Photon Manipulation of Electron Transportation in Chlamydomonas Reinhardtii Algae Using Semiconductor Lasers

A thesis submitted for the degree of
Doctor of Philosophy

By

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ABSTRACT

The aim of this research was to increase the rate of cell division in algae by exploring the effect of combinations of lasers of various wavelengths. Literature search has identified a gap in knowledge of the potential for increase in efficiency of the electron transition between photosystem II and photosystem I. This through the use of several wavelengths of blue and or red lasers, including 405 nm, 450, and 473 nm, 635 nm, 650 nm, 680 nm, 685 nm and 700 nm to generate photons with energies more closely matching the absorption spectra of algae receptors known as pigments.

This investigation underpins the realisation that photons emanating from a specific laser are absorbed by algae pigments because there is a much closer match between the emission spectrum of the laser and the absorption spectrum of the pigments within the photosystems of algae. This research examined all of the available laser wavelengths in particular combinations; the resultant data contributed to the assembly of a matrix that illustrates the most appropriate laser combinations that promote cell division within algae.

Chlamydomonas reinhardtii algae cells successfully grew and divided under exposure to both the blue laser, red laser and that of white light LED when each was applied individually or combined in a sequence. The order of the sequence of using the red and blue lasers in specific cases was important. The pH was maintained between 6.9 and 7.7, with temperatures maintained between 19.00 and 25.00 ºC.

For the blue lasers, the laboratory results were as follows, (irradiation time was 12 hours every time):

- 405 nm blue laser produced 1.8 x cell division of the white light LED.
- For 450 nm blue laser: the white light LED produced 1.5 x cell division of the blue laser 450 nm.
- 473 nm blue laser produced 2 x cell division of the white light LED.
- 405 nm blue laser produced 3.6 x cell division of natural day light.
- 450 nm blue laser produced 1.4 x cell division of natural day light.
- 473 nm blue laser produced 4 x cell division of natural day light.

For the red lasers, the laboratory results were as follows, (irradiation time was 12 hours every time):

- For 635 nm red laser: the white light LED produced 4 x cell division of the red laser 635 nm.
- 650 nm red laser produced 1.96 x cell division of the white light LED.
- 680 nm red laser produced 2.3 x cell division of the white light LED.
- For 685 nm red laser: white light LED produced 1.22 x cell division of the red laser 685 nm.
- 700 nm red laser produced 1.35 x cell division of the white light LED.
- For 635 nm red laser: the natural day light produced 2 x cell division of the red laser 635 nm.
- 650 nm red laser produced 3.9 x cell division of natural day light.
- 680 nm red laser produced 4.6 x cell division of natural day light.
- 685 nm red laser produced 1.6 x cell division of natural day light.
- 700 nm red laser produced 2.7 x cell division of natural day light.

For the combination of blue and red lasers, the laboratory results were as follows, (irradiation time was 12 hours every time):

- First combination: 405 nm blue laser followed by a combination of 680 nm and 700 nm red lasers produced 4.86 x cell division of the white light LED.
- Second combination: 473 nm blue laser followed by a combination of 680 nm and 700 nm red lasers produced 4.66 x cell division of the white light LED.
- Third combination: a combination of 680 nm and 700 nm red lasers produced 4.43 x cell division of the white light LED.

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ABBREVIATIONS

ADP	Adenosine diphosphate
ALA	α-linolenic acid
Ambient	The immediately surrounding atmosphere
ATP	Adenosine triphosphate
b6f	Plastoquinol-plastocyanin reductase
BChla	Bacteriochlorophyll A
PBR	Photo-Bio Reactor
C. Reinhardtii	Chlamydomonas Reinhardtii
Chl	Monomeric chlorophyll
ChlD1	Special accessory chlorophyll (serving as the primary electron donor)
CN	Cetane number
D1 and D2	Destabilised polypeptide protein subunits of PS II RC
DAQ	Digital Acquisition
DHA	Docosahexaenoic acid
Diffuser	Light scattering
EPA	Eicosapentaenoic acid
EPR	Electron Paramagnetic Resonance
eV	Electronvolt (photon energy unit)
FA	Fatty Acid
FTIR	Fourier Transformed Infra-Red
GHGs	Greenhouse Gasses
GM	Genetic modification / genetically modified

His 198 of D1	Histidine 198 of D1
IR	Infra-red
KNO_3	Potassium nitrate
LABVIEW	Software for analysing and presenting acquired data real-time
LED	Light Emitting Diode
mM	Millimolar concentration (mmol $l^{-1} = 10^{-3}$ mol l^{-1})
Mn_4Ca	Heterogeneous manganese-calcium cluster (electro-catalyst)
MTOE	Million Tonnes of Oil Equivalent
NADP+	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
NREL	National Renewable Energy Laboratory
OD	Optical density (cm^{-1})
OMEGA	The Offshore Membrane Enclosure for Growing Algae
P	Pair of chlorophyll
$P_{D1/D2}$	Chl monomer of P680 on the D1 or D2 side, respectively, coordinated by His 198/197 of D1/D2
P680	Reaction centre of PS II
P700	Reaction centre of PS I
PAR	Photosynthetically active radiation
PCE	Power conversion efficiency
PG	Phosphatidylglycerol
Ph	Pheophytin
PRK	Phosphoribulokinase
PS I	(Photosystem I, or plastocyanin-ferredoxin oxidoreductase)

PS II	(Photosystem II, or water-plastoquinone oxidoreductase)
PPFD	Photosynthetic Photon Flux Density (µmol m^{-2} s^{-1})
PUFA	Poly-Unsaturated Fatty Acid
Q	Quinone
Q_A	Stable primary Quinone acceptor of PS II RC
Q_B	Secondary Quinone acceptor of PS II RC
QH_2	Hydroquinone
$SE_{\bar{x}}$	Standard error of the mean
SQDG	Sulfoquinovosyl diacylglycerols
TAG	Triacylglyceride
TAP	Tris-acetate-phosphate (Growth medium for the algae)
TyrZ	Tyrosine
UV	Ultraviolet
VI	Virtual instrument (LabVIEW program-subroutine)

CHAPTER 1

INTRODUCTION

1.1 Background

The motivation for the research work presented in this thesis is the viability of using algae as a foundation for a renewable and sustainable energy source. Along with that, the aim and the objectives are given along with organising the structure of the thesis.

Microalgae are considered to be one of the most promising renewable/sustainable energy feedstock for biofuel refinery throughput, due to their advantageous fast growth, efficient carbon dioxide fixation, ultimately not competing for arable lands and potable water. Viability is highlighted by potential accumulation of high amounts of lipids and carbohydrates (Kuwahara et al. 2011). With the need to reduce carbon emissions underpinned by the current acute instability in distribution of the world's dwindling crude oil reserves; liquid fuels derived from plant material – biofuels – are becoming a highly attractive alternative source of energy as no single political power can uniquely monopolise it (Gustavsson et al. 2015), thus, energy will be more easily accessible by less financially and technically advantaged countries (WRI 2015). Moreover, in comparison with many forms of renewable energy such as wind, tidal, solar and liquid biofuels, microalgae cultivation requires a smaller budget to realise its potential. The oils produced via the fats in the lipids allow solar energy of high calorific values to be stored in both liquid and solid forms, hence potentially suited to use within existing engine fuel burn systems that dominate the transport infrastructure (Thornley & Adams 2017). The past

decade illustrated a diverse array of research programs in the field of laser-induced photosynthesis in plants for the purpose of increasing the energy harvesting in terms of higher growth rates and larger throughput of the photosynthesis process (Kuwahara et al. 2011).

Although the initial challenge was to identify and understand the fundamental science of light excitation within cell structures, research has been carried out with a degree of success in determining the characteristics of various plants for commercial applications such as tomatoes and wheat (Teo et al. 2014). Up to 60% of the world is covered in water, which sustains photosynthetic algae, each of these absorbs and fixes carbon dioxide through photosynthesis (Rajkumar et al. 2014).

Algae have higher growth than land plants, with some species having biomass doubling times of a few hours or even less if offered the most appropriate environmental conditions; some species can accumulate very large amounts of *triacylglycerides* (TAGs), the major feedstock for biodiesel production. High-quality agricultural land is not required to grow the biomass (Kuwahara et al. 2011).

1.2 Algae definition

'Algae' generically refers to a variety of 'aquatic photosynthetic microorganisms' from multicellular macro-algae to unicellular species (microalgae) and in general possess chlorophyll and other pigments. They are considered autotrophs, which are capable of synthesizing their own food from inorganic substances using light or chemical energy similar to plants, but they don't have leaves, stems, roots, 'conducting vessels' (xylem/phloem) and complex sex organs (Khan et al. 2018).

1.3 The diversity

Algae can be classified by size into two sets: macro-algae, also known as 'seaweed', and microalgae, which are microscopic single-celled organisms varying in size from a few micrometres to a few hundred micrometres (μm) (Milledge et al. 2016). The word microalgae is frequently used to include another type of algae, blue-green algae, which is

called the *prokaryotic cyanobacteria*, and often includes the *eukaryotic* microalgae such as diatoms and green algae, although nowadays they are not categorised as algae (García et al. 2017). Although 200,000-800,000 species of algae are in existence (Oviyaasri et al. 2017), only a few thousand algae are collected and cultivated on an industrial scale (Parmar et al. 2011). The majority of algae found worldwide are the green algae (*Chlorophyta*), diatoms algae (*Heterokontophyta*, (*Bacillariophyta*)), blue-green algae (*Cyanophyta*) and golden algae (*Heterokontophyta*) (Posters 2015). The largest group is the *Chlorophyceae* which is made up of several marine and freshwater structures. These are single cell and filamentous with an extensive geographical variety and rapid growth rates. This group of algae also consist of the most commonly studied algae like *Chlorella* spp., *Dunaliella* spp., *Haematococcus* spp. and *Chlamydomonas Reinhardtii* (Dixon & Wilken 2018) and in this thesis, these algae are collectively called *Chlamydomonas reinhardtii* selected for research study.

Unicellular microalgae have fast growth rates and high oil content of about 80% by weight (Pulz & Gross 2004). A diversity of lipids can be produced containing hydrocarbons and TAGs appropriate for producing biodiesel depending on the growth conditions and species of algae (Singh & Nigam 2011). Appropriate species of algae for biodiesel production must have fast growth rates together with high amounts of lipid content for optimum productivity (i.e. *Botryococcus braunii* has a very high amount of lipid content but the growth rates are very slow so as such it is inappropriate). Published amounts of results for lipid content may not account for the amount of lipids appropriate for fuel. For example, chlorophyll is inappropriate for producing biodiesel yet often creates a substantial proportion of lipid extracted from microalgae.

The world population has been increased from two billion in the middle of the 20th century to seven billion in the 21st century (Avni & Blázquez 2011) with a consequent increase in demand for energy. Energy is of prime importance to both economic and social development (Zhang et al. 2011). The issues of global warming, environmental and health concerns, decreasing fossil fuel reserves, rising fuel prices and energy security has encouraged research into the most efficient consumption of fuel in conjunction with investigations of alternative energy sources such as the renewable energy including nuclear, hydropower and other renewable sources that are considered as having zero

carbon footprint or are CO_2 free, but the growth of non-fossil energy is still not enough to meet the increasing demand for energy (IEA 2016).

Bioenergy produced from algae is considered to be an 'easy' key to energy and environmental concerns, as the carbon of the biofuel is produced by photosynthesis from CO_2 in the atmosphere and when biofuel is consumed the CO_2 is recycled to the atmosphere. Producing biodiesel from biological lipids like algae lipids is very important, as it can be directly employed for use in current transport liquid fuel (Mondal et al. 2017).

Remarks and issues regarding the traditional biofuel crops shown in Table 1.1 include the usage of high volumes of freshwater, low energy balances, poor yields and agricultural resource dislocation. Localised influences on climate change caused by modification of land use are generally ignored in life cycle analyses (Perugini et al. 2017).

Table 1.1 Advantages and disadvantages of different biofuel sources. *Adapted from Carriquiry et al. (2011).*

Source	**Advantages**	**Disadvantages**
Food crops	Established harvesting and processing technologies	Impact food prices by competing for all resources Seasonal harvests
Agricultural residues	Do not impact food prices Prevent changes in GHG emissions due to direct/indirect changes to land use New revenue for farmers	Excess removal will have an impact on soil, crop production and environment Need specially designed harvest equipment
Energy crops	Low water and nutritional input Can improve wildlife habitat Reduce soil erosion Improve soil properties Wide geographical distribution Can grow in poor quality soil and dry climate	May compete for agricultural land Seasonal harvests Take 2-3 years to reach maximum productivity Consistent high yield unachievable without high input costs Need development to become optimal
Microalgae	High yield potential Can be grown in both saline water and wastewater Can be harvested all year Do not require arable land	In early stages of development

The early positivity regarding biofuels has reduced, caused by the aforementioned disadvantages appertaining to resource displacement and incapability to exchange fossil fuels. To have a clear idea about the inconvenience of biodiesel crops (for example, the UK consumed 25 billion litres of diesel fuel in 2008) and to provide this as biodiesel produced from oilseed, it would have need of 4.2×10^7 acres of land (plantation land) (which represents more than half of the UK land area) (Scott et al. 2010). *Bio-methane*

and other biogases produced by anaerobic digestion of plant matter together represent about 70% of the renewable energy used for transportation, electricity and heating in the UK as shown in Figure 1.1 (ECCC 2016). Currently, the interest in studying the microalgae is encouraged greatly because of the potential profits gained from algae as a biofuel source; For example, in Ireland fuels from macro-algae (seaweed) could receive double green certificates, due to their high sustainability performance. On the other hand, in the UK while energy crop projects were economically supported by the UK Government, algae biomass was not eligible for support, because of the lack of clarity in classifying energetic algae whether it is an energy crop or a waste resource (Arvaniti 2015).

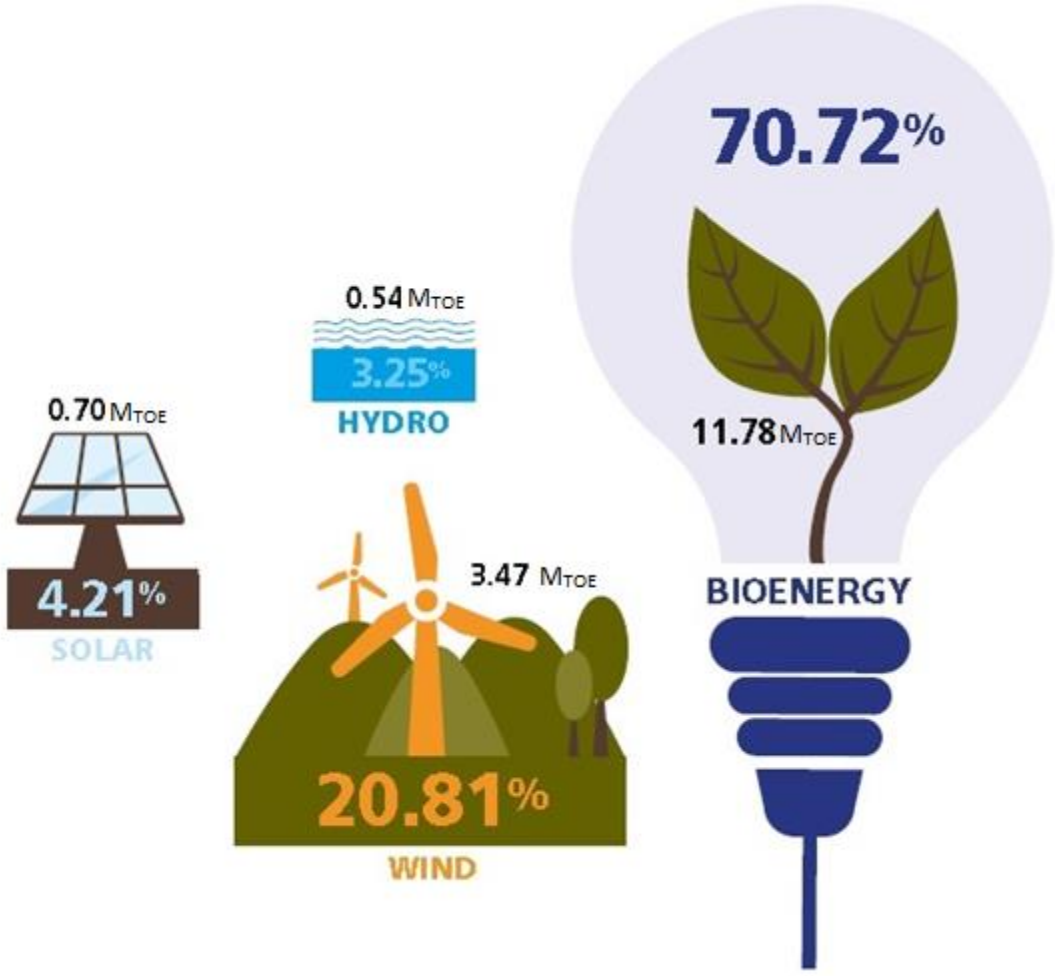

Figure 1.1 Sources of the UK's renewable energy – MTOE. *Source: (ECCC 2016).*

Microalgae have all the characteristics of plants, in addition to high productivities linked with large-scale production of microbes. Algae are capable of accumulating meaningfully

more lipid than traditional crops (20-80% by weight), their biomass yields are high and can be produced from non-freshwater sources and also algae do not need arable land nor do they compete with food security (Greene et al. 2016). Algae are able to utilize flue gas as a source of carbon dioxide and could be harvested every day (i.e. nonseasonal). The estimated yield of oil produced from algae is about 20,000-80,000 litres per acre, nearly 7-31 times larger than the majority of productive oil crops (Ali 2017). Furthermore, microalgae increase 'cleaner burning' biodiesel (Milano et al. 2016). Algae cultivation has become an important sector in the renewable energy sources that are potentially sustainable and capable of meeting future demand. The production of algae bioenergy can be classified by regions; 78% in the USA, 13% in Europe and 9% in the other regions. The production technology can be divided into three types; 52% using the closed bioreactors, 26% using open ponds, and only 22% using natural environmental conditions (Gendy & El-temtamy 2013).

Photosynthesis is a means of growth in algae or plants, which involves enabling the algae or plant to produce carbohydrates through the use of photons, CO_2, H_2O and chlorophyll. The chlorophyll is a green colour pigment present in the *Grana* of chloroplast and traps photon energy converting it into a chemical form of energy, *adenosine triphosphate* (ATP) (Johnson 2016). However, in order for the algae or plants to grow as efficiently as possible, it is essential that the wavelengths of the incident photons have to be within the *Photosynthetically Active Radiation* (PAR), blue and red lights between wavelengths of 400 nm and 700 nm, as algae or plants absorb them more efficiently than other wavelengths to advance their growth by stimulating chemical reactions (Margit Olle 2013). Bio-energy, produced from the biomass of algae or plants, is a convenient source of renewable energy since the algae or plants convert the light energy into chemical energy. Indoor farming requires artificial sources of light to provide energy as a means for growth (Junginger et al. 2010).

The way in which light affects plant or algae growth is evaluated using the (PAR) measure. It has been proven in 1972 by K. McCree (Ashdown 2017) that the number of photons influences the photosynthetic response more than energy. This is expected because photosynthesis is a photochemical conversion where each molecule is activated by the absorption of one photon in the primary photochemical process. PAR is the number of moles of photons in the radiant energy between wavelengths of 400 nm and 700 nm

(Kume 2018). One mole of photons is 6.0222 x 10^{23} photons, which is Avogadro's number (William 2003).

The *Photosynthetic Photon Flux Density* (PPFD), "the number of photosynthetically active photons that fall on a given surface each second", meaning the photon irradiance, is expressed in moles per square metre and per second (in the past, Einstein's per square metre and per second) (Mõttus et al. 2011), the µmol m^{-2} s^{-1} unit is used in this thesis to reflect the photon-electron interaction among this study. Also, using LUX or foot-candle meters to measure the light intensity of horticultural lighting systems gives varying measurements depending on the emission spectrum of the light source, even if the same intensity of PAR is used (Mõttus et al. 2011), while PPFD measurements are independent of any particular plant or algae species. The conversion of PPFD is:

1 µmol m^{-2} s^{-1} = 0.342 W m^{-2} = 54 Lux (Ashdown 2017).

1.4 State of the art in algae growth enhancement

There are numerous industrial and scientific efforts to improve algae cultivation so as to improve its cost-effectiveness and present it as a viable source for biodiesel fuel in addition to other fields of applications. Man-made production of algae tends to mimic the natural environments to achieve optimal growth conditions. The following paragraphs will present some of the state of the art research and industrial algae cultivation efficiency enhancement techniques and applications.

Kuwahara et al. (2011) has studied the effect of using red (655 nm and 680 nm) and blue (474 nm) lasers as an artificial light source. The diode lasers being a monochromatic and coherent light source in addition to having high *power conversion efficiency* (PCE) (70%) (Schultz et al. 2009) were employed to grow the *C. reinhardtii*, a type of green algae most suited to its enhanced growth appertaining to lipids and has an absorption spectrum that matches the emission spectrum of a variety of diode lasers. A unitary and mixed red and blue laser light of different wavelengths in comparison to white light source which was used was successful in yielding extra growth in the algae culture. Kuwahara made a significant finding where supplementing either red with blue laser resulted in increased algae cell count that showed an increase in growth rate at an average of 2.41 fold when

compared with those obtained using 655 nm, 680 nm wavelength red lasers and white-light.

Zhang et al. (2017) investigated inducing enhanced growth rate and lipid accumulation in *Chorella pacifica* sea water microalgae using laser sources. These sources were Nd: YAG laser of wavelength 1064 nm at 800 mW, He-Ne laser of wavelength 808 nm at 6W and semiconductor laser wavelength 632.8 nm at 40 mW. The algae samples were exposed to pre-specified irradiation periods from the mentioned laser sources. After a cultivation period of 20 days, enhancements in growth rate were observed and evaluated against the control sample which was not irradiated with laser. The results produced showed yield figures for different laser sources and irradiation periods. With maximum results obtained for the He-Ne, Nd:YAG, and semiconductor lasers within an irradiation time of 4 minutes. The total algae chlorophyll content detected was maximum when an irradiation time of 4 minutes was selected for the He-Ne and Nd:YAG lasers, whereas the semiconductor laser maximum for total chlorophyll content was observed when the irradiation time was set to 8 minutes. The optical density measurement was used to indicate algae cell density in test samples. This technique depends on the extent of light scattered by the algae culture instead of the amount of light absorbed. In addition to increasing the algae cell growth rate, the irradiation with laser sources has increased the algae lipid accumulation. This factor introduced algae as an alternative biofuel source of material for biodiesel production.

After studying this work, it can be concluded that it is evident that irradiating *Chlorella Pacifica* algae with laser light has given positive results on algae cells growth rate, yield, and lipid contents. Researchers have conducted extensive analysis on the effect of laser as a tool for promoting positive mutation effects that can be attributed directly to laser irradiation. However, the intensity of the laser sources was not controlled in order to study the intensity effect on the whole observed parameters affecting the cultivation process. The rationale for selecting the laser power was not explained.

Dobrowolski at el (2012) used laser stimulation in a wastewater treatment process to obtain increment of bioremediation abilities, soil reclamation and increment of energetic food crops. This is due to the evidence suggesting that plant biomass has risen as a result of laser stimulation, which also caused more effective extraction of biogenic elements

from polluted water and soil. In 1978, the **Dobrowolski** bioremediation method for the removal of many environmental pollutants was suggested to develop bioremediation processes in environmental biotechnology using laser stimulation of different species of plants, soil bacteria and fungi (Dobrowolski et al. 2015). These improvements cover the increase of plant biomass grown in contaminated soils and purifying sewage and soil reclamation.

Singh S.P. and Singh P. (2015) have conducted a study on the effect of temperature and light on the growth of several algae species. Different algae species were grown at different temperatures, photoperiods and light intensities. The study resulted in setting the ideal environmental conditions that promote efficient algae growth. The reported results showed that green algae containing major light-harvesting pigments of *chlorophyll-A and chlorophyll-B* were sensitive to blue and red light. Better growth rate (1.73 per day) was achieved when green algae like *C. reinhardtii* were irradiated with a combination of red and blue light with intensities up to a certain limit (from 33 to 400 µmol m^{-2} s^{-1}). Several temperature settings (from 0 to 35°C) were tested with the aim of obtaining a better growth rate. On observing *cyanobacteria* blue-green algae, it was found that the optimum temperatures for photosynthesis were in the range 0 to 20°C during winter and 20 to 30°C during summer. For other algae species, favourable temperature for growth sits between 22 and 35°C with the optimum temperature range for growth appears between 20 and 30°C. Light irradiation varies between 33 to 400 µmol m^{-2} s^{-1}. Maximum growth rate of 1.73 per day was found for *Selenarstrum minutum* to occur at 35°C and 420 µmol m^{-2} s^{-1} irradiance, while minimum growth was reported for *Botryococcus braunii KMITL* 2 strain at a temperature of 25°C and 200 µmol m^{-2} s^{-1} irradiance.

Wagner et al. (2016) worked on enhancing growth and energy conversion in microalgae to promote high-efficiency cultivation for life-supporting systems in outer space applications. This work aimed to quantify biomass yields when using colour illumination compared to white light. *C. reinhardii* algae were cultivated in plate reactors with regard to photoconversion efficiency. Highest photoconversion efficiency was obtained under low photon flux density when algae were illuminated with red and blue light of 680 nm and 447 nm wavelengths at a 90 to 10% molar ratio. At higher photon flux densities,

saturation effects were evident due to light absorption characteristics and the linear part of the photosynthesis-irradiance curve.

Vejrazka et al. (2012) investigated the effect of attenuated flashing light on photosynthetic efficiency of *C. reinhardtii* in a PBR with alternate light and dark zones in order to experience variation in photon flux density, which is called light or dark cycle. The study observed how these light or dark cycles affect biomass yield in microalgae cultivation. They have implemented a controlled short light path on laboratory-based turbidostat-operated PBRs equipped with LED light source controlled by a square wave signal to mimic light or dark cycles with frequencies ranging from 1 to 100 Hz. Biomass density was attuned so that the photon flux density leaving the PBR was equal to the compensation point of photosynthesis. Algae were acclimated to a sub-saturating incident photon flux density of 220 µmol m^{-2} s^{-1} of continuous light. On using a duty cycle of 0.5, it was found that a light or dark cycle of 1 and 10 Hz resulted in 10% lower biomass yield. On the other hand, a 100 Hz light or dark cycle resulted in a 35% higher biomass yield than that obtained using continuously illuminated samples due to Calvin cycle, details of Calvin cycle are given in Chapter 2. The results obtained confirmed that the interaction between light or dark cycle frequency, culture density, and incident photon flux density played an effective role in the overall productivity of a PBR. This has application in biomass yield maximisation techniques for space application and in the use of artificial light sources in biomass cultivation.

Ramanna et al. (2017) evaluated light enhancement strategies to lead to improved microalgae biomass productivity. One of the drawbacks for commercialisation of microalgae-based biofuels and products is limited biomass productivity. A considerable amount of research has been conducted on enhancing microalgae biomass production due to its potential sustainability and variety of applications. The traditional methods followed in order to improve biomass productivity are limited to adaptation of cultivation conditions. In some researches, genetic engineering is considered as a means to improve biomass productivity (Radakovits et al. 2010). Photons are one of the essential factors that govern microalgae growth. Research on the adaptation and manipulation of natural light rather than the adaptation of microalgae has been very limited. Microalgae utilise only a small fraction of light wavelengths (blue and red from the wide spectrum of solar radiation) for photosynthesis. In order to enhance microalgae biomass, it is required to

enhance photosynthetic efficiency. This enhancement can be attained by manipulating the light spectrum to achieve an optimal balance between photosynthesis and photoprotection. Manipulation of incident irradiance may be viable for increased light harvesting by algae. This enhancement not only reduces unused wavelengths but also concentrates the wavelengths in the range utilised by algae. This would result in maximum utilisation of light spectrum by microalgae. This work crucially analysed different light manipulation techniques that can modify the spectrum of light received by the algae in order to improve biomass productivity. Although sunlight can be considered to be an economically viable source of light, controlling the beneficial components of the spectrum is currently not realisable. Artificial light sources (especially semiconductor LEDs) can overcome some of the hurdles of sunlight utilisation as controlling light emissions from these devices is easily achievable. However, the cost and energy limitations need to be overcome. The one approach that can be considered novel is light spectrum adaptation by the shifting of unusable wavelengths to PAR by wavelength filters. Realising this concept, in reality, will depend on practical applications and economic feasibility. Although optical light filters lead to increased algae productivity by reflecting excessive ultraviolet (UV) radiation, fluorescent paints are able to convert UV to visible light, thus increasing the light intensity by the addition of photons. Improved photosynthetic photon flux densities within the PAR range due to the concentration of scattered and unusable light by organic dyes has comparatively the best potential for successful application. A combination of the light adaptation techniques can be considered to overcome individual limitations. These new concepts have potential applications in next-generation microalgae cultivation systems.

1.5 Algae cultivation for industrial and commercial purposes

A program implemented by the US *National Renewable Energy Laboratory* (NREL) following the oil crisis of the 1970s led to developing renewable transport liquid fuels produced from algae, the program ran from 1978 to 1996 and its cost was $25.05 million. The main conclusion of those studies was, that "in principle and practice large-scale microalgae production is not limited by design, engineering, or net energy considerations and could be economically competitive with other renewable energy sources" (Nrel 1998a). During the recent decade, a great amount of attention is directed at considering

microalgae biomass production as a good potential source of biofuel because of the issues regarding the high price of fuel and global warming. There are several companies working on the production of biofuel from algae, but none of them so far are providing commercial-scale amounts at competitive costs, and the procedure of producing fuel from algae would seem to be uneconomic (Sills et al. 2013). Estimations of the decrease in production cost required for the biofuel from algae to become economic differ, starting from a factor of 5 up to 2 orders of magnitude (de-Bashan & Bashan 2010). Approximately 70 years of research on microalgae fuels, and more than $2 billion of private sector investment since 2000 (Christenson & Sims 2011) have not formed economically viable amounts of algae fuel and this indicates that there are major engineering and technical challenges to be resolved before economic-scale algae biofuel products can be achieved.

Research has focused on producing energy cost effectively from wet algae because of the large amount of water in algae (about 80 to 90% of macro-algae). To obtain energy from the biomass this water has to be evaporated which requires more energy than the energy contained within it (Iersel 2008). Production of bioethanol and biogas from seaweed is being explored in South America, Europe and Asia while producing bio-butanol from macro-algae is attracting investment and research interest in the USA (CCC 2011). Using macro-algae at a commercial-scale production of fuels is limited as the cost of energy production is high, with estimations telling that biogas production from seaweed might be 7-15 times more expensive than natural gas (Milledge et al. 2014).

Biodiesel involves complete combustion when compared to fossil fuel diesel, as biodiesel has higher oxygen content (usually 10 to 12%) and higher Cetane Number (CN), resulting in better combustion, lower particulate materials and hydrocarbon emissions (Ajala et al. 2015). During engine tests biodiesel produced by algae typically gave similar results to conventional diesel, with low concentrations of Sulphur (Ramesha et al. 2016) and enhanced engine performance (Mostafa & El-Gendy 2017). Varying the cultivation conditions can affect the content of microalgae (more discussion in Chapter 2). The most broadly studied algae, which are oil-rich, are green algae and recent studies are being directed on manipulating metabolic paths, for algae production that synthesises ‘designer fuels’ (Ferenczi et al. 2017).

Further to direct use of algae biomass, waste biomass of algae can generate bio-methane and bio-ethanol by fermentation (Nguyen et al. 2016) and some of its species can produce hydrogen through activity of a *chloroplastic hydrogenase*, which is powered by reactions of reduction that took place during photosynthesis. There are several studies being conducted to develop a process so that algae can produce H_2 under certain growth conditions (Sharma & Arya 2017). A small number of species have displayed the ability for hydrocarbon production (Kosourov et al. 2007) and a few cyanobacteria are able to secrete ethanol; *Oscillatoria* sp., *Microcystis* sp., *Cyanothece* sp. and *Spirulina* sp. (Luo et al. 2010). Genetic engineering might be required in order to increase the efficiency on several sectors of the metabolism, which is not easily achievable (Bonente et al. 2011).

1.6 Other algae products

Algae have been used for food production for human consumption and for fertilising soil. Seaweeds were also used by some cultures as food; for example Dillisk in Ireland, Dulse in Scotland, Laver in Wales, Nori in Japan and Limu in Hawaii. The main markets for these seaweeds as food are in Asia. In 2006 algae production in China was more than 70% of the world's production with aquaculture about 15.1 million wet tonnes of the yearly world production, and harvest from wild stocks about 1.1 million wet tonnes (Roesijadi et al. 2010). The industry of macro-algae is currently 100 times larger in terms of wet tonnes than the industry of microalgae (Milledge et al. 2016). Microalgae can be efficient 'sunlight-driven microbial factories' for some other applications. It is also understood that the extracted biodiesel from algae may only be competitive with the traditional diesel and economically viable when the valuable secondary products are co-produced (Mondal et al. 2017). Human usage of microalgae has been around for thousands of years; for example the Chinese used *Nostoc* and *Aphanizomenon* to survive food crisis. Chad and Mexico used *Spirulina* as a source of food (Chisti 2006). There is a long history of using algae for therapeutic purposes, but using algae to produce biologically active substances, especially investigation for antibiotic activity, started only in the 1950s. The majority of research works up until the 1980s were dedicated to macro-algae (Borowitzka 1995). Screening of 15,000 natural marine products has been taking place for biological activity and 45 natural products derived from marine sources have been tested for medical purposes in preclinical and clinical experiments. Only two

products have been established as registered drugs, one from a sea squirt and the other from a marine snail, with none as yet from microalgae (Milledge et al. 2016). The world market for microalgae biomass to generate non-fuel products is expanding. Microalgae produce vitamins, which elevates their importance as a nutritional food for people and animals (Khan et al. 2018).

1.7 Aim

The aim of this research is to demonstrate that the growth rate of algae can be enhanced using blue and red lasers as coherent sources of light employed both singularly and in several combinations compared to white light LED and natural light.

1.8 Objectives

The objectives of this thesis are as follows:

1. Review current publicly available and specialist academic literature on algae growth.

2. Identify the mechanism of efficient transfer of energy between the photons of lasers and the electrons within photosystem II (Photosystem II, or water-plastoquinone oxidoreductase) and photosystem I (PS I, or plastocyanin-ferredoxin oxidoreductase).

3. Design and build three custom bioreactors to perform the experimentation work of this research for automated real-time monitoring system.

4. Investigate the influence of using blue, red and different combinations of diode lasers having wavelengths of 405 nm, 450 nm, 473 nm, 635 nm, 650 nm, 680 nm, 685 nm and 700 nm in comparison with white light LED (LTW-2S3D8 from Lite-on Inc) to verify the effective improvement in growth of *C. reinhardtii* algae species using laboratory scale PBRs.

5. Compare the growth efficiency and viability of implementing different wavelengths of blue and red diode lasers in specific combinations with the use of published data and data produced in this research and lay down recommendations for the best models and strategies that can be followed in order to yield optimised growth of algae and identify what future research is required.

1.9 Summary of methodology

In this study, blue and red laser irradiation was experimentally tested to determine if faster algae growth might be achieved when compared with that of white light LED irradiation. Eight individual lasers having the following wavelengths (405 nm, 450 nm, 473 nm, 635 nm, 650 nm, 680 nm, 685 nm and 700 nm) were employed in different combinations to generate photons with energies corresponding to the absorption spectra of pigments within *C. reinhardtii* algae. There were eleven different experimental combinations of blue and red lasers; this was with a view to enhancing the transportation system and accelerating the electrons travelling within the pathway between PS II and PS I, thus anticipating an increase in the rate of cell division, as discussed in detail in Chapter 4.

The approach to the work was in two phases:

The first phase: Analogue – in that the equipment was physically set up to facilitate each of the control parameters and measurements to be taken manually at plotted time points throughout the pre-set time frames. There were many variations being explored at this time to include the multiplicity of positions for the lasers in relation to the samples in the search for accuracy of photon throughput relating to cell division. The algae were sub-cultured using two samples at all times, and cells counted throughout the whole series of experiments by way of using the Bio-Chemistry Analyser in correlation to Beer's law.

The second phase: The first PBR provided irradiation sourced from white light LED for 60 hours; it generated samples suitably excited to a state whereby they are ready for exposure to combinations of laser irradiation and or further exposure to white light LED. A second PBR in operation used white light LED, this received a sample from bioreactor 1 and continued irradiation of white light LED for 12 hours as a reference. The third PBR received its samples from bioreactor 1 and employed the combinations of laser irradiation

for 12 hours in terms of each culture. The power of the lasers and LED was measured by using a power meter and an attenuator was used to establish a power input of 1 mW with a standard deviation of ±0.05 mW for both the laser and the LED.

Consideration was given to the temperature as well as the pH of the nutrient solution; firstly, the temperature was maintained between 19 and 25 ºC, secondly, the pH was measured at each stage and recorded accordingly. The laser of 473 nm wavelength generated a recognised amount of heat, therefore, additional ventilation was necessary to ensure accurate measurements (Abed 2015). Continual mixing to maintain uniformity of nutrient with the cell population was achieved by way of an electrical adjustable flow rate air pump, this to provide air bubbles in order to stimulate cell division in the PBR used during the experimental work of this research.

To improve the reliability of the data gathered and its continuity of accuracy, [DAQ NI USB 6008 & LABVIEW in combination] were employed. Individual control modules were written which collected data continually in real time, thus gave outcomes that could be more finely tuned through manipulation of input (Das et al. 2011). The programs responded to the output signals from the sensors strategically placed throughout the laser and LED bioreactors.

1.10 Outline of the thesis

This thesis was developed in order to determine the level of current achievement regarding the research of algae cultivation and investigate the advantages of using the diode lasers as sources of light energy in order to reduce the limitations that are impeding the commercialisation of this renewable energy source. Chapter-2 contains a thorough survey about photonic effects on accelerated algae growth along with outlining the current obstacles confronted and the feasible means and ways to overcome some of them. Chapter-3 outlines the available mechanisms of describing the interaction between photons and electrons of plants and algae pigments. Chapter-4 describes the experimentation setup and explains the experimental work conducted to assess the practicality of using blue and red diode lasers as sources of light, this to improve algae growth or production. Chapter-5 contains the outcomes of the experimental work in

addition to analytical investigation of the data gathered from the experimental algae PBRs. Batches of two or three algae samples were exposed in parallel to eight different wavelength laser sources as well as to white light LED and natural daylight conditions. The final contents of this chapter are the results and findings of the research work as well as our general theory that suggests a physical mechanism for reducing the photons' energy by successive quantized transitions of electrons among a variety of absorption spectra of pigments within the PS II and PS I. Chapter-6 presents the conclusions drawn based upon the work and outcomes applicable to this research. Sequentially, there are recommendations that may be followed as a guideline to instigation of future research and development that can develop benefits from the results of this work and its proposed strategies. That have been followed in the preparation of this thesis.

1.11 Contribution to knowledge

In this research novel findings have been verified and the following achieved:

- Obtained a matrix of blue and red diode laser outcomes employed both singularly and throughout an extensive array of several combinations that can contribute to better photosynthesis and growth rate in algae thereby resulting in higher productivity rates. This research examined all of the available laser wavelengths in particular combinations; the resultant data contributed to the assembly of a matrix that illustrates most appropriate laser combinations that promote growth of algae.
- Improved the cell division rate of algae by about 5 times that of white light LED using certain combinations of blue and red laser wavelengths as sources of light.
- Identified the scanning sequence of the diode lasers and quantified the amount of growth of algae at every stage of using blue and or red lasers.
- Identified and monitored the speed of cell division when compared to the ambient cell division rate.
- Introduced the blue laser of wavelength 405 nm as a very cheap alternative to the expensive blue laser of wavelength 473 nm as both wavelengths produced a similar amount of the growth rate of algae.
- Identified the mechanism of electron transfer through the relationship between diode laser generated photon energy and electron activity in PS II and PS I. This investigation underpins the realisation that photons emanating from a specific diode laser are absorbed by algae pigments because there is potentially a much closer matching between the emission spectra of the lasers and the peaks of the absorption spectra of the pigments of algae.

- Established a process to pump air bubbles as a fuel to mix the algae in order to allow the photons to reach the cells of algae homogeneously and also maintain the pH level at the recommended range (6 to 8).

1.12 List of publications

- Al-yasiri, S., Balachandran, W. & Hooker, D., 2017. Red Laser Excitation of Electrons for Growth Enhancement of Chlamydomonas Reinhardtii Algae *Journal of Advances in Biochemistry and its Applications*, 1(1), pp.12–19.

- Alyasiri, Sadiq et al., 2018. Blue laser excitation of electrons within the pathway between photosystem II and I for growth enhancement of chlamydomonas reinhardtii algae. *5th International Conference on Renewable Energy: Generation and Applications (ICREGA)*, pp. 25-29. Available at: https://ieeexplore.ieee.org/document/8337587/

- Al-yasiri, S., Balachandran, W., Hooker, D., 2018. Blue and Red Lasers Combinations for Growth Enhancement of Chlamydomonas Reinhardtii Algae. *The Professional Engineer Journal*, 95, pp.17-21.

CHAPTER 2

PHOTONIC EFFECTS ON ACCELERATED ALGAE GROWTH

2.1 Introduction

In this chapter, an in-depth literature survey was carried out on significant information regarding the topics related to the work done in this research, in addition to that, recent relevant research papers and studies are covered as well. A critical review of the published literature is presented.

Photosystem I and Photosystem II were defined and the factors affecting the division of cells within algae are explained including methodology details of cultivation. The details of problems and achievable solutions facing the cultivation enhancement of algae are illustrated.

2.2 Photosystem I and Photosystem II

Photosynthesis initially occurs in PS II where photons strike the photosynthetic pigments such as *chlorophyll-A* or *chlorophyll-B,* carotenoids or phycobilins and are absorbed by their electrons within an antenna complex (light harvesting complex) (Caffarri et al. 2014). The photons then are passed to *P680* reaction centre (Cardona et al. 2012); which has *chlorophyll-A* that absorbs photons of light at a wavelength of 680 nm. The electron that absorbed the entire energy of the photon is now excited to its highest energy level. This electron then leaves the reaction centre as a result of the donor ionisation and is

captured by a coenzyme called “The primary electron acceptor”, which is a *pheophytin* molecule found within PS II. The electron leaves a hole within *P680*, and the hole now needs to be occupied by a replacement electron in its ground state of energy; This is taken from the water at this point by the splitting up of hydrogen ions and molecular oxygen from the water (Engel 2011). After the electron has reached this high state of energy within the primary electron acceptor, it leaves the primary acceptor by ionisation and starts to move on down the electron transfer chain, firstly by being absorbed into *fluoroquinolone* and then onto the *b6f* cytochrome complex, also known as plastoquinol-plastocyanin reductase, which moves the electrons from PS II to PS I and on to *plastocyanin*. As the electron is transported from PS II to PS I, it loses energy to produce ATP as shown in Figure 2.1 (Engel 2011).

The photosynthetic pigments at PS I absorb the incident photons and pass them on through its own light gathering complex to reach the reaction centre *P700* which absorbs light at 700 nm. The electron that has passed on through the electron transfer chain, having lost part of its energy to the production of ATP, is re-excited by the photon energy absorbed by the pigments of *P700* and moved up to a higher state once again on its journey to the second electron acceptor. This re-excited electron then leaves the reaction centre and is absorbed by the *ferredoxin* complex. From the *ferredoxin* evolves NADP+ (Reductase), this then requires two hydrogen ions to complete the production of *nicotinamide adenine dinucleotide phosphate hydrogen* (NADPH) (Foyer et al. 2012). NADPH and ATP provide electrons and phosphate within the Calvin cycle to reduce carbon dioxide and produce a sugar complex, thus producing a glucose molecule (Disselnkötter & Rendall 2017).

Several models have been proposed to explain the electron passing sequence followed by the photosynthesis process from PS II to PS I at a quantum level; the following models are exemplar of this approach:

Forster's model (Chenu & Scholes 2015) depicts that the excitation energy taken from the photon and carried by the electron which is now at an excited state can be transferred from site to site through a series of incoherent jumps, but in the light of the conducted experimentation, it is suggested that the leaps in energy gained are in fact quantised to the levels appropriate to the particular protein that the electron has entered, thus supports their

findings of the electron jump from higher energy to lower energy levels thereby following a potential funnel similar to that which is believed to aid protein folding. In this manner, the excited electron energy can be transmitted through the membrane (Hoyer et al. 2012) as explained in detail in section 3.3.

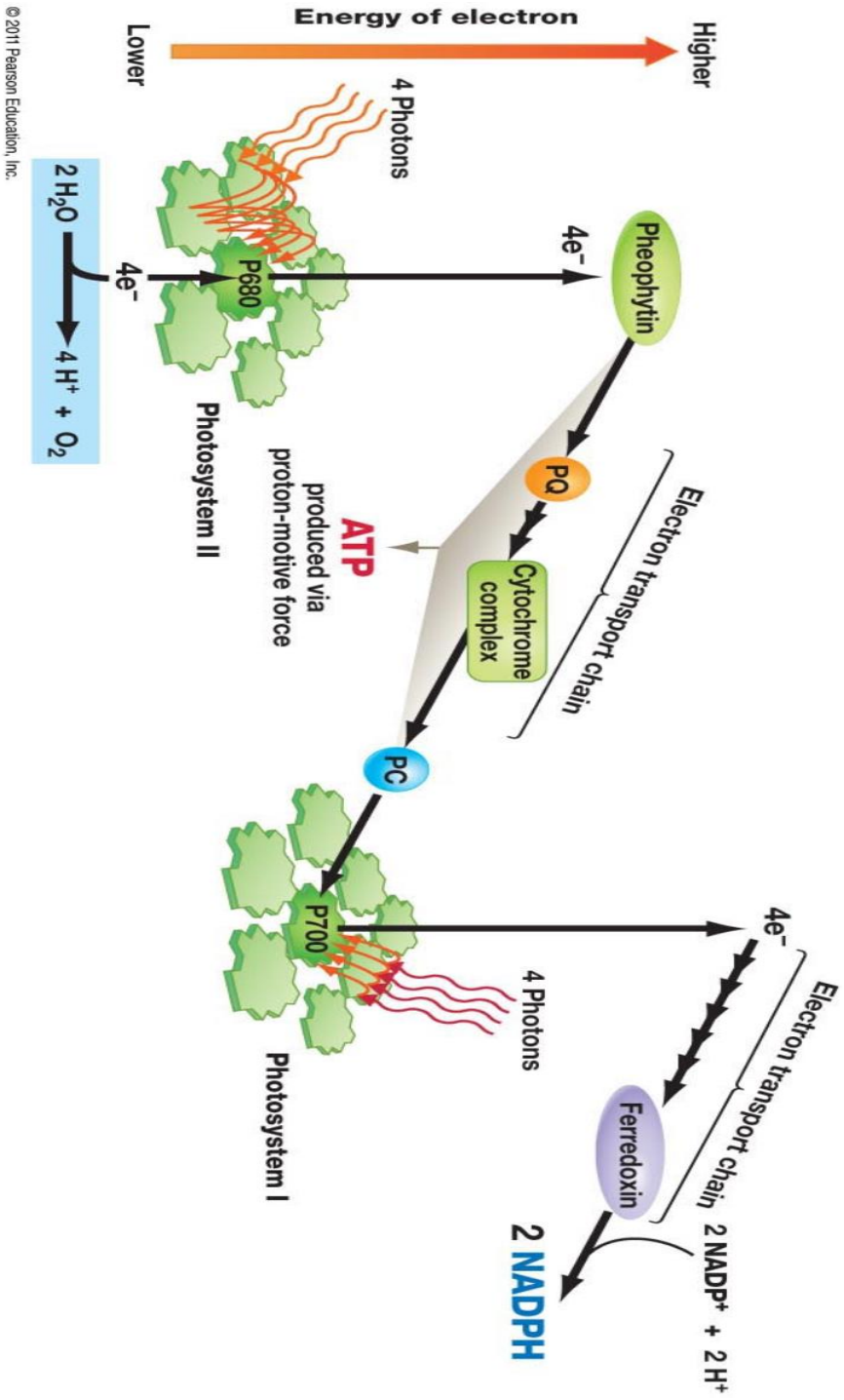

Figure 2.1 PS I and PS II *© 2011, Pearson Education, Inc.*
https://photosynthesis4all.wordpress.com/portfolio/history-of-photosynthesis-2/

During the light-dependent reaction, the chloroplast is receiving a steady flow of photons, NADPH and ATP molecules are being rapidly provided to the metabolic pathways in the stroma, which fuels the Calvin cycle reactions as illustrated in Figure 2.2 (Rizzo et al. 2014).

