Development of luminescent photobioreactors for improved cultivation of microalgae

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Abstract

This study investigated the effects of light quality, culture density, and carbon dioxide aeration on the biomass production, lipid accumulation, elemental contents, and photosynthetic pigment production of microalgae. The microalgae *Chlorella vulgaris* and *Gloeothece membranacea* were cultivated either in static (flask) or aerated and mixed (photobioreactor) cultivation modes. The highest biomass productivities in the static mode for both microalgae strains were achieved using violet luminescent filters which emitted the photosynthetic active radiation ranges. In the static mode, red wavelength ranges had a major influence on pigmentation of *C.vulgaris*, although they were the least efficient light condition for cultivation of *G.membranacea*. Red light was particularly inefficient to support growth in static cultures. Luminescent bubble column photobioreactors (PBRs) were constructed and used for the aerated mode in the blue, green, yellow, orange, and red wavelength-emitting ranges. Biomass production in both strains was enhanced in the red PBR. Lipid accumulation was significantly enhanced in high culture density groups, low light intensity, and 15% CO_2 aeration conditions which all represented a form of stress to the culture. The highest biomass density of 2.12 and 2.52 g L^{-1} were achieved at high light intensity in the red PBR for *C.vulgaris* and *G.membranacea* respectively. The blue PBR was the least efficient light condition for biopigment production, whilst it improved lipid accumulation. Chlorophyll production in *C.vulgaris* was promoted by the green PBR, although it was less influenced by the light condition or culture density in *G.membranacea* cultures. Phycobiliproteins were the dominant pigments in *G.membranacea* and red light favoured synthesis of these pigments. Compared to *C.vulgaris*, the *G.membranacea* cells showed higher tolerance to 15% CO_2 aeration by growing in this condition over a period of two weeks. At the 15% CO_2 aeration condition *G.membranacea* accumulated the highest lipid content of 36.6% in the blue PBR. However, due to their lower carbon content and biomass productivity, *G.membranacea* was less efficient than *C.vulgaris* in sequestrating carbon dioxide. *C.vulgaris* sequestrated carbon dioxide up to 363 mg L^{-1} day^{-1} in the red PBR when aerated with 15% CO_2. These results have significant implications for improved photobioreactor design for cultivation of microalgae under natural light. The proposed photobioreactor design can also tailor and improve the composition of lipids and photosynthetic compounds, using variation in light.

DEDICATION

This thesis is dedicated to the memory of my dear father whose greatest dream was to see me achieving higher education and completing my PhD. He is no longer with us but his powerful presence in my life helped me to reach this stage and finish my PhD successfully.

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Contents

List of Figures

List of Tables

List of Publications

- Mohsenpour S.F, Richards B, Willoughby N. (2012). Spectral conversion of light for enhanced microalgae growth rates and photosynthetic pigment production. *Bioresource Technology*, 125, 75-81.

- Mohsenpour S.F, Willoughby N. (2013). Luminescent photobioreactor design for improved algal growth and photosynthetic pigment production through spectral conversion of light. *Bioresource Technology*, 142, 147-153.

- Mohsenpour S.F, Willoughby N. (December 2013). 7th International Algae Congress, Hamburg, Germany. Spectral conversion of light for improved microalgae cultivation

Chapter 1: Introduction

1.1 Introduction

Microalgae are photosynthetic organisms capable of sequestrating carbon dioxide and converting solar energy into various chemical compounds (Chisti, 2007). Attempts have been made for decades to produce microalgae on a technological scale. Cultivation of microalgae as a sustainable process brings numerous advantages to different industrial sectors. The growth requirements of microalgae such as carbon dioxide, light, and water, target the most prominent industrial processes in which environmental impacts and renewable energy generation are of great concern.

Global warming and the impact of carbon dioxide emissions on climate change have drawn further attention onto microalgae. As the dry biomass of microalgae can contain up to 50% carbon, they can potentially sequestrate CO_2 in optimised conditions. During the 1970's the importance of finding alternative energy sources instead of fossil fuels attracted global interest towards biological applications of solar energy (Becker, 1994a). Microalgae require sunlight for photosynthesis and the quality of light can significantly influence the synthesis of their biochemical compounds.

Furthermore, increased awareness regarding waste disposal hazards and environmental pollution can be linked to the development of microalgae production technologies. Waste water industry could potentially benefit from the use of microalgae. Depending on the species, microalgae are capable of absorbing various heavy metals and toxic components and the waste water can be used as culture media bringing advantages to the renewable energy sector.

