sample.

Table 4.3. Pairwise comparison of dry weight using Bonferroni P-value in Post-hoc Tukey HSD test with monochromatic and dichromatic LED light quality.

Treatments	***White***	***Green***	***Blue***	***Red***	***R1/B3***	***R3/B1***
White		0.45	0.001**	0.018*	0.0096**	0.0036**
Green			0.001**	0.001**	0.001**	0.001**
Blue				0.43	0.62	0.899
Red					0.899	0.899
R1/B3						0.899

Statistically significant values are $p<0.05$* and $p<0.01$**.

4.2 Green LED Light Supplementation

R: B (1:3) was used as a control treatment in the second part to evaluate the effect of green light supplementation on the growth rate and chlorophyll concentration in *S. quadricauda* based on the maximum growth rate recorded all over experiment I.

Continuous green light supplementation with a constant percentage (4%) in amount of 2, 4, 6, 8, and 10 µmol/ m^{-2} s^{-1} as an expense of red and blue light intensity in the ratio of 1:3 as in the treatment of R:B (1:3).

4.2.1 Growth Rate

The growth curve demonstrated that the highest growth rate, is with 6 µmol/ m^{-2} s^{-1} green light supplementation (treatment 3), and the lowest growth rate, is with 2 µmol/ m^{-2} s^{-1} green light supplementation (treatment 1). Although there are no static differences between any of the treatments (See figure 4.4, table 4.4). Seems that supplementation of green light is compensated red and blue light expenses although green light alone has an suppressive effects on the growth of cultures.
The arrangement of the growth rate is as the following:

Treatment 3> treatment 5> treatment 2> control> treatment 4> treatment 1

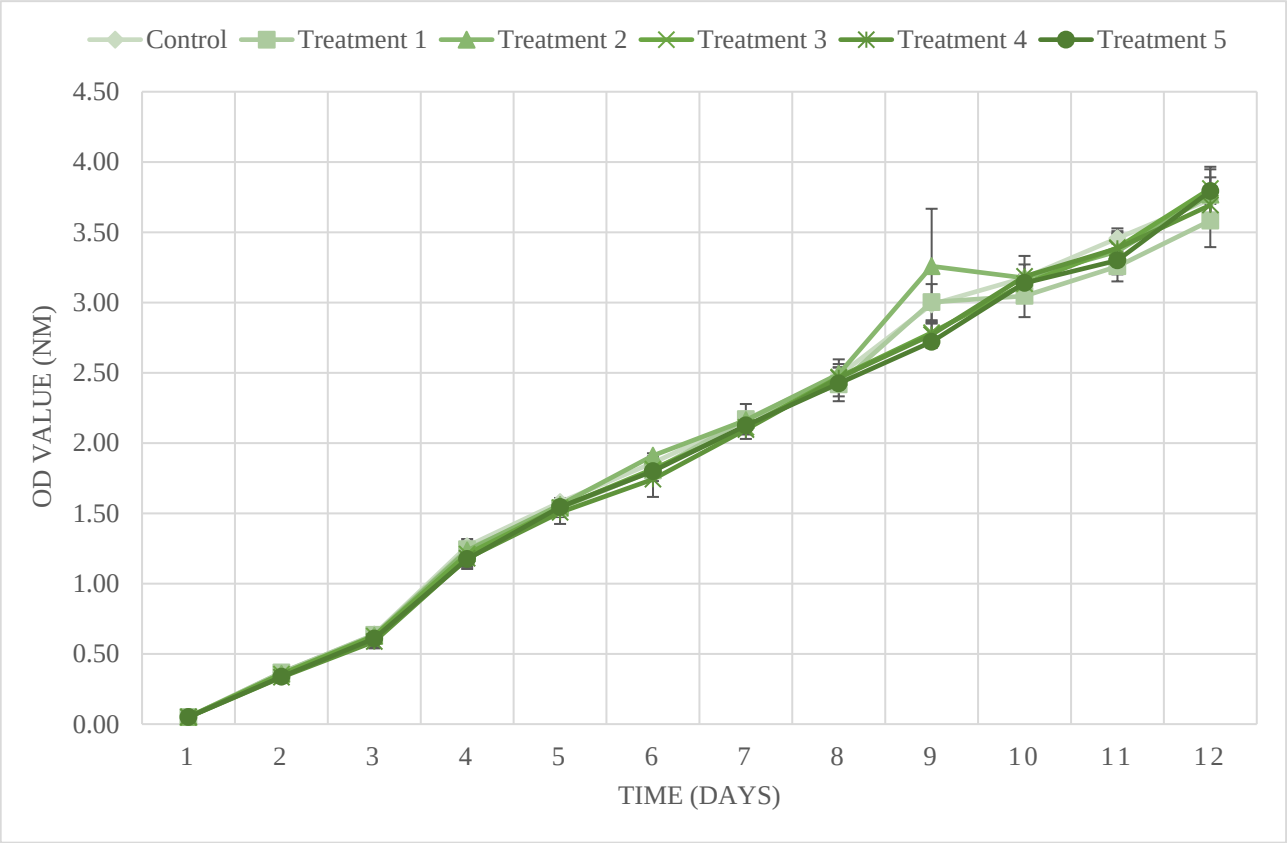


Figure 4.4. *S. quadricauda* growth curves under 6 different green LED light supplementation treatments by measuring the changing of the optical density (Absorbance) at 669 nm for 12 days experimental period with starting concentration at 0.05.

Table 4.4. Pairwise comparison of growth rate using Bonferroni P-value in Post-hoc Tukey HSD test with green light supplementation LED light quality.

Treatments	***Control***	***Treatment 1***	***Treatment 2***	***Treatment 3***	***Treatment 4***	***Treatment 5***
Control		0.739	0.899	0.899	0.899	0.899
Treatment 1			0.587	0.394	0.899	0.472
Treatment 2				0.899	0.899	0.899
Treatment 3					0.896	0.899
Treatment 4						0.899

Statistically significant values are $p<0.05$* and $p<0.01$**.

4.2.2 Chlorophyll Measurements

Chlorophyll a and b are consistent with the growth rate, which showed that treatment 3 is the highest chlorophyll concentration while the lowest was treatment 1. Thus, no statistical differences are shown.

The chl-a and chl-b are arranged as the following:

Treatment 3> control >treatment 2> treatment 4> treatment 5> treatment 1

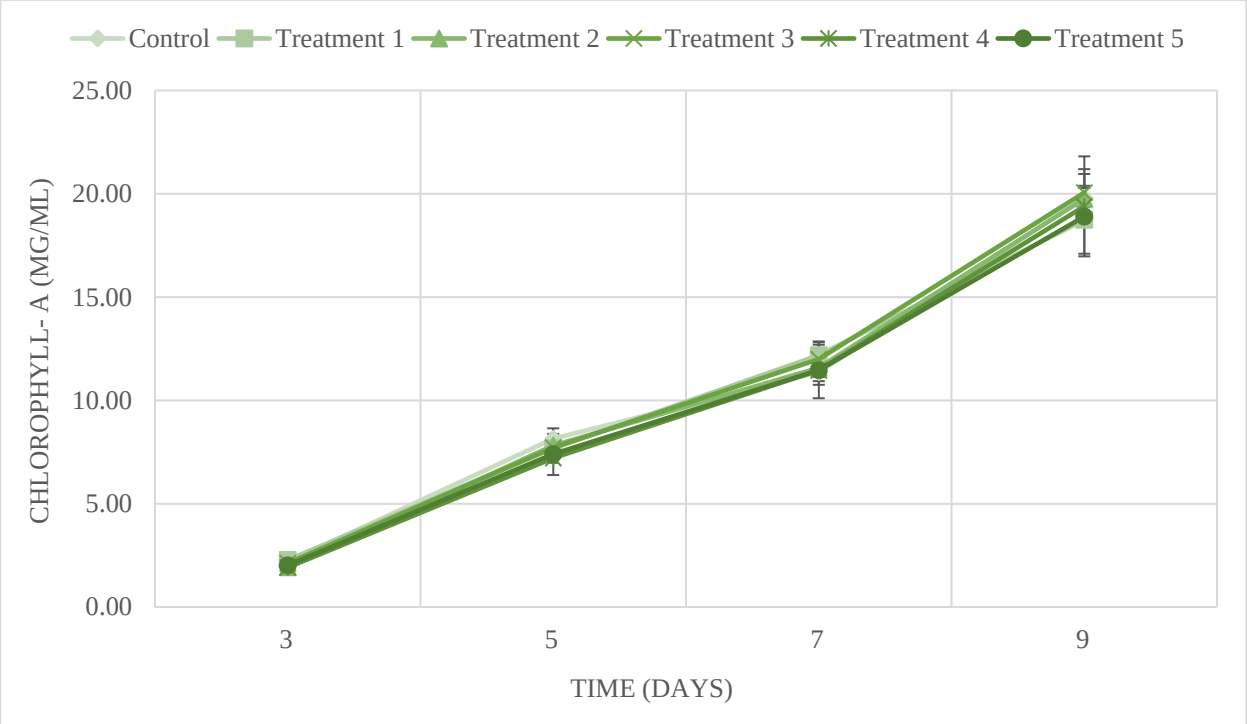


Figure 4.5. a) Chlorophyll a concentration (µg/mL) of *S. quadricauda* under 6 different experimental groups of green LED light supplementation treatments.