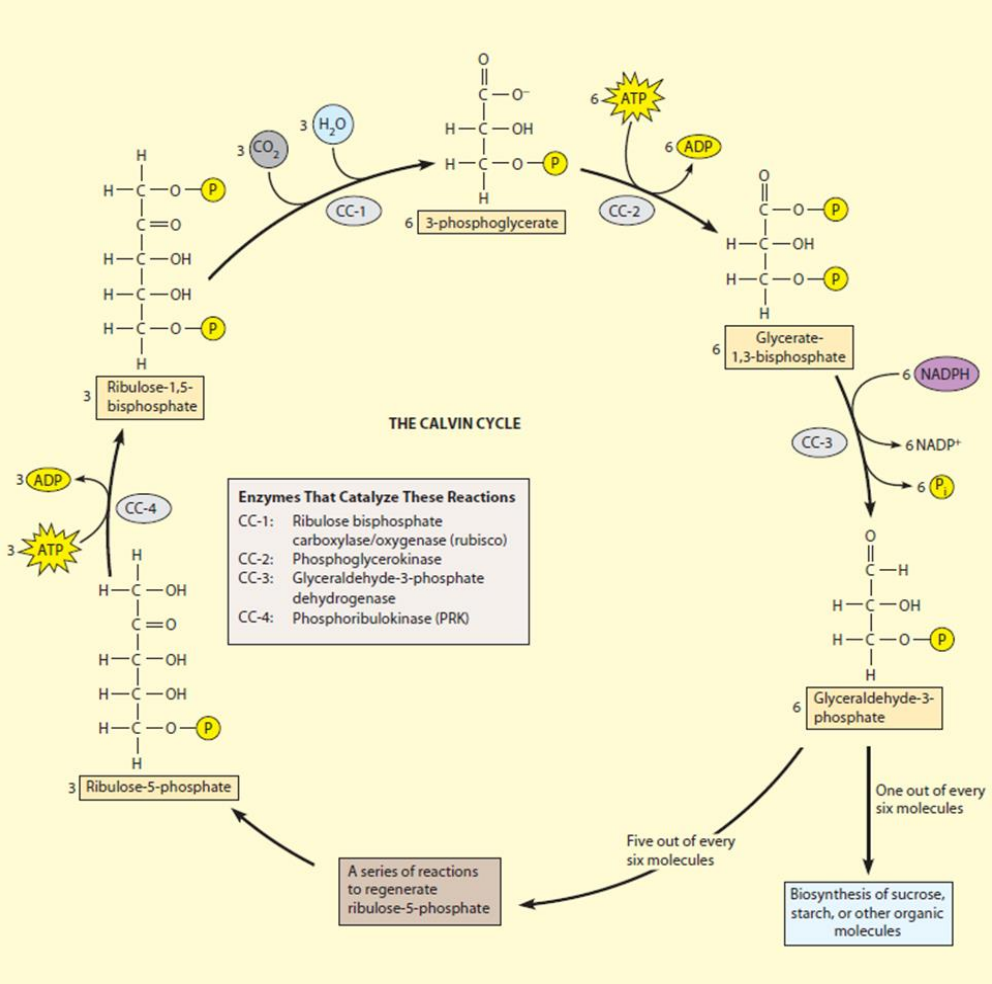

Figure 2.2 Calvin cycle. *Source: (Rizzo et al. 2014).*

The Calvin cycle includes a series of molecular reactions that reduce CO_2 to produce the *carbohydrate Glyceraldehyde-3-phosphate*. This cycle consists of three steps:

1. Carbon fixation (*carboxylation*) step; in this step, 3 carbon dioxide molecules are attached to 3 x 5-carbon sugar (*Ribulose-1, 5-biphosphate*) resulting in a 3 x 6-carbon molecules that splits into 6 x 3 carbon molecules (*3-phosphoglycerate*) by using an enzyme called *Ribulose bisphosphate carboxylase/oxygenase (Rubisco)*.

2. Reduction and energy input step: a sequence of reactions using a duality of electrons and phosphate flow from NADPH and ATP to reduce carbon dioxide; ATP is broken down, by using an enzyme called *Phosphoglycerokinase*, and converting each *3-phosphoglycerate* into *Glycerate-1, 3-bisphosphate* by giving it a phosphate; NADPH gives up electrons to convert each *Glycerate-1, 3-bisphosphate* into *Glyceraldehyde-3-phosphate* with more energy by using the enzyme *Glyceraldehyde-3-phosphate dehydrogenase*. 3 carbon sugar intermediates are being generated, the 1 x *Glyceraldehyde-3-phosphate* exit Calvin cycle to be used by algae to make glucose, fatty acids or glycerol. The leftover *Glyceraldehyde-3-phosphate* is then recycled.

3. Regeneration step: in this step *Ribulose-1, 5-biphosphate* is regenerated. 5 x *Glyceraldehyde-3-phosphate* are converted back into 3 x *Ribulose-5-phosphate* molecules and those will be *phosphorated*, by using 3 ATP and the *Phosphoribulokinase* (PRK) as a catalyst, to regenerate *Ribulose-1, 5-biphosphate* to be used in the next Calvin cycle (Rizzo et al. 2014).

The Calvin cycle has to run 6 times and used 18 ATP molecules and 12 NADPH molecules in order to produce 1 molecule of glucose. It is possible for these molecules to shed their phosphate and add fructose to produce sucrose, this being the molecule that algae cells use to transport carbohydrates throughout their systems. Glucose phosphate is the starting point for the generation of both cellulose and starch. The difference between the starch and the cellulose is the link between the glucose units. In starch, the oxygen atom is joining neighbouring glucose units – points down relative to the plains of the rings, a configuration known as "*Alpha Linkage"*. In cellulose, the oxygen atoms are almost parallel with the planes of the rings but pointing slightly upwards, this arrangement is called "*Beta Linkage*". This difference in linkage causes the differences in properties of Starch and Cellulose and is known as the *Glycosidic linkage* (Stick & Williams 2009).

This work looks into a gap in the scientific knowledge that pays particular attention to the potential to increase the efficiency of the electron transition between PS II and PS I through irradiating algae with several wavelengths individually and in combination of blue and red lasers including 405 nm, 445 nm, 473 nm, 638 nm, 650 nm, 680 nm, 685 nm

and 700 nm to generate photons with energies more closely matching the absorption spectra of receptors known as pigments (Jiang & Wen 2015).

2.3 History of producing renewable energy from algae

Producing renewable energy from algae is not a new idea as it has been conducted since the previous century. Research carried out on the concept of extracting energy from the biomass of microalgae has continuously been most predominant near times of energy insecurity (Nrel 1998b). This has been applied to both cases of freshwater and saltwater algae (Cheng et al. 2011). Research involving investigation of the potential of extracting energy from freshwater algae was first started in the 1950s (Bogan 1961). The first step was initiated as a result of thoughts on using the biomass of algae in the treatment of wastewater where the biomass was used as a source of oxygen in the oxidation process of bacteria (Bogan 1961). Research investigating the algae biomass anaerobic digestion from wastewater was first published in 1957 (Golueke et al. 1957). Studies carried out on investigations of energy extracting potentials from aquatic biomass became more evident in the 1970s because of the oil crisis in that decade. Researchers were mainly focusing on producing methane (Sialve et al. 2009) although attention began to encompass biodiesel production. The *Aquatic Species Program* focused on researching the production of biofuel from oil-rich algae (Nrel 1998b). Although the program lasted twenty years and some good results were gained, the program was slowly phased out because the US recovered from the oil crisis. Harvesting bioenergy from macro-algae (seaweed) has followed the same pathway, it also went on receiving less care because the macroalgae cultivation was less flexible and the diversity of possible energy production was limited (Bruton et al. 2009). The *Marine Biomass Program* was started in the 1970s to investigate the possibility of cultivating macro-algae and transforming the biomass into biogas. At the end of the 1970s and similar to the research on microalgae, there was a decline in interest associated with this area caused by returning to fuel security and fund reduction. Fear of climate change and oil security played a big role in research resurgence towards the transformation of the biomass of algae to bio-energy (Birch & Calvert 2015). Currently, research is extensive, having included several different species of algae both macro and micro in addition to a large diversity of bio-energy products (Magdalena et al. 2018; Khan et al. 2017).

2.4 Factors and conditions affecting algae cultivation

Technologies such as open ponds and PBRs are most generally used for growing algae (Olvera-Gonzalez et al. 2013). There are a variety of growing conditions that play a vital role in optimising the algae growth process, where the nutrients and the medium in which the algae is cultured affect the final density of lipids, the composition of energy production as well as the algae growth rate.

2.4.1 Nutrients of growing medium

In this research, a standard Tris-acetate-phosphate (TAP) medium was used to grow *C. reinhardtii* algae. Test tubes were used in order to get laboratory scale samples (discussed in detail in Chapter 4). The availability of nutrients has a major impact on algae growth and propagation and wider effects on the lipid and fatty acids composition. When they are limited, environmental stress conditions cause a progressive decline in cell division rate. Unexpectedly, fatty acid active biosynthesis is continued in some species of algae under similar conditions, assuming that there is enough carbon dioxide and light accessible for photosynthesis (Sakurai et al. 2014). When the growth rate of algae slows down, (as a result of no need for the synthesis of new compounds for the membrane) the cells then start diverting and depositing fatty acids to TAG. As a result of such conditions, producing TAG acts as a defence mechanism. Under normal conditions of algae growing, the production of ATP and NADPH by photosynthesis will then be consumed during the process of generating biomass in Calvin cycle, then ADP and NADP+ ultimately being available again to be used in photosystems II and I as acceptors of electrons and hydrogen ions in photosynthesis (detailed discussion is provided in Chapter 3). Growth and proliferation of algae cells will be impaired if lack of nutrients takes place. As a result, the pool of NADP+, the main acceptor of electrons during photosynthesis, can become depleted. In the presence of light, photosynthesis cannot be stopped completely. This can put the cell in a possibly dangerous situation that may result in damaging its components. Under growth-limiting conditions, increasing the biosynthesis and production of fatty acids by consuming NADPH and then storing them in TAGs will lead to replenishing the pool of NADP+ (Smith & Gilmour 2018). Starvation of nutrient is one of the best techniques for lipid induction in microalgae production of TAGs and has been stated for many species as illustrated in Table 2.1. As examples, a percentage increase in accumulation of TAGs and a percentage decrease of lipids was observed in all of the

cultures when the *Stephanodiscus minutulus* was grown under limitation of silicon, nitrogen or phosphorus (Das et al. 2017). Nitrogen majorly affects the metabolism of lipid in algae. During the nitrogen deficiency a general pattern of lipids accumulation, particularly TAG, has been noticed in many strains of several microalgae (Fakhry & El Maghraby 2015). The lipid produced was enhanced immensely in many green microalgae, diatoms and cyanobacteria as a result of nitrogen stress, where a study performed by Minhas et al. (2016) explained this. Rodolfi (2009) submitted a study of an extensive model of lipid production by nitrogen and phosphorus tarvation on some green algae and diatoms. Silicon deficiency conditions produced larger amounts of neutral lipids (mainly TAGs) and fatty acids in the growing of diatom *Cyclotella cryptic* (Wang & Seibert 2017). Contrastingly, nitrogen deficiency caused little rise in the amount of TAG (from 69 to 75% of the overall amounts of lipids) and a small portion of phospholipids (from 6 to 8%) for the microalga *Phaeodactylum tricornutum* (Das et al. 2017). A percentage increase in lipids of 53% and 30%, has been recorded when *Scenedesmus* sp. was subjected to phosphorus or nitrogen limitation respectively (Das et al. 2017). Under low nitrogen concentration conditions lipid content of *Chlorella vulgaris* algae could be considerably increased by 40% (El-Kassas 2013). There was a 2.5 fold rise in the amount of lipid of *Chlamydomonas vulgaris* under manipulated culture conditions of 1 mM KNO_3, 1.0% CO_2, 60 μmol m^{-2} s^{-1} (where μmol m^{-2} s^{-1} is the unit of light intensity) and at 25 °C (Chen 2013). Furthermore, in *Chlorella*, the amount of lipid was also increased with silicon deficiency (Adams 2013) and iron supplementation (Liu & Wang 2008). Additionally, in *Chlamydomonas vulgaris*, changing the normal nutrient media to nitrogen depletion media progressively changed the structure of lipid from free fatty acid-rich lipids to lipids in TAGs form (Widjaja et al. 2009). Nitrogen deficiency in microalgae affects the fatty acid metabolism as well as the pigment composition. The pigment arrangement in microalgae and the fatty acid metabolism are both influenced by nitrogen deficiency. There was a significant proportional increase of carotenoid and chlorophyll contents for growing *Parietochloris incisa* in nitrogen-replete medium (Solovchenko et al. 2013). In *Chaetoceros* sp., *P. tricornutum*, *Pavlova lutheri* and *Isochrysis galbana*, increased lipid content has been recorded; mostly as TAGs, but decreased lipid amount in *Tetraselmis* sp. and *Nannochloris atomus* due to phosphorus limitation (Das et al. 2017). Phosphorus deficiency was also the cause of increasing the overall production of TAGs from 6.5% to 39.3% with a steady decrease in the

concentration of *eicosapentaenoic acid* (EPA), the total amount of cellular lipid content increased, mostly because of the accumulation of TAG in *Monodus subterraneus* (Das et al. 2017). Under phosphate deficiency conditions, the replacement of phospholipids by non-phosphorus glycolipids and betaine lipids was recorded (Sebastian et al. 2016).

In *C. reinhardtii* green algae, sulphur depletion causes a decrease in *sulfoquinovosyl*

Table 2.1 Nutrients which have been used in induction of lipids in microalgae

Microalgae species or strain	Nutrient stress	Changes in lipid profile after induction	Reference
Chlamydomonas reinhardtii, Scenedesmus subspicatus	Nitrogen limitation	Increase in total lipids (lipid: amide ratio)	(Dean et al. 2010)
Nannochloropsis oculata	Nitrogen limitation	Total lipid increased by 15.31%	(Converti et al. 2009)
Chlorella vulgaris	Nitrogen limitation	Total lipid increased by 16.41%	(Converti et al. 2009)
Chlorella sp.	Nitrogen limitation	Lipid productivity of 53.96 ± 0.63 mg/l day	(Praveenkumar et al. 2011)
Phaeodactylum tricornutum	Nitrogen limitation	TAG levels increased from 69 to 75%	(Alonso et al. 2000)
Dunaliella tertiolecta	Nitrogen limitation	Five times increase in lipid	(Chen et al. 2011)
Chlorella vulgaris	Presence of Nitrogen	Lipids increased by 40%	(Illman et al. 2000)
Chlorella vulgaris	Nitrogen limitation	Increase in TAG	(Widjaja et al. 2009)
Chlorella sp.	Nutrient-deprived conditions (nitrogen, phosphate potassium, iron, and all three combined)	Total lipid production of 49.16 ± 1.36 mg/l day	(Griffiths & Harrison 2009)
Chlorella sp.	Urea limitation	Total lipid productivity of 0.124 g/ l day	(Hsieh & Wu 2009)
Neochloris oleoabundans	Ammonium nitrate presence	Lipid productivity of 0.133 g /l day	(Li et al. 2008)
Scenedesmus sp., *Coelastrella sp.*	Combined effect of pH and N-limitation	Increase in TAG	(Gardner et al. 2011)
Monodus subterraneus	Phosphorus limitation	Increase in TAG	(Khozin-goldberg & Cohen 2006)
Scenedesmus sp	Nitrogen and phosphorus starvation	Lipids increased 30% and 53%, respectively	(Xin et al. 2010)
Chlorella sp.	Silicon deficiency	N/A	(Griffiths & Harrison 2009)
Chlorella kessleri	Phosphorus limitation	Increase in unsaturated FAs	(El-Sheek et al. 1994)
Chlamydomonas reinhardtii	Sulphur limitation	PG was increased by 2-fold	(Sate et al. 2000)
Chlamydomonas reinhardtii	Sulphur limitation	Increase in TAG	(Matthew et al. 2009)
Cyclotella cryptica	Silicon starvation	Increased in total lipids from 27.6% to 54.1%	(Nrel 1998b)

diacylglycerols (SQDG), while *phosphatidylglycerol* (PG) was increased two-fold, providing a compensatory mechanism as sulphur enriched lipids are replaced by phospholipids. Growing *C. reinhardtii* in a phosphorus limited media displayed a percentage decrease of 40% in PG and also encouraged an increase in the SQDG content. In this way, the total of the concentrations of SQDG and PG are kept constant when there is sulphur and phosphorus deficiency (Okazaki et al. 2013; Nakajima et al. 2018). Other studies have also demonstrated that an increase in total lipid amount in the green algae *C. reinhardtii* and *Chlorella* sp. was caused by sulphur deficiency (Sate et al. 2000). Based upon the ground of the literature reviewed, nitrogen starvation is applied and studied in nearly all the microalgae that are suitable for producing biofuel (Table 1.1). Regulating the nitrogen stress on microalgae can be done by removing the source of nitrogen in the culture media. In addition, all the microalgae types studied so far (Table 2.1), appear to increase the production of TAGs under nitrogen stress conditions. However, producing high lipid under nitrogen stress conditions may take two to five days and the growth rates are slow with a small number of cell counts, therefore affecting the total amount of biomass and lipid productivity as shown by Matthew (2009).

2.4.2 Photon wavelength

Appropriate light wavelength is highly important in maintaining algae growth and survival. The reaction centres in PS I and PS II are enhanced biologically to enable the absorption of the light photons of wavelengths 680 nm and 700 nm, which have energies match with the absorption spectrum peaks of the reaction centres (Caffarri et al. 2014). Researchers have previously attempted to optimise the wavelength by using merely cool white light or LED. Thus, in this research, all the available laser wavelengths were considered and compared with white light LED to select a model of wavelengths combination for maximum algae production.

2.4.3 Photon flux

It has been established by researchers that the most hydrogen and energy can be produced when algae are grown under very low light intensities of 30-40 $\mu mol\ m^{-2}\ s^{-1}$ PAR (Bayro-Kaiser & Nelson 2017). This means that as a result of the algae being grown under a higher light intensity, a larger amount of oxygen can be produced, hence the difficulty in enforcing anaerobic conditions by sulphur deprivation. This information is reinforced by

detailed research suggesting the optimal light intensities for hydrogen and energy production as follows at different stages: 25 µmol m^{-2} s^{-1} PAR during the growing stage, 110 µmol m^{-2} s^{-1} PAR in the early sulphur deprivation and oxygen consumption stages and 20 µmol m^{-2} s^{-1} PAR later (Tsygankov et al. 2006).

Open pond bioreactors use sunlight, which is uncontrollable; therefore, algae studies are done indoors using artificial sources of light. Different sources of illumination are available; the cheapest ones are fluorescent lamps and tungsten halogen lamps. LEDs are used as well because their emission spectra are narrower than the emission spectrum of both fluorescent lamps and tungsten halogen lamps (Elvidge et al. 2010). Very few Researchers have studied the growth of algae under laser illumination (Kuwahara et al. 2011) and the research conducted in this thesis is the first unique one that studied the growth of algae under the illumination of all available semiconductors lasers having emission spectra that coincides with the absorption spectrum of different types of pigment within the algae structure (discussed in detail in Chapters 4 and 5).

The emission spectrum of white light LED (LTW-2S3D8 from Lite-on Inc) used in this research is illustrated in Figure 2.3.

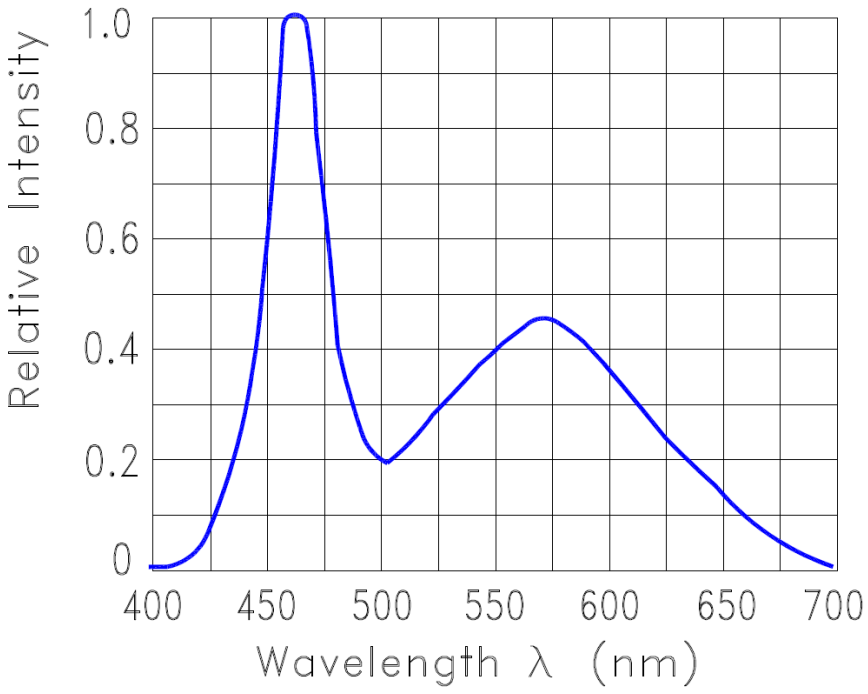

Figure 2.3 Relative intensity on energy basis of white light LED (LTW-2S3D8). *Source: (LITE-ON Technology Corp. / Optoelectronics 2012).*

A comparison between the emission spectrum of different sources of light is illustrated in Figure 2.4.

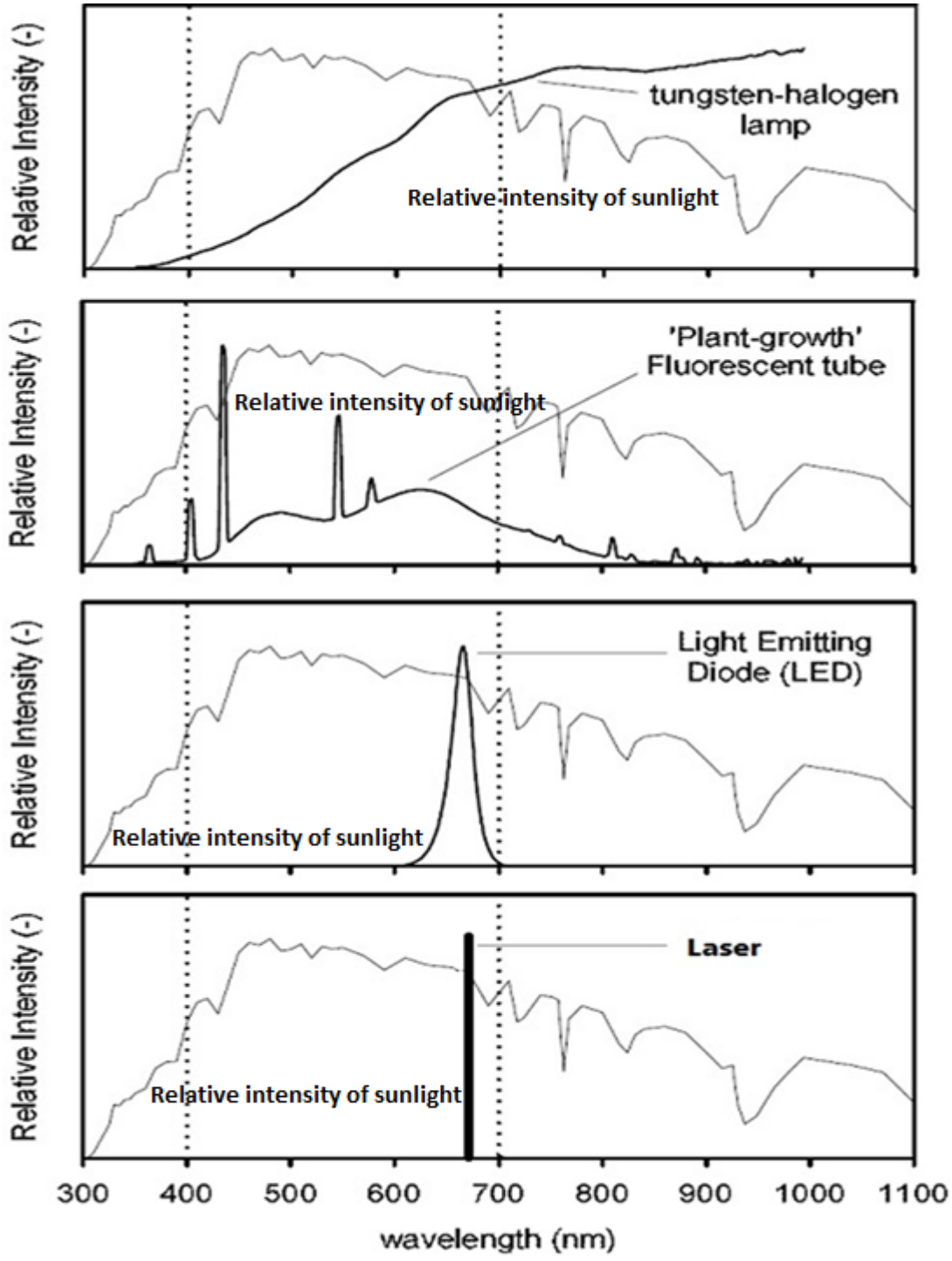

Figure 2.4 Relative intensity on energy basis of different sources of light. *Adapted from Janssen (2002).*

Figure 2.4 reflects the relative intensity on energy basis of tungsten halogen lamp (Philips Halotone, 300 W, 230 V, R7s), Fluorescent tube (Osram-Sylvania, Britegro 2003, 36 W), LED (Kingbright, L-53SRC-F) and Laser (680 nm, 0.1 mW diode laser). Relative intensity of sunlight is presented in the background as a reference.

2.4.4 Algae photoinhibition

Photoinhibition is caused by high light emission intensity. This condition reduces the rate of algae growth. The activity of photosynthesis varies during daytime; the lowest photosynthetic activity was detected between 12:00 and 2:00 pm when sunlight intensity is usually highest (Alagoas et al. 2017). When observing the production of biomass of *Isochrysis galbana* marine microalgae under different light intensities ranging from 820 to 3270 μmol m^{-2} s^{-1} showed a maximum growth rate at 1630 μmol m^{-2} s^{-1}. Any light intensity above this value will lead to decrease in biomass production due to photoinhibition (Bougaran et al. 2012). Although the production of biomass at a light intensity of 3270 μmol m^{-2} s^{-1} was higher than that at 820 μmol m^{-2} s^{-1} (19.2 mg l^{-1} h^{-1} and 16.6 mg l^{-1} h^{-1} respectively), the quantum yields were reduced when algae were exposed to light intensity above 820 μmol m^{-2} s^{-1} (Grima et al. 1997) as shown in Table 2.2.

Table 2.2 Quantum yield for different light intensities of photons. *Source: (Grima et al. 1997).*

Light Intensity (μmol m^{-2} s^{-1})	Quantum Yield (g biomass/energy mol)
820	0.603
1620	0.428
3270	0.087

2.4.5 pH values

The most appropriate pH values for algae culture range from 6 to 8, although this varies under different types of media (Simosa 2016). Throughout the cultivation process, these values fluctuate as a result of the variation of CO_2 concentrations. However, Lam and Lee's experiment (Lam & Lee 2012a; Lam & Lee 2012b) suggested otherwise, where they cultivated *Chlorella vulgaris* in media with different values of pH (3, 4, 5, 6, 7, 8 and 9), with results indicating the lack of change in the aspects of algae growth. Also, evaporation may be causing the salinity of water to increase throughout the production of algae (Jiang et al. 2018), where a high salinity may damage the algae cells since it might

alter the osmotic pressure of water between the media and the algae cells, consequently causing a change in the structure of the cells (Mata et al. 2010).

2.4.6 Temperature

The rate of algae growth is advanced to its optimal level due to an increase in temperature levels until reaching a critical temperature where the growth will decrease. The temperature may not always be possible to manage in open systems and outdoor cultures as various factors affect this, such as the surrounding temperature, humidity, and solar irradiance. For instance, changes in the surrounding temperature throughout the day can result in temperature variations during daytime of about 20°C, which could ultimately influence algae productivity (Nguyen et al. 2016; George et al. 2018). It takes longer for temperature variations of air to affect large amounts of water quantities; therefore, the air temperature needed for optimal culturing of algae (Malek et al. 2016). Temperature could affect photosynthesis and result in photoinhibition during low light intensities and sub-optimal temperature levels (Tait & Schiel 2013). The optimum range for algae growth should be maintained, as a higher temperature may kill the algae cells, though a lower temperature may not, excluding freezing temperature levels. As well as that, higher temperatures in the dark may lead to biomass loss (Weissman & Goebel 1987); therefore, reaching optimum temperature levels rapidly in the day and reducing these levels in the dark would lead to a high productivity in daytime and a decreased biomass loss at night.

A decrease in temperature levels (30 to 12 °C) could increase the production of unsaturated fatty acids, whereas an increased temperature (30 to 60 °C) could cause more saturated fatty acids to be produced (Zili et al. 2014). This condition has been experimented in several cyanobacteria and microalgae (Renaud et al. 2002); a change in the physical properties of the cell membrane had been observed as the lipid structure changed in order to maintain ordinary functions, like ion permeability, photosynthetic and respiratory processes, in working order (Kuhlbrandt 2015). When algae culture temperature drops from 30 to 12 °C, the percentage level of unsaturated lipids content in algae cells increases significantly by 20% (Singh & Kumar 2017). For *Ochromonas danica*, the cell count per unit volume of medium rises due to a raised incubation temperature from 15 to 30 °C. This also increases the amount of lipid (Zhongye 2013). Moreover, for *Botryococcus braunii* and *Chlorella vulgaris*, the amount of unsaturated fatty acids within the cells are reduced as a result of higher temperature levels (Juneja et

al. 2013). In *Nannochloropsis salina*, when temperature rises, the growth rate increases and lipid production increases as well (Ma et al. 2016). Whereas in the diatom *P. tricornutum*, a temperature decrease from 25 to 10 °C led to a yield elevation of 120% (1.2 fold) compared with the normal production; this represents yields of EPA and PUFA per unit dry mass of 2.6 and 4.9%, respectively (Jiang & Gao 2004). Studying the effects of decreasing temperatures in *Chlorella ellipsoidea* revealed an increase in the amount of unsaturated fatty acids by two-fold. Alongside this finding, it was noticed that in a low temperature-adapted strain of *Chlorella ellipsoidea*, there was an increase in ALA and unsaturated PG (Satpati et al. 2016). On observing *Pavlova lutheri* grown at 15 °C, an increase in the relative amount of EPA and DHA was found when compared with those grown at 25 °C. This was accompanied by major alterations in their acidic lipid and fatty acid contents (Guedes et al. 2011). In thermophilic cyanobacterium *Synechococcus lividus*, growing the cells at lower temperatures led to a rise in the more fluid lipids in all types of lipids (Pittera et al. 2018). In *Cyanobacterium, Spirulina platensis, Botryococcus braunii* and eukaryotic microalgae *Chlorella vulgaris*, an increase in temperature levels resulted in a larger amount of saturated fatty acids and a reduced amount of more unsaturated fatty acids (Ma et al. 2016). Whereas, when *Chlorella sorokiniana* was grown at different temperature levels, there wasn't a major change in the amount of lipid (Li 2014). In *C. reinhardtii*, as the temperature increased up to 35 °C, the amount of the starch content can be supplementarily increased. It is essential to note that all of these findings were recorded at a limited scale in the laboratories as it's difficult to obtain conclusions regarding the effects of temperature change on producing lipids by algae cultivation at industrial scales (Markina 2014).

2.4.7 Gas circulation

The biomass of algae is made of about 45–50% of carbon, therefore the small percentage of carbon dioxide in air (nearly 0.033%) will rapidly limit the growth of algae if supplementary carbon is not provided (Singh & Dhar 2011). Balancing CO_2 with air is generally achieved by injecting it into the cultures of algae using gas exchange vessels and air pumps in PBRs, and this method was adopted for the experimentation carried out throughout the conducted research. Adding CO_2 creates a buffer in the algae culture for the effects of changing pH (Sushchik et al. 2003; Simosa 2016). Several methods were developed to increase the levels of CO_2 in open systems of algae cultures including

producing bubbles through air stones, injecting air into deep sumps, dome exchangers made of plastic with perforated pipes, CO_2 trapping underneath floating gas exchangers, and controlling high alkalinities in the water of the culture system (Bagul 2011). Maintaining a certain level of oxygen concentrations is also vital as exceeding the saturation level in the cultures of algae could lead to photooxidative damage occurring to chlorophyll at the reaction centres which leads to inhibition of photosynthesis and thus productivity would be reduced (Ugwu et al. 2007; Sousa 2013). Existence of crossing points between the culture system and atmospheric air in open pond cultures is not a problem as the concentrations of oxygen will remain the same as that of ambient air. However, in laboratory scale or closed PBRs, air exchange facilities are essential.

2.4.8 Mixing

Mixing is one of the facilities implemented to increase the amount of biomass production in PBRs as it provides sufficient transfer of CO_2 and O_2 in addition to sufficient light intensity distribution and uniform pH levels (6 to 8). It is also needed to prevent sedimentation of algae and avoid attachment of cells to the wall of the bioreactor (Yang et al. 2017). Poor mixing rate leads to three phase (solid-liquid-gas) system of cells in the bioreactor; this condition tends to decrease gas transfer to biomass. However, mixing too thoroughly can damage the cells in the biomass (Raeesossadati et al. 2014).

2.4.9 Contamination

Contamination by undesired species is a serious disadvantage in the open pond systems. If extreme care is not taken, some unwanted species may predictably be produced and can harshly decrease yields of algae production. If a large number of those undesired species exist in a pond, they end up displacing the algae desired to be cultivated. It is very difficult to eliminate those unwanted species (Wang & Seibert 2017) as they include pathogens and predators like bacteria, fungus, protozoans, aquatic invertebrates, other algae and even viruses (Kotasthane 2017). However, microalgae cultures are kept quite pure by using specific relevant culture medium which is optimised to the organisms (Huang et al. 2017). *Dunaliella salina* can be grown in open system "relative pure cultures" by using a high saline environment (Oren 2014). Producers keep *Chlorella* cultures pure by using large doses of inoculants, and by processing short batches every

time with harvesting complete before main contamination can occur (Benemann 2008; Hong & Xu 2013).

2.5 Cultivation techniques

Cultivating microalgae can occur in open ponds and PBRs (Narala et al. 2016). Selecting the appropriate cultivation system is a very important factor that is affected by the expense and efficiency of the process of biofuel production from algae (Khan et al. 2018). A range of variations exists among the cultivation systems of biomass; the most commonly used techniques for algae cultivation are discussed briefly in this section.

2.5.1 Open pond culture

Cultivation of algae in outdoor large-scale open pond systems is well established (Davis et al. 2016) and has many forms and shapes with defined advantages and disadvantages. For scientific research and industrial purposes; shallow big ponds, raceway ponds, closed ponds, and circular ponds are used (Kumar et al. 2015). The critical factor for selecting the type of pond is the area of the pool existence, and because of limited control in open ponds, they become local climate dependent on their functions (Corley 2011). Open ponds depend on particular key growth parameters; these are light intensity, pH level, temperature, and concentrations of CO_2 and O_2. Contamination is another important factor as discussed in section 2.4.9. It affects the efficiency of algae growth under certain conditions of cultivation (Harun et al. 2010). Moreover, in order to compare open and closed systems; the cost of the cultivation system is of vital importance. Also, the cost of construction, operation and maintaining of open ponds are lower than PBRs, and open pond systems are simpler than the others (Narala et al. 2016).

2.5.1.1 Cultivation under indoor artificial conditions

Greenhouse algae can be grown in open pond cultures under indoor artificial conditions. Different PBR shapes and forms are used; stirred tanks, tubular bubble column, flat panel and horizontal tubular. By using stirred tanks and tubular bubble column *Chlorella vulgaris* algae has been cultured and produced a biomass of 0.6-0.7 g l^{-1} (Castellanos 2013). Improvement has been shown in producing a biomass from *Spirulina platensis*

algae when it was cultured indoors by using a tubular Camargue PBR located in 60 m^2 greenhouse, the other conditions are illumination of 430 µmol m^{-2} s^{-1}, and nutrient media supplementation during days 12, 21 and 27. It produced a biomass of 520 mg l^{-1} day^{-1} between day 10 and 11. The same species of algae produced a biomass of only 20 mg l^{-1} day^{-1} between day 5 and 10 when cultured using an indoor tubular Camargue PBR under the same conditions (Delrue et al. 2017).

2.5.1.2 Cultivation under outdoor conditions

The photosynthesis quantum yields of sunlight under outdoor open pond conditions are less than 5%, and the optimum production of a biomass is 30–40 dry gl^{-1} day^{-1} (Yadala & Cremaschi 2016). For example: *Tetraselmis suecica* algae produced a biomass of 0.49-0.56 g l^{-1} day^{-1} under outdoor open pond conditions (Chini et al. 2006), whereas *Anabaena variabilis* (cyanobacteria) algae generated a biomass of 0.40 g l^{-1} day^{-1} in winter and 1.13 g l^{-1} day^{-1} during summer, the conditions were a 35 cm depth of photosynthetic activity and temperatures maintained between 30-35°C (Fontes et al. 1987). Recently *Tetraselmis suecica* and *Chlorella* sp. obtained algae production of 0.18 and 0. 24 g l^{-1} day^{-1} respectively (Moheimani 2013).

2.5.2 Bioreactors

The disadvantages of open systems are contamination, limitation of suitable species, low volumetric yields, uncontrolled environmental conditions, evaporation, and the requirement of a large land area. Inefficiency of open systems has stimulated the construction of closed PBRs. The cost-effectiveness, design and efficiency of using microalgae to produce biofuel have been considered by several studies (Mondal et al. 2017). Details of various closed systems have been explained by many workers as PBRs can be better controlled than open pond systems (Narala et al. 2016). Their controlled conditions offer higher amount of algae cultivating yields. Although productivity is a very important factor in the technology of bioreactors, comparing productivity of different bioreactors is not easy because of the variation in strains of algae and the scale of production (Burns 2012). There are mainly flat and tubular PBRs, where tubular ones consist of transparent tubes and are more suited for open algae cultivation due to having large surface area of illumination. These tubes can be coiling or straight lines. They are adjustable depending on the specification of cultivation system (Cañedo & Lizárraga

2016). Geometry of these tubes is also an important factor; tubular PBRs can be of horizontal, vertical, or inclined shape. Many studies recommended flat-plate type PBRs as they offer great photosynthetic efficiency, high cell density, minimum energy consumption, and big size of mass transfer capacity (Huang et al. 2017). High cell mass requires maximum penetration of light and this can be achieved by using well designed

Table 2.3 Comparison of closed systems versus open ponds. *Adapted from SCHOTT (2017).*

Factor	Closed system	Open pond
Quality of biomass	High	Low
Productivity	High	Low
Production flexibility	High	Low
Investment costs	Relatively high	Low
Risk of contamination	Low	High
Water consumption	Low	High

V-shape and inclined PBRs, this includes using glass or thick transparent PVC materials in inclined or V-shapes (Özçimen et al. 2012). Table 2.3 illustrates comparison of closed systems versus open ponds.

2.5.3 Fermenters

The majority of microalgae grow phototrophically, while some use organic materials as the only sources of energy and carbon and grow heterotrophically. Although, heterotrophic mode cultivation needs satisfactory amount of oxygen for organic substances catabolism 'breaking down' (Altenhofen da Silva et al. 2016), this well-established type of cultivation has many advantages compared to algae's phototrophic growth (Zheng 2013). These contain a lot of existing knowledge with regards to fermentation techniques, lower costs of harvesting, high possibility of process control to assure production consistency and reproducibility, light supplies elimination, and independence from climate and weather conditions (Grebe et al. 2014). Generally, this

technique has been established to increase the total amount of lipids in algae in comparison to phototrophic growth mode (Morales-Sánchez et al. 2013). *Chlorella* algae have produced lipids of 57.9% of the cellular dry weight through heterotrophic growth and was 3.4 times higher than that achieved by phototrophic technique (Sakarika 2016). Mixotrophic offers a superior alternate growth mode for some species of algae like *Chlorella* that can utilise both chemical substrates and light energy, as both productivity and biomass increases have been achieved. Using mixotrophic growth mode in culturing *Chlorella* algae showed productivities of 43.70 mg l^{-1} day^{-1}, which is 1.64 times those arising using photosynthetic cultivation mode (Cui et al. 2017).

2.6 Commercial production of macro and micro algae

Japan and Taiwan began producing algae industrially in the 1950s (M et al. 2012). Many products of algae like *Chlorella* sp., *Dunaliella* sp., *Nannochloris* sp., and *Spirulina* sp. were produced by about 110 commercial companies in Asia Pacific area. Japan is currently the world leader in *Chlorella* microalgae consumption. In 1996, Japan consumed 2000 tonnes of *Chlorella* sp., 1057 tonnes of them were local products and 943 tonnes were imported (Lee 1997). The large-scale microalgae cultivation started in the 1960s in Japan, in which *Chlorella* microalgae were used as a food additive. In the last years, the production of *Spirulina* and *Chlorella* microalgae has increased, and currently it is practiced by most countries. The annual production is about 7,500 tons of dry biomass (5,000 and 2,500 tons of *Spirulina* and *Chlorella* microalgae, respectively) (Andrade 2018). *Chlorella* sp. biomass is useful as food supplements, pharmaceutical products and cosmetics (Ariede et al. 2017). The other important use is in producing oil for biodiesel (Lyon 2013). The most important species of algae for producing biodiesel is *Nannochloropsis salina* because its oil-producing capability exceeds 50% of its biomass (Lyon 2013). Commercial production of algae biodiesel can replace fossil fuel diesel without competing plants in using soils for agriculture (Rocca et al. 2015).

2.7 Barriers to successful production of algae

2.7.1 The research gap

Considerations of the energy balance, global warming and carbon dioxide emissions for a biofuel yield are essential (IEA 2017). The published literature about algae during the last three decades has increased exponentially, with the mainstream of published statistics arising out of the USA, Germany, Japan, UK, and China (majority published in Journal of Applied Phycology, Bioresource Technology, and Biotechnology and Bioengineering) with research benefits worldwide (Khan et al. 2018). Research interests include characteristics of algae species, designing, building, and operating systems for biomass production, modelling of biomass production, using home wastewater for algae cultivation, assessments of local synergistic opportunities, hydrothermal and catalytic processing of oil and biomass, residual biomass for extracting energy, and environmental and economic impacts (LCA, resultant energy conversion, and production economics) with reviews and analysis of earlier publications (Michailos 2017). Algae produced biofuels have been debated to derive only as much energy as the energy used from fossil fuels in making them (Khan et al. 2017). However, several studies often do not precisely consider large-scale aquaculture and overvalue the amount of fossil fuel input (Kempchen 2018). There is also substantial 'hype' of biofuel recovery from algae making claims that often beat the accurate physical and thermodynamic limitations (Chen et al. 2018). Several studies and information making claims to the low energy balances are credited to portions of their designated systems. These are often caused by irrational extrapolation from reports and do not account for several 'hidden' costs of energy. For example, a research modelled the energy productivity of microalgae as a function of whole yearly solar irradiance (Tredici et al. 2015); obviously, algae nearer to 0º latitude were determined as more productive (about twice as productive as 60º latitude). However, the seasonal and daily effects of temperature changes (which significantly affect amounts of carbon fixation) were not being considered (Tait & Schiel 2013). This type of hidden costs can significantly add to the economic, energy and environmental biofuel price. Some other common hidden costs are insecticides, transportation of final products, herbicides, and greatest significance of all fertilisers (Carvalho 2017). In addition to the mentioned hidden costs are dissolving organic materials given off by microalgae during cultivation. This condition results in a substantial loss of biomass (fixed carbon) (Lara-

Gil et al. 2016). Large-scale cultivation of algae is still mostly in the research and development phase and assessment of costs is still challenging as there are several ways to culture algae depending on algae species (Khan et al. 2018). As a result, energy inputs precise determination is not direct at the current stage of research and is basically subjective.

For biodiesel produced from algae to compete with fossil diesel, its cost is required to be less than $0.75 per litre (Cao et al. 2009) and to produce more valuable co-products which are important for commercial viability (other products of algae were discussed in section 1.6). India's Institute of Chemical Technology aimed to reduce the cost of producing oils from algae from about $7 per litre to roughly $0.28 per litre (Singh et al. 2018). A refinery for hybrid biofuel could be a very good solution for offsetting energy and economic costs (Brown 2018). Genetic modification (GM) is considered to have the greatest impact on producing oil from algae together with advances in water treatment and fuel extraction (Nazari 2015). Reviewing all aspects is vital for optimising a specific process (Morweiser et al. 2010). As this covers a collection of disciplines, it is essential to boost collaborations between different sectors including the industrial, scientific, and legislative communities.