Microalgae are also potential sources of high-value components such as natural pigments, polyunsaturated fatty acids, proteins and polysaccharides that are widely used in food, pharmaceutical and cosmetics industries (Chen et al., 2011).

Several research studies have investigated the most efficient cultivation systems for a sustainable microalgae production and introduced different photobioreactor designs. The study presented here is mainly focused on light optimisation as a critical factor for growth of green and blue-green algae in closed cultivation systems. Following a review of recent studies in the relevant field, this research study investigates the impact of

spectral conversion of light on the selected microalgae strains and discusses various growth patterns obtained.

Luminescent bubble column photobioreactors (PBRs) were constructed in different spectral ranges to obtain a comparative study. Luminescent PBR were made of Poly methyl methacrylate (PMMA) doped with luminescent dyes or fluorophores. Luminescent dyes have been extensively used for construction of luminescent solar cells (LSCs). LSCs are non-tracking solar concentrators capable of absorbing and redirecting sunlight by fluorophores into waveguide materials. As luminescent dyes are commercially available in a wide range of visible-emitting or infra-red-emitting, their use in photobioreactors can bring potential advantages to microalgae cultivation. The organic visible-emitting dyes were selected in this study as they tailor light into the photosynthetic active radiation ranges. By incorporating luminescent dyes into materials for photobioreactor construction, the tailoring of spectral quality of light can be combined with improved mixing and gas transfer.

This work aims to demonstrate luminescent photobioreactors can be used to enhance the efficiency of outdoor cultivation systems in areas of sub-optimal sunlight exposure without the need for artificial lighting systems, offering a cheaper and more energy-efficient route to large-scale microalgal culture and CO_2 mitigation.

1.2 Aims and objectives

The study was focused on investigating the potential of luminescent photobioreactors for algal biotechnology, and to identify future research directions. The performance of luminescent PBRs was evaluated by a series of experiments looking at their effects of culture density, carbon dioxide aeration, and light quality through spectral conversion of light on the biomass production, lipid accumulation, elemental contents, and photosynthetic pigment production of microalgae. Spectral conversion of light was achieved by using luminescent material in different forms and ranges (flat panels or cylinders). Visible-emitting fluorophores provided photosynthetic active radiation ranges essential for growth of microalgae and their influences on various aspects of cultivation was evaluated by:

- Critical comparison of growth patterns and biomass production in different cultivation modes.
- Determination of photosynthetic pigments content for investigation of the effect of specific wavelengths on photosynthetic quality.

- Evaluation of cell lipid content as a measure for biofuel production.
- Analysis of carbon dioxide consumption through elemental analysis and productivity rates.

Data analysis obtained from the experiments provided an understanding of cell growth rate and biomass productivity which was then used to determine the carbon dioxide sequestration rate. Different cultivation modes including static and aerated cultivations under similar illumination methods, also provided a broader understanding on complex behaviour of microalgae to environmental changes.

Moreover, analysis of the bio-pigment content of the selected microalgae strains were used to illustrate the influence of specific light wavelengths on photosynthesis. The experiments focused on bio-pigmentation to explore the techniques in which a target pigment such as chlorophyll or phycobiliprotiens are induced.

The determination of cell lipid content in different luminescent photobioreactors was also undertaken to demonstrate the influence of different cultivation conditions as well as the combined effect of light wavelength and intensity on cells.

1.3 Outline of thesis

Chapter 2 reviews the issues related to microalgae biomass production, including an overview on global warming and CO_2 biofixation, a description of photosynthesis, microalgae and the selected strains for this study (including cultivation systems, illumination strategies, harvesting techniques and their use in biotechnology).
Chapter 3 describes the materials and methods used in the study. This includes a description of the cultures of *Chlorella vulgaris* and *Gloeothece membranacea,* the equipments used for experiments and the analytical methods applied for growth monitoring.

Chapter 4 presents the results of the static cultivation of microalgae carried out in the laboratory and discusses the growth patterns achieved under different light conditions.

Chapter 5 contains the results and discussions of the experiments carried out in luminescent photobioreactors including biomass and lipid production, and elemental analysis obtained for various culture conditions.

Chapter 6 presents the results obtained from cultivation in photobioreactors under various concentrations of carbon dioxide and examines the effect of CO_2 aeration on growth of microalgae, CO_2 fixation rate, total lipids, and elemental content.