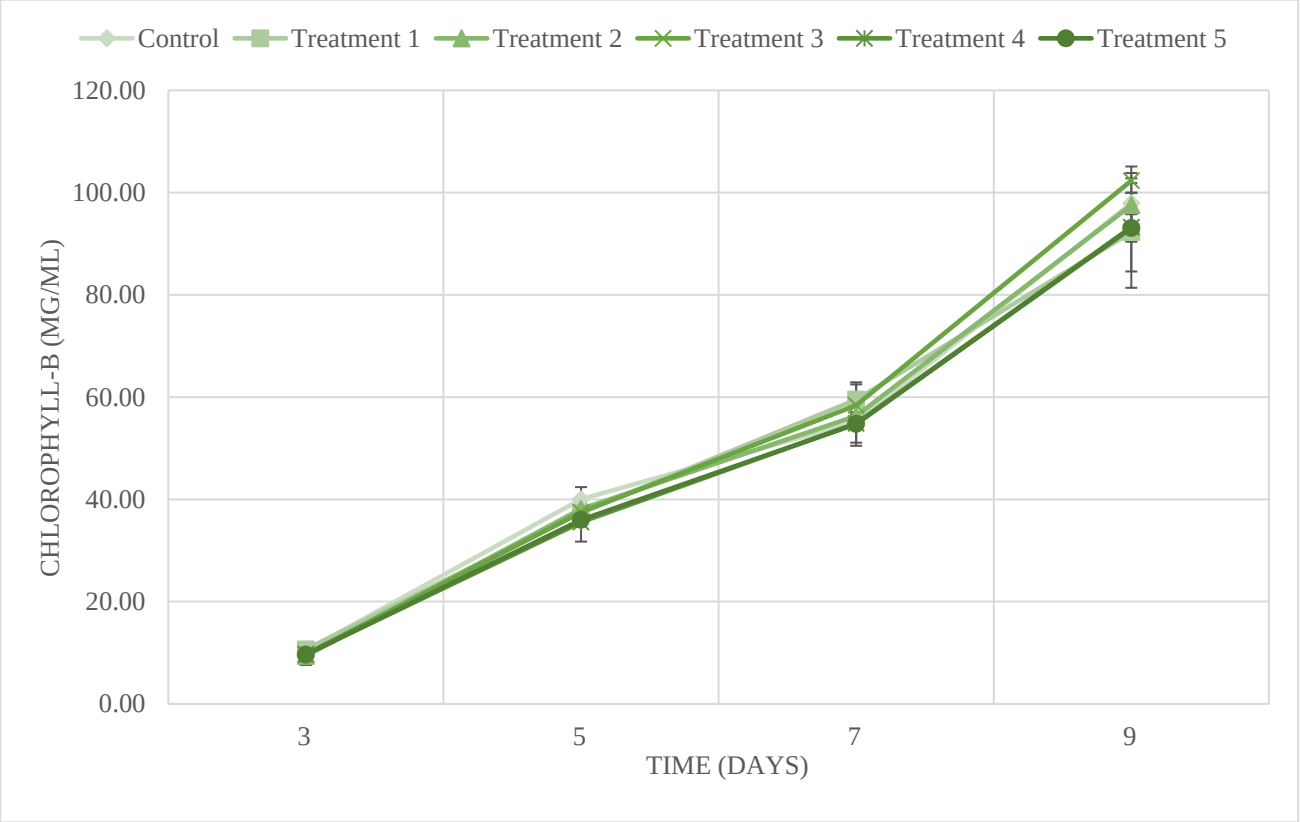


Figure 4.5. b) Chlorophyll b concentration (µg/mL) of *S. quadricauda* under 6 different experimental groups of green LED light supplementation treatments.

Table 4.5. Pairwise comparison of chlorophyll-a using Bonferroni P-value in Post-hoc Tukey HSD test with green light supplementation LED light quality.

Treatments	***Control***	***Treatment 1***	***Treatment 2***	***Treatment 3***	***Treatment 4***	***Treatment 5***
Control		0.847	0.899	0.899	0.899	0.899
Treatment 1			0.849	0.899	0.893	0.899
Treatment 2				0.899	0.899	0.899
Treatment 3					0.899	0.899
Treatment 4						0.899

Statistically significant values are p<0.05* and p<0.01**.

4.2.3 Biomass Productivity

According to the results, treatment 2 and treatment 4 are equal and showed a higher amount of dry weight than other treatments (Figure 4.6). The other treatments are ordered as: treatment 1> treatment 3> treatment 5> control. None of the results showed statistical differences from each other (Table 4.6)