2.8 Proposal solutions for increasing viability

Currently, the industrial cultivation systems of algae are aimed to get products of high value rather than bulky cheap biomass. Future design of bioreactors will concentrate on finding solutions for increasing efficiency of light sources, nutrient, dewatering and gas transfer (Show et al. 2017) (Table 2.4).

Table 2.4 Summary of proposed solutions to issues surrounding microalgae biodiesel production. *Adapted from Beilen (2010).*

Issue	Potential solution
Light	Pulsing LEDs, micro lensed PBRs or Lasers
Temperature	IR glass/plastic, use extremophilic species, warmed using cooling water
Nutrient	Wastewater, utilise flue gas
Dewatering	Filamentous species, self-flocculating/settling species
Oil extraction	GM of excretory pathways
Costs	Co-production of a valuable product, novel PBR designs

Several studies suggested focusing on developing research about technologies that can improve the yield of algae production without increasing production cost, and there are some promising studies regarding decreasing the production cost by novel harvesting technologies (Roux et al. 2017).

Using pulsed sources of light with a frequency of more than 1Hz can lead to reducing the wasted illumination energy and photoinhibition, this can increase the efficiency of photosynthesis and improve the productivity of algae by about five times in comparison with continuous illumination (Sforza et al. 2014). This is achievable by using pulsed LEDs as they are controllable in terms of selecting the desirable wavelengths, light intensities, and operating duty cycles (Olvera-Gonzalez et al. 2013), or by converting sunlight to a 'stroboscope' using the 'microlensing' technique of a closed PBR's surface (Sforza et al. 2014). High biomass densities have been achieved by using any of the two methods in comparison with a normal PBR.

When sunlight is used in cultivation systems, temperature or light fluctuations are likely to cause spatial and temporal patterns of algae growth (Bearham et al. 2013). In some regions, sunlight is very intense, therefore productivity of algae can be slowed down due to increase in temperature (Roy et al. 2016). Infrared reflectors plastic or glass are used to block the IR radiation, which represents about 40% of the amount of sunlight radiation, but this will add more cost to the production (Stanghellini et al. 2011). On the other hand, low temperatures in Europe affect algae production in the open pond systems and make it viable only during the period of June to October every year (Sandefur et al. 2011). A proposed solution for keeping open pond cultures warm is using warmed water produced from power plants (Morweiser et al. 2010). Mixed algae communities are more productive and may increase the biomass production yield (Tossavainen et al. 2018) and may also lead to reduction of nutrient need for microalgae growth. For example, nitrogen *biofertilisation* using a strain of hyper-ammonium-excreting bacteria. Using *Azotobacter vinelandii*, which is a hyper-ammonium-excreting bacterial strain led to *biofertilisation* of nitrogen, thus supported the algae growth with rich oil yield without the requirement of nitrate addition (Ortiz-Marquez et al. 2012). Coupling the cultivation of algae to other production processes can help in the reduction of capital cost. For example, production of one tonne of biomass from algae consumes three tonnes of CO_2 and helps in reducing the cost of decreasing the CO_2 concentration (Davis et al. 2016).This also increases the

rate of algae growth and produces more accumulation of biomass (He et al. 2012). Wastewater can be used as a source of nutrients (phosphates and nitrates) for algae growth, this represents a cheap method of treating wastewater, recovering resources and offsetting fertiliser costs (Kendrick 2011). Commercialisation of the growth of algae to produce biofuels can be achieved using wastewater as it contains nutrients needed for microalgae growth (Christenson & Sims 2011). This method showed remarkable improvements in accumulation of lipid when using CO_2 produced by flue gas (Prathima Devi & Venkata Mohan 2012). Reducing the cost of production can be done by self-flocculating, filamentous or settling species of algae (Lv et al. 2018). Immobilisation techniques like texturing metal sheets are commercially used for algae attachment, for example textured stainless steel sheets were successfully used for immobilising and attaching *Scenedesmus dimorphus* algae onto the metal sheets (Shen et al. 2014). Using polystyrene showed similar good results as a supporting substance (Johnson & Wen 2010).

Designing low-cost PBRs can be attractive and would increase the demand for energy produced by algae. Many special models are employed commercially on small scales using permeable membranes. For example, *Subitech Germany* (a flat panel airlift reactor) and the *Novagreen* (a V-shaped bag reactor) (Morweiser et al. 2010). The *Offshore Membrane Enclosure for Growing Algae* (OMEGA) is one of the prominent permeable membrane designs, which utilises sunlight, flue CO_2 and wastewater instead of using fertilisers and freshwater.

2.9 Production of microalgae with wastewater treatment

Wastewater treatment is the essential facility that keeps human communities clean. Removing the main sources of water pollutants and impurities such as nitrogen, turbidity, phosphorus and dairy matter involves spending large amounts of financial resources and work. Some of these pollutants are useful nutrients like phosphorus and nitrogen and can be extracted from wastewaters in different ways. Nitrogen gas extraction can be achieved by denitrification where nitrate is reduced to nitrogen gas (Gersten 2017). Phosphorus can be removed as a precipitate via ferric chloride. Though, both nutrients can be removed by growing algae using the wastewater and then removing the biomass that contains

nitrogen and phosphorus. This technique was first established by Oswald et al. in the year (1957). In 2007, Singh and Dhar also investigated the use of microalgae in the treatment of sewage as a more cost-effective method to remove phosphorous and nitrogen from it (Patel 2015). Traditional techniques of wastewater treatment require large amounts of energy for mechanical aeration in order to deliver the needed oxygen for consuming nutrients in wastewater by aerobic bacteria, while microalgae can provide all of that efficiently and cheaply by photosynthesis (Martínez 2016). Approximately, removing one kilogram of biological oxygen from wastewater using an activated sludge method involves spending one kilowatt-hour of electricity used for aeration and producing one kilogram of carbon dioxide from electrical power generation. On the other hand, using photosynthetic oxygenation with microalgae to remove the same amount of biological oxygen requires zero energy inputs and extracts sufficient amount of biomass that produces enough methane to generate one kilowatt-hour of electricity (Kabariya & Ramani 2018).

2.10 Chapter summary

This chapter reviewed available literature through a detailed survey in addition to explanations of important factors affecting algae growth. Photosynthesis system I and II were discussed with emphasis on the Calvin cycle. It paid particular attention to factors and conditions affecting algae cultivation. This section outlines specific nutrient requirement for inducement of lipid production; in addition to the optimal intensity of light required for better yields, proper pH values, temperature ranges sustaining maximum growth rates and other parametric value range and settings. Modern techniques for open pond and indoor algae cultivation were described together with reference to solutions for barriers restricting successful production of algae in order to achieve optimum production for biofuel.

Chapter 3

MECHANISM OF ELECTRON TRANSFER PROCESSES IN PS I AND PS II OF PLANTS AND ALGAE

3.1 Introduction

This chapter contains a description of the interaction mechanism between photons and electrons of plants and algae pigments. Later on in Chapter 5, using the findings of the work done in this research, a general theory regarding this matter is laid out. It suggests a physical mechanism explaining the reduction of the photons' energy by successive quantised transitions of electrons among a variety of pigment' absorption spectra that match the emission spectra of the incident photons within the photosystems.

The motivation for this chapter is to comprehend the role of laser coherence on the optimisation of efficient energy transfer between electrons in different energy states. This leads to emitting photons which have energies matching the absorption spectra of the chlorophyll at the reaction centres of PS II and PS I. This then uses the released heat energy in the build-up of a high potential difference between the ***stroma*** and ***lumen*** (Figure 3.1) which is utilised in producing the ATP and NADPH.

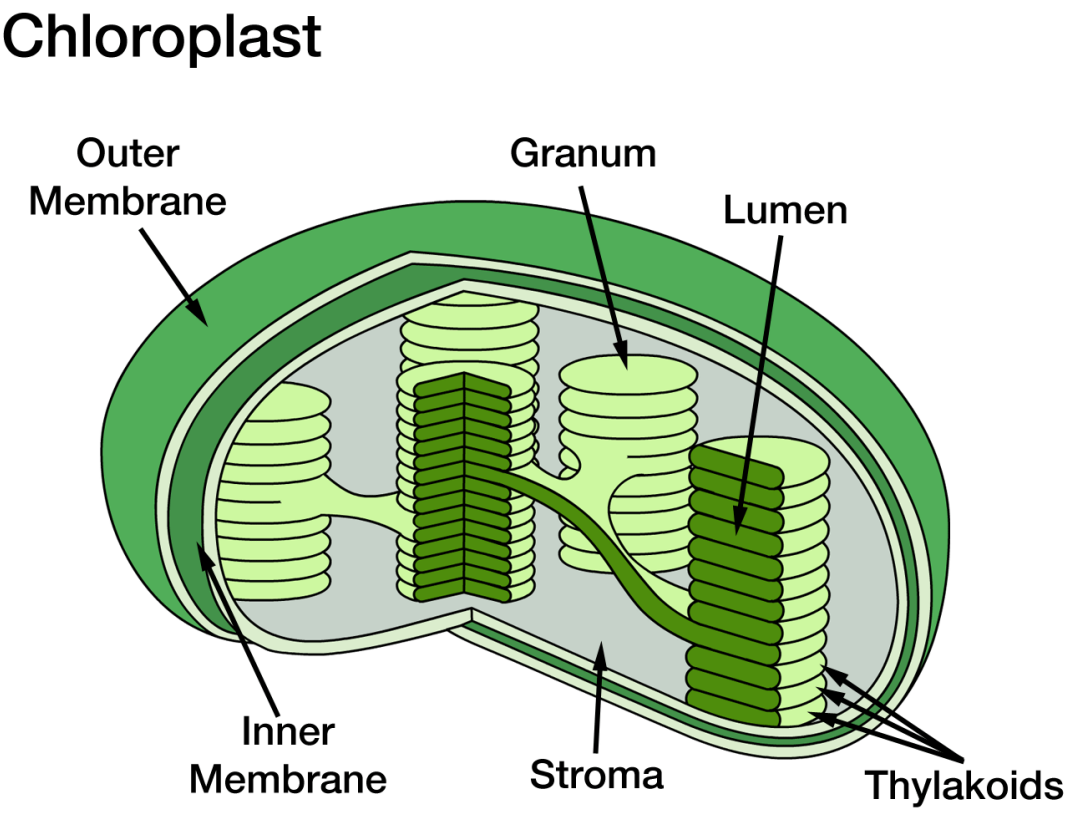

Figure 3.1 Chloroplast structure. *Source: (Yadufashije 2018).*

3.2 The biological explanation of photon-electron interaction

Algae and plants utilise several types of pigments for harvesting light. Some of which are chlorophyll or bacteriochlorophyll, these are found in all photosynthetic membranes (Semchonok 2016). Incident light is absorbed by the light-absorbing molecules (chromophores). Thereafter, their electrons are shifted from ground state of energy to an excited state, thus storing the majority of energy (photons) in these molecules. This energy, however, is only stored briefly as the electrons at the excited states return to the ground state through releasing energy by spontaneous emission (fluorescence or phosphorescence) or by non-radiative processes (heat emission) as the lifetime the electron can remain in the excited levels is very short; for example, this lifetime in chlorophyll is about 6 ns when dissolved in de-oxygenated diethyl ether at ambient temperature and about 4 ns in the living body of plants or algae (Fassioli et al. 2013).

The excitation energy released by the excited electrons is then transferred to reaction centres (RC) where the energy is stored by means of charge separation. This process must be very fast and happens during the lifetime (10 ns) of the excited state of the electrons in order for the excitation energy to reach the RC rather than being lost via the spontaneous emission or non-radiative decay. The natural efficiency of energy transfer from the chromophores to the reaction centres is very high (more than 90 %) (Wientjes et al. 2013). This high efficiency is facilitated by the arrangement of chlorophyll molecules and other chromophores in a way that avoids concentration quenching, and by using different types of chromophore in photon energy harvesting within the antenna complexes (Demmig-adams & Iii 2014). The energy absorbed by these pigments is transferred to *chlorophyll-A* with high efficiency (Clegg et al. 2010).

Despite the simple structure of pigment molecules, carotenoids have complex electronic characteristics; as the lowest singlet state of energy is not allowed and the lifetime of the excited level which has a very short lifetime (~10 ps) (Liguori et al. 2017), these unusual properties make carotenoids very efficient in quenching both triplet and singlet excited levels (Wagner 2018). They can also transfer charge with chlorophyll, forming efficient traps for electron excitation energy (Demmig-adams & Iii 2014).

Non-photochemical quenching is a feature of light harvesting complexes where they respond to the excess of absorbed light conditions by modifying chromophores and dissipating excitation energy as heat instead of fluorescence. This can decrease the efficiency of energy transfer to the reaction centre by a factor of 2–10 within several seconds to minutes (Matuszyńska et al. 2016). In algae and higher plants, many factors like the complex electronic structure of chromophores (chlorophyll and carotenoids) rendered the photon-electron interaction mechanisms in photosynthesis to remain incompletely understood (Demmig-adams & Iii 2014).

3.3 Light harvesting and excitation energy transfer, the FORSTER mechanism

The FORSTER mechanism theory was proposed by Theodor Forster (Clegg et al. 2010), it states that: if two pigments are close to each other and one of them is at its excited state, then the excitation energy may transfer to the neighbouring pigment by Coulomb

interaction between electrons of the molecules of the two pigments as shown in Figure 3.2.

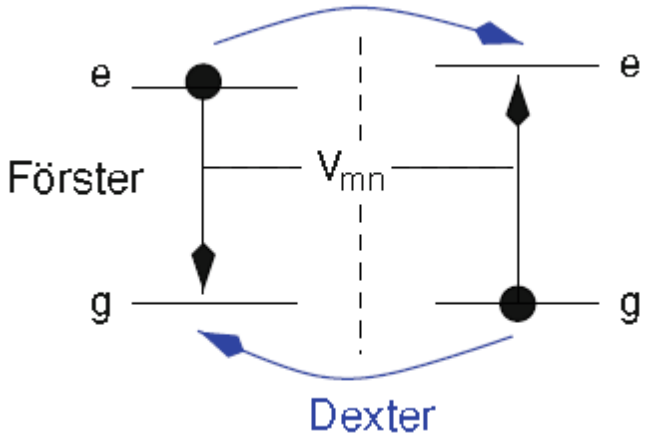

Figure 3.2 Forster mechanism. *Source: (Clegg et al. 2010; Renger 2009).*

The excitation energy transfer between two interacting pigments represented by their ground (g) and excited (e) state levels either by the Forster mechanism (Forster 1948) that is based on the Coulomb coupling between the optical transition densities of the two pigments, or by a simultaneous electron exchange between the excited and the ground states of the two pigments, as suggested by Dexter (1953) (Şener et al. 2012).

While different types of pigments absorb photons of different wavelengths and convert their energy to electron excitation, it is the chlorophyll molecules that are responsible for the migration of the electron excitation energy in photosynthesis units, with about ~300 chlorophyll molecules in each unit (Clegg et al. 2010). This process includes accepting the electron excitation energy from a neighbouring chromophore and forwarding it to the next one. As a result, electron excitation energy is trapped by the RC (Caycedo-Soler et al. 2017).

The electronic properties and structures of the molecules of chlorophyll are modelled by a simple two-level system (Mallus et al. 2016). Electron excitation energy migration between chromophores in the antenna complexes can be explained by the 'random walk' model with hops (single excitation energy transfer event) occurring only between the

nearest neighbouring chromophores organized into a square lattice (Zhang et al. 2016). Electron excitation energy transportation may also be conducted by the pigment-protein coupling. By tuning the site energies of the pigments, the proteins can lead the electron excitation energy to the RC (Renger 2009).

3.4 Charge separation in PS I and PS II reaction centres

After commencing photon absorption process by chlorophyll in PSII, an electron is returned from the excited state to the ground state or to another lower state by emitting a photon and or heat. If the absorbed photon was in the red (mainly 680 nm) region of visible spectrum, it will be moved to the first excited singlet state. This process provides the energy needed for photochemistry process which requires the absorption of a red photon (e.g. the energy provided by a photon of 680 nm wavelength is 1.82 eV). The energy of the shorter wavelength of a blue photon is sufficient for the absorbing electron to reach the higher excited singlet state, but this state decays rapidly and loses some energy by emitting a photon and or heat to return to the longer-lived first excited state (Cardona et al. 2012).

All types of chlorophyll are bound to two types of protein (reaction centre and antenna proteins). The proportion of chlorophyll in antenna proteins is higher than its proportion in the reaction centre proteins; therefore, different types of chlorophyll play an important role in light energy collection. When an incident photon is absorbed by antenna in chlorophyll, an electron at the ground state of chlorophyll molecule will be excited, and then it will rapidly pass on the excitation energy to an adjacent chlorophyll molecule to which it is electronically coupled. This excitation energy coupling can happen between several types of chlorophyll molecules within the same protein or in different adjacent proteins which are close to each other during the lifetime of the excited state (Redeckas et al. 2017). The pigments' coupling process continues until the excitation energy transfers to a particular chlorophyll in the reaction centre (known as the primary electron donor), then an electron is transferred to the first electron acceptor, resulting in the first charge separation.

This process forms a pair of more stable oppositely charged ions: a highly oxidising cation and a highly reducing anion. Several rapid electron transfer reactions happen, out from the anion and into the cation. These reactions lead to increasing the distance between the ions and decreasing the standard free energy (Rutherford & Faller 2003) as shown in Figure 3.3.

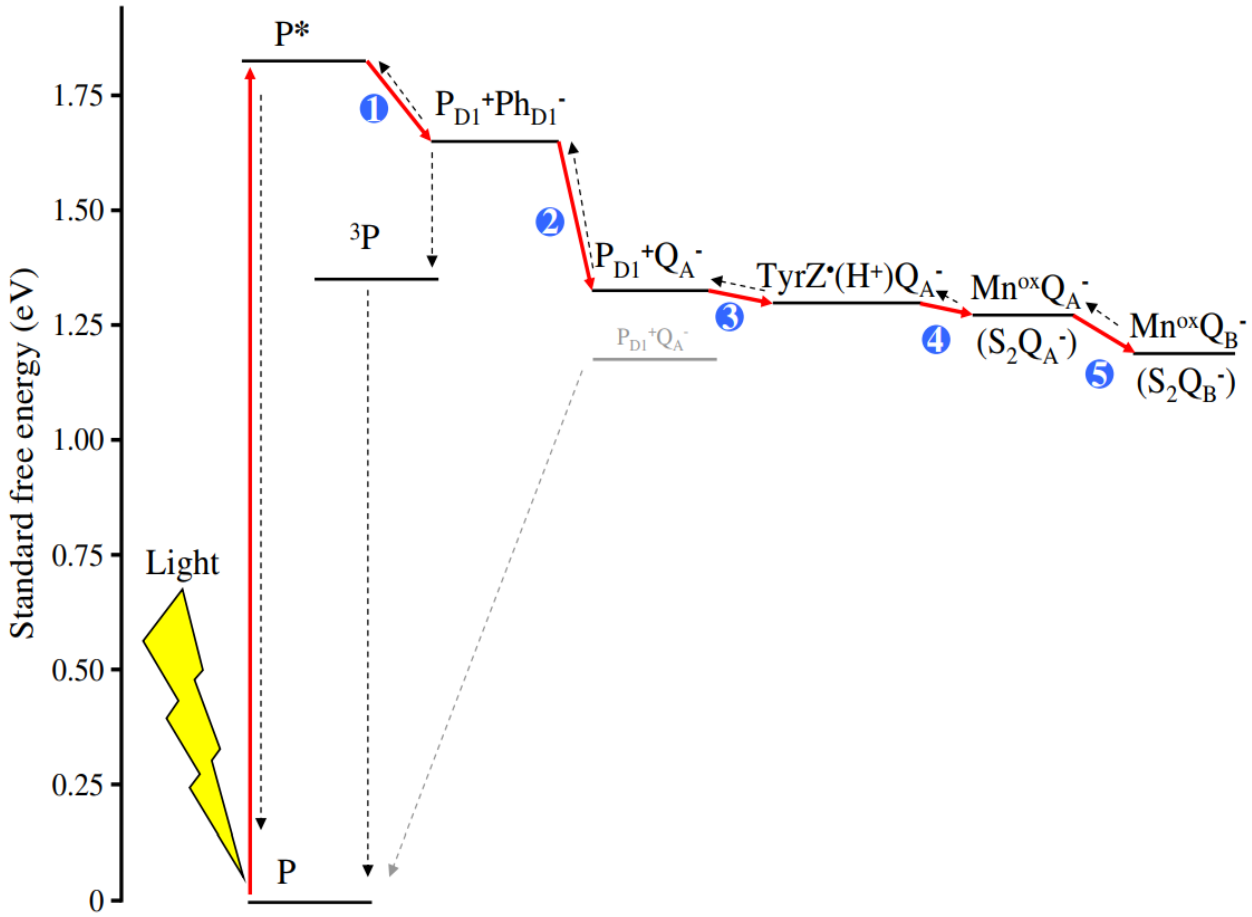

Figure 3.3 An energy scheme of the electron transfer steps in PS II occurring after the absorption of one red photon and assuming that all centres are in the dark-adapted stable state, S1. *Source: (Cardona et al. 2012).*

In the reaction centre of PS I, electrons are transferred through 1-electron redox carriers from a 1-electron donor (plastocyanin) to a 1-electron acceptor (ferredoxin) (Caffarri et al. 2014).

The reaction centre of PS II is more complicated, as the source of electrons varies and more than one electron is needed (Caffarri et al. 2014). It has 2-electron regime on one side and 4-electron regime on the other, which are taken from water molecules (Barry et al. 2017). Quinone (Q) is the final electron acceptor in the electron transfer chain and it's

a 2-electron acceptor, therefore a fully reduced quinone, hydroquinone (QH_2) is released through the membrane. This requires it to undergo two sequential 1-electron reduction along with the allied protonation reactions before its reduction is complete (Cardona et al. 2012).

Although the central pigments pheophytins (Ph) and chlorophyll molecules (Chl) have overlapping absorption spectra, they are not identical and it's not easy to identify the contributions of each pigment to specific absorptions (Shelaev et al. 2011) as the absorption characteristics are affected by the isolation procedures particularly in the preparation of the smallest and most used subunits of PS II RC, which are destabilised polypeptide proteins "D1 and D2". The longest wavelength pigment in PS II is thought to be the special accessory chlorophyll (ChlD1) serving as the primary electron donor (Shelaev et al. 2011), therefore, it has been considered to be the main electron donor (Kawashima and Ishikita 2018). At low temperature, ChlD1 is the site of the triplet state (Caffarri et al. 2014).

Figure 3.3 shows the PS II cofactors involved in charge separation and the numbers in the blue circles indicate the order of the reactions. After absorption of the red photon, the first excited singlet state is 1.83 eV above the ground state. Excitation of the core 'special pair' pigments (P), which are all weakly coupled, leads to the formation of PD1+ PhD1 as the first easily identifiable radical pair; this is designated step 1. After each subsequent step, some energy is lost, with a major loss after the formation of the PD1+ QA radical pair. PD1+ is able to oxidise the redox active tyrosine (TyrZ), forming the neutral radical TyrZ• with proton transfer to the nearby Histidine (His) 190 (step 3). The TyrZ• oxidises the Mn_4Ca cluster by one electron forming the S_2 singlet state (step 4). Finally, electron transfer between two plastoquinones (from Q_A to Q_B) occurs (step 5). The absorption of 4 photons is necessary to complete a cycle of water oxidation and the reduction of two plastoquinone molecules. Back reactions are shown as broken lines. The triplet state, 3P, is formed from a triplet form of PD1+ PhD1, which is mainly formed by the back reaction, but for simplicity, this is not distinguished from the singlet radical pair in the figure. The back reaction from the high potential form of Q_A- (in inactive centres) is shown in grey (Cardona et al. 2012).

The first photon absorption and excitation of the chlorophyll at the reaction centre would form charge separation and electron transfer occurring with an even chance on either side of the reaction centre (Kawashima and Ishikita 2018).

3.5 Photon calculations

Generating one molecule of sugar in the Calvin cycle requires 24 photons of 680 nm wavelength and 36 photons of 700 nm wavelength. The photosynthetic process consists of three processes: Photosystem I (cyclic and non-cyclic), Photosystem II and the Calvin cycle (Kommareddy et al, 2003).

1. Photosystem I, non-cyclic electron flow path:

2 Chlorophyll + 2 photon (700 nm) -> 2 Chlorophyll + NADPH + H_2O

Cyclic electron flow path:

2 Chlorophyll + 2 photon (700 nm) -> 2 Chlorophyll + ATP + H_2O

2. Photosystem II:

H_2O + 2 photon (680 nm) + 2 Chlorophyll -> 2 Chlorophyll + ATP + 1/2 O_2 + $2H^+$

3. Calvin cycle:

6 CO_2 + 12 NADPH + 18 ATP + 12 H_2O -> $C_6H_{12}O_6$

The photosynthesis process is the sum of the three processes balanced chemically as:

12 PS II + 12 PS I (non-cyclic) + 6 PS I (cyclic) + Calvin cycle

6 CO_2 + 24 H_2O + 60 photon -> $C_6H_{12}O_6$ + 6 O_2 + 18 H_2O

Inputs (Carbon dioxide consumption) -> Outputs (Oxygen output)

The last equation shows only the inputs and outputs to the photosynthetic process. The heat of reaction is 2814 kJ, which means it's an endothermic reaction. The energy is provided by 24 moles of 680 nm photons and 36 moles of 700 nm photons (10389 kJ).

The efficiency of photosynthesis is (2814 kJ x 100% / 10389 kJ) = 27% (Kommareddy et al, 2003).

3.6 Chlorophyll-A molecular excitation

Absorption process takes place when an incident photon is absorbed by an electron at the ground state of a *chlorophyll-A* molecule and then transferred to any of the excited states depending on the energy of the photon. If the wavelength of the incident photon is 680 nm, then the electron will be excited to the first singlet excited state of PS II; the same will happen for PS I if the wavelength is 700 nm. For absorbed photons with wavelengths in the blue light region will make the electron shift to the second or higher states as these photons have sufficient energy to move the electron from the ground state to higher excited states. The photon energy has to be exactly equal to the energy difference between any two states of energy, otherwise, the photon will be rejected (reflected or transmitted). The electron at the excited states will either go back to its ground state by spontaneous emission (fluorescence), emit heat and move to another lower state or change spin and transfer to a triplet state (phosphorescence) (Bowsher et al. 2008; Caffarri et al. 2014).

The non-radiative relaxation rate can be controlled by the photosynthetic machinery; it can increase this relaxation rate under high-intensity light stress conditions by modifying electronic properties and interaction of chromophores or decrease this rate under optimal conditions as there is no need to reduce the energy of the photon when energy transfer efficiency reaches >90 % (Kommareddy et al, 2003).

The triplet state is a very reactive excited state. Therefore, the electrons at this energy state will transfer their excitation energy to the stable oxygen molecules and fall to the ground state. The excited molecules of oxygen will then react with fatty acids to produce lipid peroxides, which are very destructive to the algae cell membrane and could cause fatality. Therefore, the best efficient absorption for photosynthesis has to be between the ground state and the first singlet excited state (Mirkovic et al. 2017). If the electron is found at the first singlet excited state, it will be transferred to the first electron acceptor for a photochemical process and be used in photosynthesis, the required energy for this process will be provided by photons having wavelengths of 680 nm or 700 nm. Incident

photons of wavelengths in the range (600 – 700) nm would provide the necessary energy to excite an electron from ground state to the first singlet state where it may well be used in photosynthesis without large fluorescence or heat loss. The lost heat is essential for building the potential difference between the stroma and the lumen in order to help in the H^+ transfer, while fluorescence is crucial in getting photons with lower wavelengths to match the absorption spectra of chlorophyll in PS II or PS I (Betterle 2011).

Incident photons of wavelengths in the range (380-480) nm are suitable for utilisation in improving the efficiency of carbon fixation in addition to the whole photosynthesis process as they match the absorption spectra of b-carotene antennas, therefore, they can be absorbed by electrons at the ground state and transfer them to higher excited states. Then, the electrons at the excited states have to lose some energy through fluorescence or heat in order to move from the higher excited states to the first singlet excited state before participating in photosynthesis as illustrated in Figure 3.4 (Kommareddy et al, 2003).

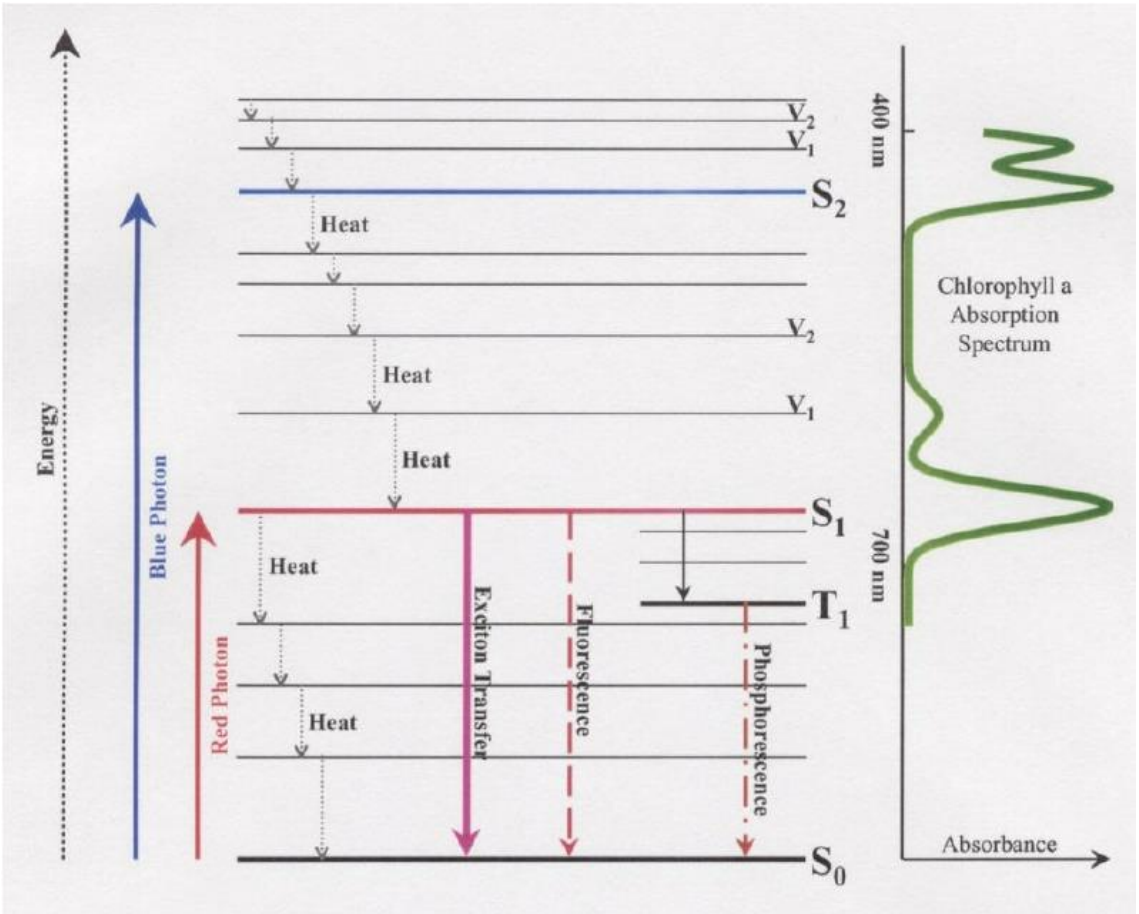

Figure 3.4 The energy states of chlorophyll-A. *Source: (Kommareddy et al, 2003).*

3.7 Comparison of low and high intensity of light sources

Light intensity plays a vital role in maintaining the good conditions of algae growth. Increasing intensity of a light source used for algae growth will increase the number of incident photons and therefore the population of electrons found at the excited levels will increase as well. Therefore, the probability of getting a triplet state transition will be augmented. The triplet state may force algae to undergo photo-oxidation process, which is not recommended as it will lead to increase in algae cells fatality as mentioned in the previous section. Increasing light intensity to levels more than those needed could result in depressed photosynthetic rate due to photo-oxidation and or photo-respiration resulting from the higher population of electrons at the excited states (Al-Qasmi et al, 2012; Singh and Singh, 2015).

On the other hand, decreasing light intensities less than required to excite the electrons found in pigments to the first singlet state would lead to a decreased photosynthesis rate caused by the low population of electrons at the excited states and thus low biomass production (Xu et al. 2016).

3.8 Chapter summary

Photosynthesis is a process that converts photons energy into chemical energy. This process includes two stages: light reaction and dark reaction. The light reaction occurs in two energy harvesting complexes: photosystem II and photosystem I, using the light energy of photons within a small difference in wavelengths (680 nm and 700 nm) respectively. The dark reaction takes place in the Calvin cycle in order to produce glucose using the energy generated from the light reaction.

This chapter discussed the published mechanism models on how ultrafast energy transfer occurs during the light harvesting by plants and algae. The recent work done in this research, particularly the role of the coherent laser light in enhancing the algae growth rate by optimising the energy interaction between photons and electrons within PS II and PS I, will be discussed in detail in Chapter 5.

Chapter 4

EXPERIMENTAL ARRANGEMENT

4.1 Introduction

In this chapter, the experimental arrangement implemented to study the effects of using lasers as coherent light sources on the growth of algae is explained along with a quantum study of laser photon absorption by algae cells.

A full description of the experimental setup configurations used is modelled upon that expounded in our published paper (Al-yasiri et al. 2017) where three PBRs were designed. These bioreactors employed a variety of light sources delivering light at several wavelengths using blue and red semiconductor lasers and broad spectrum light emitting diodes. The bioreactors were fed with *C. reinhardtii* algae samples. An air mixing pump was used to standardise the initial distribution of cells at the start of every experiment and continued throughout. To the best of this thesis author's knowledge, this is the first study that used quantum investigation with regards to all the available semiconductor lasers wavelengths in order to study their effect on improving the growth of algae cells. Each experiment was repeated several times (n=10) using different samples to ascertain the reproducibility of the obtained results.

4.2 Experimental design and setup

An in-depth investigation was initially carried out by reference to the limited amount of research that has already been performed in this area, most of which had an emphasis on

the biology of chlorophyll as opposed to the quantum physics of using photon excitation of electrons through photon energy absorption. Particular attention was paid to a published work emanating from Arizona that put more emphasis on the electron pathways (Kuwahara et al. 2011).

Diode lasers are more beneficial than traditional lighting sources in that they increase the photon flux density through their coherence, LEDs show "efficiency droop" with time (Neumann et al. 2011). A comparison of energy efficiency and lifespan between different sources of light is illustrated in Figure 4.1.

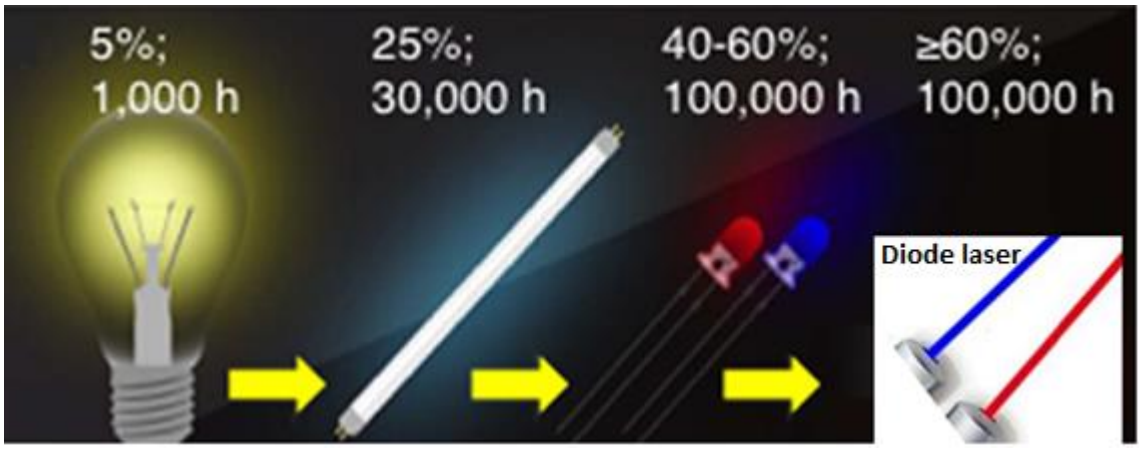

Figure 4.1 Comparison of energy efficiency and lifespan between lasers and conventional light sources. *Adapted from Gómez et al. (2013).*

Also, the matching of the emission spectra of lasers radiation with the absorption spectra of plants and algae causes them to contribute to the biological environment in a certain way; Lasers have a more advanced input power density-to-optical light output than LEDs, hence diode lasers have enhanced PCE (85%) (Wierer et al. 2014). Due to this PCE enhancement, laser radiation causes seeds to advance and develop more quickly at all vegetation stages, which reduces the plant's development time and stimulates the production of more flowers in each plant, with an improved quality and quantity of production (Niculiţă et al. 2008). As a result, using lasers in lighting devices can conserve the space and costs, especially since using smaller electronic chips will be possible (Wierer et al. 2014). Specifically, unlike regular light sources, increasing the photon flux density is possible with laser devices due to their ability to function at higher current densities, long lifespan and suitability for directional emissions. It is also possible to tune the laser beams (Ooi et al. 2016), especially as they are chromatic, to match the peaks of

the absorption spectra of different plant and algae species at alternating stages of the growth lifecycle to benefit the economically relevant traits (Tsao et al. 2014).

This research pays particular attention to the absorption spectrum characteristics of the chlorophyll pigments in relation to the sympathetic emission spectra of the lasers employed both singularly and throughout an extensive array of many combinations.

The influence of diode lasers in improving the growth of algae must be assessed based upon consolidated experimental conditions that can finally be compared with the white light LED; therefore the need for controlled conditions to implement the laboratory experiments is important. The layout sketch and detailed dimensions of this setup are given in Figure 4.2 and Figure 4.3.

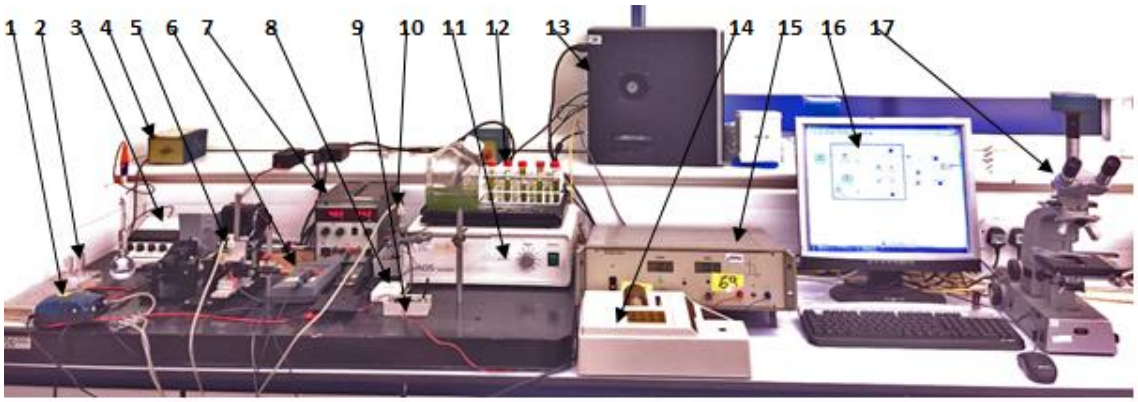

Figure 4.2 The PBRs experimental set-up and the monitoring devices

The labels of Figure 4.2 are as follows:

1. Air pump 2. The preliminary PBR (white LED, electric current stabilizer and the algae samples) 3. pH meter 4. Power supply for the white LED of the preliminary PBR 5. The laser's PBR (lasers, electric current stabilizer, the algae sample and the photo diode) 6. Light meter 7. Power supply for the lasers 8. DAQ 9. The electric current stabilizer for the LEDs 10. The LED's PBR (LEDs, the algae sample and the photo diode) 11. The mechanical mixer 12. Produced Algae 13. PC 14. Bio-Chemistry Analyser 15. Power supply for the LEDs at the LED's PBR 16. LABVIEW package 17. Microscope.

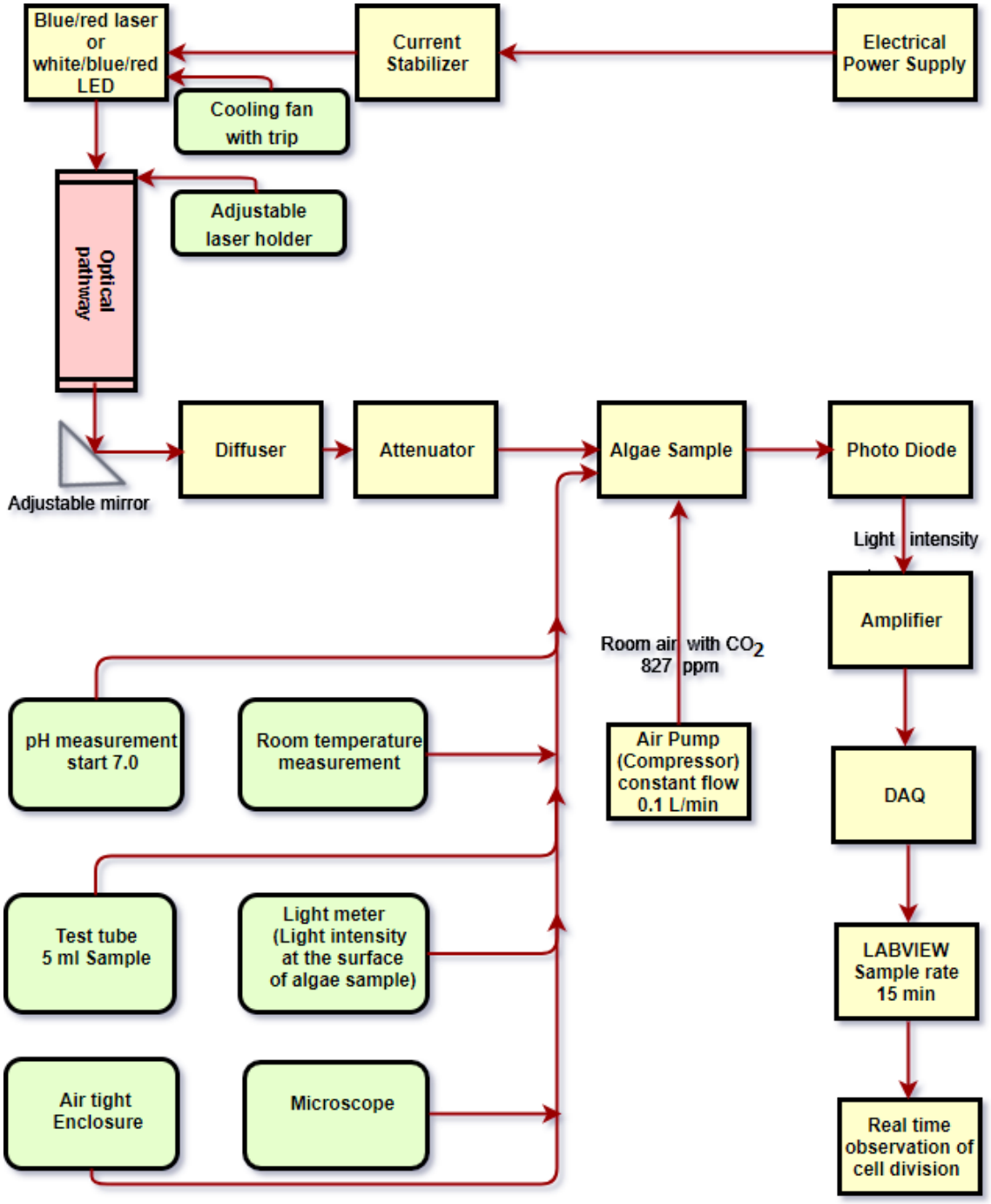

Figure 4.3 Block diagram of the PBR experimental set-up for illuminating the culture using lasers and white light LED

The block diagram in Figure 4.3 shows the laser or LED light pathway from source to target. The light beam emanating from the light source passed through the diffuser, then the PBR sample, and ultimately received by a photodiode, the output of which is amplified by a precision signal amplifier and then via the NI USB 6008 data acquisition system onto the personal computer where it gets processed by the processing and data

logging configuration for LABVIEW package. The quantity of light permitted to reach the photodiode was the initial photon quantity generated by the laser and emanating from the diffuser, minus those photons absorbed by the number of cells suspended in the nutrient medium, which was situated within the light pathway of the PBR.

4.3 Bioreactors

Three PBRs were custom designed and built to perform the experimentation involved with this research as shown in Figure 4.4 and Figure 4.5. A monochromatic light produced by semiconductor lasers and a white light LED were employed as light sources in the conducted experiments. The PBRs were filled with *C. reinhardtii* algae samples (Sams Research Services Ltd, Scottish Marine Institute).

Eight wavelengths of blue and red lasers were used. Figure 4.4 illustrates the experimental setup of the Laser PBR. Stability of the laser diode emission was maintained through a combination of controlled current via a stabiliser circuit.

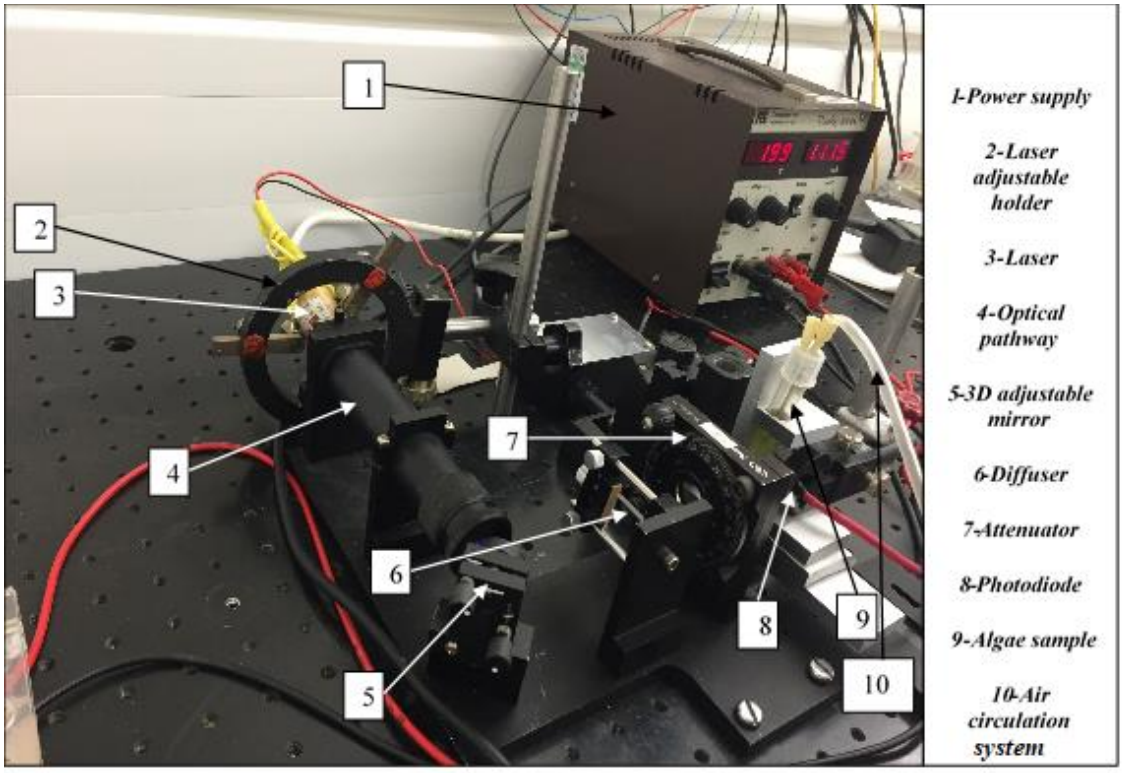

Figure 4.4 The experimental setup - Laser PBR

Table 4.1 shows the devices used, their colour, wavelength, and manufacturer part code. The power of the lasers and the LEDs irradiating the samples of algae was measured using an optical power meter (Ophir Nova). An attenuator was used to control the input power to the lasers and LEDs to be limited to 1 mW with a standard deviation of ±0.05 mW.