Chapter 7 describes various pigmentation patterns produced by microalgae under the corresponding cultivation conditions presented in Chapters 5 and 6.

Chapter 8 summarises the main findings of the research and outlines suggestions for further work.

Chapter 2: Literature Review

2.1 Introduction

This chapter critically reviews the literature related to microalgae cultivation and illustrates the background information required for this research study. It starts by giving an overview on global warming and carbon dioxide bioremediation, followed by a description of photosynthesis, microalgae (including cultivation and harvesting techniques), biotechnological and environmental applications. The chapter then finishes by reviewing the potential of microalgae for food, pharmaceuticals and biofuel production.

2.2 Global warming and carbon dioxide bioremediation

Increasing levels of carbon dioxide concentration in the atmosphere and its major impacts on global warming has become a great concern in the past decades. Consequently, various measures for mitigating CO_2 emission have been introduced. Fossil fuels produce about 80% of global energy demands and are one of the major causes of environmental pollution and global climate change to date (Chen et al., 2011). Natural processes called sinks can balance and distribute the total amount of carbon in the atmosphere, biosphere and lithosphere (Marini, 2007). These sinks such as forests, oceans and soil capture CO_2 from the atmosphere in different ways, such as photosynthesis process in plants and algae or CO_2 dissolved in the waters of oceans. In total these sinks store up to 38×10^{12} tonnes of carbon dioxide. Although natural sinks play an important role in balancing the amount of carbon dioxide, human activities such as the combustion of fossil fuels, electricity generation, mineral and metal production and using petroleum-based products emit high amounts of CO_2 to the atmosphere. The development of several methods of carbon capture has been studied in recent decades. In 2008, the UK Department for Environment, Food and Rural Affairs (DEFRA) published some figures which showed the amount of carbon dioxide emissions per annum in the UK (Chu et al., 2013). This was initiated by the draft climate change bill presented to the UK parliament, aiming to cut 60% greenhouse gas emissions by 2050. Various strategies to reduce CO_2 emissions have been introduced. The proposed CO_2 mitigation strategies can be categorised into three major approaches:

- Direct CO_2 injection into the ocean or to the underground (Herzog, 2001, Israelsson et al., 2009).
- Chemical reaction-based approaches; the examples are amine coating activated carbon (Plaza et al., 2007), washing with alkaline solutions (Diao et al., 2004), and multi-walled carbon nanotubes (Su et al., 2009).
- Biological carbon dioxide mitigation by means of CO_2 conversion to organic compounds (Skjanes et al., 2007).

Carbon dioxide bioremediation can be achieved by means of photosynthesis in terrestrial plants or photosynthetic organisms (Ho et al., 2011). CO_2 sequestration by plants is considered as an inadequate strategy since plants have slow growth rates and their contribution to CO_2 fixation is currently limited to 3-6% of total fossil fuel emissions (Skjanes et al., 2007, Anjos et al., 2013, Kumar et al., 2011). In comparison, higher growth rates and photosynthetic efficiency makes microalgae capable of fixating carbon dioxide 10-50 times more than terrestrial plants (Chisti, 2007, Dragone et al., 2011). Microalgae biomass also brings some additional benefits from CO_2 reduction by being useful in various industrial products such as medications, nutritious foods, cosmetics as well as biofuels (Ho et al., 2011). Carbon dioxide sequestration by microalgae is a sustainable process which can be achieved in combination with various environmental protecting processes such as biosorption of heavy metals (Cetinkaya Donmez et al., 1999) and waste water treatment (Mallick, 2002).

Depending on the species and cultivation conditions, microalgae can accumulate lipid and oil up to 50-70% of their dry weight (Chisti, 2007). The oil produced by microalgae contains the suitable fatty acids for synthesis of biodiesel (Sidler, 2004). However, high scale production of microalgae is highly influenced by major technical challenges and economical feasibility. The following section will illustrate microalgae culture systems and the major biological processes associated with them. This will lead to an in depth investigation of different factors affecting microalgae cultivation and examination of the efficiency of the methods suggested.

2.3 Microalgae

Microalgae are photosynthetic organisms with the ability to convert carbon dioxide to potential sources of food, biofuels, and high-value bioactives (Walker et al., 2005). Varied evolutionary origins of microalgae are reflected in their profound diversity of

ecology and colonized habitats, cell size and cellular structure, morphology, photosynthetic pigments, storage and structural polysaccharides, and type of life history (Barsanti and Gualtieri, 2006a). Microalgae are highly diversified microorganisms and include both prokaryote and eukaryote species.