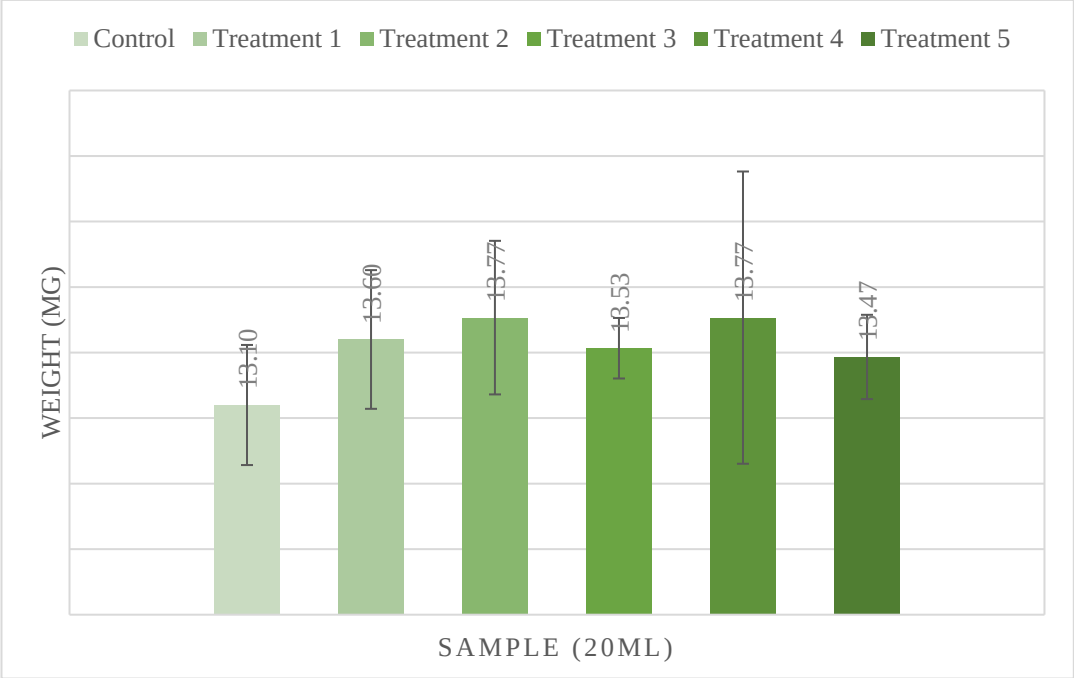


Figure 4.6. Dry weight measurements (mg) of *S. quadricauda* with standard deviation under green LED light supplementation on the last day (day 12) of the experiment by using 20 ml of the sample.

Table 4.6. Pairwise comparison of dry weight using Bonferroni P-value in Post-hoc Tukey HSD test with green light supplementation LED light quality.

Treatments	***Control***	***Treatment 1***	***Treatment 2***	***Treatment 3***	***Treatment 4***	***Treatment 5***
Control		0.847	0.738	0.899	0.738	0.899
Treatment 1			0.849	0.899	0.893	0.899
Treatment 2				0.899	0.899	0.899
Treatment 3					0.899	0.899
Treatment 4						0.899

Statistically significant values are $p<0.05$* and $p<0.01$**.

5. DISCUSSION

Microalgae have recently gained significant interest on a global scale, due to their broad application possibilities in the renewable energy, biopharmaceutical, and nutraceutical industries.

Many species of microalgae have been found to be richest sources of diverse biologically active compounds, *S. quadricauda* is one of the potential sources of biodiesel production due to high lipid content. Microalgae-based biofuel extraction is thought to be an effective approach, for increasing the yield of production, many parameters could be applied.

Light has shown a unique pattern of influence on the growth and biomass production of algae, these effects are dependent on algae species. The variations in microalgal culture growth under various illumination profiles highlight the need to research how various radiation spectrum areas affect microalgal growth.

5.1 Effect of Monochromatic and Dichromatic LED Light on the Growth *S. quadricauda*

Previous studies regarding the optimal spectrum for enhancing algae growth have shown that blue and red-light combinations are more effective in promoting microalgae growth than monochromatic or full-spectrum white light (74,75). The ratio of blue and red combinations was found to be species-dependent, as it was presented in previous studies, the ratio of red:blue was highest growth at 5:5 in *chlorella* species (75), For *Haematococcus lacustris* (86), and for *Dunaliella salina* (70), the highest biomass was under the ratio of 1:3. A study by Kurt et al. (87) on *Botryococcocus braunii* showed that mixing 20% blue light and 80% red exhibit the best biomass production.

In the present study, red: blue light combinations have shown the highest growth rates for *S. quadricauda* with significant variation, followed by blue> red> white > green (Fig. 4.1). These are consistent with Kim et al. (74). which showed mixed red and blue light have a higher production in *Scenedesmus* species than other monochromatic.

The synchronized application of both red and blue light, which provides the required wavelength ranges for chlorophyll (64), and accessory pigment (23). which will enhance the efficiency of the photosynthesis process. Hence, microalgae were produced at higher rates than when using simply monochromatic wavelength (70,74,75,86,87).