Table 4.1 Specifications of diode Lasers implemented in the experimental setup

LASER Colour	Wavelength	Manufacturer Part Code
Blue	405 nm	WSLD-405-020m^{-1}, 405nm 10mW 5.6mm 4.8V Wavespectrum
Blue	450 nm	Single Mode LD/TO-38 I Cut pack/PL-450B, 450nm 80mW 2.2V OSRAM
Blue	473 nm	PGL-H-473-2mW, 473nm 2mW 5.6mm 3V ACAL BFI UK Limited
Red	635 nm	SANYO DL-3148-034 635nm 5mW 5.6mm 2.2V N-type Diode Laser, LASERLANDS
Red	650 nm	D650-5I, TO-18, 650nm 5mW 5.6mm 2.2V Single Transversal Mode, USLASERS
Red	680 nm	DA-LD680-10-TO-18, 680nm 10mW 5.6mm 2.2V JIANGSU DAAO OPTOELECTRICAL LTD
Red	685 nm	WSLP-685-005m-PM-PD, 685nm 5mw 5.6mm 2.4V Wavespectrum
Red	700 nm	QLD-700-50S, 700nm 50mW 5.6mm 2.5V JIANGSU DAAO OPTOELECTRICAL LTD

4.4 Controlling light sources

This study aims to investigate the effects of the illuminating light properties on the growth of the algae culture. Lasers and white light LED sources were used which required different experimental set-ups for each light source.

During all conducted experiments, other light sources in the room were turned off and the brightness of the computer monitor was dimmed and directed away from the experimentation setup. The lab has opaque windows; hence, daylight had no effect on the experiments. The light intensity measuring photodiode was appropriately positioned in relation to the algae samples and connected to a precision electronic amplifier as explained in section 4.7 to be processed under LABVIEW package which serves as a data logger that records real-time observations of the cell divisions.

A top view of the experimental setup shows the PBR and the algae sample being illuminated using white light LED is shown in Figure 4.5.

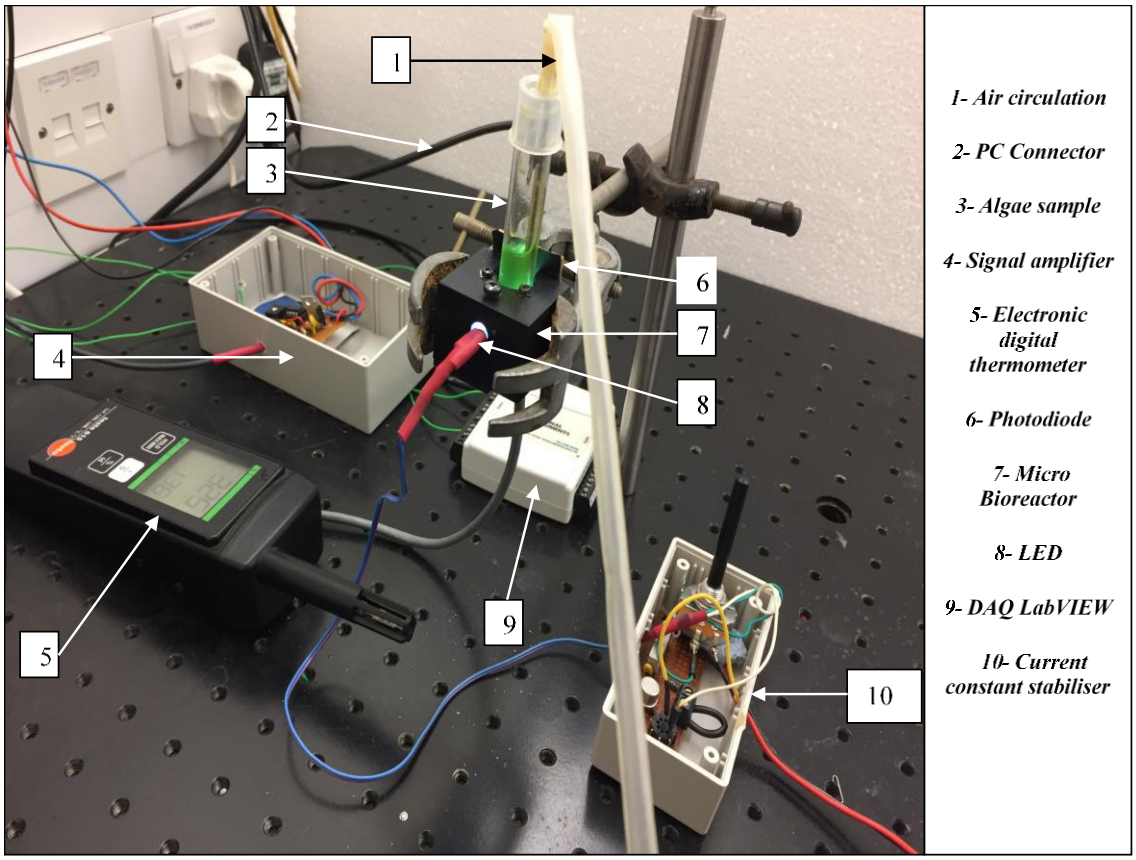

Figure 4.5 The experimental setup using white light LED source in PBR

A significantly different set-up was required when laser light was utilised to illuminate algae samples. The PBR had an entirely different arrangement due to the employment of a diffuser and an attenuator in order to regulate the distribution of laser light among the whole algae samples as shown previously in Figure 4.4. For the duration of each experimental run, and between runs, the system was sealed in an airtight enclosure, in this way, the air pump provides air and mixes the algae samples.

4.5 Experimentation materials

This section contains a thorough description of the various materials used in conducting the research related experiments.

4.5.1 Chemicals

Unless otherwise stated, all chemicals were sourced from Life Technologies Limited. The *C.reinhardtii* culture was enhanced due to the nutrients (C, N, S and trace elements) supplied by Tris-Acetate-Phosphate (TAP) Growth Media. This nutrient optimises the sustenance and reproduction of *C. reinhardtii* as it contains all the necessary salts and trace elements required for optimal algae growth.

Algae require sufficient amounts of carbon to make organic molecules, which were provided by *Tris* or *trisaminomethane* "$(HOCH_2)_3CNH_2$" as it is considered an ideal source of carbon (Estime et al. 2017).

4.6 Cell culture and growth conditions

The investigation carried out prior to implementation of any activity stage within this study identified the appropriate materials, methods and processes. Several highly equitable academic studies referred to in this study clearly illustrate that *C. reinhardtii* (Figure 4.6) was the ideal strain of algae that can be chosen as a vehicle to be further investigated in terms of the aims of this research because this species had been broadly studied and would serve as a good model for other algae (Mondal et al. 2017; Venkanna 2017; Scranton et al. 2017). Also, *C. reinhardtii* contains both *chlorophyll-A and chlorophyll-B* as well as associated pigments that have absorption spectra, which are

matching the emission spectra of the red and blue lasers. In addition, *C. reinhardtii* algae have receptors of blue light that play a role in controlling the production of enzymes (Zou et al. 2017) and triggering specific steps in the process of algae reproduction and the formation of gametes (Müller et al. 2017).

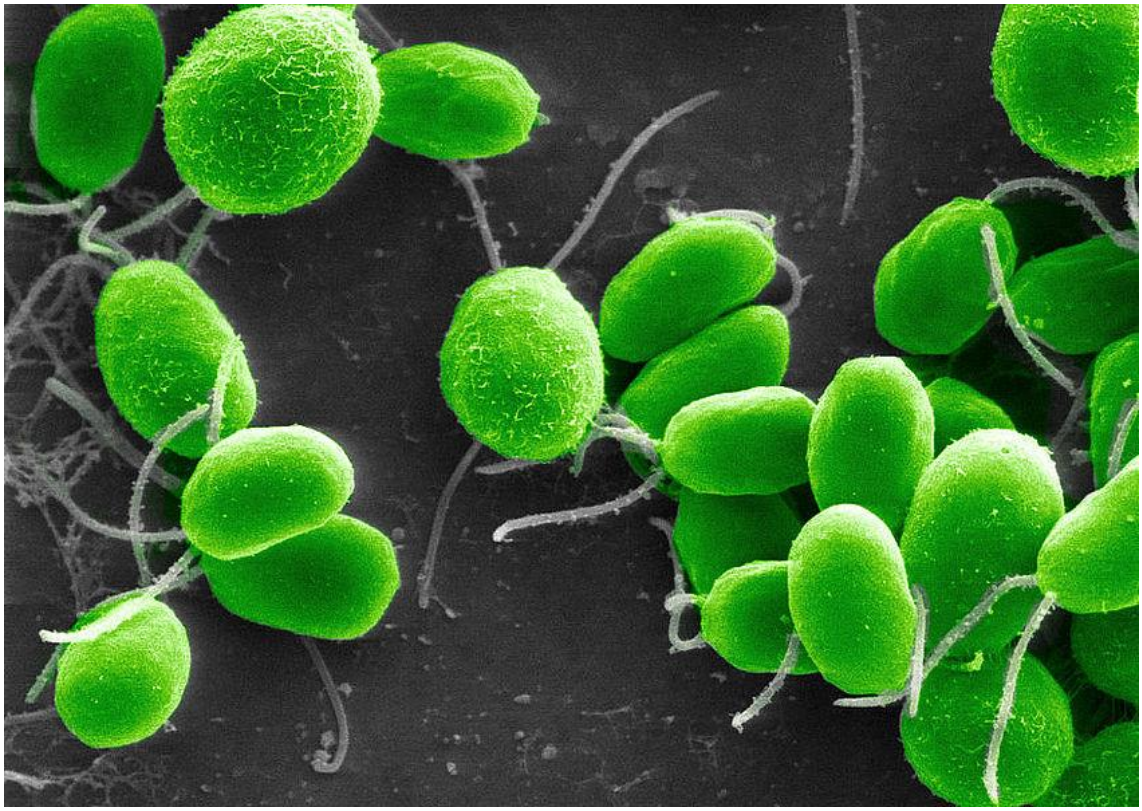

Figure 4.6 Chlamydomonas reinhardtii - model system for alternative energy resources.
Source: (Molecular Systems Biology University of Vienna 2018)

Algae samples of *C. reinhardtii* (strain CCAP 11/32A) were selected for conducting all the experiments within this research. These algae samples were purchased from specialist supplier (the Culture Collection of Algae and Protozoa (CCAP) Oban, Scotland) to ensure uniformity of product, consistency of quality parameters and avoidance of microbe contamination. They had been grown under heterotrophic conditions in TAP medium and continuously mixed in order to keep them homogeneous (Saifuddin et al. 2017).

The cultures of algae cells were stored in 50 ml skirted centrifuge tubes in TAP medium with continuous mechanical shaking. These cells become photosynthetic by illuminating them using a white fluorescent light on 12 hours light and 12 hours dark cycle (using a

12:12 hours timer) with an intensity of 1800 lux (33.3 µmol m^{-2} s^{-1}) measured next to the tubes of algae culture.

Every four weeks, it was essential that the primary cultures were sub-cultured to maintain young, motile and healthy cells. This time frame was the most suitable time to allow cells to divide sufficiently in order to become concentrated enough for sub-culturing. During sub-culturing, the TAP media was added to optimise cell growth. The samples were typically left for 60 hours under white light LED illumination before cells were used in the experiments to ensure the same starting threshold for all cells as a baseline of photon excitation of electron within the pathways between the RCs of PS II and PS I. A 5 millilitres test tube volume of culture was used in each experiment unless otherwise stated.

4.6.1 Growth phases of algae cultures

There are five phases to algae growth in batch cultures, which are lag, exponential, declining relative growth, stationary and death phase (Lim et al. 2012; Zhang et al. 2015) as shown in Figure 4.7.

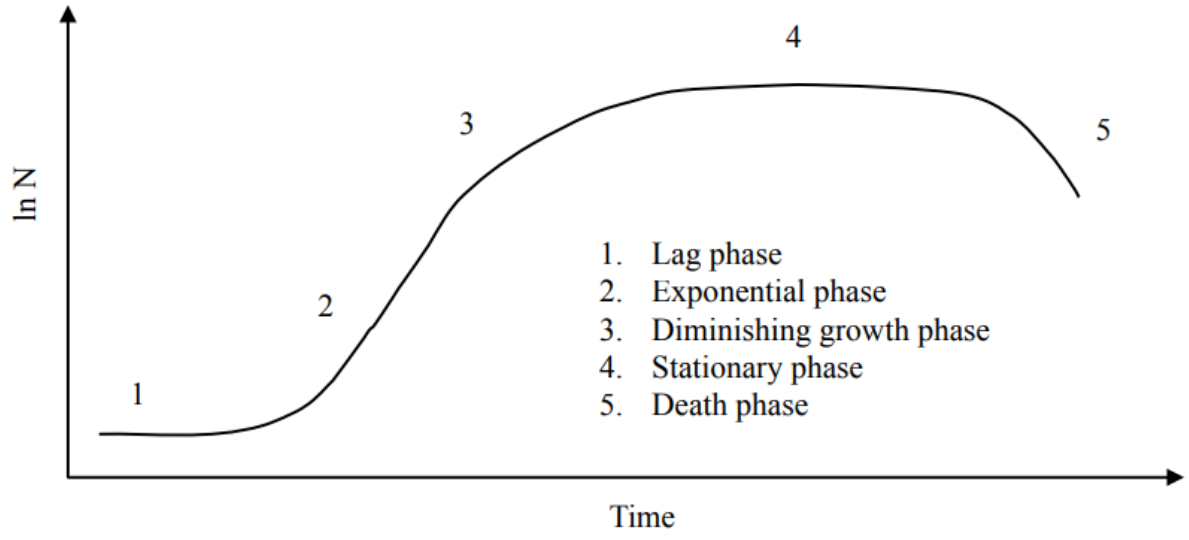

Figure 4.7: Growth curve of microalgae. The characteristic relation between the number of cells (N) and time during microalgae cultivations. *Adapted from Lavens & Sorgeloos (1996).*

During the lag phase, the algae cannot divide, but are merely maturing and preparing for the division process. Thus, it is not possible to note down any growth yet as the growth is very slow. In this phase, the algae are adjusting to the surrounding environment and they need to become accustomed to the growth conditions (Lavens & Sorgeloos 1996).

The second stage, the exponential phase, consists of a steep growth gradient as the algae begin to multiply quickly, thus consuming nutrients at a faster rate and presenting optimal growth rate. At this stage, there is rapid growth and often cell division (Lim et al. 2012), and the growth rate is affected by temperature, light intensity, pH and nutrients (Al-safaar et al. 2016).

Phase 3 is the diminishing or declining relative growth phase where the algae growth rate decreases due to a limit in the resources needed for growth, as a result, there is a decrease in the rate of cell division (Lim et al. 2012).

During phase 4, which is the stationary phase, there is an equilibrium between the growth rate and the natural rate of cell death, causing an overall balanced cell density, which represents the maximum cell density capacity. The death phase begins and the cultivation stops due to lack of resources (Lavens&Sorgeloos 1996).

Monitoring the culture growth and cell activity over time is essential in order to interpret the cultivation phases, which is possible by drawing a growth curve based on the experimental data (Figure 4.8). This curve demonstrates the growth of C. reinhardtii grown in TAP medium and its trend matches the mechanism of the 5 cultivation phases.

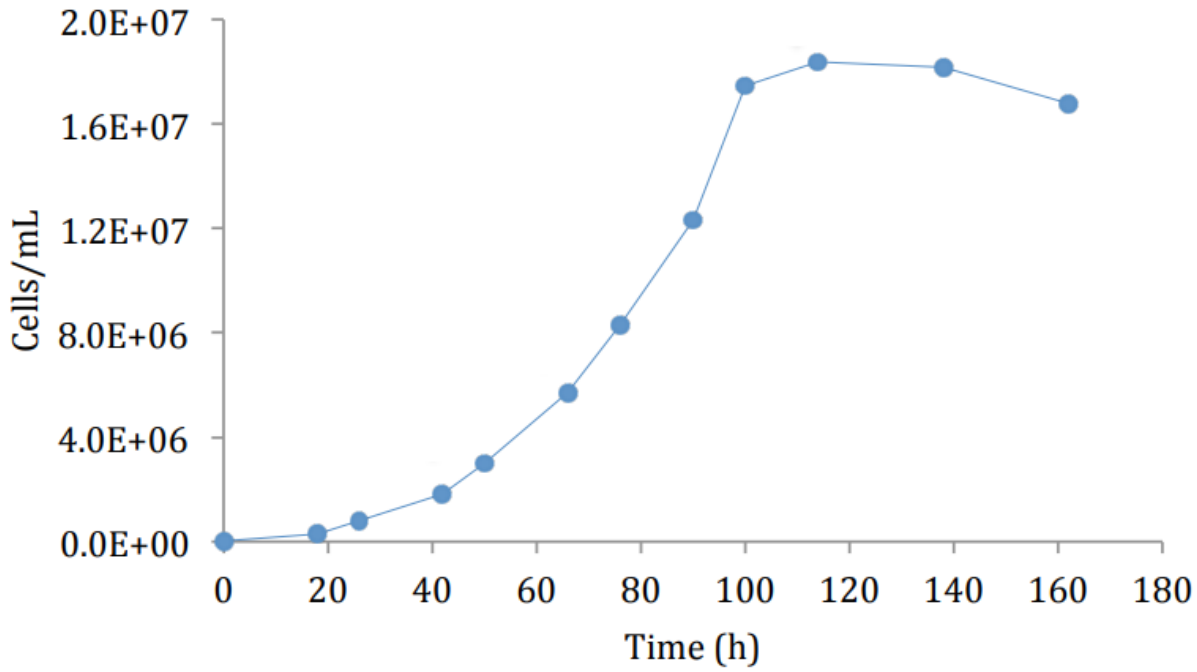

Figure 4.8. Growth curve of C. reinhardtii. *Adapted from Basu (2015).*

When considering the effect of nutrients, light and temperature on cell growth activity, it is important to investigate the growth rate at the exponential phase (Zhang et al. 2015). The activity of cell growth rate is high during early exponential phase (42 h), however, harvesting the algae culture in this early stage is not recommended as the cell density is still low (Basu 2015; Kodama & Fujishima 2016). This led to the conclusion that the suitable harvesting time of the cultures should be during the mid-late exponential phase (90 h). The exponential phase lasts for 7 days with the maximum growth noted during day 0 to day 4 (Lim et al. 2012). Therefore, throughout this research, it was assured that these recommendations were applied as the work progressed in the experimental phases for 84 hours in total.

4.7 Algae culture mixing

Normally, culture mixing was performed manually as per previous studies such as reference Bees & Hill (1997) and reference Czirók et al. (2000). A set of mixing routines was used every time. However, there is an element of human error involved in this method since it is practically difficult to mix in exactly the same way in every case. This state results in a heterogeneous mixture of cells that varies between experiments. In order to standardise mixing between experimental runs, an automated mixing device has been implemented in this study. It consists of an automatic tunable rotating mixer (AQS Shaker Manufacturing Ltd) covered by an opaque box to avoid any external source of light from reaching the algae samples. The light source is positioned in front of the samples to distribute light directly and evenly. The mechanical mixer works by running an electric motor, which causes the flat head attachment to oscillate uniformly in a circular motion, creating a rotation in the culture that mixes algae cells. It has been found that a slow and steady mixing pattern was the best way to create a homogeneous mixture of algae cells and keep the cells alive. It ensures the prevention of different initial conditions caused by the mixing pattern in each experiment.

4.8 Instrumentation devices used throughout the experimental work

The instrumentation devices used in conducting the experimental part of this research are a microscope, an electronic temperature meter, a pH electrode and meter, and a light meter. All measurements were taken every 12 hours in order to record the conditions of each experiment at its beginning and end. This interval provided possibilities to check the influence of light source in improving algae growth rate.

The observed growth rate of algae while using lasers or white light LED as a light source was recorded and monitored at regular intervals using computer-based data logging system. The system consisted of a photodiode sensor, drive and amplification circuit, data logging system (LABVIEW software and DAQ device), and recording/display system (computer set and monitor). The system was used to log the output signals received from photosensor through the data acquisition system. The data acquisition system consisted of the DAQ NI USB 6008 from National Instruments. It is a multifunction interface device comprising 8 single-ended or 4 differential analogue input channels, two analogue output channels, and 12 digital input/output lines. The recording/display system was a windows based desktop computer. A USB communication cable was used to interface the multi I/O device with the computer.

This setup works under the control of LABVIEW software version 2014 SP1; it is a windows based icon driven data acquisition, processing and measurement system control software package that has the capability to save data into excel sheet files, which is well-suited with the DAQ NI USB 6008. LABVIEW desktop can be configured to monitor and display the outcomes of the measurement process.

The measurements displayed by LABVIEW were in real time taken at 15-minute intervals. This arrangement produced a monitoring mechanism that could be more finely tuned through manipulation of input. These figures refer to the light passing through the diffuser, bioreactor sample, and then received by a photodiode, the output of which was augmented by the signal amplifier and processed within the computer using LABVIEW platform. The quantity of light that reached the photodiode was the initial photon count generated by the laser and diffused, minus those absorbed by the cells suspended in the nutrient medium, which was situated within the light pathway of the bioreactor.

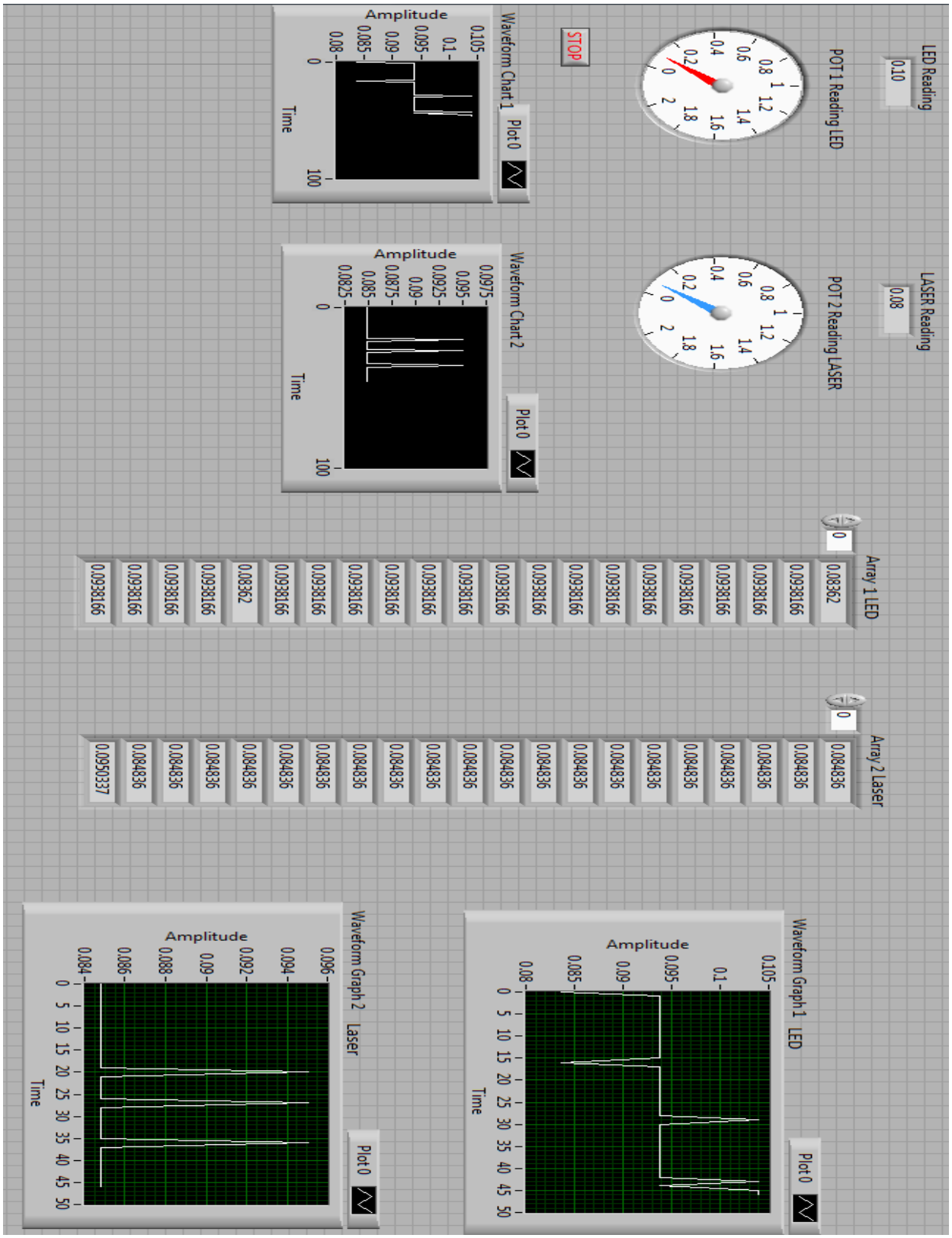

Figure 4.9 The virtual instrument (VI) for LABVIEW

The real-time data were collected using the configuration described in the preceding paragraph and the use of virtual instrument (VI) control modules were implemented to collect data continuously in real time. The programs responded to the output signals from the sensors that were strategically placed throughout the PBRs used in the experimental work as shown in Figure 4.9.

Two electronic amplifiers were designed and constructed to cover all the instrumentation devices used in this work and the configuration of each amplifier and the channels are detailed in section 4.10.

The following sections provide a brief description of each device.

4.8.1 Intensity measurements of the light sources

The illumination source used for the experiment consisted of either a red or blue coherent, diffused laser or a white light LED. By using an optical diffuser and a polarisation attenuator, the light from each direction was always set to the same power rating, giving uniform lighting over the entire surface of algae samples. Light flux was measured at each intensity setting at the surface of the algae samples using a light meter. This ensured that there was no influence of other sources of light by restricting all other light sources in the laboratory.

4.8.2 Temperature and pH measurements

The 5 ml test tubes were kept under 'ideal' algae growth conditions as recommended in the reviewed literature: at room temperature and with a starting pH of 7.0 (Huang et al. 2017). It was mandatory to monitor the pH very carefully every 12 hours. Temperatures were recorded every 3 hours, including the room temperature. Those measurements were made using a highly accurate Testo 615 digital thermo-hydrometer with a temperature measurement range 0 to 50 °C with specific error (as specified by manufacturer) of ± 0.7 $^{\circ}$C.

The pH level in the test tube was measured through a P13 Glass combination pH microelectrode and an analogue pH meter. It uses distilled water as the electrolyte and was calibrated using standard buffer solutions (Duracal® buffers with pH 7.00 and pH 4.01) and kept in a solution of a pH of 3-4.

4.8.3 CO_2 measurements

An electrical adjustable rate compressor was used to provide air bubbles in order to promote algae growth, the air pump (Adjustable Air Pump 270 180 L/H Free Tube Line+Stone 5W) was used for the purpose mentioned. The Vernier CO_2-BTA (470001-856) gas sensor was used to measure the concentration of CO_2 gas in the air flow. These two devices in combination with a four-way air flow splitter with lever control valve were used to control the amount of air delivered to the PBRs used in conducting the experimental work of this research. Carbon dioxide gas was measured using CO_2 Gas Sensor operating in two ranges 0 to 10,000 ppm and 0 to 100,000 ppm. In order to run controlled experiments with small samples of CO_2 gas, a 250 ml respiration chamber with probe attachment was implemented. It was calibrated using a 250 ml Nalgene Bottle while setting its measurement span to the lower range (0-10,000) ppm to provide more sensitivity.

It was necessary that the air flow was set to a constant 0.1 l/min x 60 x 12 = 72 l/12h rate in order to accurately calculate the total mass of CO_2 injected into the system over a period of 12 hours. The laboratory had a CO_2 concentration of 827ppm, equivalent to 0.827 g/l. Therefore, the total mass of CO_2 injected every 12 hours at a constant air flow was calculated as follows:

0.827g/l x 72 l/12hours = 59.544 gCO_2/12h

4.9 Driving the white light LED

In this section, the choice of driving circuit for the white light LED was considered. The consideration had to be made between either constant voltage or constant current drive for the white light LED. This decision is influenced by junction characteristics. It can be noticed from Figure 4.10 (Schubert 2006) that the LED forward bias voltage shows clear dependency on temperature, which leads to the conclusion that constant voltage drive is not capable of providing stable emission from the LED device as the passing current tended to heat the junction, which led to changing forward voltage resulting in dimmer operation. Different considerations played a role in designing the DC drive circuit of an LED operated under steady-state conditions. These considerations included the simplicity

and cost of the drive circuit, the power efficiency and the compensation of the temperature dependence of the light intensity emitted from the LED. In the conducted research, it was more important that the light intensity emitted from the white light LED device remains stable during the course of experimentation.

A basic drive circuit for the white light LED is a ***constant-voltage supply*** such as a battery or the rectified AC output of a transformer. However, two drawbacks to constant-voltage drives of LEDs were noted: the first is the diode forward current relies exponentially on bias voltage; therefore, a small variation in drive voltage can result in a large change in current, which will produce a large change in the emission intensity. The second drawback observed and noted was the threshold voltage of a diode, thus displayed great dependency on temperature, so any temperature change resulted in a significant change in current.

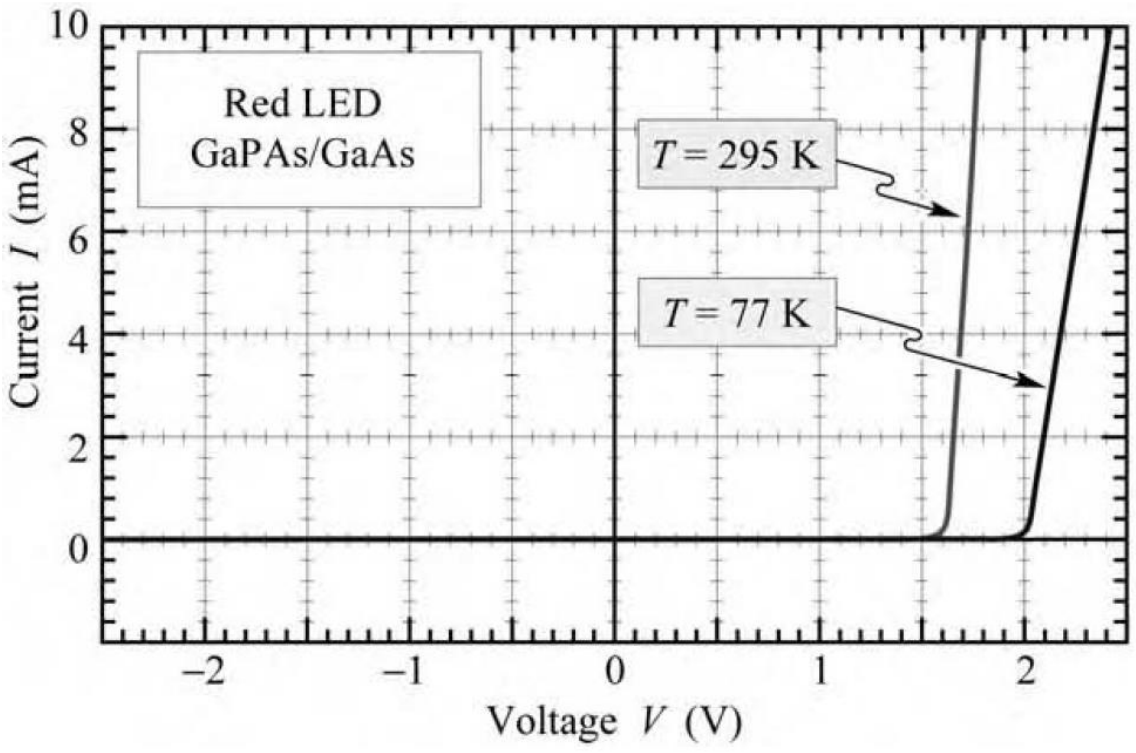

Figure 4.10 The typical LED forward voltage variation with temperature. *Source: (Schubert 2006).*

When a diode was driven with a constant current, the emission intensity decreased as the operating temperature of the device increased. The emission intensity of LEDs generally decreases with increasing temperature due to non-radiative recombination (Schubert

2006). In addition, the threshold voltage decreases with increasing temperature. It should be noted that the electrical-to-optical power-conversion efficiency drops as well.

The temperature dependence of white light LED intensity was an important factor for LEDs used as a light source in the experiments carried out. These experiments were conducted under a temperature controlled environment and the LEDs used in the experimentation were operated at a fraction of their power handling. This meant that the device reached a steady state temperature which could be considered constant for the practical application in hand. Therefore, the constant current LED drive circuit ensures stable light emission as required to conduct the experiments.

A constant-current drive circuit may consist of a transistor with the LED as a load. A constant-current drive circuit (Malvino & Bates 2006) allows one to drive the LED irrespective of the diode threshold voltage and the diode temperature. However, a constant-current drive circuit does not compensate for the decrease of LED emission at elevated temperatures, which was not the case in the application in hand. Figure 4.11 shows the constant current LED drive circuit.

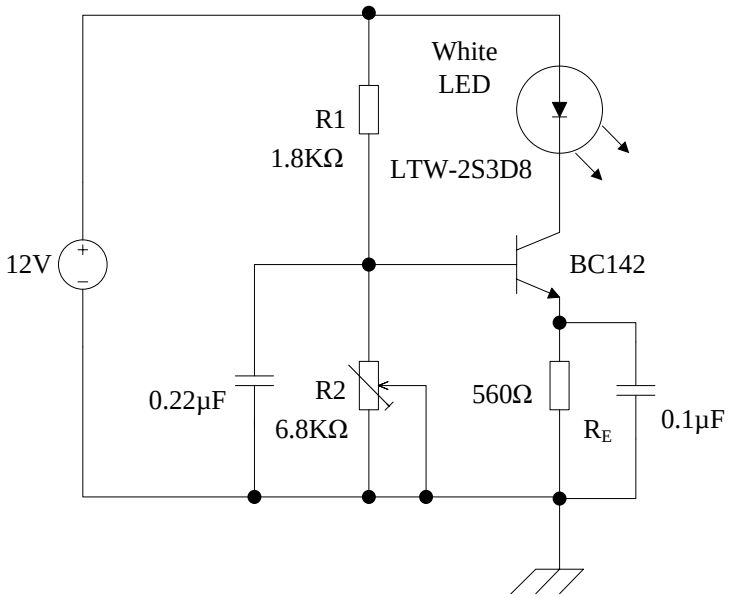

Figure 4.11 The constant current LED drive circuit. *Source: (Malvino & Bates 2006).*

This circuit was designed around the white light LED LTW-2S3D8 from Lite-on Inc. The maximum drive current for this LED was 20 mA, which produced a light intensity of

17000 mcd. The BC142 transistor was selected to drive this white light LED, which had a continuous current rating of 1A.

In this design, the white light LED was driven at reduced drive current with a maximum of 15 mA.

Therefore, for a maximum current of 15 mA the emitter current was found from Eq. (4.1) (Malvino & Bates 2006):

$$R_E = \frac{V_{CC} - (Vf_{LED} + VCE_{SAT})}{I_E} \tag{4.1}$$

Where V_{CC} = 12 V, VCE_{SAT}= 0.15 V, Vf_{LED} = 3.3 Volts and I_E =15 mA

Applying these values to equation 4.1 resulted in

R_E= 570 Ω. The nearest standard value was 560 Ω.

The bias base current required was:

$$I_B = \frac{I_C}{\beta} \tag{4.2}$$

Where $I_C \approx I_E$=15 mA, and β=100 for BC142 thus:

I_B= 0.15 mA

So, a 1.5 mA current is assumed to be passing through it, resulting in a total value of the potential divider resistor network (R_1 + R_2) of:

$$(R_1 + R_2) = \frac{V_{CC}}{10I_E} = \frac{12}{1.5 * 10^{-3}} = 8\ k\Omega \tag{4.3}$$

Now to find R_1 and R_2 it was required to calculate the voltage at the base of the transistor (V_{BB}) from:

$$V_{BB} = V_{BE} + V_E \tag{4.4}$$

Where V_{BE} = 0.83 V (from BC142 data sheet) and V_E= 8.4 V which made

V_{BB}= 9.24 V

Neglecting I_B, R_2 was calculated to be

$$R_2 = \frac{V_{BB}}{I_{DIV}} = \frac{9.24}{1.5 * 10^{-3}} = 6.15\ k\Omega \quad (4.5)$$

R_2 was chosen from the nearest standard value of (6.8 kΩ). This resistor was chosen to be of a variable type so that the drive current of the LED was able to be controlled in order to maintain the light intensity.

R_1 was calculated from:

$$R_1 = \frac{V_{CC} - V_{BB}}{I_{DIV}} = \frac{12 - 9.24}{1.5 * 10^{-3}} = 1.85\ k\Omega \quad (4.6)$$

The nearest standard value was selected to be (1.8 kΩ)

Thus, the bias circuit for the white light LED drive was complete. The addition of a 0.22 µF parallel to R_2, and 0.1 µF in parallel with R_E, enabled better circuit stability and noise immunity.

This circuit was capable of varying white light LED current from 0 mA to 15 mA, which resulted in variable light output intensity. This was mandatory in conducting the required experimentation done through the path taken in the research in hand.

4.10 The light intensity meter

When conducting the experiments, the light emitted from several light sources was required to provide fixed values of luminous intensity. These values were verified using a special PIN photodiode, namely the VBPW34S from Vishay Semiconductors (Vishay 2002), chosen for its suitability for this application due to having high photosensitivity, high irradiant sensitivity and linear reverse current versus irradiance, as shown in Figure 4.12 which was taken from the device data sheet.

In order to obtain an output that could be measured accurately using high accuracy voltmeters, it was required to change the reverse light current to voltage using an operational amplifier special circuit that performs the current-to-voltage conversion.

The circuit diagram shown in Figure 4.13 (Fiore 2018) represents the current-to-voltage conversion circuit required to properly tune the light sources in order to provide identical luminous intensities. The photodiode was biased from the 5 V reference supply using78L05 integrated circuit that had a temperature compensated buried Zener reference that provided a stable 5 V output dedicated to drive the photodiode (Analog Devices 2006). From the datasheet of the VBPW34S, this device provided a reverse current with linearly proportional irradiance energy with a dark current of 0.8 µA and an output of 50 µA per mW/cm^2.

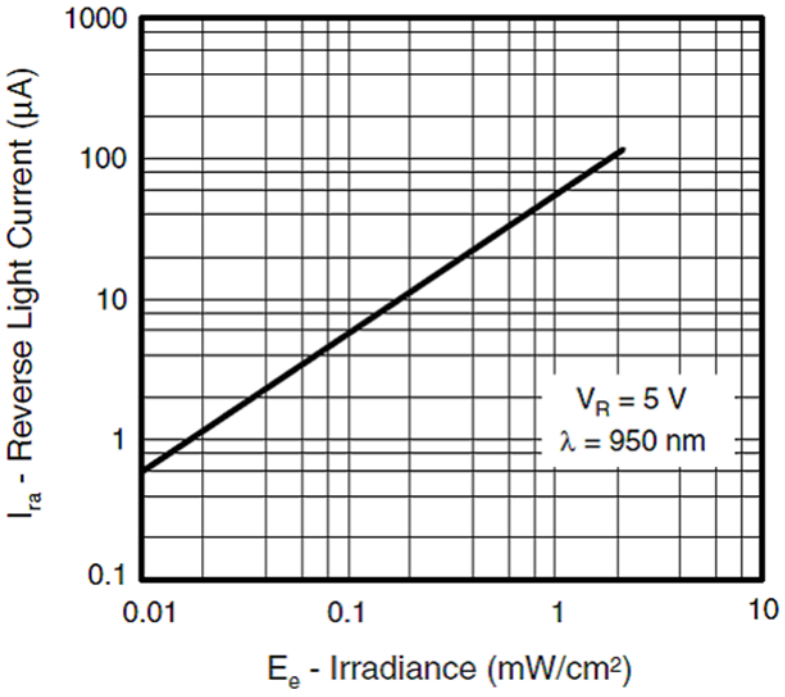

Figure 4.12 Reverse light current versus irradiance for VBPW34S photodiode. *Source: (Vishay 2002).*

The reverse current from the VBPW34S photodiode passed through a 10 kΩ precision metal oxide resistor having 1% tolerance. This resistor converted the reverse current from the photodiode to voltage and the resultant figure of sensitivity became:

$$V_{OUT(PhotoSensor)} = I_{OUT(hhotosensor)} * R_{(precision)} = 500 mVper \cdot mW / cm^2 \quad (4.7)$$

The OP-27 precision operational amplifier (Analog Devices 2006) was used as a non-inverting amplifier with a gain given by:

$$Av_{(OP-AMP)} = 1 + \frac{R_f}{R_1} \tag{4.8}$$

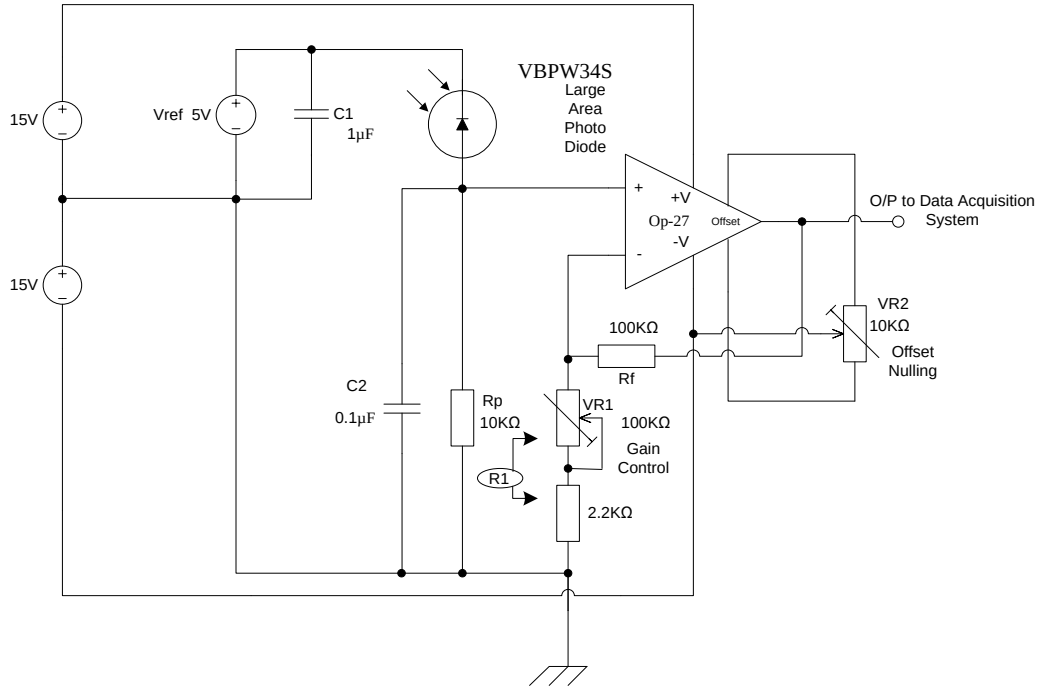

Figure 4.13 The photodiode drive and current-to-voltage conversion circuit. *Source: (Fiore 2018).*

This gave a maximum gain of 46.5 and a minimum of ≈2. Gain could be altered by varying VR_1 which was a multi-turn precision trimming resistor.

VR_2 was used to null any offset observed at the output when there was no incident light falling on the photodiode. It was used to null the offset appearing at the op-amp output when the dark current flowed through R_P (the precision conversion resistor) so that the output of the circuit became 0 Volts for 0 mW/cm^2 irradiation. C_1 provided stability and noise immunity for the reference voltage supply, and C_2 provided noise immunity to the photodiode output.

4.11 Experimental procedure

The *C. reinhardtii* algae were sub-cultured and initially diluted to an optical density (OD) of 0.1(cm^{-1}) using two samples at all times, and cells counted throughout the whole series of experiments by way of measuring the OD at 750 nm using the Bio-Chemistry Analyser (Stat Fax 1904 Plus). A correlation curve of algae cell concentration was firstly established using regression i.e. Y = 36.6X – 6.99 (n=10), where Y is the algae cell concentration and X is the OD_{750}. Growth of the algae cells in the 5 ml test tube occurred under both laser irradiation and white light LED irradiation and was maintained at the ideal growth conditions of temperature and pH (room temperature with a starting pH 7.0), as recommended by Huang et al. (2017). In the first PBR (the preliminary) two identical algae samples were initially exposed to 24 and 48 hours (this irradiation duration was later increased to 60 hours as it gave better results) of white light LED having a controlled power input of 1 mW measured at the algae samples with a standard deviation of ±0.05 mW, followed by 12 hours of darkness. The second (the laser) and third (the white light LED) PBRs received one sample of algae each, from the first PBR, this commenced with a regular 12 hour light period. The source of light for the second PBR was the blue and red lasers including the wavelengths 405 nm, 450 nm, 473 nm, 635 nm, 650 nm, 680 nm, 685 nm and 700 nm, whereas the source of light for the third PBR was the white light LED. The blue lasers were selected based on the finding that microalgae showed better growth rate when cultured under the blue light condition compared with white light (Atta et al. 2013) as the intensity of the blue light enables deep penetration through the batch culture thus enhancing the cell division and growth rates of microalgae (Teo et al. 2014). The red lasers were selected based on the finding that the maximum photon utilisation efficiency for growth of the *C. reinhardtii* algae is centred at 674 nm (Darko et al. 2014) and the red light's wavelengths (650 nm to 665 nm) perfectly fit with the absorption spectrum peak (λ_{max}) of chlorophyll (Yacobi et al. 2015), *C. reinhardtii* algae was successfully grown under the 655 nm and 680 nm red lasers irradiation (Kuwahara et al. 2011). Consideration was given to the temperature as well as the pH of the nutrient solution as they directly affect the growth rate of algae by changing the structure of nutrients (Singh & Singh 2015). The optimum temperature range for growth of algae was recorded between 20 and 30 ºC (Singh & Singh 2015) and the growth of algae could be significantly decreased only if the pH exceeds the 6.5-8.2 range (Kosourov et al. 2003), as pH can affect the activity of some enzymes (Ying 2014); firstly the temperature was

maintained between 19 and 25 ºC, secondly, the pH was measured at each stage and recorded accordingly. In order to maintain the uniformity of nutrient with the cell population it was necessary to have continuous mixing, thus, an air pump was used to give off air, which includes CO_2 gas that was useful to keep the pH at a (6.9-7.7) level, as the algae growth rate was not greatly influenced by the (6.9-7.7) pH fluctuations (Svaldenis 2014). The cell concentration was calculated based upon a collection of generated data as per Figure 5.1. The specific growth rate (μ) of algae, determined to be in the exponential growth phase, was calculated using the method of Guillard by means of a least-squares fit of a straight line to the logarithmically transformed data as illustrated in Eq. (4.9) (Nagasoe et al. 2006):

The specific growth rate (μ) of algae:

$$\mu = \frac{\ln(N_f / N_i)}{t_f - t_i} \tag{4.9}$$

Where N_f and N_i were the cell number per millilitre at time t_f and t_i respectively. The time taken for duplicating the number of cell division rate (k), was calculated according to Eq. (4.10) (Nagasoe et al. 2006):

The doubling time (k):

$$k = \frac{ln2}{\mu} \tag{4.10}$$

The growth rate (M; divisions/hour) was then obtained using Eq. (4.11) (Nagasoe et al. 2006):

The growth rate (M; divisions/hour):

$$M = \frac{1}{k} \tag{4.11}$$

The real-time data was collected using a combination of (DAQ NI USB 6008 & LABVIEW) and individual control modules codes were written for this purpose. The measurements displayed by LABVIEW were at 15-minute time intervals, thus produced a real-time monitoring mechanism. The programs responded to the output signals from the sensors strategically placed throughout the laser beam and the LED PBRs.