Microalgae are classified by biologists based on their lifecycle, basic cellular structure and pigmentation (Sheehan et al., 1998). There is no definitive classification system which could include all algae species because of the constant changes of revision in new genetic and ultrastructural evidence (Barsanti and Gualtieri, 2006a). Classification of the different algae groups based on the work of Van Den Hoek et al. (1995) is presented in Table 2.1.

Division	**Common name**	**Habitat**			
		Marine	**Freshwater**	**Terrestrial**	**Symbiotic**
Cyanophyta	Blue-Green algae	Yes	Yes	Yes	Yes
Prochlorophyta	N.A.*	Yes	N.D.**	N.D.**	Yes
Glaucophyta	N.A.*	N.D.**	Yes	Yes	Yes
Rhodophyta	Red algae	Yes	Yes	Yes	Yes
Heterokontophyta	Golden algae Yellow-Green algae Diatoms Brown algae	Yes	Yes	Yes	Yes
Haptophyta	Coccolithophorids	Yes	Yes	Yes	Yes
Cryptophyta	Cryptomonads	Yes	Yes	N.D.**	Yes
Chlorarachniophyta	N.A.*	Yes	N.D.**	N.D.**	Yes
Dinophyta	Dinoflagellates	Yes	Yes	N.D.**	Yes
Euglenophyta	Euglenoids	Yes	Yes	Yes	Yes
Chlorophyta	Green algae	Yes	Yes	Yes	Yes

Table 2.1, Classification of different algae groups (Van Den Hoek et al., 1996).* N.A.: (Not Applicable); **N.D. (Not Determined).

Based on this classification, prokaryotic algae are grouped into two divisions and eukaryotic algae are classified into nine divisions (Van Den Hoek et al., 1996). Aquatic microalgae can be found in freshwater spring, salt lakes, open water bodies, and even under ice in polar areas with high tolerance to a broad diversity of environmental conditions such as light intensity, temperature, pH, turbidity, and O_2 and CO_2 concentrations. In deep oceans the darkest zone recorded in which microalgae could grow was 268 m below sea level with a light intensity only 0.0005% of the light at the surface water. At this depth, the red part of sunlight spectrum is filtered out because it has lower energy content, presenting further penetration into the water. Although at this water depth the solar energy required for photosynthesis is insufficient, some microalgae species can grow and survive. Possession of accessory pigments capable of absorbing light wavelengths different from those of the green chlorophylls, give the ability to microalgae to transfer this absorbed light energy to chlorophyll-a. Chlorophyll-a is the only molecule capable of conversion of solar energy into chemical energy. Alternatively, microalgae species growing in high irradiance habitat typically contain pigments that protect them against photo-damage. The wide variety of colours found in different microalgae species are based on their accessory and protective pigments.

Algae are characteristically aquatic organisms, although they can be found in soil and some species are adapted to grow in uncommon habitats, such as attached on stones, mud, or sand, animals, and other algae and plants (Myers, 1976). Algal cells typically contain a nucleus (with the exception of cyanobacteria), chloroplast, and semisolid cytoplasm. Blue-green algae (Cyanobacteria) have no distinct chloroplast organisation or nucleus. The pigments such as chlorophyll and carotenoid are localised in the chloroplast. The cytoplasm contains an outer layer which is bounded by a permeable plasma membrane. The permeability of the membrane is a distinctive limiting characteristic which varies with different algal species. Algae are mainly unicellular. The others form colonial organisation in microscopic size, macroscopic mass, extensive filaments or flat sheets.

Methods of reproduction can be vegetative by single cell divisions or fragmentation of a colony, sexual by the union of gametes or asexual by producing motile spore. Cultivation of those species reproducing by vegetative or asexual modes provides a fast

and economical way for growing algae. However, it restricts genetic variability while sexual reproduction allows genetic recombination.

Microalgae biomass contains 50% carbon dry weight on average (Sanchez Miron et al., 2003). This carbon is mainly derived from carbon dioxide which is available from the air or from waste streams of factories.