A certain combination of red and blue LED light sources may combine their benefits while minimizing their limitations. Ruyter (66). stated that the exposure to blue light may repair the cell damage caused by red light. This might explain our result of the dominated red fraction (R3/B1) was significantly higher than dominated blue (R1/B3), while monochromatic red was significantly lower than blue light. The red light may stimulate PSII in microalgae while the blue light may affect the PSI (88). In dominated red fraction (R3/B1) result, the red light may induce the PSII and transfer the electrons by light harvesting complexes toward PSI, then the PSI and PSII will be stimulated by the red light. While the blue light effect may be focused on fixing the cell damage caused by red light and also stimulating PSI (66,88). This could be reasonable to explain why the dominated red (R3/B1) is higher than dominated blue (R1/B3). But in monochromatic red, there is no blue light to fix the damage, so the growth will be lower than blue monochromatic light and R3/B1.

The blue light might have a high energy for photosynthesis activation, this excess energy may trigger the formation of reactive oxygen species which may cause photoinhibition (70), the red light has a long wavelength which help in avoiding this photoinhibition by the combination with other wavelegths (89).
A study by Fu et al.(70) showed that mixing red and blue lights would be efficient to decrease the photoinhibition on the growth of *Dunaliella salina*. In present work, it is possible that adding red light might lower the photoinhibition that may occur under blue light like in *Chlorella pyrenoidosa* study (89).

Monochromatic blue and red light have a positive impact on growth rate due to the higher efficiency of chlorophyll to absorb their wavelengths. Our present study showed *S. quadricauda* has a significantly higher growth rate under blue light than red light and other monochromatic light, these results were similar to the study

by Leonardi et al. (90) also reported that the highest biomass production in *S. quadricauda* species is under the monochromatic blue. Ruyter (66). demonstrated the effect of blue light on the photosynthetic enzymes in *Scenedesmus species* by the activation of particular enzymes, especially those that are available in the Calvin cycle (ALA synthase) and various photosynthetic enzymes. Likewise, *Nannochloropsis sp.* by Das et al. (71). showed that the highest growth rates will be achieved under short-wavelength (blue light), which is efficient to increase the light-harvesting complex resulting in higher energy for photosynthesis.

On the other hand, red light has optimal growth for many other species (91), which has long wavelength providing high growth rate with smaller cell size by triggering the cell cycle in many microalgae (50). In *Chlorella vulgaris* which conducted by Ge et al. (72), Yan et al. (92) and in *Chlorella pyrenoidosa* by Matthijs et al. (89), they presented that the red wavelength was the optimum. While in *Nannochloropsis sp.* (71) the red light was the lowest compared to blue light, which is in line with our study on *S. quadricauda.* Confirming the fact that diverse microalgae have various primary and accessory pigments available in different ratios.

Monochromatic green light did not exhibit a positive effect in either growth rate or biomass production for *Scenedesmus* species. This finding is consistent with previous authors, Baba et al. (93), Yan et al. (87) on *Botryococcocus braunii* and *Chlorella vulgaris* species respectively, found that green light has lower results compared to red and blue light. This due to limited capacity of absorption by chlorophyll (95). Therefore, it appears that exposure to the green wavelength caused slow growth, which if prolonged would have resulted in the stasis of cells.

5.2 Effect of Monochromatic and Dichromatic LED Light on Chlorophyll Concentration *S. quadricauda*

The chlorophyll concentration under the monochromatic green was significantly the highest, the reason for this might be explained by the chromatic adaptation process (96,97). Which is a classical algae strategy in low light conditions is to increase chlorophyll per cell, to increase light absorption in order to enhance photosynthesis. Accordingly, the result in significant changes in cell pigmentation was observed as cells adapted to their new light environment.

Likewise, blue monochromatic and dichromatic lights enhanced the chlorophyll concentration (Fig 4.2), these results are consistent with the study by by Leonardi et al. (90) on *Scenedesmus* species presented that increasing blue fraction in red and blue combination treatments, will be resulted in high chlorophyll content. A study by Ra et al. (98) exhibited that four microalgae cultivated under mixed LED had the highest chl a and chl b concentrations. thus, combining blue and red LEDs is essential for producing photosynthetic pigments.