4.12 Data analysis

All experiments were repeated 10 times using individual random samples chosen from *C. reinhardtii* algae in order to ensure reproducibility of results, enabling us to make inferences about all samples of that algae. The level of experimental repetition was (n = 10) and the data were represented as the mean ± 1.96 calculated standard error of the mean of ten independent random samples. Controls performed throughout the research were: a specific chosen type of algae (*C. reinhardtii*), light intensity, light source exposure times, temperature, pH, nutrient gas (air) and nutrient solid (TAP medium), these were the independent variables that consistently remained unchanged. The independent variable was the wavelength of the light source (laser or LED) and the dependent variable was the cell growth of random samples of *C. reinhardtii*. In order to measure the margin of error, statistical treatment of data performed on comparisons included finding the mean, sample standard deviation, s, population standard deviation, σ and standard error of the mean ($SE_{\bar{x}}$).

4.13 Chapter summary

This chapter described in detail the materials and methods used in the experimental work of this thesis.

Eight individual lasers having the following wavelengths (405 nm, 450 nm, 473 nm, 635 nm, 650 nm, 680 nm, 685 nm, and 700 nm) were employed in different combinations to generate photons with energies overlapping on the absorption spectra of pigments within *C. reinhardtii* algae; this was made with a view to enhancing transportation within the electron pathway between photosystems II and I, thus anticipating increase in rate of cell division.

Chapter 5 presents the experimental results and comparisons made between the results obtained while using both blue and red lasers combined and individually as a light source for the PBRs.

CHAPTER 5

EXPERIMENTAL RESULTS AND DISCUSSION

5.1 Introduction

In this chapter, the experimental results are presented as investigated. This includes describing the manipulation of contributory components and their effect on accelerated algae growth, this through the relationship between semiconductor laser generated photon energy and the electron activity during the initial take-up of hydrogen ions from H_2O in photosystem II. Blue and or red lasers of wavelengths 405 nm, 450 nm, 473 nm, 635 nm, 650 nm, 680 nm, 685 nm, and 700 nm were used to generate photons with energies (wavelengths) more closely matching the absorption spectra of algae receptors known as pigments (Jiang & Wen 2015). Based on the reviewed theoretical literature in Chapter 3 and the findings of this chapter, the influence of laser wavelength scanning sequence used in this study is correlated with the experimentally observed algae growth.

5.2 Correlation between the biochemical and microscopic cell count

Figure 5.1 shows the relationship between absorption of light in an algae sample (measured by a biochemical spectrum analyser) and the number of actual algae cells (measured under a microscope). The graph shows a directly proportional relationship between absorption and cell concentration.

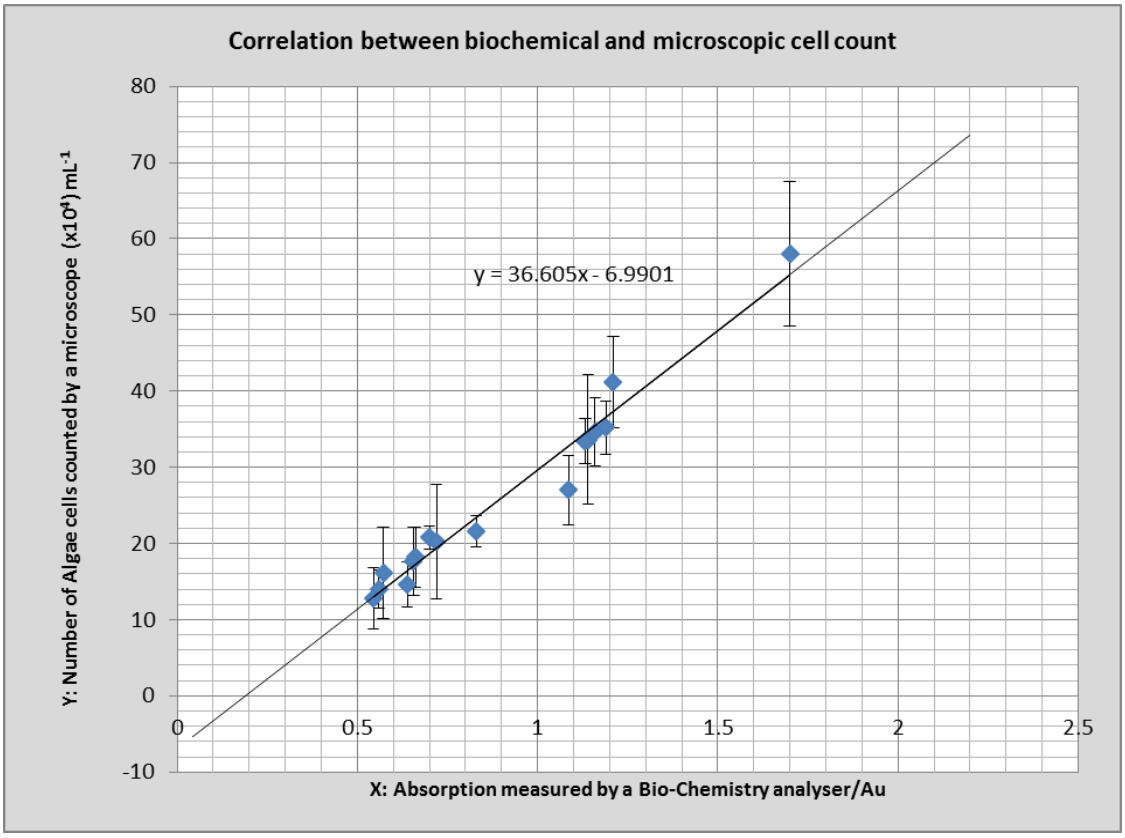

Figure 5.1 Correlation between the biochemical and microscopic cell count within the medium

Figure 5.1 illustrates the correlation between mean values of biochemical and microscopic cell count within the medium. Error bars indicate 1.96xSE$\bar{x}$ of the mean from the ten independent samples (n=10, SE$\bar{x}$: standard error).

5.3 Comparison of algae growth rate difference using the blue lasers and white light LED as a reference

All calculations were carried out after each sample was firstly routed to PBR number 1 and subjected to 60 hours of timed exposure to white light LED emission followed by 12 hours of total darkness. This light exposure/dark periods combination helped in obtaining stable results that repetitively demonstrated high degree of uniformity.

These baseline samples were then divided into separate groups of equal volume and distributed to other PBRs. These PBRs are then exposed to different light sources in a manner outlined in the methodology. The absorption outcomes of using the Blue lasers

of 405 nm, 445 nm and 473 nm wavelengths as compared with those obtained using white light LED in isolation as a reference are shown in Table 5.1 and Figure 5.2, which summarise the effect of laser and LED wavelengths on *C. reinhardtii* specific growth rate and division rate.

Table 5.1 The absorption outcomes of blue lasers and white LED used in isolation as a reference

Source of light	**Initial cell number per mL (N_i) x10^5***	**Final cell number per mL (N_f) x10^5***	**μ x10^{-2} (h^{-1})**	**k (h)**	**M x10^{-2} (h^{-1})**
Laser 405 nm	3.005 ± 0.067	4.199 ± 0.196	2.79	24.88	4.02
White light LED	2.994 ± 0.083	3.701 ± 0.213	1.77	39.27	2.55
Laser 450 nm	2.841 ± 0.094	3.284 ± 0.217	1.21	57.41	1.74
White light LED	2.954 ± 0.115	3.620 ± 0.183	1.69	40.90	2.44
Laser 473 nm	3.049 ± 0.140	4.481 ± 0.221	3.21	21.61	4.63
White light LED	3.027 ± 0.212	3.716 ± 0.172	1.71	40.61	2.46

Table 5.1 illustrates the mean values of absorption outcome, specific growth rate (μ), doubling time (k) and the division rate (M; divisions/hour) of ten individual samples of C. reinhardtii cultivated under the emission of different wavelengths of blue lasers (405 nm, 450 nm or 473 nm) and white light LED. * Each value is represented as the mean ± 1.96x$SE_{\bar{x}}$ (n=10, $SE_{\bar{x}}$: standard error).

Blue lasers tend to stimulate gene transcription and improve the regulation of activated enzymes (Richmond & Hu 2013). This finding clarified the reason behind the higher growth rates accomplished when microalgae were cultivated under certain wavelengths of the blue laser (Teo et al. 2014). It also showed the effect of wavelengths on the specific growth rate and division rate of algae cells. The highest specific growth rate of 3.21x10^{-2} h^{-1} was attained under the irradiance of 473 nm blue laser. This was accompanied with a division rate of 4.63x10^{-2} h^{-1}. Improved results were also obtained when *C. reinhardtii* samples were cultured under the irradiance of the 405 nm blue laser, but the specific

growth, division rate, and maximum cell concentration were much higher when compared to those obtained with 450 nm blue laser.

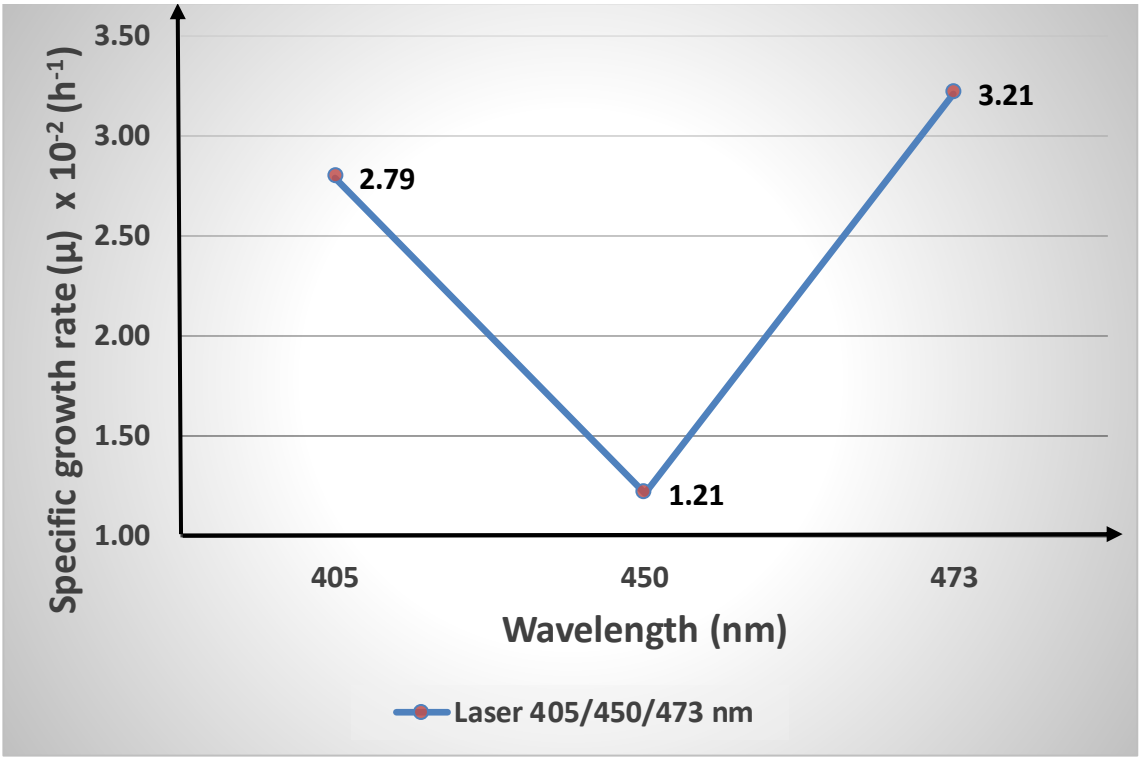

Figure 5.2 The specific growth rate (μ) of C. reinhardtii irradiated by blue lasers

Figure 5.2 reflects the mean values of specific growth rate (μ) of ten individual samples of C. reinhardtii cultivated under the emission of different wavelengths of blue lasers (405 nm, 450 nm or 473 nm).

Microalgae showed better growth rate when cultured under the blue laser light irradiation compared with those achieved with white light LED and obtained results were with close conformity with those reported by Kuwahara (2011) as the exposure of *C. reinhardtii* to blue laser light results in obtaining high cell densities and high hydrogen ions production rates through the batch culture thus enhancing the cell division and growth rates (Lee et al. 1994; Venkanna 2017). The best results were achieved using the lasers of wavelengths 473 nm and 405 nm. Prior to the 12 hours of darkness, the cell growth gains were 38.36% (2 times that of the white light LED) and 32.24% (1.8 times that of the white light LED) respectively and were calculated by using equation (5.1). This represents an increase of $1.43x10^5$ and $1.19x10^5$ cells per ml respectively based upon a collection of generated data as per Figure 5.1. Whilst the 450 nm blue laser had no enhancement as the white light

LED increased the rate of cell division by 1.5 times that of the laser of 450 nm wavelength as illustrated in Figure 5.3.

$$Cell\ growth\ gain = \frac{N_f - N_i}{N_i}\ x\ 100\% \qquad (5.1)$$

When used in isolation, both blue lasers and white light LED proved to be advantageous when compared to that of the natural daylight. The algae growth rate enhancement obtained with white light LED was about 2 times that of the natural daylight, thus in comparison to the samples under natural daylight cycles, the enhancement obtained with the laser of wavelengths 405 nm, 450 nm and 473 nm were 3.6, 1.4 and 4 times respectively.

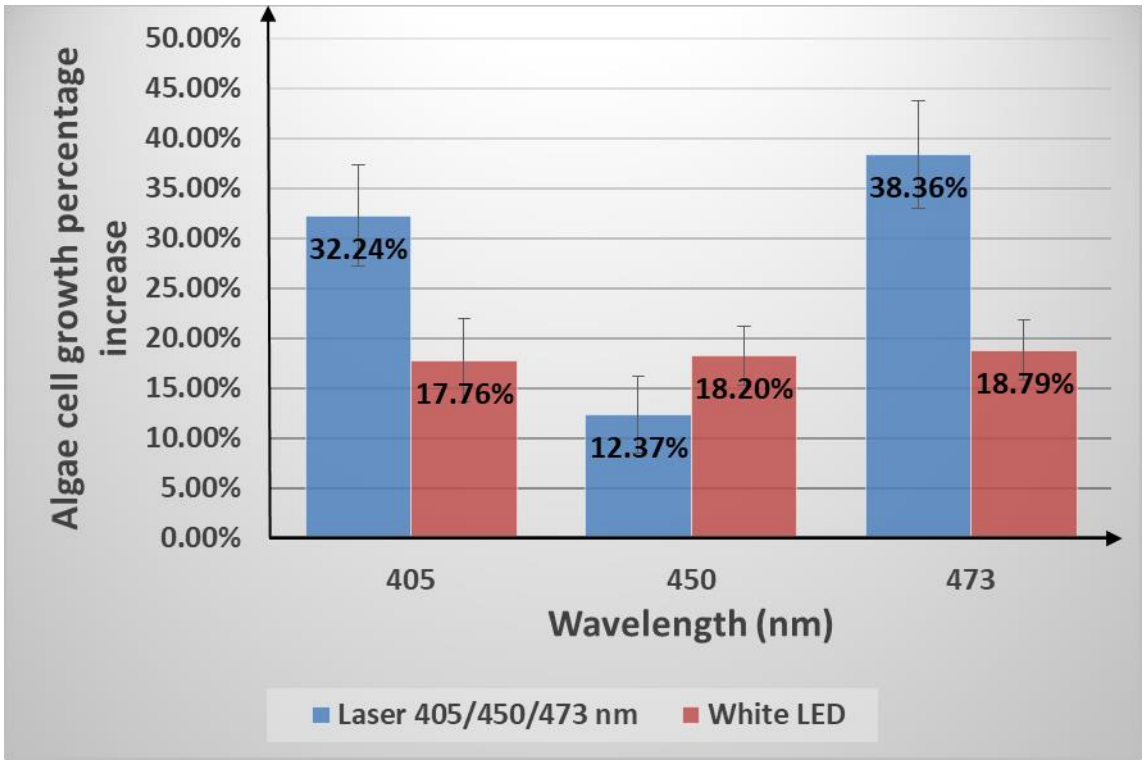

Figure 5.3 The percentage increase of C. reinhardtii irradiated by blue lasers

Figure 5.3 illustrates the means of the cell count percentage increase of ten independent samples of C. reinhardtii over 12 hours of growth period while exposed to a white light LED as a reference and blue lasers 405 nm, 450 nm or 473 nm. The close correlation of the three individual values for LED (brown colour) indicates that the samples were created with high accuracy and the pipetting was consistent. Error bars indicate (±1.96

SE$\bar{x}$), (n=10, SE$\bar{x}$: standard error).

5.4 Comparison of algae rate of growth using the red lasers and white light LED as a reference

When using red lasers, the best growth rates were obtained while irradiating algae samples with lasers of wavelengths 650 nm, 680 nm and 700 nm, the cell growth gains were 36.67%, 44.53% and 28.99% respectively and were calculated using equation (5.1) on page 84.

The aggregate gain in growth rate obtained using red laser of wavelengths 650 nm, 680 nm and 700 nm was 1.95, 2.39 and 1.50 times that achieved using white light LED respectively. This represents an increase of $1.12x10^5$, $1.2x10^5$ and $9.66x10^4$ cells per ml respectively prior to the 12 hours of darkness based upon a collection of generated data as per Figure 5.4. The lasers of wavelengths 635 nm or 685 nm produced no improvement in growth rate compared to that of the white light LED. The gain in growth rate was 4 times of that achieved using laser of 635 nm wavelength, and 1.22 times of that obtained with laser of 685 nm wavelength, once again measured prior to 12 hours of darkness.

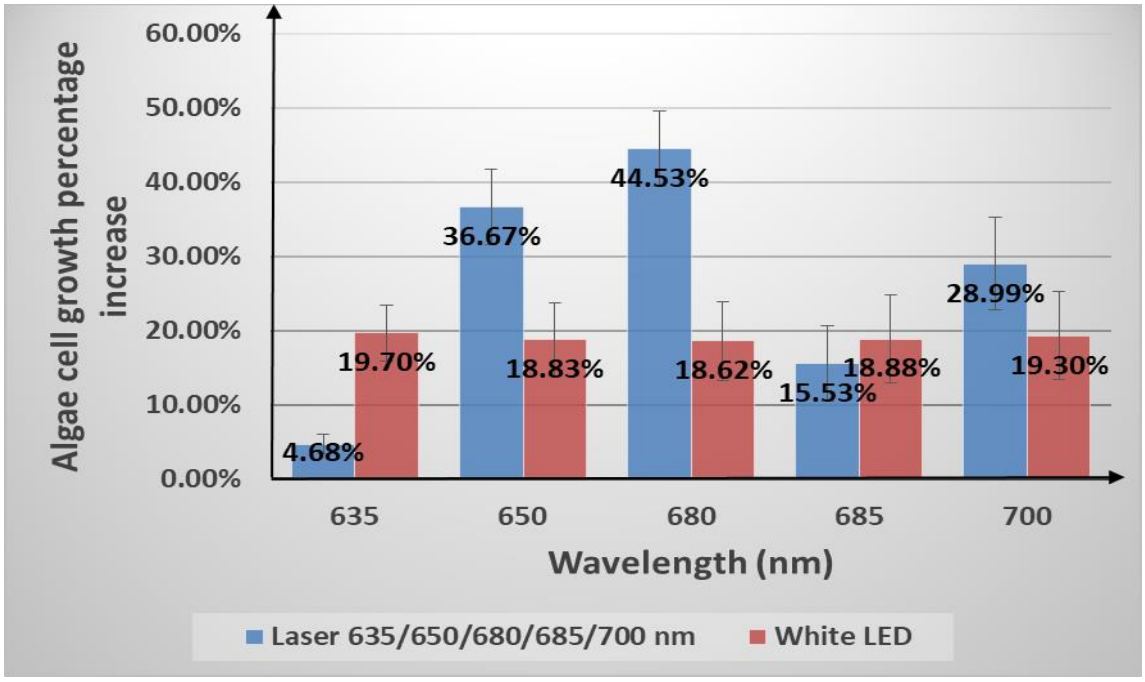

Figure 5.4 The percentage increase of C. reinhardtii irradiated by red lasers

Figure 5.4 reflects the means of the cell count percentage increase of ten independent samples of *C. reinhardtii* over 12 hours period while exposed to a white light LED as a reference and red lasers of wavelengths 635 nm, 650 nm, 680 nm, 685 nm and 700 nm. The close correlation of the five individual values for LED (brown colour) indicates that the samples were created with high accuracy and the pipetting was consistent. Error bars indicate (±1.96 SE$\bar{x}$), (n=10, SE$\bar{x}$: standard error).

When used in isolation, both red lasers and white light LED proved to be advantageous when compared to that of the natural daylight. In comparison to the samples of algae under natural daylight cycles, the enhancement for the laser of wavelengths 650 nm, 680 nm, 685 nm and 700 nm were 3.9, 4.6, 1.6 and 2.7 times respectively. Whereas the laser of wavelength 635 nm had no enhancement, and the natural daylight showed an increase in cell division rate by 2 times of that achievable with 635 nm wavelength laser as shown in Figure 5.4.

The absorption outcomes of using the red lasers of wavelengths 635 nm, 650 nm, 680 nm, 685 nm and 700 nm and white light LED used in isolation as a reference are shown in Table 5.2 and Figure 5.5, which summarise the effect of laser and LED wavelengths on *C. reinhardtii* specific growth rate and division rate.

Table 5.2 The absorption outcomes of red lasers and white LED used in isolation as a reference

Source of light	**Initial cell number per mL (N_i) x10^5***	**Final cell number per mL (N_f) x10^5***	**μ x10^{-2} (h^{-1})**	**k (h)**	**M x10^{-2} (h^{-1})**
Laser (635 nm)	2.972±0.077	3.148±0.159	0.48	144.83	0.69
White light LED	2.943±0.119	3.635±0.223	1.76	39.40	2.54
Laser (650 nm)	3.027±0.098	4.385±0.127	3.09	22.44	4.46
White light LED	3.024±0.115	3.716±0.135	1.72	40.37	2.48
Laser (680 nm)	3.024±0.100	4.440±0.140	3.20	21.65	4.62
White light LED	3.053±0.100	3.792±0.185	1.81	38.35	2.61
Laser (685 nm)	3.009±0.082	3.580±0.163	1.45	47.87	2.09
White light LED	2.994±0.085	3.683±0.147	1.72	40.21	2.49
Laser (700 nm)	3.031±0.094	3.997±0.202	2.31	30.06	3.33
White light LED	3.042±0.121	3.683±0.155	1.59	43.53	2.30

Table 5.2 illustrates the mean values of absorption outcome, specific growth rate (μ), doubling time (k) and the division rate (M; divisions/hour) of ten individual samples of

C. reinhardtii cultivated under the emission of different wavelengths of red lasers (635 nm, 650 nm, 680 nm, 685 nm or 700 nm) and white light LED. * Each value is represented as the mean ± 1.96xSE$\bar{x}$, (n=10, SE$\bar{x}$: standard error).

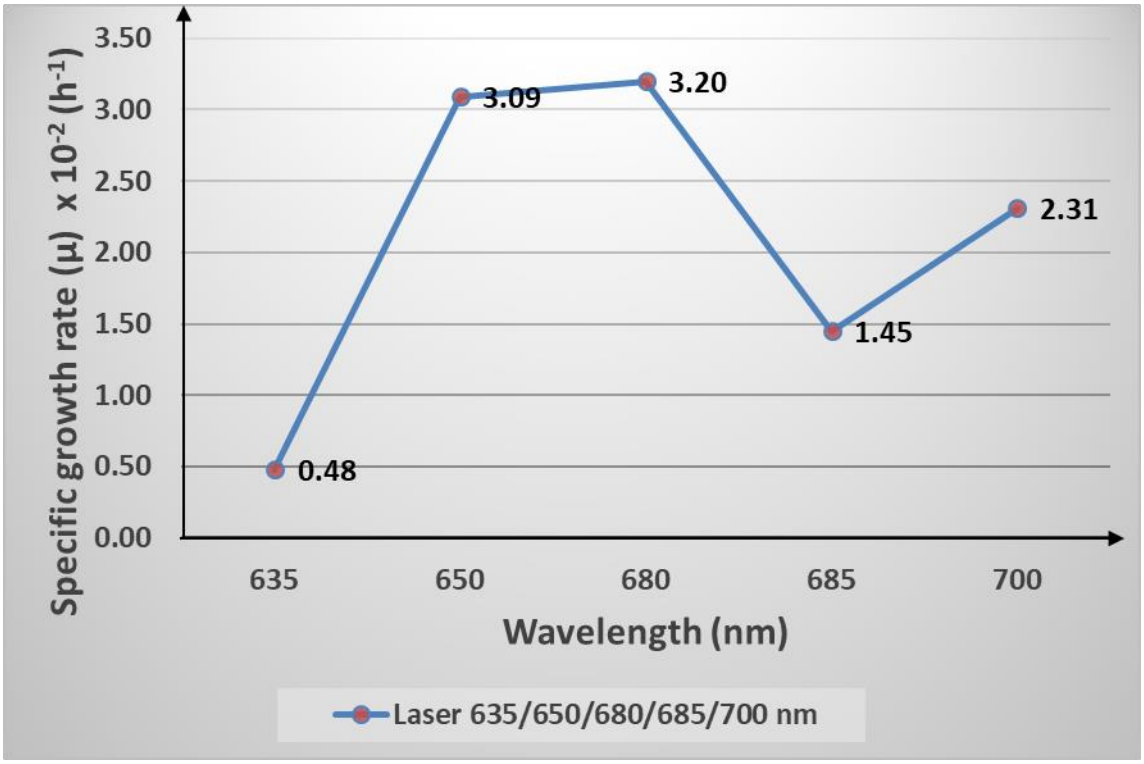

Figure 5.5 The specific growth rate (μ) of C. reinhardtii irradiated by red lasers

Figure 5.5 illustrates the mean values of specific growth rate (μ) of ten individual samples of *C. reinhardtii* cultivated under the emission of different wavelengths of red lasers (635 nm, 650 nm, 680 nm, 685 nm or 700 nm).

The highest specific growth rate of 3.20×10^{-2} h^{-1} was attained under the irradiance of 680 nm red laser with a division rate of 4.62×10^{-2} h^{-1}. Similar results were obtained when *C. reinhardtii* was cultured under the irradiance of the 650 nm or 700 nm red laser; the specific growth, division rate, and maximum cell concentration were much higher compared to that achieved with 635 nm or 685 nm red laser. Microalgae showed better growth rate when cultured under the red laser light condition compared with the white light LED, as it obtained high hydrogen ions production rates through the *C. reinhardtii* culture thus enhancing the cell division and growth rates of microalgae, and these laboratory results are similar to the results mentioned by Lee et al. (1994) and de Mooij et al. (2016).

5.5 The pH results

The effect that nutrients had on various physical properties was observed as mentioned in Table 5.3. Recorded research results showed a clear correlation between the change in pH of the nutrients and the growth rate of algae (Ying 2014).

Table 5.3 Growth medium pH value change when exposed to blue or red lasers and white light LED before and after 12 hours period

Light exposure (12 hours)	Opening pH	Closing pH
Blue laser (405 nm)	7.0	7.6
White light LED	7.0	7.3
Blue laser (450 nm)	7.0	7.2
White light LED	7.0	7.4
Blue laser (473 nm)	7.0	7.7
White light LED	7.0	7.4
Red laser (635 nm)	7.0	7.1
White light LED	7.0	7.3
Red laser (650 nm)	7.0	7.7
White light LED	7.0	7.4
Red laser (680 nm)	6.9	7.7
White light LED	6.9	7.3
Red laser (685 nm)	7.0	7.3
White light LED	7.0	7.4
Red laser (700 nm)	7.0	7.5
White light LED	7.2	7.6

The pH was maintained between 6.9 and 7.7 by use of an air pump whereby the influence was the CO_2 within that air supply, while test environment temperature was maintained between 19.00 and 25.00 ºC. The results of the two styles of irradiation being laser and or white light LED displayed distinct similarities in terms of initial baseline pH, temperature range and patterns of timeline exposure. The pH figure in both instances

followed a trend of increase of pH after the period of darkness. This profile was repeated throughout the periods of irradiation with varied wavelengths by each of LED and lasers.

The effect of nutrients of various physical properties, (organic and inorganic) are recorded research results showed a clear correlation between the change in pH of the nutrients and the rate of growth of algae. The experimental results exhibited in Table 5.3 illustrate that there was indeed a clear correlation between the growth of cells and the criticality of the nutrient medium in terms of its pH.

5.6 Comparison of growth rate of algae using different combinations of blue and red lasers and white light LED as a reference

Time periods for application of blue laser, red laser or LED were 12 hours for each experiment and the intensity ratio of any combination was 1:1. Eight wavelengths of blue and red lasers were used as follows:

5.6.1 The laboratory results obtained from using the blue laser of wavelength 405 nm followed by different combinations of red lasers

The absorption outcomes of using the blue laser of wavelength 405 nm when followed by the red laser of wavelengths 650 nm, 680 nm and 700 nm, and white light LED used in isolation as a reference are shown in Table 5.4 and Figure 5.6, which summarise the effect of laser and LED wavelengths on *C. reinhardtii* specific growth rate and division rate.

Table 5.4 reflects the mean values of absorption outcome, specific growth rate (μ), doubling time (k) and the division rate (M; divisions/hour) of ten individual samples of *C. reinhardtii* cultivated under the emission of different wavelengths of blue and red lasers (405 nm, 650 nm, 680 nm or 700 nm) and white light LED. * Each value is represented as the mean ± 1.96x$SE\bar{x}$, (n=10, $SE\bar{x}$: standard error).

The highest specific growth rate of $4.48x10^{-2}$ h^{-1} was attained under the irradiance of 405 nm blue laser followed by a combination of 680 nm and 700 nm red lasers. This combination yielded a division rate of $6.47x10^{-2}$ h^{-1}; the specific growth, division rate and maximum cell concentration were much higher compared to that of any result obtained

using individual blue or red laser irradiation. In this study, it was discovered that using the laser of wavelength 405 nm provided a cheap and an efficient alternative to the expensive blue laser of wavelength 473 nm as both laser wavelengths obtained similar good results of algae growth rate improvement.

Table 5.4 The absorption outcomes of blue laser of wavelength 405 nm and red lasers and white LED used in isolation as a reference

Source of light	Initial cell number per mL (N_i) x10^5*	Final cell number per mL (N_f) x10^5*	μ x10^{-2} (h^{-1})	k (h)	M x10^{-2} (h^{-1})
Laser (405 nm)	2.910±0.063	4.151±0.123	2.46	28.15	3.55
White light LED	2.936±0.051	3.664±0.129	1.52	45.54	2.20
Laser (405 nm followed by 650 nm)	3.002±0.056	5.985±0.189	2.88	24.11	4.15
White light LED	3.014±0.048	4.622±0.222	1.78	38.90	2.57
Laser (405 nm followed by 680 nm)	2.956±0.076	6.039±0.236	2.98	23.28	3.44
White light LED	2.875±0.081	4.364±0.166	1.74	39.86	2.44
Laser (405 nm followed by 700 nm)	3.013±0.037	5.339±0.161	2.38	29.07	4.34
White light LED	3.004±0.070	4.406±0.188	1.69	41.07	2.90
Laser (405 nm followed by 680 nm then 700 nm)	3.020±0.109	7.737±0.312	2.61	26.52	3.77
White light LED	2.949±0.077	5.441±0.235	1.70	40.74	2.45
Laser (405 nm followed by 700 nm then 680 nm)	3.021±0.114	7.981±0.357	2.70	25.68	3.89
White light LED	3.003±0.054	5.459±0.286	1.66	41.76	2.39
Laser (680+700) nm	3.038±0.072	5.846±0.214	2.73	25.42	3.93
White light LED	2.987±0.103	3.705±0.254	1.70	38.64	2.59
Laser (405 nm followed by 680+700 nm)	2.964±0.078	8.689±0.218	4.48	15.47	6.47
White light LED	3.059±0.077	4.264±0.190	1.38	50.10	2.00

Figure 5.6 reflects the mean values of specific growth rate (μ) of ten individual samples of *C. reinhardtii* cultivated under the emission of different wavelengths of blue and red lasers (405 nm, 650 nm, 680 nm or 700 nm).

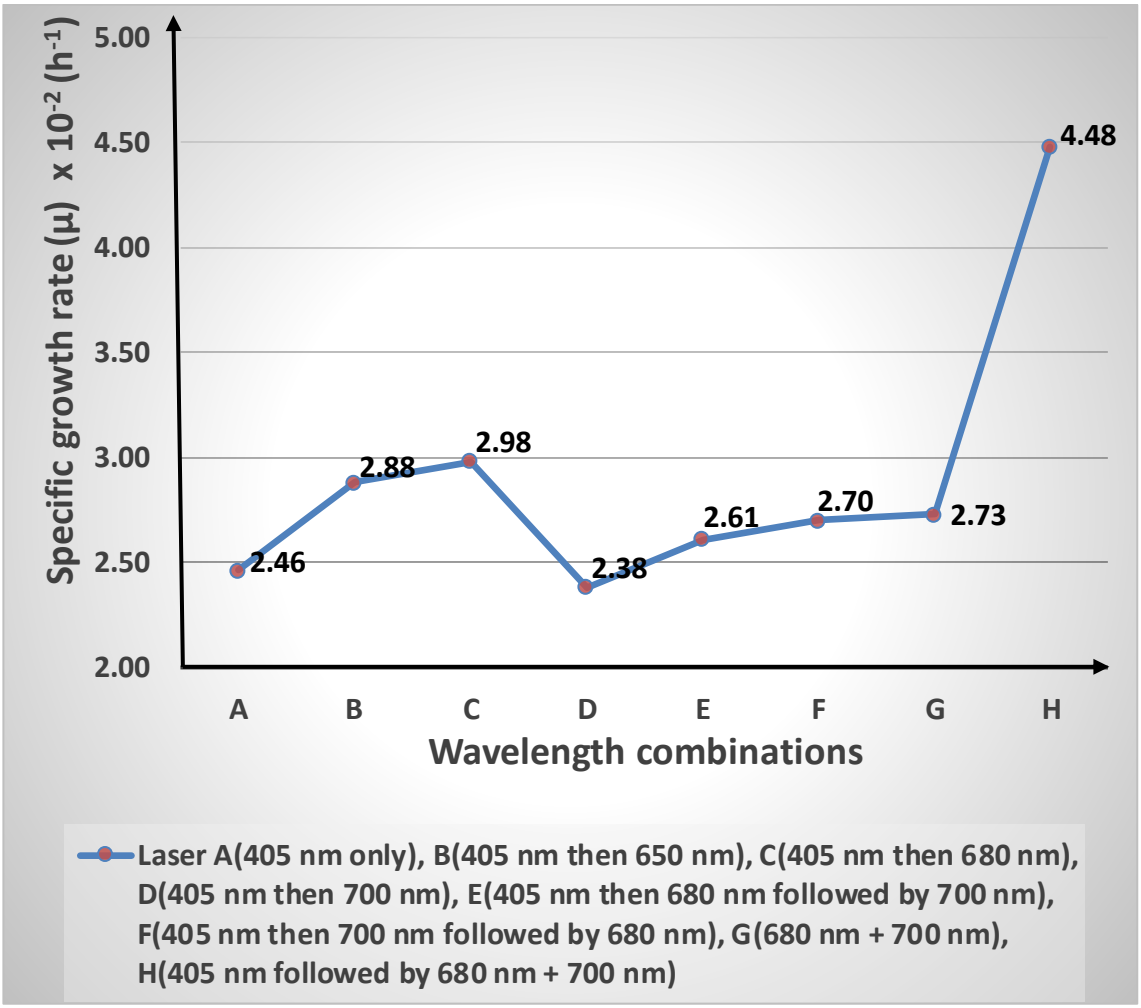

Figure 5.6 The specific growth rate (μ) of C. reinhardtii irradiated by blue laser of wavelength 405 nm and red lasers

The best result was obtained when algae samples were irradiated with the blue laser of wavelength 405 nm followed by a combination of 680 nm and 700 nm red lasers. Prior to the 12 hours of darkness, cell growth gain was 156.65% (4.86 times that of the white light LED) and was calculated by using the equation (5.1) on page 84 as illustrated in Figure 5.7.

Microalgae showed better growth rate when cultured under the combination of red lasers irradiation compared with white light LED light as the photons of red light obtain higher cell densities and higher oxygen production rates through the batch culture thus enhancing cell division and growth rates of microalgae (Lee et al. 1994; de Mooij et al. 2016).

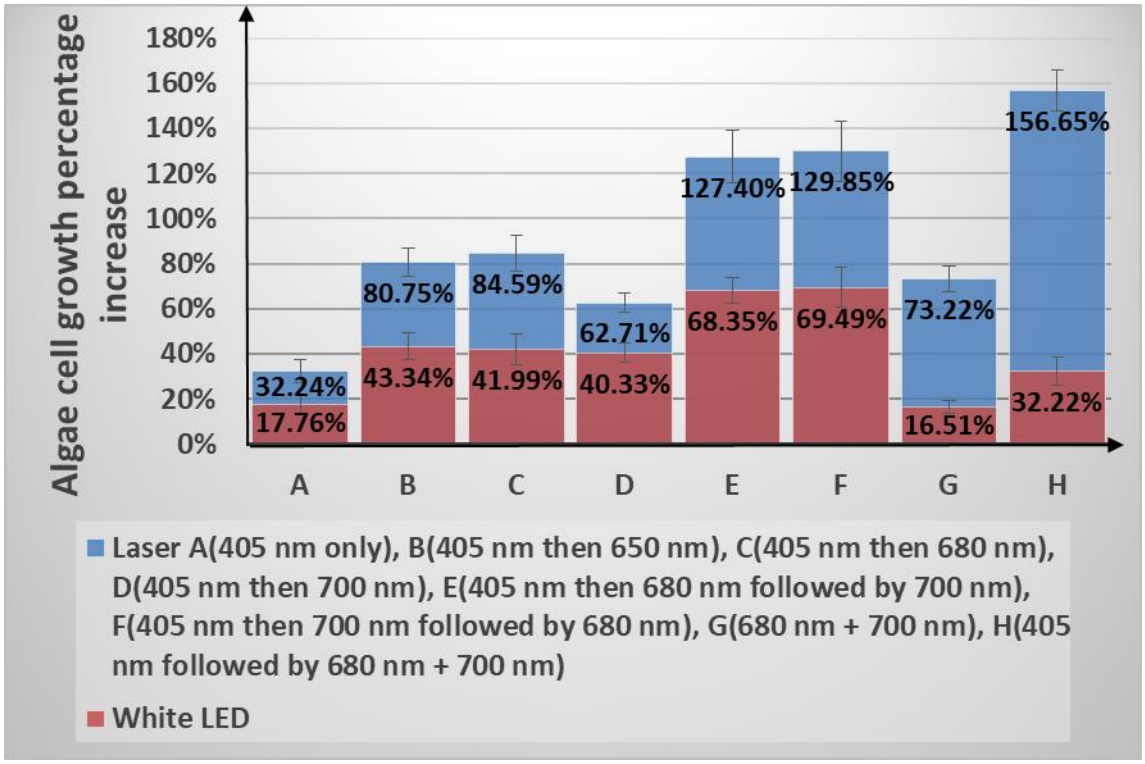

Figure 5.7 The percentage increase of C. reinhardtii irradiated by blue laser of wavelength 405 nm and red lasers

Figure 5.7 reflects the means of the cell count percentage increase of ten independent samples of *C. reinhardtii* over 12 hours of growth period while exposed to a white light LED as a reference and blue laser of wavelength 405 nm and different combinations of red laser of wavelengths 650 nm, 680 nm and 700 nm. Error bars indicate (±1.96 $SE\bar{x}$), (n=10, $SE\bar{x}$: standard error).

The results obtained using a combination of red and blue laser wavelengths were better than those achieved using any singular source of the blue or red laser wavelengths. The laboratory results of using the blue laser of wavelength 405 nm when followed by either 650 nm or 680 nm red lasers were similar to the results obtained by Kuwahara (2011). However, using a combination of 680 nm and 700 nm wavelengths after using the blue laser of wavelength 405 nm, showed improvement in the growth rate of algae by two times than those reported by Kuwahara (2011).

5.6.2 The absorption outcomes of using the blue laser of wavelength 473 nm followed by different combinations of red lasers

The results obtained from using the blue laser of wavelength 473 nm when followed by

the red laser of wavelengths 650 nm, 680 nm and 700 nm and white light LED used in isolation as a reference are shown in Table 5.5 and Figure 5.8, which summarise the effect of laser and LED wavelengths on *C. reinhardtii* specific growth rate and division rate.

The highest specific growth rate of $4.56x10^{-2}$ h^{-1} was attained under the irradiance of 473 nm blue laser followed by a combination of 680 nm and 700 nm red lasers. This combination yielded a division rate of $6.58x10^{-2}$ h^{-1}. The specific growth, division rate and maximum cell concentration were much higher compared to that of any result obtained using individual blue or red laser irradiation.

Table 5.5 The absorption outcomes of blue laser of wavelength 473 nm and red lasers and white LED used in isolation as a reference

Source of light	Initial cell number per mL (N_i) x10^5*	Final cell number per mL (N_f) x10^5*	μ x10^{-2} (h^{-1})	k (h)	M x10^{-2} (h^{-1})
Laser (473 nm)	3.049±0.140	4.481±0.221	3.21	21.61	4.63
White light LED	3.027±0.212	3.716±0.172	1.71	40.61	2.46
Laser (473 nm followed by 650 nm)	3.013±0.125	6.197±0.371	3.01	23.07	4.34
White light LED	3.004±0.082	4.866±0.265	2.01	34.48	2.90
Laser (473 nm followed by 680 nm)	3.042±0.058	6.321±0.233	3.05	22.74	4.40
White light LED	2.886±0.084	4.478±0.196	1.88	36.97	2.71
Laser (473 nm followed by 700 nm)	3.043±0.059	5.849±0.203	2.72	25.46	3.93
White light LED	3.023±0.089	4.483±0.231	1.64	42.24	2.37
Laser (473 nm followed by 680 nm then 700 nm)	3.064±0.112	8.430±0.225	2.81	24.65	4.06
White light LED	2.960±0.096	5.402±0.334	1.67	41.48	2.41
Laser (473 nm followed by 700 nm then 680 nm)	2.826±0.110	8.262±0.416	2.98	23.26	4.30
White light LED	2.883±0.163	5.371±0.274	1.73	40.11	2.49
Laser (680+700) nm	3.038±0.072	5.846±0.214	2.73	25.42	3.93
White light LED	2.987±0.103	3.705±0.254	1.70	38.64	2.59
Laser (473 nm followed by 680+700 nm)	3.035±0.095	9.064±0.269	4.56	15.20	6.58
White light LED	3.042±0.064	4.339±0.195	1.48	46.84	2.14

Table 5.5 illustrates the mean values of absorption outcome, specific growth rate (μ), doubling time (k) and the division rate (M; divisions/hour) of ten individual samples of *C. reinhardtii* cultivated under the emission of different wavelengths of blue and red lasers (473 nm, 650 nm, 680 nm or 700 nm) and white light LED. * Each value is represented as the mean ± 1.96xSE$\bar{x}$, (n=10, SE$\bar{x}$: standard error).