A mole of CO_2 has a mass of 44 grams including 12 grams of carbon. Based on these premises, carbon dioxide consumption by each gram of microalgae can be obtained from equation 2.1 as below:

$$\frac{44\ (g\ CO_2/mol)}{12\ (g\ Carbon/mol)} \times \frac{0.50\ (\text{g Carbon})}{(g\ algae\ biomass)} = 1.83\ (g\ CO_2/\ g\ algae\ biomass) \qquad (2.1)$$

Equation (2.1) shows that one gram of algal dry cell weight can fix approximately 1.83 grams of carbon dioxide. Brennan and Owende (2010) reported that 54.9-67.7 tonnes of carbon dioxide per hectare can be fixed annually by 30-37 tonnes of algae per hectare, cultivated in raceway ponds. This confirms the validity of equation (2.1).

A number of criteria have been considered for selecting the microalgae strains;

- Environmental adaptation: the selected strain should have reasonable tolerance to different environmental conditions in order to be cultivated in various geographical areas.
- Productivity: biomass production in the active phase of growth must be accommodated by consistent growth rates to achieve uniform cultures with high biomass productivity.
- Temperature: species with high optimum temperatures for growth are not appropriate for cultivation in Northern Europe areas since obtaining the suitable temperature requires high energy consumption.
- Toxicity: it is crucial to select a non-toxic strain to avoid any possible risk during work with cyanobacteria and to use it in downstream stages as food or animal feed.

2.3.1 *Green algae (Chlorophyta)*

Green algae are eukaryotic organisms with a great range of somatic differentiation. Green algae are ubiquitous in marine, freshwater, and terrestrial habitats (Barsanti and Gualtieri, 2006a). Their photosynthetic pigments are mainly chlorophylls a and b. In

addition β- and γ-carotene, several xanthophylls as accessory pigments, and chlorophyll c are present (Van Den Hoek et al., 1996). Starch is the most important storage polysaccharide in chlorophyta.

The basis of classification of green algae is on the different level of their thallus organization. The thallus is composed of filaments or plates of cells and ranges in size in forms such as unicellular, colonial, and filamentous structure. Their simple structure lacks specialized tissues such as a stem, leaves, and conducting tissue typically found in higher plants. Their chloroplasts are green because they are not masked by accessory pigments and are enclosed by a double membrane (South and Whittick, 1987). Chloroplasts are photosynthetic compartments which contain the pigments for light absorbing and transferring the energy through a series of enzymatic and photochemical reactions (Barsanti and Gualtieri, 2006a).

2.3.1.1 *Chlorella vulgaris*

Chlorella vulgaris is a single celled, eukaryotic green algae with an average diameter of 3 µm (De Grooth et al., 1985). *C.vulgaris* is a strain of *Chlorella* which is a common inhabitant of fresh waters. Different *Chlorella* strains are typically tolerant to high temperatures and can grow between 15 and 40^{o}C in various cultivation conditions (Mohan et al., 2009). The hemicellulotic cell wall of *Chlorella* is the cause of its cell rigidity. In flagellate species the cell is surrounded by glycoprotein and in non-flagellate ones firm polysaccharide walls protect the main body of cells. Autotrophic cultivation of *Chlorella* strains (generally in open systems) can be obtained in inorganic mediums while heterotrophic and mixotrophic condition is achieved by adding acetic acid and glucose.

Chlorella has been used in production of various industrial products and it is one of the most cultivated eukaryotic microalgae (Mohan et al., 2009). Being a rich source of protein, lipid, polysaccharides, vitamins, carotenoids, minerals, and antioxidants has made *Chlorella* an interesting algae species that have been studied for decades. For this reason C.vulgaris was selected as one of the microalgae strains in this study, so that the results obtained from this study could be compared with other research studies in the field.

2.3.2 *Blue-green algae (Cyanophyta)*

Cyanophyta or blue-green algae are non-motile Gram-negative Eubacteria (Carr and Whitton, 1982). Blue-green algae, also known as cyanobacteria are the main

components of phytoplankton communities in oceans and freshwater. They are different from eukaryotic algae, structurally and biochemically. Lack of chloroplasts in their cells, variation in pigment complement, and lower content of cell chlorophyll-a are the major differences between prokaryotic cyanobacteria and eukaryotic algae (Agusti, 1991). Agustí (1991) showed that some species of cyanobacteria can grow as large planktonic colonies. Consequently, their absorption of light can be influenced by increasing the pigment packaging within the colony. These organisms dominate the ocean eco-systems and it is reported that there are approximately 10^{24} cyanobacterial cells in the oceans (Barsanti and Gualtieri, 2006a). The main photosynthetic pigments in cyanobacteria include blue and red phycobiliproetins, chlorophyll a, and carotenoides. Phycobiliproteins including phycoerythrin, phycocyanin, allophycocyanin, and phycoerythrocyanin are water soluble pigments which absorb portions of light energy in the *Photosynthetic Active Radiation* (PAR) range that are poorly utilized by chlorophylls (Sidler, 2004). Cyanophytan starch and lipids are two valuable photosynthetic products of cyanobacteria both with high carbon content (Graham, 2000).