The effect of monochromatic blue light on increasing the chlorophyll concentration while red light was the lowest, this might be explained by the photosynthetic adaptation mechanism that is stated by Hermsmeier et al. (99) which mentioned that red light suppressed cab-gene expression while blue light increased it. Cab gene is the gene encoding protein, which is responsible of chl a and chl b in LHC (90).

Thielmann and Galland (100) also supported that blue light affects the photoreceptor region (chromophore) which is effective to enhance the synthesis of chlorophyll in *Scenedesmus* sp. Humbeck et al. (101) also stated a high chlorophyll content under blue light compared to red in *Scenedesmus* cells thus it will alter light-harvesting complex content.

Our results of dichromatic combination (R1/B3, R3/B1) showed chlorophyll concentration in order of R3/B1> Blue> R1/B3 with no statistical differences between them. This is probably due to the fact of, that in order to activate the chromophore of the photoreceptor, the presence of photons must be sufficient (102).

This would be attained in our experiment by either blue monochromatic or with a combination, consequently, the same amount of chlorophyll concentration would be achieved since the blue photons was sufficient in the three treatments to trigger the chromophore.

Using low light intensity (50 mol/ m-2 s -1) in our study may contribute in high chlorophyll accumulation. It was presented in previous studies on some species that increasing the light intensity, chlorophyll concentration will be decreased (103). Like in *chlorella* species presented by, He et al. (104), also in *Scenedesmus dimorphous* shown by Ferreira et al. (105) that low light intensity treatment had higher chlorophyll content compared to the control.

5.3 Effect of Green LED Light Supplementation on the *S. quadricauda*

Green light was thought to be the undiscovered wavelength region for efficient photosynthesis, but recent studies have reported that it may contribute a significant role in the photosynthesis as similar as the other colors (for example, blue light) since it has a good penetration (106,107). Nevertheless, green light alone is not sufficient for most algae species and plants, the combination of green light with other wavelength is a novel strategy for future research to consider.

Several studies have been investigated the effect of supplementing green light to red and blue lights on plant. The effects of green wavelength remain poorly understood due to limited data on microalgae species, more research on microalgae will be necessary to learn more about how green light affects growth.

In this study, we applied a gradient green light supplementation dose of 2, 4, 6, 8, and 10 μmol/m^2 as an expense of red and blue light based on their proportions. light intensity was maintained at 50 mol photons m^{-2} s^{-1} for all treatments. The aim of this experiment was to test if adding green light by decreasing red and blue light intensity has any negative or positive effects on growth of *S. quadricauda.*

As was mentioned on lettuce by Kim et al. (108), more plant growth produced in 24% addition of green light mixed with red and blue light. where Nguyen et al. (109) reported the growth rate of spinach will be slowed by supplementing the green to red and blue light. In *Gracilaria chilensis* (red algae),

demonstrated that the pigments are affected by green light then the excitation energy will be transferred to chl-a (110).

Our results of dry weight, growth rate and chlorophyll concentration on *S. quadricauda* exhibited no significant differences had been seen with any of the results, it means that there is no positive or negative effect of green light supplementation in *S. quadricauda.* This results are consistent with Hernández and Kubota (80) study that stated the growth in cucumber was not affected by the addition of green light to the R and B spectra.

Johkan et al. (111) indicated that the plant could use the green light with a high intensity for photosynthesis, since the intensity promote the absorption of green light, inconsistent to our study while we have used low amount of intensity (50 mol/ m^{-2} s^{-1}), which may be the reason why we could not observe the actual effect of green light on *S. quadricauda* species.