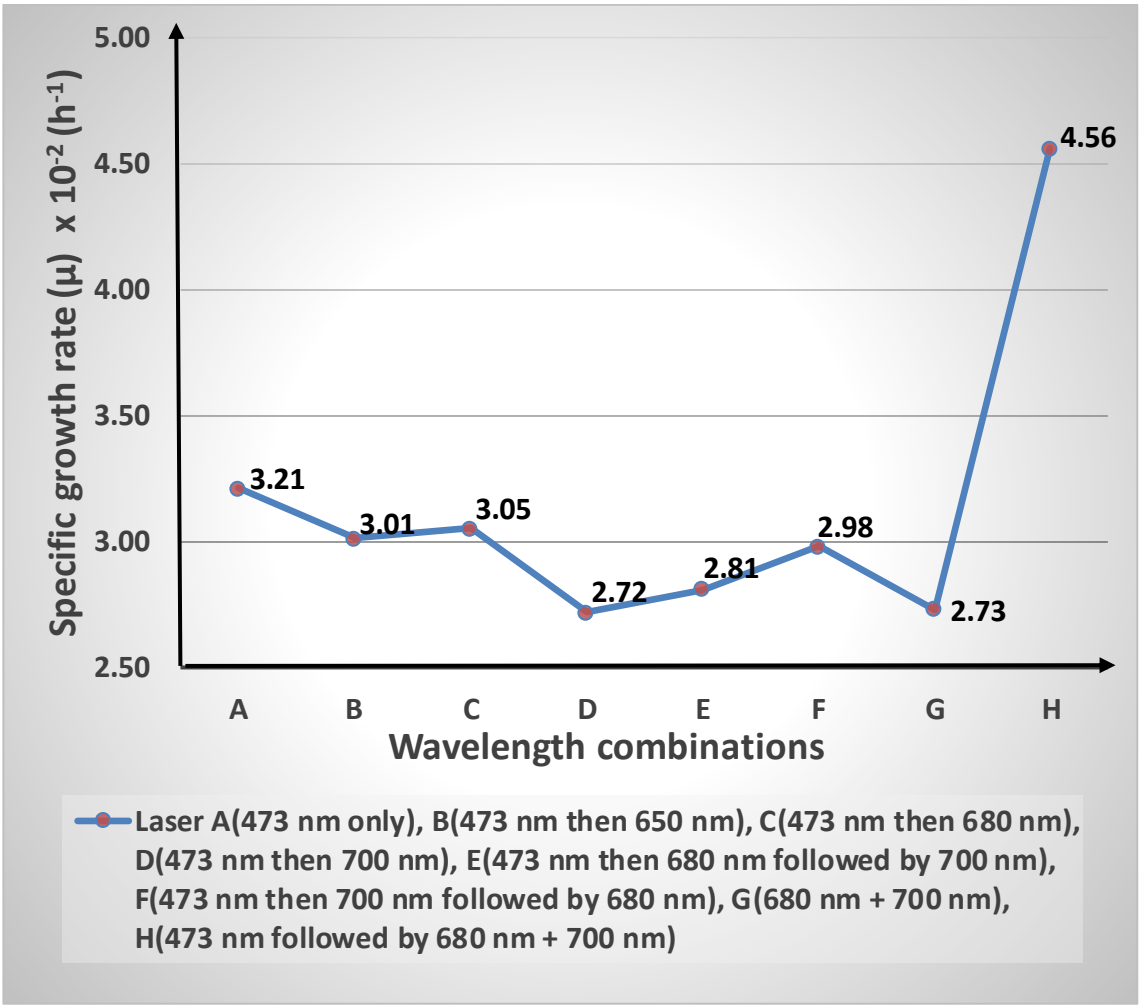

Figure 5.8 The specific growth rate (μ) of C. reinhardtii irradiated by blue laser of wavelength 473 nm and red lasers

Figure 5.8 illustrates the mean values of specific growth rate (μ) of ten individual samples of *C. reinhardtii* cultivated under the emission of different wavelengths of blue and red lasers (473 nm, 650 nm, 680 nm or 700 nm).

The best laboratory results were obtained when algae samples were irradiated with the blue laser of wavelength 473 nm followed by a red lasers combination of wavelengths 680 nm and 700 nm. Prior to the 12 hours of darkness, the cell growth gain was 161.8%

(4.66 times that of the white light LED) and was calculated by using the equation (5.1) on page 84 as illustrated in Figure 5.9.

When the blue laser of wavelength 473 nm was used and followed by the red lasers this gave improvements in the growth rate of algae; the results obtained using the blue laser of wavelength 473 nm and followed by the red laser of wavelengths 680 nm or 650 nm were similar to those recorded by Kuwahara (2011), while supplementing either red wavelengths with a combination of 680 nm and 700 nm wavelengths this resulted in increasing the algae growth rate by twice that of those obtained by Kuwahara (2011). Using the blue laser of wavelength 473 nm followed by the red laser of wavelength 680 nm then followed by 700 nm obtained 1.4 fold growth rate improvement, Similar results were obtained when *C. reinhardtii* algae were cultured under the irradiance of the 473 nm blue laser followed by a red laser wavelength of 700 nm then followed by a 680 nm red laser. The order of the sequence of using the red lasers after using the blue laser in this specific case was not important. Improved results of 30-40% were obtained when *C. reinhardtii* algae were cultured under the irradiance of the 473 nm blue laser followed by a combination of 680 nm and 700 nm red lasers, this enhancement was due to the fact that both PS II and PS I were activated by using the wavelengths 680 nm and 700 in the

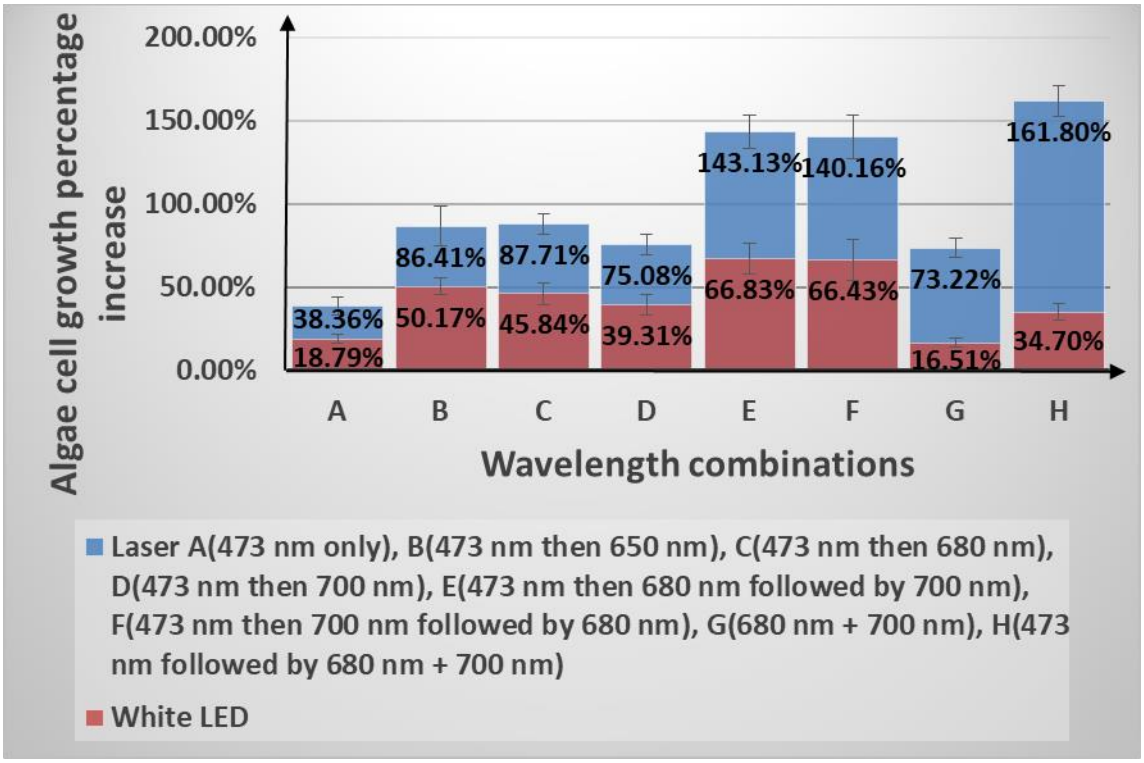

Figure 5.9 The percentage increase of C. reinhardtii irradiated by blue laser of wavelength 473 nm and red lasers

same time, this is called Emerson effect (Lysenko et al. 2014).

Figure 5.9 reflects the means of the cell count percentage increase of ten independent samples of *C. reinhardtii* over 12 hours of growth period while exposed to a white light LED as a reference and blue laser of wavelength 473 nm and different combinations of red laser of wavelengths 650 nm, 680 nm and 700 nm. Error bars indicate (±1.96 SE$\bar{x}$), (n=10, SE$\bar{x}$: standard error).

5.6.3 The laboratory results obtained from using the red laser of wavelength 650 nm followed by different wavelengths of blue lasers

The results obtained from using the red laser of wavelength 650 nm when followed by the blue laser of wavelength 405 nm or 473 nm and white light LED used in isolation as a reference are shown in Table 5.6 and Figure 5.10, which summarise the effect of laser and LED wavelengths on *C. reinhardtii* specific growth rate and division rate.

Table 5.6 The absorption outcomes of red laser of wavelength 650 nm and blue lasers and white LED used in isolation as a reference

Source of light	Initial cell number per mL (N_i) x10^5*	Final cell number per mL (N_f) x10^5*	μ x10^{-2} (h^{-1})	k (h)	M x10^{-2} (h^{-1})
Laser (650 nm)	3.027±0.098	4.385±0.127	3.09	22.45	4.46
White light LED	3.024±0.115	3.716±0.135	1.72	40.37	2.48
Laser (650 nm followed by 405 nm)	2.999±0.048	5.819±0.164	2.76	25.10	3.98
White light LED	3.121±0.079	4.392±0.160	1.42	48.71	2.05
Laser (650 nm followed by 473 nm)	3.016±0.075	6.427±0.213	3.15	21.99	4.55
White light LED	2.882±0.076	4.440±0.282	1.80	38.48	2.60

Table 5.6 reflects the mean values of absorption outcome, specific growth rate (μ), doubling time (k) and the division rate (M; divisions/hour) of ten individual samples of *C. reinhardtii* cultivated under the emission of different wavelengths of red and blue lasers (650 nm, 405 nm or 473 nm) and white light LED. * Each value is represented as the mean ± 1.96xSE$\bar{x}$, (n=10, SE$\bar{x}$: standard error).

Figure 5.10 reflects the mean values of specific growth rate (μ) of ten individual samples of *C. reinhardtii* cultivated under the emission of different wavelengths of red and blue

lasers (650 nm, 405 nm or 473 nm).

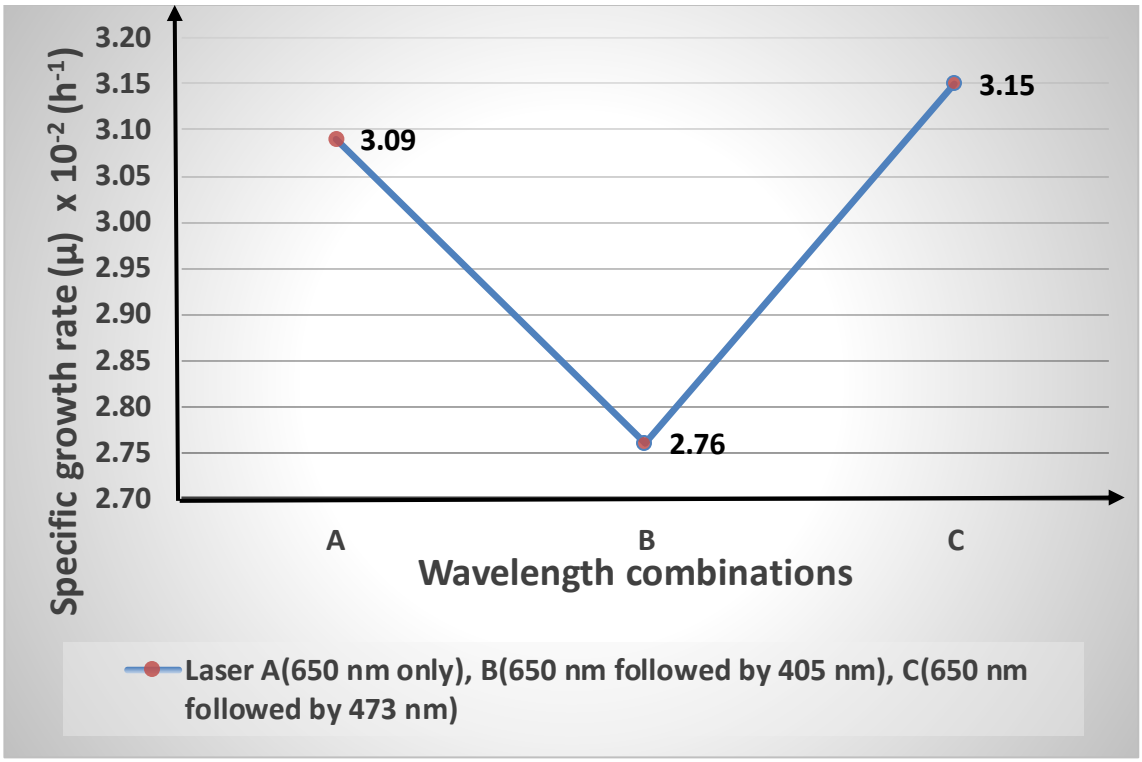

Figure 5.10 The specific growth rate (μ) of C. reinhardtii irradiated by red laser of wavelength 650 nm and blue lasers

The highest specific growth rate of $3.15x10^{-2}$ h^{-1} was attained under the irradiance of 650 nm red laser followed by the blue laser of 473 nm. This combination yielded a division rate of $4.55x10^{-2}$ h^{-1}. These results gave improvement of 2.12 fold in the growth rate of algae compared to white light LED results as shown in Figure 5.11, which were better than the obtained results when *C. reinhardtii* algae were cultured under the irradiance of the 473 nm blue laser followed by a 650 nm red laser (1.7 fold); the order of the sequence when the red and blue lasers were used in this specific case was important., both results were near to those recorded by Kuwahara (2011).

Figure 5.11 illustrates the means of the cell count percentage increase of ten independent samples of *C. reinhardtii* over 12 hours of growth period while exposed to a white light LED as a reference and red laser of wavelength 650 nm and blue laser of wavelengths 405 nm and 473 nm. Error bars indicate (±1.96 $SE\bar{x}$), (n=10, $SE\bar{x}$: standard error).

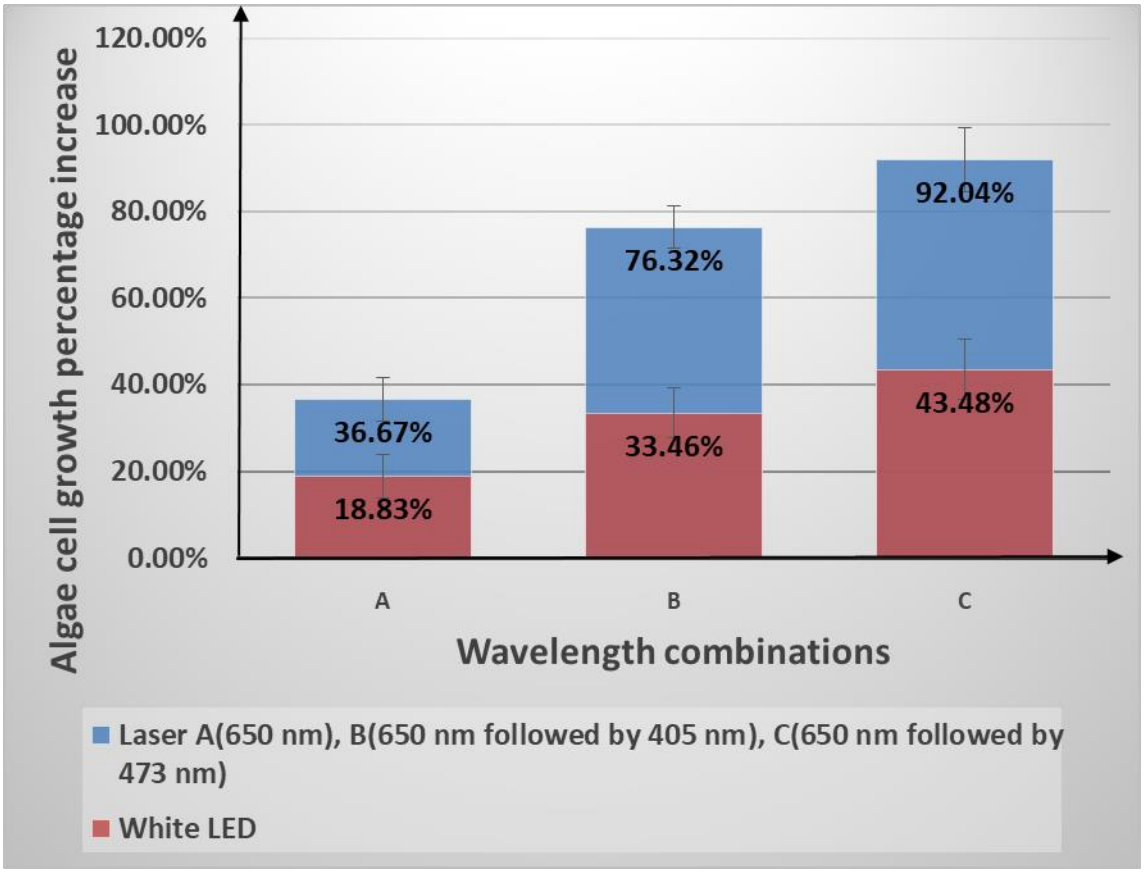

Figure 5.11 The percentage increase of C. reinhardtii irradiated by red laser of wavelength 650 nm and blue lasers

5.6.4 The laboratory results obtained from using the red laser wavelength 680 nm followed by different wavelengths of blue lasers

The results obtained from using the red laser of wavelength 680 nm followed by the blue laser of wavelength 405 nm or 473 nm and white light LED used in isolation as a reference are shown in Table 5.7 and Figure 5.12, which summarise the effect of laser and LED wavelengths on *C. reinhardtii* specific growth rate and division rate.

Table 5.7 illustrates the mean values of absorption outcome, specific growth rate (μ), doubling time (k) and the division rate (M; divisions/hour) of ten individual samples of *C. reinhardtii* cultivated under the emission of different wavelengths of red and blue lasers (680 nm, 405 nm or 473 nm) and white light LED. * Each value is represented as the mean ± 1.96xSE$\bar{x}$, (n=10, SE$\bar{x}$: standard error).

Table 5.7 The absorption outcomes of red laser of wavelength 680 nm and blue lasers and white LED used in isolation as a reference

Source of light	Initial cell number per mL (N_i) x10^5*	Final cell number per mL (N_f) x10^5*	μ x10^{-2} (h^{-1})	k (h)	M x10^{-2} (h^{-1})
Laser (680 nm)	3.024±0.100	4.440±0.140	3.20	21.65	4.62
White light LED	3.053±0.100	3.792±0.185	1.81	38.35	2.61
Laser (680 nm followed by 405 nm)	2.944±0.057	6.108±0.143	3.04	22.80	4.39
White light LED	2.957±0.105	4.532±0.199	1.78	38.96	2.57
Laser (680 nm followed by 473 nm)	2.956±0.036	6.365±0.188	3.19	21.69	4.61
White light LED	2.537±0.068	3.718±0.288	1.59	43.51	2.30

Figure 5.12 illustrates the mean values of specific growth rate (μ) of ten individual samples of *C. reinhardtii* cultivated under the emission of different wavelengths of red and blue lasers (680 nm, 405 nm or 473 nm).

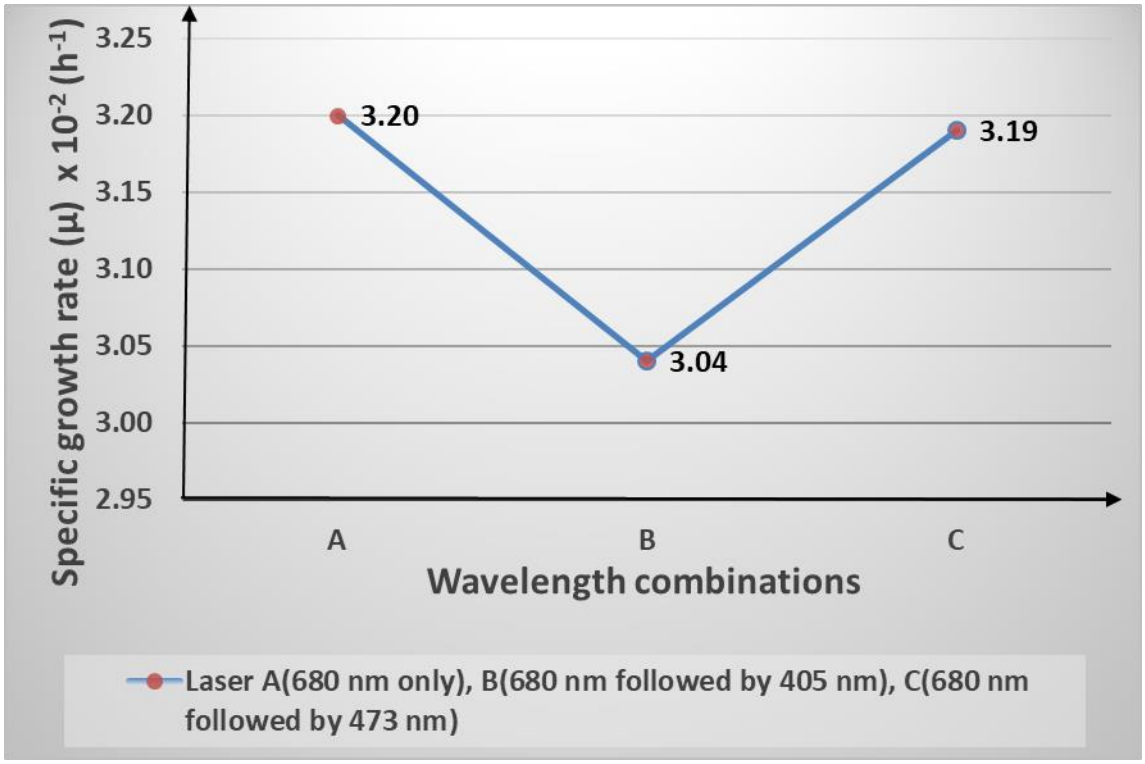

Figure 5.12 The specific growth rate (μ) of C. reinhardtii irradiated by red laser of wavelength 680 nm and blue lasers

The highest specific growth rate of 3.19x10^{-2} h^{-1} (2.55 fold) was attained under the

irradiance of 680 nm red laser followed by the blue laser of 473 nm, this combination yielded a division rate of 4.61×10^{-2} h^{-1} as shown in Figure 5.13. Lower results (1.91 fold) were obtained when *C. reinhardtii* algae were cultured under the irradiance of the 473 nm blue laser followed by a 680 nm red laser; the order of the sequence when the red and blue lasers were used in this specific case was important. Both results were similar to those recorded by Kuwahara (2011).

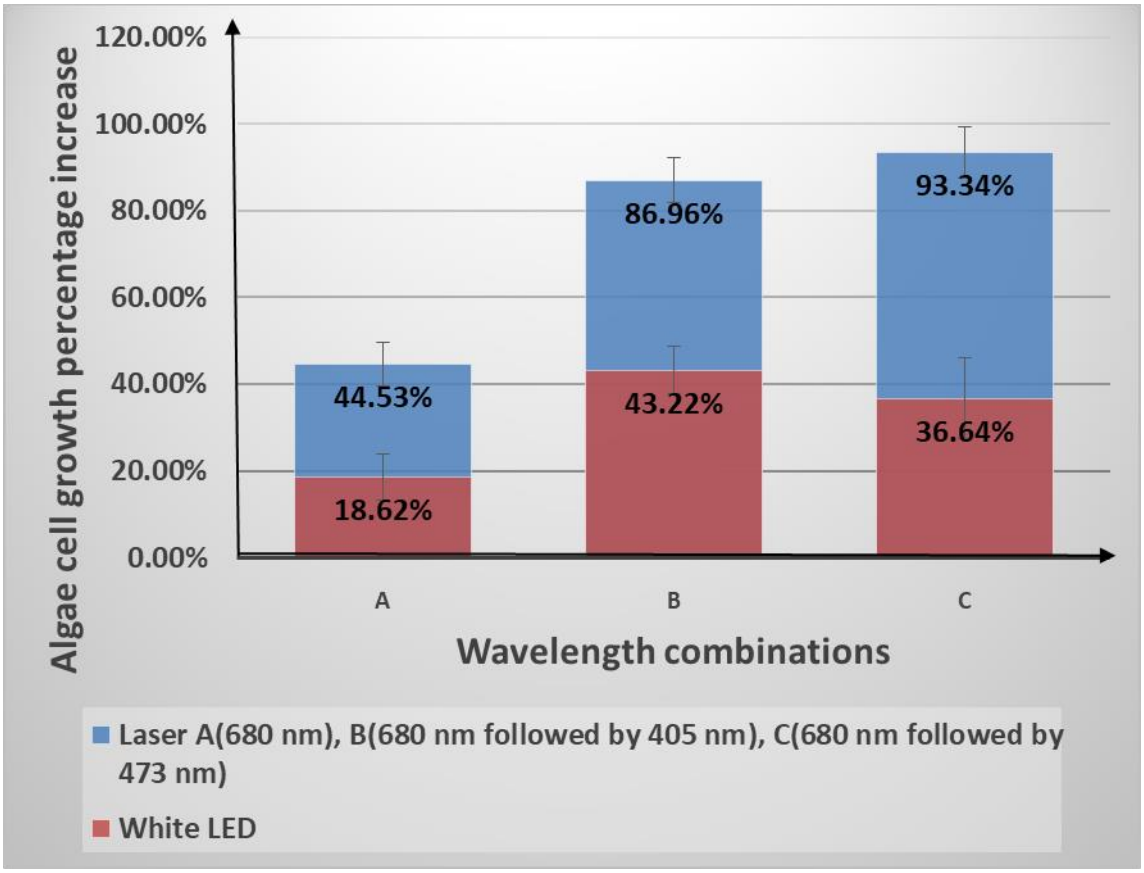

Figure 5.13 The percentage increase of C. reinhardtii irradiated by red laser of wavelength 680 nm and blue lasers

Figure 5.13 reflects the means of the cell count percentage increase of ten independent samples of *C. reinhardtii* over 12 hours of growth period while exposed to a white light LED as a reference and red laser of wavelength 680 nm and blue laser of wavelengths 405 nm and 473 nm. Error bars indicate (±1.96 $SE\bar{x}$), (n=10, $SE\bar{x}$: standard error).

5.6.5 The laboratory results obtained from using the red laser wavelength 700 nm followed by different combinations of blue lasers

The absorption outcomes of using the red laser of wavelength 700 nm when followed by the blue laser of wavelength 405 nm or 473 nm and white light LED used in isolation as a reference are shown in Table 5.8 and Figure 5.14, which summarise the effect of laser and LED wavelengths on *C. reinhardtii* specific growth rate and division rate.

Table 5.8 The absorption outcomes of red laser of wavelength 700 nm and blue lasers and white LED used in isolation as a reference

Source of light	Initial cell number per mL (N_i) x10^5*	Final cell number per mL (N_f) x10^5*	μ x10^{-2} (h^{-1})	k (h)	M x10^{-2} (h^{-1})
Laser (700 nm)	3.031±0.094	3.997±0.202	2.31	30.06	3.33
White light LED	3.042±0.121	3.683±0.155	1.59	43.53	2.30
Laser (700 nm followed by 405 nm)	2.994±0.053	5.424±0.123	2.47	27.99	3.57
White light LED	3.117±0.045	4.499±0.161	1.53	45.35	2.21
Laser (700 nm followed by 473 nm)	2.957±0.088	5.618±0.226	2.67	25.92	3.86
White light LED	3.065±0.059	4.720±0.188	1.42	38.54	2.60

Table 5.8 reflects the mean values of absorption outcome, specific growth rate (μ), doubling time (k) and the division rate (M; divisions/hour) of ten individual samples of *C. reinhardtii* cultivated under the emission of different wavelengths of red and blue lasers (700 nm, 405 nm or 473 nm) and white light LED. * Each value is represented as the mean ± 1.96x$SE\bar{x}$, (n=10, $SE\bar{x}$: standard error).

Figure 5.14 reflects the mean values of specific growth rate (μ) of ten individual samples of *C. reinhardtii* cultivated under the emission of different wavelengths of red and blue lasers (700 nm, 405 nm or 473 nm).

The highest specific growth rate of 2.67x10^{-2} h^{-1} (1.66 fold) was attained under the irradiance of 700 nm red laser followed by the blue laser of 473 nm, this combination yielded a division rate of 3.86x10^{-2} h^{-1} as shown in Figure 5.15. Better results were obtained when *C. reinhardtii* algae were cultured under the irradiance of the 473 nm blue laser followed by a 700 nm red laser (1.91 fold); the order of the sequence when the red and blue lasers were used in this specific case was important.

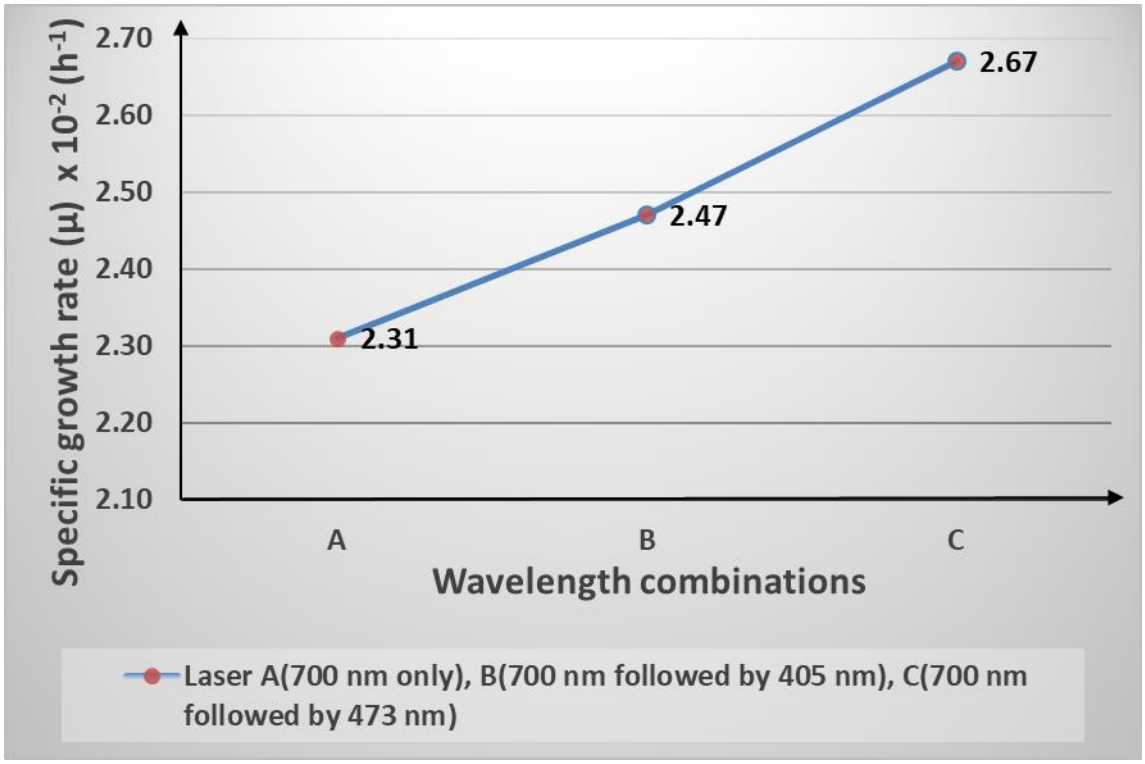

Figure 5.14 The specific growth rate (μ) of C. reinhardtii irradiated by red laser of wavelength 700 nm and blue lasers

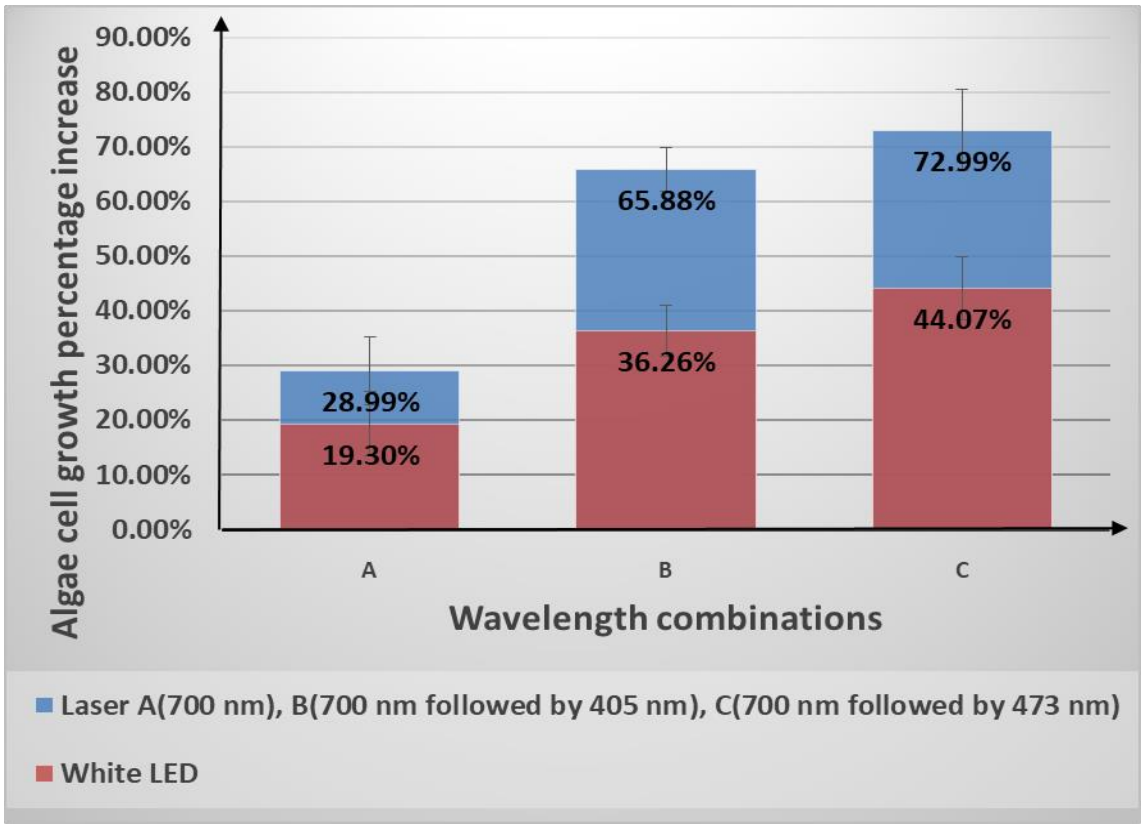

Figure 5.15 The percentage increase of C. reinhardtii irradiated by red laser of wavelength 700 nm and blue lasers

Figure 5.15 illustrates the means of the cell count percentage increase of ten independent samples of *C. reinhardtii* over 12 hours of growth period while exposed to a white light LED as a reference and red laser of wavelength 700 nm and blue laser of wavelengths 405 nm and 473 nm. Error bars indicate (±1.96 SE$\bar{x}$), (n=10, SE$\bar{x}$: standard error).

5.7 Coherence study: comparison of algae growth rate differences using the blue laser wavelength 473 nm and red laser wavelength 680 nm when the blue and red LEDs of the same wavelengths are used as a reference

The absorption outcomes of using the blue laser of 473 nm wavelength and the red laser of 680 nm wavelengths as compared with those obtained using blue and red LEDs of wavelengths 473 nm and 680 nm respectively as a reference are shown in Table 5.9 and Figure 5.16, which summarise the effect of different laser wavelengths on *C. reinhardtii* specific growth rate and division rate. Microalgae showed better growth rate when cultured under the blue or red laser light irradiation compared with those achieved with blue or red LED.

Table 5.9 The absorption outcomes of blue or red laser and blue or red LED used in isolation as a reference

Source of light	Initial cell number per mL (N_i) x10^5*	Final cell number per mL (N_f) x10^5*	μ x10^{-2} (h^{-1})	k (h)	M x10^{-2} (h^{-1})
Laser 473 nm	3.009 ± 0.041	4.395 ± 0.104	3.16	21.96	4.55
Blue LED 473 nm	2.963 ± 0.061	3.743 ± 0.131	1.95	35.61	2.81
Laser 680 nm	2.981 ± 0.053	4.445 ± 0.097	3.33	20.81	4.80
Red LED 680 nm	2.960 ± 0.046	3.573 ± 0.088	1.57	44.21	2.26

Table 5.9 illustrates the mean values of absorption outcome, specific growth rate (μ), doubling time (k) and the division rate (M; divisions/hour) of ten individual samples of *C. reinhardtii* cultivated under the emission of two wavelengths of blue and red lasers (473 nm or 680 nm) and two wavelengths of LEDs (473 nm or 680 nm) respectively as a reference. * Each value is represented as the mean ± 1.96xSE$\bar{x}$, (n=10, SE$\bar{x}$: standard error).

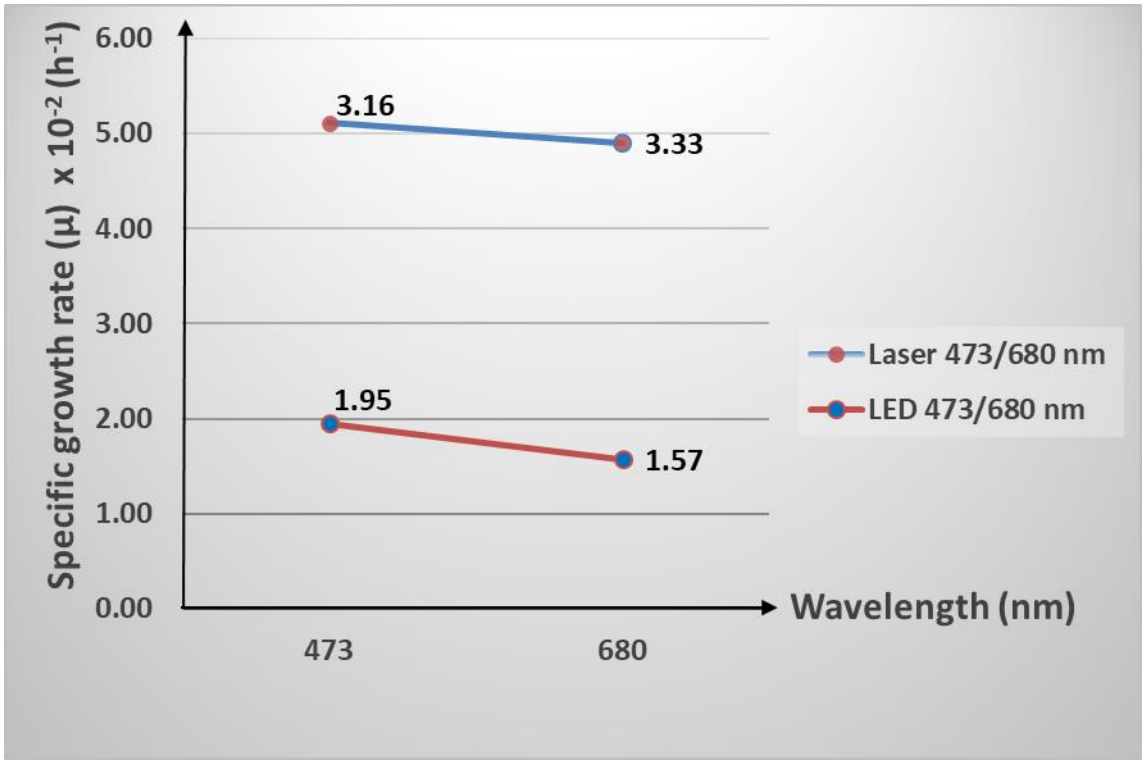

Figure 5.16 The specific growth rate (μ) of C. reinhardtii irradiated by blue or red laser and blue or red LED used in isolation as a reference

Figure 5.16 illustrates the mean values of specific growth rate (μ) of ten individual samples of *C. reinhardtii* cultivated under the emission of two wavelengths of blue and red lasers (473 nm or 680 nm) and two wavelengths of LEDs (473 nm or 680 nm).

The highest specific growth rate of 3.33 x10^{-2} h^{-1} (5 fold) was attained under the irradiance of 680 nm red laser. This combination yielded a division rate of 4.80x10^{-2} h^{-1} as shown in Figure 5.17, this growth rate enhancement of algae implies that although both the laser and LED has the same wavelength and intensity, the advantage is in the laser having a greater coherence (Kuwahara et al. 2011). In comparison to the white light LED, the blue LED of wavelength 473 nm obtained better results (1.15 fold), while the red LED of wavelength 680 nm obtained lower growth rate results (0.9 fold). These results were in close conformity with those reported by Das (2011).

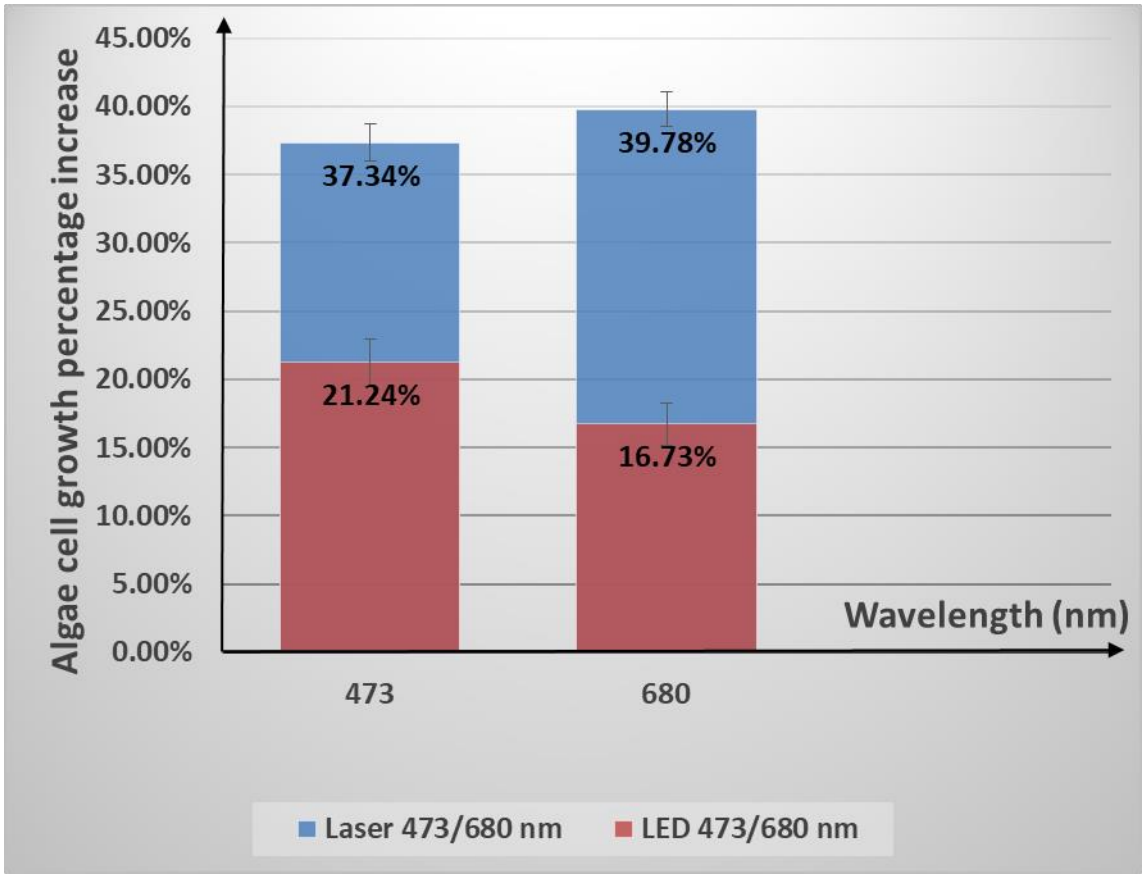

Figure 5.17 The percentage increase of C. reinhardtii irradiated by blue or red laser and blue or red LED

Figure 5.17 illustrates the means of the cell count percentage increase of ten independent samples of *C. reinhardtii* over 12 hours of growth period while exposed to a blue (473 nm) or red (680 nm) LED as a reference and blue laser of wavelength 473 nm and red laser of wavelength 680 nm. Error bars indicate (±1.96 SE$\bar{x}$), (n=10, SE$\bar{x}$: standard error).

5.8 Matching of the absorption spectra of chromophores with the emission spectra of blue and red lasers

Green algae contain *chlorophyll-A and chlorophyll-B* in the ratio of (3 *chlorophyll-A* to 1 of *chlorophyll-B*) (Pappas et al. 2016).

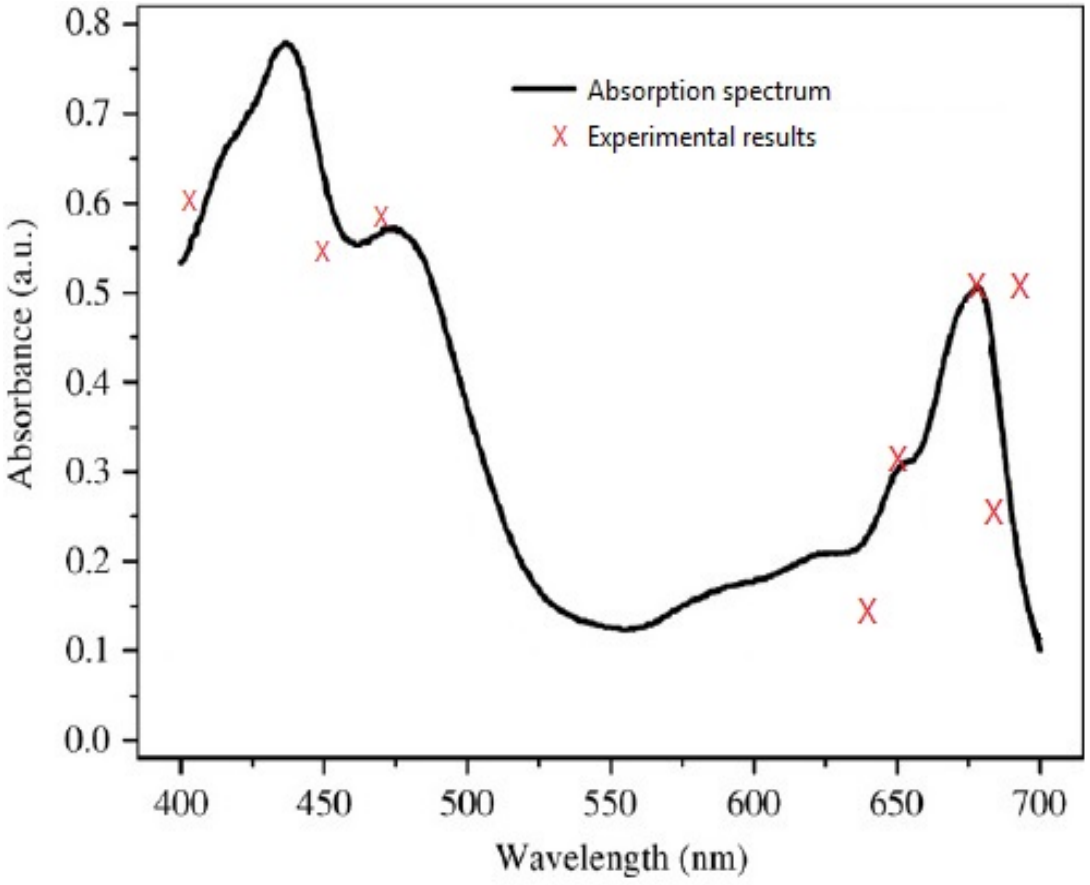

Figure 5.18 Absorption spectra of the thylakoid membrane system of an untreated C. reinhardtii cell. *Adapted from Rodriguez et al. (2007).*

Figure 5.18illustrates the absorption spectra of the thylakoid membrane system of an untreated C. reinhardtii cell (black line) and the experimental results (red crosses) are shown together with their maxima for the laser of wavelengths 405 nm, 445 nm, 473 nm, 635 nm, 650 nm, 680 nm, 685 nm and 700 nm respectively.