2.3.2.1 *Gloeothece membranacea*

Gloeothece membranacea is a unicellular freshwater cyanobacterium. This strain of cyanobacteria typically occupies the subsurface spaces within sandstone and limestone rocks. Compared to other cyanobacterial strains such as *Spirulina*, studies about the characteristics of *G.membranacea* have been limited to some laboratory work. *G.membranacea* was a local cyanobacterial strain which was selected in this study because of its environmental adaptation and response to growth with available light conditions in Scotland and Northern Europe. One of the other reasons for this choice was the potential of freshwater cyanobacterial species in industrial applications and waste water treatments.

2.4 Photosynthesis

Photosynthesis is a natural mechanism of converting light energy into organic chemical energy which occurs in plants, algae and some bacteria species (Sparks, 2004). Photosynthesis, also known as photoautotrophism, consists of both physical and chemical reactions which encompasses two main groups (Barsanti and Gualtieri, 2006a).

In the first reaction group, known as “light dependent reactions”, the light energy absorbed by the photosynthetic organism breaks down water molecules to oxygen and hydrogen. Light dependent reactions are about absorption and transfer of photon energy and generation of chemical energy. The second reaction group, called “dark reactions”, involves conversion of inorganic molecules into organic matters (carbohydrates).

Approximately half of the incident sunlight intensity received on the earth belongs to the Photosynthetic Active Radiation (PAR) range. The PAR is the portion of light spectrum in the range of 400-700 nm, in which microalgae are able to use the light as a source of energy to produce biomass and oxygen (Madamwar et al., 2000).

Infrared (IR) and ultraviolet (UV) radiations are not suitable for photosynthesis process. Infrared photons contain lower energy and longer wavelengths (compared to the PAR range). When microalgae cells are exposed to infrared radiation, the low energy absorbed by pigment molecules conflicts the formation and development of the photosynthetic system of the cells. On the other hand, high energy content of UV photons can dislocate electrons from the electronic cloud and damage the molecular bonds in photosynthetic pigments (Barsanti and Gualtieri, 2006c).

The portions of solar energy which are suitable for biochemical processes are often lower and within narrow wavelength ranges when distributed in water. Photosynthesis processes in algae convert the energy of PAR into biologically utilizable energy through oxidation and reduction reactions. During photosynthesis carbon is converted from the highly oxidised states to significantly reduced compounds using the light energy.

Equation 2.2 specifies light as a substrate, chlorophyll-a as catalytic agent and $(CH_2O)_n$ as organic matter in the form of carbohydrates:

$$nCO_2 + nH_2O + light \xrightarrow{Chl\text{-}a} (CH_2O)_n + nO_2 \qquad (2.2)$$

Oxidation-reduction is the process of electron transfer from a donor molecule to an acceptor molecule. During the reaction, the acceptor is reduced and the donor is oxidized. A compound which was electrically neutral will become charged negatively and take a proton from water to return to electrical neutrality (Sparks, 2004).

Photosynthetic organisms capture light energy by different kinds of pigment molecules and convert the absorbed light energy into chemical energy. In light dependent reactions

the pigments (mainly the chlorophylls) absorb light from the sun or other illumination sources and create excited pigments.

A complex of various pigments and protein molecules which are assembled together to convert the light energy through a series of reactions is called the reaction centre (RC). The excitation energy can travel through the light-harvesting antenna molecules to the RC where an electron is lost. The lost electron can be replaced by an electron from the environment. Cyanobacteria and other photosynthetic organisms use the excited electrons to form nicotinamide adenine dinucleotide (NADH). At the same time, protons on the other side of the cellular membrane create a proton concentration gradient. This provides the energy to generate adenosine triphosphate (ATP) through the phosphorylation of adenosine diphosphate (Barsanti and Gualtieri, 2006a, Sparks, 2004). In the light independent reactions also known as the Calvin cycle, the energy of photosynthesis (i.e., ATP and NADPH) is used to convert various inorganic compounds to organic matters (see Figure 2.1).