These results concluded the growth rate of *S. quadricauda* under green light supplementation with red: blue combinations seemed to be dependent on the concentration of green light. (Figure 3.4)

A study of Kalmaoğlu (112) reported that 10% green light supplemented with red and blue light on *Haematococcus pluvialis* will increase the RubisCo (Enzyme in CBB cycle) which will facilitate the carbon assimilation. Additionally, it was demonstrated that green light stimulates photosynthesis in plants (113) by causing carbon fixtation in deep leaves rather than by red or blue light (114). Also, Terashima et al. (81) and smith et al. (115) presented that green light drives photosynthesis more efficiently than red light and can reach deeper into leaves than red or blue light. It may have a role on affecting the protein in photosynthesis and thus affecting the growth.

Green light caused the maximum production and biomass concentration in *Spirulina platensis* in a closed system, according to a study by Ravelonandro et al. (84). Where in our study there is no positive effect of green light on *S. quadricauda.*

Sepúlveda-Ugarte et al. (110) reported that the presence of phycobiliproteins in phycobilisomes have an interesting effect by transforming the excitation energy from pigments into chlorophyll a, the green light affect these proteins and increase the yield of photosynthesis.

Scenedesmus species has a limited level of phycobiliproteins (116), unlike the *spirulina* species which showed the higher growth under the green light (117). Hohmann-Marriott and Blankenship (65) stated that green algae are unable to fully use green light since their phycobilin (The light-harvesting pigment that absorbs green to red wavelengths of light) was lost during growth.

Green wavelength more effectively promotes biomass production in the dense microalgal cultures of *Scenedesmus bijuga* and *Scenedesmus quadricauda* examined by Mattos et al. (118) and Niizawa et al. (83) respectively, this would be achieved by using high-density microalgal culture. Even weakly absorbed chlorophyll wavelengths will allow deeper light penetration and uniform light distribution, stimulating greater biomass development. In contracts, the density used in the present study was low.

The green light supplementation did not show any negative effect as we decrease the amount of red and blue light, the cause might be linked to the fact that green light increased *Scenedesmus's* chlorophyll content (first experiment). The supplementation of the green light will increase the chlorophyll a and b, which they will absorb more red and blue light than in the control treatment. Since chlorophyll data and the growth rate are consistent in tr-3 (highest growth rate and chl) and tr-1 (lowest growth rate and chl). (Figure 4.4, figure 4.5)

Therefore, the supplementation of the green light with expense of R and B light combination will not affect the result, due to the fact of green decrease the growth rate in *Scenedesmus* in contrast it will increase chlorophyll concentration, so it may compensate the results.

6. CONCLUSIONS AND RECOMMENDATIONS

In this study, the effect of different radiation profiles on microalgal growth was analyzed. *Scenedesmus quadricauda* responds differently to lighting conditions.

The combined mixture of red and blue light is considered to be the most important spectral wavelength in almost all species in which providing the absorption spectrum of chlorophyll a and b, as well as some types of accessory pigments. *S. quadricauda* resulted in a high growth rate and chlorophyll concentration.

S. quadricauda cells with blue radiation profiles have higher chlorophyll concentrations, maybe due to the presence of a blue light photoreceptor.

The green light has a significant role in some plants by affection physiochemical processes, however, using green light in combination with red and green could be an important approach since using it alone is not sufficient.

Thus, to determine whether *S. quadricauda* growth would be impacted negatively or positively by increasing green light while reducing red and blue light intensity.

Our results showed that supplementation would not affect negatively the growth of *Scenedesmus* microalgae, considering the effect of green could not be observed may be due to:

1. The microalgae are not dense
2. The intensity was low (not enough illumination)

Additional research on *S. quadricauda* using green light supplementation with red and blue combination has to be conducted with other light intensities.

Before coming to a definitive conclusion regarding how green light affects algae, it is obvious that more investigation is required to comprehend how various photosynthetic organisms react to this region of the electromagnetic spectrum.

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