The ideal laser arrangement had wavelengths corresponding to the highest algae light absorption without the pigments being shed (Erickson et al. 2015). *Chlorophyll-A* had two absorption peaks, the first being about 430 nm (blue and or violet colour) and the

second around 660 nm (deep red). *Chlorophyll-B* absorption peaks being near 460 nm (blue) and 630 nm (red) (Devaraja, S. et al. 2017).

The results of this experiment obtained using laser of wavelengths 405 nm, 450 nm, 473 nm, 650 nm, 680 nm and 685 nm showed a good matching of the emission spectra of these lasers with the absorption spectra of algae pigments as illustrated in Figure 5.18. Both *chlorophyll-A* and *chlorophyll-B* showed better absorption of blue laser than red laser.

5.9 Discussion

In this thesis, the efficiency of using lasers as sources of energy to optimise the growth and multiplication rate of green algae was investigated using three PBRs under 12hours: 12hours light-dark cycles, where monocultures could be maintained for the given time. As light energy was the crucial limiting factor of the growth of algae, it played a key role in the PBR design. The light exposure arrangements included white light LED which was used in the preparation PBR, different wavelength semiconductor lasers in the main PBR, and white light LED in the reference PBR.

The following points display the findings made through investigating the obtained results of the conducted experimentation work (Table 5.10).

- The most interesting result achieved by this research was attributed to the fact that particular attention was paid to the blue laser of wavelength 405 nm as a very cheap alternative to the expensive blue laser of wavelength 473 nm and both wavelengths produced similar amounts of growth rate improvement in algae samples under investigation.

- The laboratory results obtained from the experimental work of this research showed that the blue laser of wavelengths 405 nm or 473 nm followed by a combination of 680 nm and 700 nm red lasers developed the highest obtained growth rate throughout the conduction of this study as shown in Table 5.10. The blue laser of wavelength 445 nm or the red laser of wavelengths 635 nm or 685 nm has always developed less growth rate than that obtained by the other

combinations of light sources. These results were in good conformity with and about twice better than those obtained in the research made by Kuwahara (2011).

Table 5.10 Growth rate increase using different combinations of blue and red lasers with comparison to white light LED

Source of light	Growth rate increase with comparison to white light LED
Laser (405 nm followed by 680 nm and 700 nm)	4.86 times
Laser (473 nm followed by 680 nm and 700 nm)	4.66 times
Laser (680 nm and 700 nm)	4.43 times
Laser (680 nm followed by 473 nm)	2.55 times
Laser (650 nm followed by 405 nm)	2.28 times
Laser (473 nm followed by 680 nm then 700 nm)	2.14 times
Laser (650 nm followed by 473 nm)	2.12 times
Laser (473 nm followed by 700 nm then 680 nm)	2.11 times
Laser (405 nm followed by 680 nm)	2.01 times
Laser (680 nm followed by 405 nm)	2.01 times
Laser (473 nm followed by 680 nm)	1.91 times
Laser (473 nm followed by 700 nm)	1.91 times
Laser (405 nm followed by 700 nm then 680 nm)	1.87 times
Laser (405 nm followed by 650 nm)	1.86 times
Laser (405 nm followed by 680 nm then 700 nm)	1.86 times
Laser (700 nm followed by 405 nm)	1.82 times
Laser (473 nm followed by 650 nm)	1.72 times
Laser (700 nm followed by 473 nm)	1.66 times
Laser (405 nm followed by 700 nm)	1.55 times

- Evidence would suggest that alternating light and dark cycles were the most practical mode to dilute strong light and could result in efficient light utilisation by algae cultures (Khan et al. 2018), however such a lighting system is very difficult to arrange in large-scale bioreactors (Singh & Sharma 2012). In this

study, the use of an air pump ensured a continual circular rotation of the algae within the nutrient to ensure its exposure to both light and dark was equal and thus had a positive effect on improving the photosynthetic efficiency of algae growth.

- Since the photosynthetic elements of algae are the chloroplasts, light reaching the rest of the algae body was not utilised for photosynthesis, therefore increases in standing biomass increased light shadowing. The shadowing effect resulted in light being blocked that was not utilised for photosynthetic process by algae located nearer to the light source. This condition resulted in less light reaching those cells of algae located further away from the light source. This effect caused loss of photons, therefore the amount of energy lost was greater at shorter wavelengths when light photons had more energy (Das et al. 2011). Since blue light of wavelength 405 nm has more energy per photon ($6.68x10^{-19}$ J) than other light sources, for example, red light of wavelength 665 nm ($3.18x10^{-19}$ J), losses of light energy caused by light shadowing was greatest under blue light exposure. Therefore, the light source combination of red-blue lasers wavelengths performed better than blue light laser alone because the red light losses due to shadowing effect were less than those measured using blue, and red light complements. This resulted in a higher standing biomass.

- The pH number in the instances of using the laser having wavelengths of 405 nm, 473 nm, 650 nm, 680 nm or 700 nm followed a trend of increase after the period of darkness indicating that the algae in these PBRs were consuming more carbon dioxide and growing faster in number of cells, quantity of biomass, and size. As a result, the green algae became a more viable solution to eliminate CO_2 emission from power plants. This applies to algae use to clean the environment by utilising CO_2.

-

i) The lower valued light intensity readings by the photodiode detector for the wavelengths 405 nm or 473 nm of blue laser and 650 nm, 680 nm and 700 nm for the red laser indicate the high amount of photons absorbed by algae cells in comparison with the white light LED.

ii) In order to explain this, there was a distinctive need to illustrate the correlation between the high coherence of monochromatic lasers as well as the increased populations at the excited states and enhanced level of electron activity in photosystem II where hydrogen ions were gathered from water.

iii) White light has a broad frequency spectrum. Algae cells absorb a narrow spectrum of light. Hence monochromatic light that matches this narrow spectrum is more readily absorbed with higher energy efficiency.

iv) The employment of various lasers generating monochromatic light increased the consistency of relevant photons in the hope of accelerating the uptake of hydrogen ions from the water. When one of the wavelengths above generated a photon within a given energy, it collided with an electron within an antenna pigment molecule having a matching absorption spectrum within the reaction centre of photosystems II (Krupnik et al. 2013). A ground state electron of *chlorophyll-A* within PS II absorbed this photon and moved from the ground state to the highest state of energy, then passed to the primary electron acceptor as the photon's energy is sufficient to ionise its donor. The electron then lost its energy by emitting heat and a photon having a longer wavelength than the previously absorbed photon - at this point, the electron has outlived its life at the excited state of energy. This new photon was the result of a spontaneous emission process, whereby the electron returned to its ground state; this photon was absorbed by an alternative electron at ground state within another acceptor whereby the cycle continually repeats itself until getting a photon of a wavelength of 700 nm (Mcclure 2013).

v) Ultimately, the energy of this photon is the same as the gap between two states of energy of a special pigment within the PS I reaction centre. An electron within an antenna pigment molecule would absorb the energy of the photon, and would then be excited to the highest state of energy and passed to the primary electron acceptor at PS I, as the photon's energy is sufficient to ionise its donor. The electron is then transferred by the electron carriers within the electron transport chain to $NADP^+$ in order to produce NADPH.

vi) The result of these sequential transitions produced energy in order to force the hydrogen ions to move through the thylakoid membrane from the stroma to the thylakoid interior (*Lumen*), building a potential difference which is required in the production of ATP. Also, it provided high energy electrons which were required for the process of converting $NADP^+$ into NADPH. Both ATP and NADPH are used in making sugars in the Calvin cycle.

5.10 Investigating the theoretical model established in this work by means of quantum explanation of photon-electron interaction in PS I and PS II

The hypothesis that claims electronic coherences can enhance the quantum efficiency in electron energy transfer by coupling the electron states to vibrational states of energy is supported by means of the recorded results obtained by (Fassioli et al. 2013). Other experimental results gave alternative interpretations by proving that the excitations caused by coherent laser light sources are 'vibronic' in nature (Tiwari et al. 2013; Chenu et al. 2013). These theories suggest that some vibrational states are mixed with the electronic states (Singh et al. 2015), therefore, the lifetime of coherence of the electronic states increases due to the mixing of electronic and vibrational states of the chromophores within PS II and PS I. In other studies, vibrational and electron states mixing was studied using a vibronic model to explain the nature of coherence in photosynthetic systems. These studies found that vibrational states are attributed to electronic potential energy surfaces of the individual BChla's (Yuen-Zhou et al. 2012). Some of the studies presented numerical results indicating the existence of a long-lived coherence (Zhang & Wang 2016). Franck-Condon factor weighted Coulombic interactions between the vibrational and electron excited states of the pigments create vibronic states for the whole photosynthetic complex. The excited states of the vibronic model are neither purely vibrational nor purely electronic in nature as they have mixed electronic and vibrational electronic characteristics (Chenu et al. 2013).

The excitation energy transportation in the pigment antennas is very fast and efficient, typically with quantum yields of nearly 100% (Hildner et al. 2013). Although, the electronic structure of the pigment antennas are well defined, and despite the vast improvements in spectroscopy techniques, the complete understanding of the role of

coherence and the mechanism of photon-electron excitation and energy transfer still remains shadowy (Lee et al. 2018).

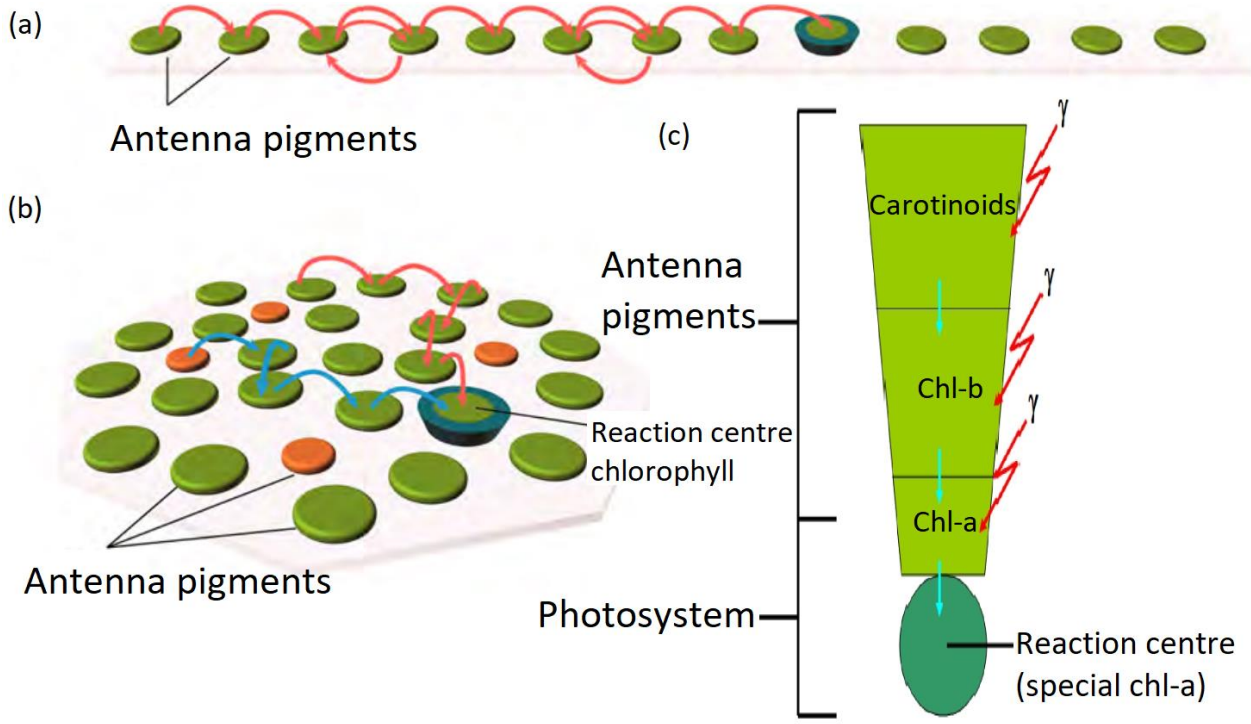

Figure 5.19 Schematic of models of chromophore arrangement and energy transfer to reaction centres. *Adapted from Mirkovic et al. (2017).*

Figure 5.19 illustrates a schematic depicting excitation-transfer pathways in models where chromophores are arranged in (a) a linear (one-dimensional) format and (b) a two-dimensional planar distribution. The inefficiency of the one-dimensional model compared to the two-dimensional arrangement is manifested in the larger number of steps needed for energy transfer to the reaction centre. (c) Energy collection in an antenna system depicted as an energy funnel. Shorter wavelengths of light are absorbed by peripheral antenna complexes, followed by energy-transfer processes to the lower-energy pigments located closer to the reaction centre.

In this research, a quantised approach has been added in the attempt to explain and analyse the laser coherence as a cause of algae growth enhancement due to efficient electron transfer between PS II and PS I.

The lower light intensity readings by the photodiode detector for the laser of 473 nm wavelength resulting from experimentation done in this research indicated a high amount

of photons were absorbed by algae cells as compared with figures obtained with white LED as discussed in section 5.7. In order to explain this, there is a distinct need to illustrate the correlation between the high coherence of monochromatic lasers and the enhanced level of electron activity in photosystem II. The employment of various lasers generating monochromatic light increased the consistency of relevant photons in an attempt to accelerate the uptake of hydrogen ions from water.

When a 473 nm wavelength semiconductor laser generated photon within a given energy ran into an electron within antenna pigment molecules having a matching absorption spectrum within photosystem II (Ooi et al. 2016), the photon was absorbed by a ground state electron of one of the pigment molecules which was taken from the ground state to a higher state of energy. The electron then lost its energy by emitting heat and a photon of longer wavelength than that previously absorbed. At this point, the electron has outlived its lifetime at the excited state of energy. This new photon was the result of a spontaneous emission process, whereby the electron returned to its ground state. This newly generated photon was absorbed by an alternative electron at ground state within another pigment whereby the cycle continuously repeats itself until a photon of a wavelength of 680 nm reaches PS II. Ultimately, energy of this photon is the same as the gap between two energy levels of an electron of a special chlorophyll within the PS II reaction centre, that electron would absorb the energy of the photon, and would then be excited to the highest state of energy and passed to the primary electron acceptor as the absorbed photon's energy is sufficient to ionise the atom of that chlorophyll. The electron is then transported by the electron carriers within the electron transport chain to PS I ending its journey by emitting a photon that has a wavelength of 700 nm. The energy of this photon is matching the gap between two states of energy of an electron of a pigment within the reaction centre of PS I. The final stage is transferring the energy of this photon to the electron transport chain of PS I as this energy will be consumed in the Calvin cycle.

Using a laser wavelength of 680 nm would go through a similar mechanism until a photon of a wavelength of 700 nm reaches PS I, then the process of transferring the energy of that photon will be repeated. The result of these sequential transitions produces energy in order to force the hydrogen ions to move through the thylakoid membrane building a potential difference which is required in the production of ATP and NADPH. Using the laser having wavelengths of 680 nm or 700 nm will lead to direct absorption in the

reaction centres of PS II and PS I respectively as no heat or fluorescence will occur.

5.11 Chapter summary

This chapter described the results of the experimental work done in this thesis. Algae cultures in a "Photon Powered PBR" were irradiated with laser generated monochromatic light employing three blue lasers and five red lasers, the results were compared with those obtained for exposure to white light LED. Blue and red laser irradiation was experimentally tested to determine if faster algae growth rate could be achieved when compared with that obtained with white light LED irradiation. Eight individual lasers having wavelengths of (405 nm, 450 nm, 473 nm, 635 nm, 650 nm, 680 nm, 685 nm and 700 nm) were employed to generate photons with energies (wavelengths) overlapping on the absorption spectra of pigments within *C. reinhardtii* algae. This was done in an attempt to enhance transportation within the electron pathway between photosystems II and I, thus anticipating increase in algae cell division rate. *C. reinhardtii* algae have successfully grown and divided under exposure to both the blue and red lasers when each of these photon energy sources was applied individually. It has also exhibited accelerated growth rate and growth volume when irradiated with a combination of the mentioned photon energy sources.

Statistical analysis of experimental results was used to validate the initial assumptions, the algae growth optimisation model and the mechanism of electron transfer. These were discussed and illustrated in this chapter.

CHAPTER 6

CONCLUSIONS AND PROPOSALS FOR FUTURE WORK

6.1 Introduction

The previous chapters examined experimentally and theoretically the use of diode lasers to optimise the increase of growth and cell division within *C. reinhardtii* algae cultivated under different wavelengths of blue and red lasers in addition to white LEDs lighting.

The experimental study used eight different wavelengths of blue and red diode lasers as a light source to cultivate algae showed enhancements in terms of algae growth performance. The study demonstrated the energy exchange between the laser photons and the electrons at the reaction centre of photosystem II and photosystem I. Analytical investigations were conducted to determine the required wavelengths in each BR used in the experimental part of this research.

The results of these experiments were used to construct and validate a matrix of diode lasers in combination as a light source for algae cultivation, illustrating that a matrix of lasers were used sequentially and or simultaneously. Laboratory results with best figures were used in the construction of this model. This model was then employed to predict and optimise growth of *C. reinhardtii* algae, by choosing various parameters such as temperature, pH and light exposure time.

The results of this research and its recommendations are suggested to reduce the cost in future work for the growth of algae and may contribute to increase viability for industrial production as are summarised in this chapter overall.

6.2 Conclusions

The following points highlight the main conclusions of the work done in this research:

1. The success achieved by this research was attributed to the fact that particular attention was paid to the algae species of (*Chlamydomonas reinhardtii*) which was considered to be most suited for enhanced growth and has photosynthetic pigment absorption spectrum matching with the emission spectrum of a variety of current market available diode lasers. The growth rate enhancement achieved for this particular algae species by using the diode lasers as light sources displayed great promise when excited in the manner applied throughout this experimentation.
2. *C. reinhardtii* algae have successfully grown and divided under exposure to the emission of both blue and red lasers, and that of white light LED when each was applied individually. This finding clearly demonstrated the suitability of these photonic energy sources for algae cultivation.
3. In order to improve the growth rate of algae, using monochromatic sources of light in particular diode lasers is vital and critically effective because the difference between the peak wavelengths of PS II and PS I is just 20 nm. When using the individual blue lasers as sources of light, the best results were achieved using the 473 nm blue laser, as using it increased the rate of cell division by twice that obtained with white light LED. The 405 nm blue laser followed by achieving 1.8 times growth enhancement, whilst the 450 nm blue laser produced no enhancement, and when compared to that achieved using white light LED a rate of cell division of 1.5 times of that realised using the laser of 450 nm wavelength. In comparison to the growth rate achieved in the growth of algae under natural daylight cycles, the enhancement figures attained using the blue laser of wavelengths 405 nm, 450 nm and 473 nm were 3.6, 1.4 and 4 times respectively.

4. To our knowledge, this work represents the first research that introduced the blue laser of wavelength 405 nm as a very cheap alternative to the expensive blue laser of wavelength 473 nm and both wavelengths produced a similar growth rate of algae.
5. When deploying individual red lasers as a source of light, the best laboratory obtained growth rate enhancements were realised using the red laser of wavelengths 650 nm or 680 nm red laser. Using these light sources increased the rate of algae cell division by 1.95 and 2.39 times of that which was obtainable using white light LED respectively. These were followed by the laser of 700 nm wavelength with 1.5 times enhancement. There was an increase in the statistical difference in growth rate of *C. reinhardtii* algae under the laser having wavelengths of 650 nm, 680 nm or 700 nm against white light LED and natural daylight PBRs. However, there was never any conclusive evidence for positive outcomes from using the red laser of wavelengths 635 nm or 685 nm, as white light LED performed better (4 times that of the laser of 635 nm wavelength and 1.22 times that of the laser of 685 nm wavelength). In comparison to the samples of algae under natural daylight cycles, the red laser of wavelengths 650 nm, 680 nm, 685 nm and 700 nm showed enhancement in algae growth rate of 3.9, 4.6, 1.6 and 2.7 times respectively.
6. Better results were obtained using several combinations of blue and red lasers. The best laboratory results were obtained by using the 405 nm blue laser followed by a combination of 680 nm and 700 nm red lasers, as it increased the rate of cell division by 4.86 times of that obtained with white light LED. The second best results were attained using the 473 nm blue laser followed by a combination of 680 nm and 700 nm red lasers, which produced the second highest cell division rate improvement of 4.66 times of that achieved using white light LED. The third best laboratory results were realised using a combination of laser of wavelengths 680 nm and 700 nm, it yielded enhancement in growth rate that was 4.43 times that of white light LED. When laser diodes of wavelengths 405 nm, 473 nm, 650 nm, 680 nm or 700 nm were utilised, a greater advantage was gained in terms of the number of hydrogen ions forced through the thylakoid membrane, the result was a greater potential difference. This greater potential difference strongly suggested an increase in the quantity of ATP and NADPH. These energy-rich organic compounds are known to feed the Calvin cycle on their journey to become glucose.

7. The order of the sequence when the red and blue lasers were used in specific cases was important:

    i) Employment of red lasers having wavelengths of 650 nm or 680 nm, followed by the use the blue lasers showed improvement of the algae growth rate. In spite of that, using the red laser of wavelength 700 nm followed by blue lasers showed less growth activity.

    ii) The order of the sequence in the case of using the red lasers of wavelength 680 nm or 700 nm after using blue lasers was not important as the results were similar to each other.

8. One of the important outcomes of this experimental work was the introduction of an easy and immediate way to count the number of cells at any stage of cell division. Combination of a photodiode system with the data acquisition system (NI USB 6008) and LABVIEW software package greatly supported adoption of a real-time observed cell counting method having tightly controlled parameters to quantify the actual initial and closure algae cell numbers within accurate time frames.
9. The productive outcome of the experimental work done in this research potentially provided the bioenergy industry with an informative model of laser light combinations. This model may be used to increase the rate of algae cell division and make the production of algae under artificial light sources more efficient and viable for large-scale industrial applications.

6.3 Proposals for future work

Improving the cultivation techniques of algae requires more research to be conducted so as to realise environmental and commercial benefits. It is essential to achieve more efficient algae growth on a large-scale through developing modern techniques as technology permits that will enhance the utilisation of current findings. As a caveat, the process of developing current technology remains costly compared to the number of algae produced, thus, a more cost-effective method needs to be invented as a solution to the financial negativity of the issue. Although this economic aspect may discourage investors in this field, it is quintessential to highlight the many potential commercial benefits

applicable to industries that may include biofuel production, food, medicine and light oils for precision engineering.

The premise of this research was to improve the rate of cell division and cultivation of *C. reinhardtii,* the achieved results were very promising. A prospective technology was developed that employed a combination of blue and red diode lasers as the photonic energy source needed to more effectively manage the cultivation of algae as a biomass. While this technique promoted higher yields, it has not to date been cultured on an anticipated commercial scale. Therefore, the recommendation that can be given by this thesis is to scale up the work of this study to an industrial level by constructing fully automated bioreactors designed around the findings of the work done in this research. Furthermore, additional future research is still required, including but not limited to the following issues:

1) Studies on improving the functionality of the model given in this study by investigation of various CO_2 concentration levels, so as to establish the limits to ascertain where CO_2 concentration is no longer a factor in acceleration of cell division.
2) Broadening the model given in this thesis to include studying the effect of the electrical field in addition to different colour laser combinations outcome on improving algae growth rate.
3) Using pulsed lasers in comparison to the continuous laser to study the effect of changing the mode of light exposure on the growth of algae.
4) Investigating the growth rate of *C. reinhardtii* biomass under different pH values of the TAP medium in order to study the effect of pH on the TAP nutrients in the course of algae culture growth.
5) Studying the genetic factors of the algae species under investigation in order to discover a means to extend algae lifespan in an attempt to sustain the increase in growth rate of algae biomass.
6) Investigation of cell division speed: Does the algae produced using the model of this research having a specific speed of cell division show potential for exponential acceleration in steps generation by generation? Potentially through alteration of the keys within the structure of the DNA.

REFERENCES

Abed, R.N., 2015. The thermal management system of laser diode: A review. *ARPN Journal of Engineering and Applied Sciences*, 10(12), pp.5250–5260.

Adams, C., 2013. *Studies on nitrogen and silicon deficiency in microalgal lipid production*. Doctoral Thesis, Utah State University. Available at: http://digitalcommons.usu.edu/etd/1955. [Accessed February 4, 2018].

Ajala, O.E. et al., 2015. Biodiesel: Sustainable Energy Replacement to Petroleum-Based Diesel Fuel - A Review. *ChemBioEng Reviews*, 2(3), pp.145–156. Available at: http://doi.wiley.com/10.1002/cben.201400024. [Accessed February 4, 2018].

Alagoas, U.F. De et al., 2017. Photosynthetic capacity and water use efficiency in Ricinus communis (L .) under drought stress in semi-humid and semi-arid areas. *Annals of the Brazilian Academy of Sciences*, 89, pp.3015–3029.

Ali, M. et al., 2017. Prospects of microalgal biodiesel production in Pakistan – a review. *Renewable and Sustainable Energy Reviews*, 80, pp. 1588-1596.

Al-Qasmi, M., Raut, N., Talebi, S., Al-Rajhi, S., & Al-Barwani, T., 2012. A review of effect of light on microalgae growth. *In Proceedings of the world congress on engineering* , I, pp.4–6.

Al-safaar, A.T., Al-rubiaee, G.H. & Salman, S.K., 2016. Effect of pHCondition on the Growth and Lipid Content of Microalgae Chlorella vulgaris&Chroococcus minor. *Journal of Scientific & Engineering Research*, 7(11).

Altenhofen da Silva, M. et al., 2016. Heterotrophic growth of green microalgae Desmodesmus subspicatus in ethanol distillation wastewater (vinasse) and lipid extraction with supercritical CO_2. *Journal of Chemical Technology and Biotechnology*, 92, pp.573-579.

Al-yasiri, S., Balachandran, W. & Hooker, D., 2017. Red Laser Excitation of Electrons for Growth Enhancement of Chlamydomonas Reinhardtii Algae. *Journal of Advances in Biochemistry and its Applications*, 1(1), pp.12–19.

Alonso, Â. et al., 2000. Acyl lipid composition variation related to culture age and nitrogen concentration in continuous culture of the microalga Phaeodactylum tricornutum. *Phytochemistry*, 54, pp.461-471.

Analog Devices, Inc., 2006. *Low Noise Precision Operational Amplifier OP27 Datasheet*. USA: Analog Devices. Available http://www.analog.com/media/en/technical-documentation/data-sheets/OP27.pdf. [Accessed February 4, 2018].

Andrade, L.M., 2018. Chlorella and Spirulina Microalgae as Sources of Functional Foods, Nutraceuticals, and Food Supplements; an Overview. *MOJ Food Processing & Technology*, 6(2), pp.45–58. Available at: http://medcraveonline.com/MOJFPT/MOJFPT-06-00144.php. [Accessed February 4, 2018].

Ariede, M.B. et al., 2017. Cosmetic attributes of algae - A review. *Algal Research*, 25, pp.483–487. Available at: http://dx.doi.org/10.1016/j.algal.2017.05.019. [Accessed February 4, 2018].

Arvaniti, E., Gray, C., Higson, A. 2015. *Energy, fuels and environmental policies relevant to algae production and use in NW Europe*. Public Output report WP2A8.01 of the EnAlgae project, Swansea, October 2015, 58 pp. Available at: http://www.enalgae.eu/public-deliverables.htm. [Accessed February 4, 2018].

Ashdown, I., 2017. All Things Lighting Photometry and Photosynthesis. In: *All Things Lighting's weblog*. 10 December 2014 [viewed 23 September 2018]. Available at: https://lightinganalysts.com/photometry-and-photosynthesis/. [Accessed February 4, 2018].

Atta, M. et al., 2013. Intensity of blue LED light: A potential stimulus for biomass and lipid content in fresh water microalgae Chlorella vulgaris. *Bioresource Technology*, 148, pp.373–378. Available at: http://dx.doi.org/10.1016/j.biortech.2013.08.162. [Accessed February 4, 2018].

Avni, A. & Blázquez, M.A., 2011. Can plant biotechnology help in solving our food and energy shortage in the future? *Current Opinion in Biotechnology*, 22(2),

pp.220–223.

Bagul, S., 2011. *Evaluation and optimization of cultural conditions for maximum lipid content in microalgal biomass*. Master Thesis, Indian Agricultural Research Institute. Available at: http://krishikosh.egranth.ac.in/bitstream/1/87513/1/samdhan%20bagul.pdf. [Accessed February 4, 2018].

Barry, B.A., Brahmachari, U. & Guo, Z., 2017. Tracking Reactive Water and Hydrogen-Bonding Networks in Photosynthetic Oxygen Evolution. *Accounts of Chemical Research*, 50(8), pp.1937–1945.

Basu, A., 2015. *Relation between H ydrogen production and P hotosynthesis in THE green algae Chlamydomonas reinhardtii*. Master Thesis, Uppsala University.

Bayro-Kaiser, V. and Nelson, N., 2017. Microalgal hydrogen production: prospects of an essential technology for a clean and sustainable energy economy. *Photosynth Res*, 133(1–3), pp. 49–62. doi: 10.1007/s11120-017-0350-6.

Bearham, D., Vanderklift, M.A. & Gunson, J.R., 2013. Temperature and light explain spatial variation in growth and productivity of the kelp Ecklonia radiata. *Marine Ecology Progress Series*, 476(February), pp.59–70.

Bees, M.A. & Hill, N.A., 1997. Wavelengths of bioconvection patterns. *The Journal of experimental biology*, 200(Pt 10), pp.1515–1526. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9319416. [Accessed February 4, 2018].

Beilen, J., 2010. Why microalgal biofuels won't save the internal combustion machine. *Biofuels, Bioproducts and Biorefining*, 4(1), pp.41–52.

Benemann, J.R., 2008. *Open Ponds And Closed Photobioreactors – Comparative Economics*. 5th Annual World Congress on Industrial 5th Annual World Congress on Industrial Biotechnology & Bioprocessing, Chicago, April 2008, pp. Available at: https://www.researchgate.net/publication/228965376_Open_ponds_and_closed_photobioreactors-comparative_economics. [Accessed February 4, 2018].

Betterle, N., 2011. *Dynamics of Photosystem II during short and long term response to light intensity: a biochemical and biophysical study*. Doctoral Thesis, University of Verona. Available at: http://www.univr.it/main?ent=catalogoaol&id=351382&page=dettaglioPubblicazione&lang=it. [Accessed February 4, 2018].

Birch, K. & Calvert, K., 2015. Rethinking "drop-in" biofuels: On the political materialities of bioenergy. *Science and Technology Studies*, 28(1), pp.52–72.

Bogan, R.H., 1961. *Removal of Sewage Nutrients by Algae*. Public Health reports, 76(4), pp.301-308.

Bonente, G. et al., 2011. Mutagenesis and phenotypic selection as a strategy toward domestication of Chlamydomonas reinhardtii strains for improved performance in photobioreactors. *Photosynthesis Research*, 108(2–3), pp.107–120.

Borowitzka, M.A., 1995. Microalgae as sources of pharmaceuticals and other biologically active compounds. *Journal of Applied Phycology*, 7(1), pp.3–15.

Bougaran, G. et al., 2012. Optimizing conditions for the continuous culture of Isochrysis affinis galbana relevant to commercial hatcheries *Aquaculture journal*, 326 (January), pp.106–115.

Bowsher, C., Steer, M. & Tobin, A., 2008. Light reactions of photosynthesis. *Plant Biochemistry*, pp.65–91. Available at: http://www.ck12.org/book/CK-12-Life-Science-Concepts-For-Middle-School/section/2.12/. [Accessed February 4, 2018].

Brown, T. R., 2018. Price uncertainty, policy, and the economic feasibility of cellulosic biorefineries. *Biofuels, Bioprod. Bioref.*, 12, pp. 485-496. doi:10.1002/bbb.1865

Bruton, T. et al., 2009. A Review of the Potential of Marine Algae as a Source of Biofuel in Ireland. *Sustainable Energy Ireland Dublin*, p.88. Available at: http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:A+Review+of+the+Potential+of+Marine+Algae+as+a+Source+of+Biofuel+in+Ireland#0. [Accessed February 4, 2018].

Burns, A., 2012. Photobioreactor Design for Improved Energy Efficiency of Microalgae

Production. *Bioresource Technology*, 11(1), pp.1–180.

Caffarri, S. et al., 2014. A Comparison Between Plant Photosystem I and Photosystem II Architecture and Functioning. *Current Protein & Peptide Science*, 15(4), pp.296–331. Available at: http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1389-2037&volume=15&issue=4&spage=296. [Accessed February 4, 2018].

Cañedo, J.C.G. & Lizárraga, G.L.L., 2016. Considerations for Photobioreactor Design and Operation for Mass Cultivation of Microalgae. *Algae - Organisms for Imminent Biotechnology*. Available at: http://www.intechopen.com/books/algae-organisms-for-imminent-biotechnology/considerations-for-photobioreactor-design-and-operation-for-mass-cultivation-of-microalgae. [Accessed February 4, 2018].

Cao, J. et al., 2009. A Preliminary Study of the Effect of Surface Texture on Algae Cell Attachment for a Mechanical-Biological Energy Manufacturing System. *Journal of Manufacturing Science and Engineering*, 131(6), p.064505. Available at: http://manufacturingscience.asmedigitalcollection.asme.org/article.aspx?articleid=1456303. [Accessed February 4, 2018].

Cardona, T. et al., 2012. Charge separation in Photosystem II: A comparative and evolutionary overview. *Biochimica et Biophysica Acta - Bioenergetics*, 1817(1), pp.26–43. Available at: http://dx.doi.org/10.1016/j.bbabio.2011.07.012. [Accessed February 4, 2018].

Carriquiry, M.A., Du, X. & Timilsina, G.R., 2011. Second generation biofuels: Economics and policies. *Energy Policy*, 39(7), pp.4222–4234. Available at: http://dx.doi.org/10.1016/j.enpol.2011.04.036. [Accessed February 4, 2018].

Carvalho, F.P., 2017. Pesticides, environment, and food safety. *Food and Energy Security*, 6(2), pp.48–60.

Castellanos, C.S., 2013. *Batch and Continuous Studies of Chlorella Vulgaris in Photo-Bioreactors*. Master Thesis, The University of Western Ontario.

Caycedo-Soler, F. et al., 2017. Quantum Redirection of Antenna Absorption to

Photosynthetic Reaction Centers. *Journal of Physical Chemistry Letters*, 8(24), pp.6015–6021.

CCC, 2011. *The Renewable Energy Review*. The Committee on Climate Change, pp.1–166.

Chen, G., 2013. *The Study of Biomass Yield and Macromolecular Content of Microalgae Change as a Function of the Physiological State and Nutrient Supply Conditions*. Master Thesis, University of Kansas.

Chen, M. et al., 2011. Bioresource Technology Effect of nutrients on growth and lipid accumulation in the green algae Dunaliella tertiolecta. *Bioresource Technology*, 102(2), pp.1649–1655. Available at: http://dx.doi.org/10.1016/j.biortech.2010.09.062. [Accessed February 4, 2018].

Chen, Z. et al., 2018. Determination of microalgal lipid content and fatty acid for biofuel production. *BioMed Research International*, 2018, 1503126, pp.1-17. Available at: https://doi.org/10.1155/2018/1503126. [Accessed February 4, 2018].

Cheng, W.-L. et al., 2011. A novel household refrigerator with shape-stabilized PCM (Phase Change Material) heat storage condensers: An experimental investigation. *Energy*, 36(10), pp.5797–5804.

Chenu, A. et al., 2013. Enhancement of vibronic and ground-state vibrational coherences in 2D spectra of photosynthetic complexes. *Scientific Reports*, 3, pp.1–12.

Chenu, A. & Scholes, G.D., 2015. Coherence in Energy Transfer and Photosynthesis. *Annual Review of Physical Chemistry*, 66(1), pp.69–96. Available at: http://www.annualreviews.org/doi/10.1146/annurev-physchem-040214-121713. [Accessed February 4, 2018].

Chini, G. et al., 2006. Productivity and photosynthetic efficiency of outdoor cultures of Tetraselmis suecica in annular columns. *Aquaculture*, 261, pp.932–943.

Chisti, Y., 2006. Environmental Pollution and Monitoring MICROALGAE AS SUSTAINABLE CELL FACTORIES. *Journal of Environmental Engineering and*

Management, 5(3), pp.261–274.

Christenson, L. & Sims, R., 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnology Advances*, 29(6), pp.686–702. Available at: http://linkinghub.elsevier.com/retrieve/pii/S073497501100070X [Accessed March 20, 2017].

Clegg, R.M., Sener, M. & Govindjee, 2010. From Förster resonance energy transfer to coherent resonance energy transfer and back. *Society of Photo-Optical Instrumentation Engineers (SPIE) Conference Series*, pp.1–21. Available at: http://www.ks.uiuc.edu/~melih/Clegg2010.pdf. [Accessed February 4, 2018].

Converti, A. et al., 2009. Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris for biodiesel production. *Chemical Engineering and Processing: Process Intensification*, 48(6), pp.1146–1151.

Corley, C.E., 2011. Principles of design and operations of wastewater treatment pond systems for plant operators, engineers, and managers. *Illinois Environmental Protection Agency*, 600(August), pp.1–457. Available at: papers3://publication/uuid/69FD0F3F-7DA1-4812-8958-480E064E30AF. [Accessed February 4, 2018].

Cui, H. et al., 2017. Two-stage mixotrophic cultivation for enhancing the biomass and lipid productivity of Chlorella vulgaris. *AMB Express*, 7(1).

Czirók, A., Jánosi, I.M. & Kessler, J.O., 2000. Bioconvective dynamics: dependence on organism behaviour. *The Journal of experimental biology*, 203, pp.3345–3354. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11023854. [Accessed February 4, 2018].

Darko, E. et al., 2014. Photosynthesis under artificial light: the shift in primary and secondary metabolism. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369(1640), pp.20130243–20130243. Available at: http://rstb.royalsocietypublishing.org/cgi/doi/10.1098/rstb.2013.0243. [Accessed

February 4, 2018].

Das, P. et al., 2011. Enhanced algae growth in both phototrophic and mixotrophic culture under blue light. *Bioresource Technology*, 102(4), pp.3883–3887. Available at: http://dx.doi.org/10.1016/j.biortech.2010.11.102. [Accessed February 4, 2018].

Das, S. et al., 2017. Lipid induction in microalgae. *J. Algal Biomass Utln.*, 8(4), pp.39–50.

Davis, R. et al., 2016. Process Design and Economics for the Production of Algal Biomass: Algal Biomass Production in Open Pond Systems and Processing Through Dewatering for Downstream Conversion. *National Renewable Energy Laboratory*, (February), p.128. Available at: www.nrel.gov/publications. [Accessed February 4, 2018].

de-Bashan, L.E. & Bashan, Y., 2010. Immobilized microalgae for removing pollutants: Review of practical aspects. *Bioresource Technology*, 101(6), pp.1611–1627. Available at: http://dx.doi.org/10.1016/j.biortech.2009.09.043. [Accessed February 4, 2018].

Dean, A.P. et al., 2010. Bioresource Technology Using FTIR spectroscopy for rapid determination of lipid accumulation in response to nitrogen limitation in freshwater microalgae. *Bioresource Technology*, 101(12), pp.4499–4507. Available at: http://dx.doi.org/10.1016/j.biortech.2010.01.065. [Accessed February 4, 2018].

Delrue, F. et al., 2017. Optimization of Arthrospira platensis (Spirulina) Growth: From Laboratory Scale to Pilot Scale. *Fermentation*, 3(4), p.59. Available at: http://www.mdpi.com/2311-5637/3/4/59. [Accessed February 4, 2018].

Demmig-adams, B. et al., 2014. Non-Photochemical Quenching and Energy Dissipation in Plants, Algae and Cyanobacteria, *Advances in Photosynthesis and Respiration*, 40, pp.97-128. Available at: http://link.springer.com/10.1007/978-94-017-9032-1. [Accessed February 4, 2018].

Devaraja, S. et al., 2017. Studies on the Effect of Red, Blue and White LED Lights on

the Productivity of Chlorella Vulgaris to Treat Dye Industry Effluent. *Advances in Biotechnology & Microbiology*, 6(2), pp.1-6.

Disselnkötter, S. & Rendall, A.D., 2017. Stability of stationary solutions in models of the Calvin cycle. *Nonlinear Analysis: Real World Applications*, 34, pp.481–494. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1468121816301201. [Accessed February 4, 2018].

Dixon, C. & Wilken, L.R., 2018. Green microalgae biomolecule separations and recovery. *Bioresources and Bioprocessing*, 5(1). Available at: https://doi.org/10.1186/s40643-018-0199-3. [Accessed February 4, 2018].

Dobrowolski, J.W. et al., 2015. Integration of Local Eco-innovation with Global Problems of Protection of the Natural Environment and Bio-based Green Economy. In *PROCEEDINGS OF THE 2015 AASRI INTERNATIONAL CONFERENCE ON CIRCUITS AND SYSTEMS (CAS 2015)*. pp. 25–28.

Dobrowolski, J.W., Śliwka, M. & Mazur, R., 2012. Laser biotechnology for more efficient bioremediation, protection of aquatic ecosystems and reclamation of contaminated areas. *Journal of Chemical Technology and Biotechnology*, 87(9), pp.1354–1359.

ECCC, 2011. *The Renewable Energy Review*. House of Commons Energy and Climate Change Committee, pp.1–45.

El-Kassas, H.Y., 2013. Growth and fatty acid profile of the marine microalga Picochlorum Sp. grown under nutrient stress conditions. *Egyptian Journal of Aquatic Research*, 39(4), pp.233–239. Available at: http://dx.doi.org/10.1016/j.ejar.2013.12.007. [Accessed February 4, 2018].

El-Sheek, M.M. & Rady, A.A., 1994. Effect of Phosphorus Starvation on Growth , Photo- synthesis and Some Metabolic Processes in the Uni- cellular Green Alga Chlorella kessleri. *Phyton (Horn, Austria)*, 35, pp.139–151.

Elvidge, C.D. et al., 2010. Spectral identification of lighting type and character. *Sensors*, 10(4), pp.3961–3988.

Engel, G.S., 2011. Quantum coherence in photosynthesis. *Procedia Chemistry*, 3, pp.222–231.

Erickson, E., Wakao, S. & Niyogi, K.K., 2015. Light stress and photoprotection in Chlamydomonas reinhardtii. *Plant Journal*, 82(3), pp.449–465.

Fakhry, E.M. & El Maghraby, D.M., 2015. Lipid accumulation in response to nitrogen limitation and variation of temperature in nannochloropsis salina. *Botanical Studies*, 56(6), pp.1-8.

Fassioli, F. et al., 2013. Photosynthetic light harvesting: excitons and coherence. *Journal of The Royal Society Interface*, 11(92), pp.20130901–20130901. Available at: http://rsif.royalsocietypublishing.org/cgi/doi/10.1098/rsif.2013.0901. [Accessed February 4, 2018].

Ferenczi, A. et al., 2017. Efficient targeted DNA editing and replacement in Chlamydomonas reinhardtii using Cpf1 ribonucleoproteins and single-stranded DNA. *Proceedings of the National Academy of Sciences*, 12, p.201710597. Available at: http://www.pnas.org/lookup/doi/10.1073/pnas.1710597114. [Accessed February 4, 2018].

Fiore, J.M., 2018. Operational Amplifiers & Linear Integrated Circuits: Theory and Application / 3E. *Dissidents*, 3.0.8, pp.117–197. Available at: https://www2.mvcc.edu/users/faculty/jfiore/OpAmps/OperationalAmplifiersAndLinearICs_3E.pdf. [Accessed February 4, 2018].

Fontes, A.G. et al., 1987. Factors Affecting the Production of Biomass by a Nitrogen-fixing Blue-green Alga in Outdoor Culture. *Biomass*, 13, pp.33–43.

Foyer, C.H. et al., 2012. Photosynthetic control of electron transport and the regulation of gene expression. *Journal of Experimental Botany*, 63(4), pp.1637–1661.

García, J.L., de Vicente, M. & Galán, B., 2017. Microalgae, old sustainable food and fashion nutraceuticals. *Microbial Biotechnology*, 10(5), pp.1017–1024.

Gardner, R. et al., 2011. Medium pH and nitrate concentration effects on accumulation of triacylglycerol in two members of the chlorophyta. *Journal of Applied*

Phycology, 23(6), pp.1005–1016.

Gendy, T.S. & El-temtamy, S.A., 2013. Commercialization potential aspects of microalgae for biofuel production : An overview. *Egyptian Journal of Petroleum*, 22(1), pp.43–51. Available at: http://dx.doi.org/10.1016/j.ejpe.2012.07.001. [Accessed February 4, 2018].

George, R. et al., 2018. High midday temperature stress has stronger effects on biomass than on photosynthesis: A mesocosm experiment on four tropical seagrass species. *Ecology and Evolution*, 8(9), pp.4508–4517.

Gersten, B., 2017. *A feasibility study in the use of domestic water treatment residuals to remove phosphorus from wastewater*. Doctoral Thesis, Cardiff University.

Golueke, C.G., Oswald, W.J. & Gotaas, H.B., 1957. Anaerobic digestion of Algae. *Applied microbiology*, 5, pp.47–55.

Gómez, C. et al., 2013. Comparison of intracanopy light-emitting diode towers and overhead high-pressure sodium lamps for supplemental lighting of greenhouse-grown tomatoes. *HortTechnology*, 23(1), pp.93–98.

Greene, C. et al., 2016. Marine Microalgae: Climate, Energy, and Food Security from the Sea. *Oceanography*, 29(4), pp.10–15. Available at: https://tos.org/oceanography/article/marine-microalgae-climate-energy-and-food-security-from-the-sea. [Accessed February 4, 2018].

Grebe, A. et al., 2014. Innovations in Cell Culture Section 1: Upstream Technologies Design of Experiments with Small-Scale Bioreactor Systems for Efficient Bioprocess Development and Optimization Integrated Optical Single-Use Sensors: Moving Toward a True Single-Use Factory for. *Bioprocess International*, 12(5). Available at: https://www.sartorius.com/mediafile/BioProcess_Vol-12_Supp-5.pdf. [Accessed February 4, 2018].

Griffiths, M.J. & Harrison, S.T.L., 2009. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *J Appl Phycol* (2009) 21, pp.493–507.

Grima, E.M. et al., 1997. Evaluation of photosynthetic efficiency in microalgal cultures using averaged irradiance. *Enzyme and Microbial Technology*, 21(5), pp.375–381.

Guedes, A.C. et al., 2011. Fatty acid composition of several wild microalgae and cyanobacteria, with a focus on eicosapentaenoic, docosahexaenoic and α-linolenic acids for eventual dietary uses. *Food Research International*, 44(9), pp.2721–2729.

Gustavsson, L., Johansson, B. & Svenningsson, P.E.R., 2015. Reducing CO_2 Emissions By Substituting Biomass for Fossil Fuels. *Energy*, 20(11), pp.1079-1113.

Harun, R. et al., 2010. Bioprocess engineering of microalgae to produce a variety of consumer products. *Renewable and Sustainable Energy Reviews*, 14, pp.1037–1047.

He, L., Subramanian, V.R. & Tang, Y.J., 2012. Experimental analysis and model-based optimization of microalgae growth in photo-bioreactors using flue gas. *Biomass and Bioenergy*, 41(314), pp.131–138. Available at: http://dx.doi.org/10.1016/j.biombioe.2012.02.025. [Accessed February 4, 2018].

Hildner, R. et al., 2013. Quantum coherent energy transfer over varying pathways in single light-harvesting complexes. *Science*, 340(6139), pp.1448–1451.

Hong, Y. & Xu, K., 2013. Co-existing growth relationships of a lipid-producing alga with three microalgae. *Allelopathy Journal*, 32(2), pp.301–314.

Hoyer, S., Ishizaki, A. & Whaley, K.B., 2012. Spatial propagation of excitonic coherence enables ratcheted energy transfer. *Physical Review E - Statistical, Nonlinear, and Soft Matter Physics*, 86(4), pp.1–14.

Hsieh, C. & Wu, W., 2009. Bioresource Technology Cultivation of microalgae for oil production with a cultivation strategy of urea limitation. *Bioresource Technology*, 100(17), pp.3921–3926. Available at: http://dx.doi.org/10.1016/j.biortech.2009.03.019. [Accessed February 4, 2018].

Huang, Q. et al., 2017. Design of Photobioreactors for Mass Cultivation of Photosynthetic Organisms. *Engineering*, 3(3), pp.318–329. Available at: http://dx.doi.org/10.1016/J.ENG.2017.03.020. [Accessed February 4, 2018].

IEA, 2016. CO_2 Emissions from Fuel Combustion. *Oecd/Iea*, pp.1–155.

IEA, 2017. CO_2 Emissions from Fuel Combustion 2017 - Highlights. *International Energy Agency*, 1, pp.1–162. Available at: www.iea.org/t&c/%0Ahttps://www.iea.org/publications/freepublications/publication/CO2EmissionsfromFuelCombustionHighlights2017.pdf. [Accessed February 4, 2018].

Iersel, S. Van, 2008. Worldwide Potential of Marine. *Ecofys Bio Energy Group the Netherlands BV*, (May), pp.1-66.

Illman, A.M., Scragg, A.H. & Shales, S.W., 2000. Increase in Chlorella strains calorific values when grown in low nitrogen medium. *Ecofys Bio Energy Group the Netherlands BV*, 27, pp.631–635.

Janssen, M., 2002. '*Cultivation of microalgae : effect of light / dark cycles on biomass yield'*. Unpublished Master's thesis, Wageningen University Wageningen The Netherlands.

Jiang, L. & Wen, L., 2015. Photonic sensitive switchable materials. *Biophotonics for Medical Applications*, pp.25–51. Available at: https://www.sciencedirect.com/science/article/pii/B9780857096623000026. [Accessed 2 January 2018].

Jiang, H. & Gao, K., 2004. Effects of lowering temperature during culture on the production of polyunsaturated fatty acids in the marine diatom Phaeodactylum tricornutum (Bacillariophyceae). *Journal of Phycology*, 40(4), pp.651–654.

Jiang, L. *et al.* 2018. Lipid productivity in limnetic Chlorella is doubled by seawater added with anaerobically digested effluent from kitchen waste. *Biotechnology for Biofuels*. BioMed Central, 11(1), pp. 1–14. doi: 10.1186/s13068-018-1064-5.

Johnson, M.B. & Wen, Z., 2010. Development of an attached microalgal growth system for biofuel production. *Applied Microbiology and Biotechnology*, 85(3), pp.525–534.

Johnson, M.P., 2016. Photosynthesis. *Essays In Biochemistry*, 60(3), pp.255–273.

Available at: http://essays.biochemistry.org/cgi/doi/10.1042/EBC20160016. [Accessed February 4, 2018].

Juneja, A., Ceballos, R.M. & Murthy, G.S., 2013. Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: A review. *Energies*, 6(9), pp.4607–4638.

Junginger, M. et al., 2010. Technological learning in bioenergy systems. *Energy Policy*, 34(2006), pp.4024–4041.

Kabariya, J.H. & Ramani, V.M., 2018. Dairy Wastewater Treatment by Cyanobacteria for Removal of Nutrients with Extraction of High Value Compounds from Biomass. *International Journal of Current Microbiology and Applied Sciences*, 7(04), pp.1527–1538. Available at: https://www.ijcmas.com/abstractview.php?ID=7438&vol=7-4-2018&SNo=172. [Accessed February 4, 2018].

Kawashima, K. and Ishikita, H. 2018. Energetic insights into two electron transfer pathways in light-driven energy-converting enzymes. *Chemical Science. Royal Society of Chemistry*, 9(17), pp. 4083–4092. doi: 10.1039/c8sc00424b.

Kempchen, L. 2018. *Understanding Fragmentation In Global Governance, The Case Of Global Land Governance*. Master thesis, Wageningen University.

Kendrick, M., 2011. *Algal Bioreactors for Nutrient Removal and Biomass Production During the Tertiary Treatment of Domestic Sewage*. Doctoral Thesis, Loughborough University, p.274.

Khan, M.I., Shin, J.H. & Kim, J.D., 2018. The promising future of microalgae: Current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial Cell Factories*, 17(1), pp.1–21. Available at: https://doi.org/10.1186/s12934-018-0879-x. [Accessed February 4, 2018].

Khan, S. et al., 2017. Biodiesel Production From Algae to Overcome the Energy Crisis. *HAYATI Journal of Biosciences*, 24(4), pp.163–167. Available at:

https://doi.org/10.1016/j.hjb.2017.10.003. [Accessed February 4, 2018].

Khozin-goldberg, I. & Cohen, Z., 2006. The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water eustigmatophyte Monodus subterraneus. *Phytochemistry*, 67 (2006), pp.696–701.

Kodama, Y. & Fujishima, M., 2016. Differences in infectivity between endosymbiotic Chlorella variabilis cultivated outside host Paramecium bursaria for 50 years and those immediately isolated from host cells after one year of reendosymbiosis. *Biology Open*, 5(1), pp.55–61. Available at: http://bio.biologists.org/cgi/doi/10.1242/bio.013946. [Accessed February 4, 2018].

Kommareddy, Anil; Anderson, G., 2003. Study of Light as a parameter in the growth of algae in a Photo-Bio Reactor (PBR). *Asae*, 0300(03).

Kosourov, S. et al., 2007. A comparison of hydrogen photoproduction by sulfur-deprived Chlamydomonas reinhardtii under different growth conditions. *Journal of Biotechnology*, 128(4), pp.776–787.

Kosourov, S., Seibert, M. & Ghirardi, M.L., 2003. Effects of extracellular pH on the metabolic pathways in sulfur-deprived H2-producting Chlamydomonas reinhardtii under different growth conditions. *Plant Cell Physiology*, 44(2), pp.145–155.

Kotasthane, T., 2017. Potential of Microalgae for Sustainable Biofuel Production. *Journal of Marine Science: Research & Development*, 07(01). Available at: https://www.omicsonline.org/open-access/potential-of-microalgae-for-sustainable-biofuel-production-2155-9910-1000223.php?aid=86863. [Accessed February 4, 2018].

Krupnik, T. et al., 2013. A reaction center-dependent photoprotection mechanism in a highly robust photosystem II from an extremophilic red alga, cyanidioschyzon merolae. *Journal of Biological Chemistry*, 288(32), pp.23529–23542.

Kuhlbrandt, W., 2015. Structure and function of mitochondrial membrane protein complexes. *BMC Biology*, 13(1), pp.1–11. Available at: http://dx.doi.org/10.1186/s12915-015-0201-x. [Accessed February 4, 2018].

Kumar, K. et al., 2015. Recent trends in the mass cultivation of algae in raceway ponds. *Renewable and Sustainable Energy Reviews*, 51, pp.875–885. Available at: http://dx.doi.org/10.1016/j.rser.2015.06.033. [Accessed February 4, 2018].

Kume, A., 2018. Correction to: Importance of the green color, absorption gradient, and spectral absorption of chloroplasts for the radiative energy balance of leaves (Journal of Plant Research, (2017), 130, 3, (501-514), 10.1007/s10265-017-0910-z). *Journal of Plant Research*, 131(3), p.569.

Kuwahara, S.S. et al., 2011. Growth of the green algae Chlamydomonas reinhardtii under red and blue lasers. *Optics and Lasers in Engineering*, 49(3), pp.434–438. Available at: http://dx.doi.org/10.1016/j.optlaseng.2010.11.015. [Accessed 2 January 2018].

Lam, M.K. & Lee, K.T., 2012a. Microalgae biofuels : A critical review of issues , problems and the way forward. *Biotechnology Advances*, 30(3), pp.673–690. Available at: http://dx.doi.org/10.1016/j.biotechadv.2011.11.008. [Accessed 2 January 2018].

Lam, M.K. & Lee, K.T., 2012b. Potential of using organic fertilizer to cultivate Chlorella vulgaris for biodiesel production. *Applied Energy*, 94, pp.303–308. Available at: http://dx.doi.org/10.1016/j.apenergy.2012.01.075. [Accessed February 4, 2018].

Lara-Gil, J.A., Senés-Guerrero, C. & Pacheco, A., 2016. Cement flue gas as a potential source of nutrients during CO2mitigation by microalgae. *Algal Research*, 17, pp.285–292. Available at: http://dx.doi.org/10.1016/j.algal.2016.05.017. [Accessed February 4, 2018].

Lavens, P. & Sorgeloos, P., 1996. Manual on the production and use of live food for aquaculture. *FAO Technical Paper*, 361, pp. 295. Available at: http://www.fao.org/docrep/field/003/ab825f/AB825F00.htm#TOC. [Accessed 2 January 2018].

Lee, C. et al., 1994. High?density algal photobioreactors using light?emitting diodes. *Biotechnology and Bioengineering*, 44(10), pp.1161–1167.

Lee, H., Cheng, Y. & Fleming, G.R., 2018. Coherence Dynamics in Photosynthesis : Protein Protection of Excitonic Coherence Linked references are available on JSTOR for this article : Coherence Dynamics in Photosynthesis : Protein Protection of Excitonic Coherence. *Science*, 316(5830), pp.1462–1465.

Lee, Y.K., 1997. Commercial production of microalgae in the Asia-Pacific rim. *Journal of Applied Phycology*, 9(5), pp.403–411.

Li, T., 2014. *Lipid production, photosynthesis adjustement and carbon partitioning of Chlorella sorokiniana cultured under mixotrophic and heterotrophic conditions.* Doctoral Thesis, Washington State University.

Liguori, N. et al., 2017. Different carotenoid conformations have distinct functions in light-harvesting regulation in plants. *Nature Communications*, 8(1), pp.1–9. Available at: http://dx.doi.org/10.1038/s41467-017-02239-z. [Accessed February 4, 2018].

Lim, D.K.Y. et al., 2012. Isolation and evaluation of oil-producing microalgae from subtropical coastal and Brackish waters. *PLoS ONE*, 7(7).

LITE-ON Technology Corp. / Optoelectronics, 2012. Through Hole Lamp. , (90). Available at: http://optoelectronics.liteon.com/upload/download/DS-20-92-0237/S_110_LTL-4221N.pdf. [Accessed 2 January 2018].

Liu, Z. & Wang, G., 2008. Effect of iron on growth and lipid accumulation in Chlorella vulgaris. *Bioresource Technology*, 99, pp.4717–4722.

Luo, D. et al., 2010. Life cycle energy and greenhouse gas emissions for an ethanol production process based on blue-green algae. *Environmental Science & Technology*, 44(22), pp.8670–8677.

Lv, J. et al., 2018. The performance of a self-flocculating microalga chlorococcum sp. GD in wastewater with different ammonia concentrations. *International Journal of Environmental Research and Public Health*, 15(3), pp.1–13.

Lyon, T. et al., 2013. Environmental manipulation of select algae strains for maximal oil production. *Bios*, 84(1), pp.21-29. Available at:

http://www.jstor.org/stable/23595340. [Accessed February 4, 2018].

Lysenko, V.S. et al., 2014. Far-red spectrum of second emerson effect: A study using dual-wavelength pulse amplitude modulation fluorometry. *American Journal of Biochemistry and Biotechnology*, 10(4), pp.234–240.

M, M. et al., 2012. Phycoremediation efficiency and biomass production by micro alga Desmococcus olivaceus (Persoon et Acharius) J.R. Laundon treated on chrome-sludge from an electroplating industry-A open raceway pond study. *Int J Curr Sci*, (1999), pp.52–62.

Ma, X.N. et al., 2016. Lipid production from Nannochloropsis. *Marine Drugs*, 14(4), pp.61.

Magdalena, J.A., Ballesteros, M. & González-Fernandez, C., 2018. Efficient anaerobic digestion of microalgae biomass: Proteins as a key macromolecule. *Molecules*, 23(5), pp.1–16.

Malek, A., Zullo, L.C. & Daoutidis, P., 2016. Modeling and Dynamic Optimization of Microalgae Cultivation in Outdoor Open Ponds. *Industrial and Engineering Chemistry Research*, 55(12), pp.3327–3337.

Mallus, M.I. et al., 2016. Relation between Dephasing Time and Energy Gap Fluctuations in Biomolecular Systems. *Journal of Physical Chemistry Letters*, 7(7), pp.1102–1108.

Malvino, A. & Bates, D., 2006. *Electronic Principles*, Available at: http://www.just.edu.jo/CoursesAndLabs/Electronics 1_Phy 231/Syllabus_231.doc. [Accessed February 4, 2018].

Margit Olle, A.V., 2013. The effect of light-emitting diode lighting on greenhouse plant growth and quality. *Agricultural and Food Science*, 22(April), pp.223–234.

Markina, D., 2014. *Effects of culture conditions on the photoautotrophic growth and biochemical composition of Chlamydomonas reinhardtii , as a potential source for hydrogen production*, Doctoral Thesis, Norwegian University of Life Sciences.

Martínez, R.G., 2016. *Microalgae harvesting in wastewater treatment plants : application of natural techniques for an efficient flocculation*. Doctoral Thesis, Polytechnic University of Catalonia.

Mata, T.M., Martins, A.A. & Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews*, 14(1), pp.217–232.

Matthew, T. et al., 2009. The metabolome of Chlamydomonas reinhardtii following induction of anaerobic H2 production by sulfur depletion. *Journal of Biological Chemistry*, 284(35), pp.23415–23425.

Matuszyńska, A. et al., 2016. A mathematical model of non-photochemical quenching to study short-term light memory in plants. *Biochimica et Biophysica Acta - Bioenergetics*, 1857(12), pp.1860–1869.

Mcclure, S., 2013. *Investigation of light-harvesting dynamics of photosynthetic pigment-protein complexes using ultrafast spectroscopy*. Doctoral Thesis, University of Toronto.

Michailos, S., 2017. Process design, economic evaluation and life cycle assessment of jet fuel production from sugar cane residue. *Environmental Progress & Sustainable Energy*, (December 2017) 37(3), pp.1227-1235. Available at: http://doi.wiley.com/10.1002/ep.12840. [Accessed February 4, 2018].

Milano, J. et al., 2016. Microalgae biofuels as an alternative to fossil fuel for power generation. *Renewable and Sustainable Energy Reviews*, 58(May), pp.180–197. Available at: http://dx.doi.org/10.1016/j.rser.2015.12.150. [Accessed February 4, 2018].

Milledge, J.J. et al., 2014. Macroalgae-derived biofuel: A review of methods of energy extraction from seaweed biomass. *Energies*, 7(11), pp.7194–7222.

Milledge, J.J. & Harvey, P.J., 2016. Potential process 'hurdles' in the use of macroalgae as feedstock for biofuel production in the British Isles. *Journal of Chemical Technology and Biotechnology*, 91(8), pp.2221–2234.

Minhas, A.K. et al., 2016. A review on the assessment of stress conditions for simultaneous production of microalgal lipids and carotenoids. *Frontiers in Microbiology*, 7(MAY), pp.1–19.

Mirkovic, T. et al., 2017. Light absorption and energy transfer in the antenna complexes of photosynthetic organisms. *Chemical Reviews*, 117(2), pp.249–293.

Moheimani, N.R., 2013. Long-term outdoor growth and lipid productivity of Tetraselmis suecica, Dunaliella tertiolecta and Chlorella sp (Chlorophyta) in bag photobioreactors. *Journal of Applied Phycology*, 25(1), pp.167–176.

Molecular Systems Biology University of Vienna. 2018. *Chlamydomonas reinhardtii - model system for alternative energy resources*. [ONLINE] Available at: https://mosys.univie.ac.at/research/topics/clamydomonas-as-energy-resource/. [Accessed 2 January 2018].

Mondal, M. et al., 2017. Production of biodiesel from microalgae through biological carbon capture: a review. *3 Biotech*, 7(2), pp.1–21.

de Mooij, T. et al., 2016. Impact of light color on photobioreactor productivity. *Algal Research*, 15, pp.32–42. Available at: http://dx.doi.org/10.1016/j.algal.2016.01.015. [Accessed 2 January 2018].

Morales-Sánchez, D. et al., 2013. Heterotrophic growth of Neochloris oleoabundans using glucose as a carbon source. *Biotechnology for Biofuels*, 6(1).

Morweiser, M. et al., 2010. Developments and perspectives of photobioreactors for biofuel production. *Applied Microbiology and Biotechnology*, 87(4), pp.1291–1301.

Mostafa, S.S.M. & El-Gendy, N.S., 2017. Evaluation of fuel properties for microalgae Spirulina platensis bio-diesel and its blends with Egyptian petro-diesel. *Arabian Journal of Chemistry*, 10, pp.S2040–S2050. Available at: http://dx.doi.org/10.1016/j.arabjc.2013.07.034. [Accessed 2 January 2018].

Mõttus, M. et al., 2011. Photosynthetically Active Radiation : Measurement and Modeling. *Encyclopedia of Sustainability Science and Technology*, (September

2015), pp.7902–7932.

Müller, N. et al., 2017. A Plant Cryptochrome Controls Key Features of the Chlamydomonas Circadian Clock and Its Life Cycle. *Plant Physiology*, 174(1), pp. 185–201. doi: 10.1104/pp.17.00349.

Nagasoe, S. et al., 2006. Effects of temperature, salinity and irradiance on the growth of the red tide dinoflagellate Gyrodinium instriatum Freudenthal et Lee. *Harmful Algae*, 5(1), pp.20–25.

Nakajima, Y. et al., 2018. Thylakoid membrane lipid sulfoquinovosyl-diacylglycerol (SQDG) is required for full functioning of photosystem II in Thermosynechococcus elongatus. *Journal of Biological Chemistry*, 293, pp.14786-14797. Available at: http://www.jbc.org/lookup/doi/10.1074/jbc.RA118.004304. [Accessed 2 January 2018].

Narala, R.R. et al., 2016. Comparison of Microalgae Cultivation in Photobioreactor, Open Raceway Pond, and a Two-Stage Hybrid System. *Frontiers in Energy Research*, 4(August), pp.1–10. Available at: http://journal.frontiersin.org/Article/10.3389/fenrg.2016.00029/abstract. [Accessed 2 January 2018].

Nazari, F., 2015. Genetic Engineering of Microalgae for Enhanced Biodiesel Production Suitable Fuel Replacement of Fossil Fuel as a Novel Energy Source. *American Journal of Life Sciences*, 3(1), p.32. Available at: http://www.sciencepublishinggroup.com/journal/paperinfo.aspx?journalid=118&doi=10.11648/j.ajls.20150301.17. [Accessed 2 January 2018].

Neumann, A. et al., 2011. Four-color laser white illuminant demonstrating high color-rendering quality. *Optics Express*, 19(S4), p.A982. Available at: https://www.osapublishing.org/oe/abstract.cfm?uri=oe-19-S4-A982. [Accessed 2 January 2018].

Nguyen, M.A., Linh, A. & Hanoi, H., 2016. A review on microalgae and cyanobacteria in biofuel production. *Economies and finances, hal-01383026*. Available at: https://hal-enpc.archives-ouvertes.fr/hal-01383026/document. [Accessed 2 January

2018].

Niculiță P.et al., 2008. Testing the effect induced by the simultaneous treatment with magnetic field and laser light field, modulated at audio frequencies, on colonies of Alternaria alternata. *Romanian Biotechnological Letters*, 13(2).

Nrel, 1998a. A look back at the U. S. Department of Energy's aquatic species program: biodiesel from algae. *Report*, 328, p.291 p. Available at: http://www.biodiesel.pl/uploads/media/A_Look_Back_at_the_U.S._Department_of_Energy_s_Aquatic_Species_Program_Biodiesel_from_Algae_July_1998.pdf. [Accessed 2 January 2018].

Nrel, 1998b. A look back at the U. S. Department of Energy's aquatic species program: biodiesel from algae. *Report*, 328, p.291 p. Available at: http://www.biodiesel.pl/uploads/media/A_Look_Back_at_the_U.S._Department_of_Energy_s_Aquatic_Species_Program_Biodiesel_from_Algae_July_1998.pdf. [Accessed 2 January 2018].

Okazaki, Y. et al., 2013. A new class of plant lipid is essential for protection against phosphorus depletion. *Nature Communications*, 4, p.1510. Available at: http://dx.doi.org/10.1038/ncomms2512. [Accessed 2 January 2018].

Olvera-Gonzalez, E. et al., 2013. Intelligent lighting system for plant growth and development. *Computers and Electronics in Agriculture*, 92, pp.48–53. Available at: http://dx.doi.org/10.1016/j.compag.2012.11.012. [Accessed 2 January 2018].

Ooi, A. et al., 2016. Growth and development of Arabidopsis thaliana under single-wavelength red and blue laser light. *Scientific Reports*, 6(March), pp.1–13. Available at: http://dx.doi.org/10.1038/srep33885. [Accessed 2 January 2018].

Oren, A. (2014) 'The ecology of Dunaliella in high-salt environments', *Journal of Biological Research (Greece)*, 21(1), pp. 1–8. doi: 10.1186/s40709-014-0023-y.

Ortiz-Marquez, J.C.F. et al., 2012. Association with an ammonium-excreting bacterium allows diazotrophic culture of oil-rich eukaryotic microalgae. *Applied and environmental microbiology*, 78(7), pp.2345–52. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/22267660. [Accessed December 13, 2017].

Oviyaasri, M. et al., 2017. Extraction and Isolation of Polyunsaturated Fatty Acids from Microalgae Tetraselmis.Sp. *International Journal of Innovative Research in Science*, 6(3), pp.4810–4814. Available at: https://www.ijirset.com/upload/2017/march/273_ICPHD060.pdf. [Accessed 2 January 2018].

Özçimen, D., Gülyurt, M.Ö. & İnan, B., 2012. Algal Biorefinery for Biodiesel Production. *Biodiesel - Feedstocks, Production and Applications, Biodiesel Zhen Fang, IntechOpen*, p.34. Available at: https://www.intechopen.com/books/biodiesel-feedstocks-production-and-applications/algae-as-natural-biorefinery. [Accessed 2 January 2018].

Pappas, C. et al., 2016. Evaluation of a Raman Spectroscopic Method for the Determination of Alcohol Content in Greek Spirit Tsipouro. *Current Research in Nutrition and Food Science*, 09, pp.1–9. Available at: http://dx.doi.org/10.12944/CRNFSJ.4.Special-Issue-October.01. [Accessed 2 January 2018].

Parmar, A. et al., 2011. Cyanobacteria and microalgae: A positive prospect for biofuels. *Bioresource Technology*, 102(22), pp.10163–10172. Available at: http://dx.doi.org/10.1016/j.biortech.2011.08.030. [Accessed 2 January 2018].

Patel, B.D., 2015. Pesticides and sustainable agriculture. *International Pest Control*, 57(1), p.45.

Perugini, L. et al., 2017. Biophysical effects on temperature and precipitation due to land cover change Biophysical effects on temperature and precipitation due to land cover change. *Environ. Res. Lett.*, 12, pp.1–21.

Pittera, J. et al., 2018. Thermoacclimation and genome adaptation of the membrane lipidome in marine Synechococcus. *Environmental Microbiology*, 20(2), pp.612–631.

Posters, 2015. *European Journal of Phycology, 50(sup1)*, pp.121–217. Available at:

http://www.tandfonline.com/doi/full/10.1080/09670262.2015.1069493. [Accessed 2 January 2018].

Prathima Devi, M. & Venkata Mohan, S., 2012. CO 2 supplementation to domestic wastewater enhances microalgae lipid accumulation under mixotrophic microenvironment: Effect of sparging period and interval. *Bioresource Technology*, 112, pp.116–123. Available at: http://dx.doi.org/10.1016/j.biortech.2012.02.095. [Accessed 2 January 2018].

Praveenkumar, R., Shameera, K. & Mahalakshmi, G., 2011. Influence of nutrient deprivations on lipid accumulation in a dominant indigenous microalga Chlorella sp ., BUM11008 : Evaluation for biodiesel production. *Biomass and Bioenergy*, 37, pp.60–66. Available at: http://dx.doi.org/10.1016/j.biombioe.2011.12.035. [Accessed 2 January 2018].

Pulz, O. & Gross, W., 2004. Valuable products from biotechnology of microalgae. *Applied Microbiology and Biotechnology*, 65(6), pp.635–648.

Radakovits, R. et al., 2010. Genetic engineering of algae for enhanced biofuel production. *Eukaryotic Cell*, 9(4), pp.486–501.

Raeesossadati, M.J. et al., 2014. CO_2bioremediation by microalgae in photobioreactors: Impacts of biomass and CO_2 concentrations, light, and temperature. *Algal Research*, 6(PA), pp.78–85. Available at: http://dx.doi.org/10.1016/j.algal.2014.09.007. [Accessed 2 January 2018].

Rajkumar, R., Yaakob, Z. & Takriff, M.S., 2014. Potential of the micro and macro algae for biofuel production: A brief review. *BioResources*, 9(1), pp.1606–1633.

Ramanna, L., Rawat, I. & Bux, F., 2017. Light enhancement strategies improve microalgal biomass productivity. *Renewable and Sustainable Energy Reviews*, 80(December), pp.765–773.

Ramesha, D.K. et al., 2016. Experimental Investigation Of Biogas-Biodiesel Experimental investigation of biogas-biodiesel dual fuel combustion in a diesel engine. *De Gruiter MECDC*, 0003, pp.12–20.

Redeckas, K. et al., 2017. Unveiling the excited state energy transfer pathways in peridinin-chlorophyll a-protein by ultrafast multi-pulse transient absorption spectroscopy. *Biochimica et Biophysica Acta - Bioenergetics*, 1858(4), pp.297–307. Available at: http://dx.doi.org/10.1016/j.bbabio.2017.01.014. [Accessed 2 January 2018].

Renaud, S.M. et al., 2002. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. *Aquaculture*, 211(1–4), pp.195–214. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0044848601008754. [Accessed 2 January 2018].

Renger, T., 2009. Theory of excitation energy transfer: From structure to function. *Photosynthesis Research*, 102(2), pp.471–485.

Richmond, A. & Hu, Q., 2013. Handbook of Microalgal Culture: Applied Phycology and Biotechnology: Second Edition. *Handbook of Microalgal Culture: Applied Phycology and Biotechnology: Second Edition*, 1002, pp.1–719.

Rizzo, F. et al., 2014. Wavelength dependence of the fluorescence emission under conditions of open and closed Photosystem II reaction centres in the green alga Chlorella sorokiniana. *Biochimica et Biophysica Acta - Bioenergetics*, 1837(6), pp.726–733. Available at: http://dx.doi.org/10.1016/j.bbabio.2014.02.009. [Accessed 2 January 2018].

Rocca, S. et al., 2015. Biofuels from algae: technology options, energy balance and GHG emissions: Insights from a literature review. *EUR 27582*, pp.14-28. doi 10.2790/125847

Rodolfi, L. et al., 2009. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnology and Bioengineering, 102(1), pp.100–112.

Rodríguez, M.C. et al., 2007. Effects of chromium on photosynthetic and photoreceptive apparatus of the alga Chlamydomonas reinhardtii. *Environmental Research*, 105(2), pp.234–239.

Roesijadi, G., Jones, S.B. & Zhu, Y., 2010. Macroalgae as a Biomass Feedstock : A Preliminary Analysis. *Analysis*, (September), pp.1–50. Available at: http://www.pnl.gov/main/publications/external/technical_reports/PNNL-19944.pdf. [Accessed 2 January 2018].

Roux, J.M., Lamotte, H. & Achard, J.L., 2017. An Overview of Microalgae Lipid Extraction in a Biorefinery Framework. *Energy Procedia*, 112(October 2016), pp.680–688. Available at: http://dx.doi.org/10.1016/j.egypro.2017.03.1137. [Accessed 2 January 2018].

Roy, F. et al., 2016. Algae and Aquatic Plant. , *Rideau Valley Conservation Authority*, p.24. Available at: http://www.southfrontenac.net/en/town-hall/resources/Algae-Manual-Concise-Final.pdf. [Accessed 2 January 2018].

Rühle, T. et al., 2008. A novel screening protocol for the isolation of hydrogen producing Chlamydomonas reinhardtii strains. *BMC plant biology*, 8, pp.107–120. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2576467&tool=pmcentrez&rendertype=abstract. [Accessed 2 January 2018].

Rutherford, A.W. & Faller, P., 2003. Photosystem II: evolutionary perspectives. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 358(1429), pp.245–253. Available at: http://rstb.royalsocietypublishing.org/cgi/doi/10.1098/rstb.2002.1186. [Accessed 2 January 2018].

Saifuddin N. et al., 2017. Enhancement of Bio-Hydrogen Production in Chlamydomonas Reinhardtii by Immobilization and Co-Culturing. *International Journal of Bio-Science and Bio-Technology*, Vol. 9, No. 1, pp.35-52 http://dx.doi.org/10.14257/ibsbt.2017.9.1.03

Sakarika, M., 2016. *Kinetic study of biomass growth and lipid synthesis of the microalga Chlorella vulgaris under heterotrophic conditions and energy valorization of the produced biomass*. Master Thesis, University of Patras.

Sakurai, K., Moriyama, T. & Sato, N., 2014. Detailed identification of fatty acid

isomers sheds light on the probable precursors of triacylglycerol accumulation in photoautotrophically grown Chlamydomonas reinhardtii. *Eukaryotic Cell*, 13(2), pp.256–266.

Sandefur, H.N., Matlock, M.D. & Costello, T.A., 2011. Seasonal productivity of a periphytic algal community for biofuel feedstock generation and nutrient treatment. *Ecological Engineering*, 37(10), pp.1476–1480. Available at: http://dx.doi.org/10.1016/j.ecoleng.2011.04.002. [Accessed 2 January 2018].

Sato, N. et al., 2000. Environmental effects on acidic lipids of thylakoid membranes. *Biochemical Society transactions*, 28(6), pp.912–914.

Satpati, G.G., Gorain, P.C. & Pal, R., 2016. Efficacy of EDTA and Phosphorous on Biomass Yield and Total Lipid Accumulation in Two Green Microalgae with Special Emphasis on Neutral Lipid Detection by Flow Cytometry. *Advances in Biology*, 2016, pp.1–12. Available at: http://www.hindawi.com/journals/ab/2016/8712470/. [Accessed 2 January 2018].

SCHOTT, 2017. Open ponds vs. closed systems. pp.2–4. Available at: http://microsites.schott.com/pbr/english/systems/systems.html. [Accessed February 4, 2018].

Schultz, C.M. et al., 2009. Narrow vertical far-field 975-nm broad-area DFB lasers for wide temperature range operation. *IEEE Photonics Technology Letters*, 21(9), pp.593–595.

Schubert, E.F., 2006, *Light-Emitting Diodes*, 2nd edn, Cambridge Universtiy Press, New York.

Scott, S.A. et al., 2010. Biodiesel from algae: Challenges and prospects. *Current Opinion in Biotechnology*, 21(3), pp.277–286. Available at: http://dx.doi.org/10.1016/j.copbio.2010.03.005. [Accessed 2 January 2018].

Scranton, M.A. et al., 2017. Chlamydomonas as a model for biofuels and bio-products production. *Plant J. 2015 May*, 82(3), pp.523–531.

Sebastian, M. et al., 2016. Lipid remodelling is a widespread strategy in marine

heterotrophic bacteria upon phosphorus deficiency. *ISME Journal*, 10(4), pp.968–978.

Semchonok, D. A., 2016. *Structures of photosynthetic membrane complexes*. Doctoral Thesis, University of Groningen.

Şener, M. et al., 2012. Förster energy transfer theory as reflected in the structures of photosynthetic light harvesting systems. *NIH Public Access*, 12(3), pp.518–531.

Sforza, E. et al., 2014. Effects of light on cultivation of scenedesmus obliquus in batch and continuous flat plate photobioreactor. *Chemical Engineering Transactions*, 38, pp.211–216.

Sharma, A. & Arya, S.K., 2017. *Hydrogen from algal biomass: A review of production process. Biotechnology Reports*, 15(June), pp.63–69. Available at: http://dx.doi.org/10.1016/j.btre.2017.06.001. [Accessed 2 January 2018].

Shelaev, I. V. et al., 2011. P680(PD1PD2) and ChlD1as alternative electron donors in photosystem II core complexes and isolated reaction centers. *Journal of Photochemistry and Photobiology B: Biology*, 104(1–2), pp.44–50. Available at: http://dx.doi.org/10.1016/j.jphotobiol.2011.02.003. [Accessed 2 January 2018].

Shen, Y. et al., 2014. Influence of algae species, substrata and culture conditions on attached microalgal culture. *Bioprocess and Biosystems Engineering*, 37(3), pp.441–450.

Show, P.L. et al., 2017. A holistic approach to managing microalgae for biofuel applications. *International Journal of Molecular Sciences*, 18(1).

Sialve, B., Bernet, N. & Bernard, O., 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnology Advances*, pp.1–8. Available at: http://dx.doi.org/10.1016/j.biotechadv.2009.03.001. [Accessed 2 January 2018].

Sills, D.L. et al., 2013. Quantitative Uncertainty Analysis of Life Cycle Assessment for Algal Biofuel Production. *Environmental Science & Technology*, 47(2), pp.687–

694. Available at: http://pubs.acs.org/doi/abs/10.1021/es3029236. [Accessed March 20, 2017].

Simosa, A.E., 2016. *Factors affecting algal biomass growth and cell wall destruction*. Master Thesis, University of New Orleans.

Singh, A., Nigam, P.S. & Murphy, J.D., 2011. Mechanism and challenges in commercialisation of algal biofuels. *Bioresource Technology*, 102(1), pp.26–34. Available at: http://dx.doi.org/10.1016/j.biortech.2010.06.057. [Accessed 2 January 2018].

Singh, A.K., Singh, A.K. & Singh, S., 2018. An Overview on : Next Generation Cost Effective Biofuel Production from Algae. *International Journal of Current Microbiology and Applied Sciences*, (7), pp.4139–4149.

Singh, N., & Dhar, D., 2011. Microalgae as second generation biofuel. A review. Agronomy for Sustainable Development. *Springer Verlag/EDP Sciences/INRA*, *2011*, 31 (4), pp.605-629.

Singh, R., & Kumar, S., 2017. *Green Technologies and Environmental Sustainability*, 1st edn, Springer International Publishing. Switzerland. 10.1007/978-3-319-50654-8. doi: 10.1007/978-3-319-50654-8

Singh, R.N. & Sharma, S., 2012. Development of suitable photobioreactor for algae production - A review. *Renewable and Sustainable Energy Reviews*, 16(4), pp.2347–2353.

Singh, S.P. & Singh, P., 2015. Effect of temperature and light on the growth of algae species: A review. *Renewable and Sustainable Energy Reviews*, 50, pp.431–444. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1364032115004839. [Accessed 2 January 2018].

Singh, V.P. et al., 2015. Towards quantification of vibronic coupling in photosynthetic antenna complexes. *Journal of Chemical Physics*, 142(21), pp.1–8. Available at: http://dx.doi.org/10.1063/1.4921324. [Accessed 2 January 2018].

Smith, R.T. & Gilmour, D.J., 2018. The influence of exogenous organic carbon

assimilation and photoperiod on the carbon and lipid metabolism of Chlamydomonas reinhardtii. *Algal Research*, 31(May 2017), pp.122–137. Available at: https://doi.org/10.1016/j.algal.2018.01.020. [Accessed 2 January 2018].

Solovchenko, A. et al., 2013. Probing the effects of high-light stress on pigment and lipid metabolism in nitrogen-starving microalgae by measuring chlorophyll fluorescence transients: Studies with a δ5 desaturase mutant of Parietochloris incisa (Chlorophyta, Trebouxiophyceae). *Algal Research*, 2(3), pp.175–182. Available at: http://dx.doi.org/10.1016/j.algal.2013.01.010. [Accessed 2 January 2018].

Sousa, C.F.E., 2013. *Oxygen accumulation in photobioreactors*. Doctoral Thesis, Wageningen University. Available at: http://library.wur.nl/WebQuery/wda/lang/2029104. [Accessed 2 January 2018].

Stanghellini, C., Dai, J. & Kempkes, F., 2011. Effect of near-infrared-radiation reflective screen materials on ventilation requirement, crop transpiration and water use efficiency of a greenhouse rose crop. *Biosystems Engineering*, 110(3), pp.261–271. Available at: http://dx.doi.org/10.1016/j.biosystemseng.2011.08.002. [Accessed 2 January 2018].

Stick, R. V. & Williams, S.J., 2009. Formation of the Glycosidic Linkage. In *Carbohydrates: The Essential Molecules of Life. Elsevier*, pp. 133–202. Available at: https://www.sciencedirect.com/science/article/pii/B9780240521183000041. [Accessed 2 January 2018].

Sushchik, N.N. et al., 2003. A temperature dependence of the intra-and extracellular fatty-acid composition of green algae and cyanobacterium. *Russian journal of plant physiology*, 50(3), pp.374–380.

Svaldenis, A., 2014. *Cultivating Algae in a Photobioreactor: Synthetic wastewater nutrient removal and carbon capture efficiency using the green algae species Chlorella pyrenoidosa 35*. Degree Programme in Environmental Engineering Thesis, Helsinki Metropolia University of Applied Sciences.

Tait, L.W. & Schiel, D.R., 2013. Impacts of Temperature on Primary Productivity and Respiration in Naturally Structured Macroalgal Assemblages. *PLoS ONE*, 8(9), pp.1–10.

Teo, C.L. et al., 2014. Enhancing growth and lipid production of marine microalgae for biodiesel production via the use of different LED wavelengths. *Bioresource Technology*, 162, pp.38–44. Available at: http://dx.doi.org/10.1016/j.biortech.2014.03.113. [Accessed 2 January 2018].

Thornley, P, & Adams, 2017, *Greenhouse Gas Balances of Bioenergy Systems*, 1st edn, Elsevier Science & Technology, Saint Louis. Available from: ProQuest Ebook Central. [Accessed 2 January 2018].

Tiwari, V., Peters, W.K. & Jonas, D.M., 2013. Electronic resonance with anticorrelated pigment vibrations drives photosynthetic energy transfer outside the adiabatic framework. *Proceedings of the National Academy of Sciences of the United States of America*, 110(4), pp.1203–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23267114. [Accessed February 4, 2018].

Tossavainen, M. et al., 2018. Conversion of biowaste leachate to valuable biomass and lipids in mixed cultures of Euglena gracilis and chlorophytes. *Algal Research*, 35(July), pp.76–84.

Tredici, M.R. et al., 2015. Energy balance of algal biomass production in a 1-ha "Green Wall Panel" plant: How to produce algal biomass in a closed reactor achieving a high Net Energy Ratio. *Applied Energy*, 154, pp.1103–1111. Available at: http://dx.doi.org/10.1016/j.apenergy.2015.01.08. [Accessed 2 January 2018].

Tsao, J.Y. et al., 2014. Toward Smart and Ultra-efficient Solid-State Lighting. *Advanced Optical Materials*, 2(9), pp.809–836.

Tsygankov, A.A. et al., 2006. Hydrogen production by sulfur-deprived Chlamydomonas reinhardtii under photoautotrophic conditions. *International Journal of Hydrogen Energy*, 31(11), pp.1574–1584.

Ugwu, C.U., Aoyagi, H. & Uchiyama, H., 2007. Influence of irradiance, dissolved

oxygen concentration, and temperature on the growth of Chlorella sorokiniana. *Photosynthetica*, 45(2), pp.309–311.

Vejrazka, C. et al., 2012. Photosynthetic efficiency of chlamydomonas reinhardtii in attenuated, flashing light. *Biotechnology and Bioengineering*, 109(10), pp.2567–2574.

Venkanna, D., 2017. *Synthetic rewiring of Chlamydomonas reinhardtii to improve biological H 2 production*. Doctoral Thesis, Bielefeld University.

Vishay, 2002. Silicon PIN Photodiode. *Vishay Semiconductors*, pp.1–5.

Wagner, I., 2018. *Triplet state dynamics of pigments in photosynthetic light- harvesting complexes*. Master Thesis, Charles University.

Wagner, I., Steinweg, C. & Posten, C., 2016. Mono- and dichromatic LED illumination leads to enhanced growth and energy conversion for high-efficiency cultivation of microalgae for application in space. *Biotechnology Journal*, 11(8), pp.1060–1071.

Wang, J.K. & Seibert, M., 2017. Prospects for commercial production of diatoms. *Biotechnology for Biofuels*, 10(1), pp.1–13.

Weissman, J.C. & Goebel, R.P., 1987. *Design and analysis of microalgal open pond systems for the purpose of producing fuels: A subcontract report*, Microbial Products, tnc., Fairfield, California. Available at: http://www.osti.gov/servlets/purl/6546458-qOBOVX/. [Accessed 2 January 2018].

Widjaja, A., Chien, C.C. & Ju, Y.H., 2009. Study of increasing lipid production from fresh water microalgae Chlorella vulgaris. *Journal of the Taiwan Institute of Chemical Engineers*, 40(1), pp.13–20.

Wientjes, E., Van Amerongen, H. & Croce, R., 2013. Quantum yield of charge separation in photosystem II: Functional effect of changes in the antenna size upon light acclimation. *Journal of Physical Chemistry B*, 117(38), pp.11200–11208.

Wierer, J.J., Tsao, J.Y. & Sizov, D.S., 2014. The potential of III-nitride laser diodes for solid-state lighting. *Physica Status Solidi (C) Current Topics in Solid State*

Physics, 11(3–4), pp.674–677.

William W. Biggs, 2003. Principles Of Radiation Measurement. *Prehospital Emergency Care*, 7(1), pp.157–158. Available at: http://www.tandfonline.com/doi/full/10.1080/10903120390937364. [Accessed 2 January 2018].

WRI, 2015. *Clean Energy Access In Developing Countries: Perspectives On Policy and Regulation*. Issue Brief, World Resources Institute.

Xin, L. et al., 2010. Bioresource Technology Effects of different nitrogen and phosphorus concentrations on the growth , nutrient uptake , and lipid accumulation of a freshwater microalga Scenedesmus sp . *Bioresource Technology*, 101(14), pp.5494–5500. Available at: http://dx.doi.org/10.1016/j.biortech.2010.02.016. [Accessed 2 January 2018].

Xu, Y., Ibrahim, I.M. & Harvey, P.J., 2016. The influence of photoperiod and light intensity on the growth and photosynthesis of Dunaliella salina (chlorophyta) CCAP 19/30. *Plant Physiology and Biochemistry*, 106, pp.305–315. Available at: http://dx.doi.org/10.1016/j.plaphy.2016.05.021. [Accessed 2 January 2018].

Yadala, S. & Cremaschi, S., 2016. A Dynamic Optimization Model for Designing Open-Channel Raceway Ponds for Batch Production of Algal Biomass. *Processes*, 4(2), p.10. Available at: http://www.mdpi.com/2227-9717/4/2/10. [Accessed 2 January 2018].

Yadufashije, C., 2018. *Lecture Notes : Cell Biology*. Ideal International E- Publication, pp.13-14

Yang, J. et al., 2017. Experimental study on microalgae cultivation in novel photobioreactor of concentric double tubes with aeration pores along tube length direction. *International Journal of Green Energy*, 14(15), pp.1269–1276. Available at: https://doi.org/10.1080/15435075.2017.1402772. [Accessed 2 January 2018].

Yacobi, Y.Z. et al., 2015. Phycocyanin-specific absorption coefficient: Eliminating the effect of chlorophylls absorption. *Limnology and Oceanography: Methods*, 13(4),

pp.157–168.

Ying, K., 2014. Effects of CO_2 and pH on Growth of the Microalga Dunaliella salina. *Journal of Microbial & Biochemical Technology*, 06(03), pp.167–173. Available at: https://www.omicsonline.org/open-access/effects-of-co-and-ph-on-growth-of-the-microalga-dunaliella-salina-1948-5948.1000138.php?aid=24456. [Accessed 2 January 2018].

Yuen-Zhou, J., Krich, J.J. & Aspuru-Guzik, A., 2012. A witness for coherent electronic vs vibronic-only oscillations in ultrafast spectroscopy. *Journal of Chemical Physics*, 136(23).

Zhang, D. et al., 2015. Analysis of green algal growth via dynamic model simulation and process optimization. *Biotechnology and Bioengineering*, 112(10), pp.2025–2039. Available at: http://doi.wiley.com/10.1002/bit.25610. [Accessed 2 January 2018].

Zhang, N., Lior, N. & Jin, H., 2011. The energy situation and its sustainable development strategy in China. *Energy*, 36(6), pp.3639–3649. Available at: http://dx.doi.org/10.1016/j.energy.2011.01.035. [Accessed 2 January 2018].

Zhang, Q. et al., 2016. Förster Energy Transport in Metal-Organic Frameworks Is beyond Step-by-Step Hopping. *Journal of the American Chemical Society*, 138(16), pp.5308–5315.

Zhang, Z. & Wang, J., 2016. Origin of long-lived quantum coherence and excitation dynamics in pigment-protein complexes. *Scientific Reports*, 6, pp.1–9. Available at: http://dx.doi.org/10.1038/srep37629. [Accessed 2 January 2018].

Zheng, Y., 2013. *Two-Stage Heterotrophic and Phototrophic Culture Technology for Microalgal Biofuel Production*. Doctor of Philosophy, Washington State University.

Zhongye, L. 2013. *Microalgal Growth and Lipid Production from Organic Waste*. Doctor of Philosophy. University of Akron.

Zili, F. et al., 2014. Fatty acid composition of the thermophilic Gloeocapsa gelatinosa

under different combinations of temperature, light intensity, and NaNO3 concentration. *Journal of Applied Phycology*, (April), pp.97–107.

Zou, Y. et al., 2017. An Animal-Like Cryptochrome Controls the Chlamydomonas Sexual Cycle. *Plant Physiology*, 174(3), pp. 1334–1347. doi: 10.1104/pp.17.004