One of the common reduced substances is carbon dioxide (CO_2) which can be converted to sugars and other organic carbon based molecules. Therefore, photosynthesis is always associated with carbon fixation process (Sparks, 2004). The energy inside the cells is transformed via two large protein complexes known as photosystem I (PSI) and photosystem II (PSII). PSI and PSII are surrounded by light harvesting complexes and photons of light absorbed by photosystems induce excitation of chlorophyll-a (Barsanti and Gualtieri, 2006a).

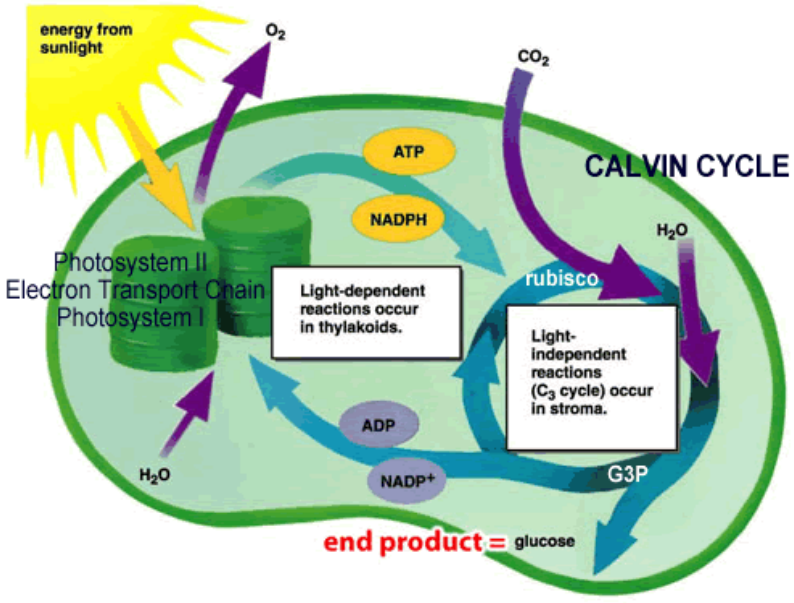


Figure 2.1, The photosynthesis cycle. Source: (Saracco, 2012)

2.5 Use of light by microalgae

Light is a form of electromagnetic radiation which has both particle and wave properties (Barsanti and Gualtieri, 2006c). Sunlight is mainly in the wavelength ranges of 300nm to 4000nm and known as the broadband or total solar radiation. Light wavelengths within this region contain different forms of energy which can be harmful or even mutagenic. Depending on the wavelength and energy levels, the total solar radiation is divide into ultra-violet radiation (UV), visible light (PAR or sight), and infra-red radiation (IR or heat).

Blue-green algae (cyanobacteria) lack a double-membrane organelle known as chloroplast and they capture light energy using chlorophyll, carotenes, and phycobiliproteins (Graham, 2000). Phycobiliproteins are the most important components of Phyco Bilisome Structures (PBS). PBS are peripheral membrane complexes in cyanobacteria that absorb light energy efficiently and transfer it to photosynthetic reaction centres. PBS are structured into two domains (Core and Rod), which contain 30% of the cellular protein in cyanobacteria (Grossman et al., 2001). Whilst the photosynthesis process in cyanobacteria occurs within the PBS, in green algae such as *Chlorella*, chloroplast encloses the photosynthetic membranes.

The rings or double bonds of the chemical structure of chlorophyll and other pigments enable them to capture light energy and convert it to the chemical components with carbon content, typically found in the cell structure, such as carbohydrates, lipids and proteins (as shown in Figure 2.2).

It must be noted that the photosynthetic pigments present in microalgae (except chlorophyll-a) have the important function of protecting photosynthetic assemblies in case of extreme illumination conditions. In addition, they transfer the absorbed light at specific wavelengths (not used by chlorophyll-a) to the essential wavelengths for the chlorophyll-a function.

Many elements of the light capture, carbon dioxide fixation systems, and electron transport in PSI and PSII, are similar in eukaryotic algae and cyanobacteria. Chlorophylls and carotenoids are carried in an internal antenna-domain in the PSI and PSII complexes. Light is mainly harvested by chlorophylls whereas carotenoids and other accessory pigments generally protect the system against excess light energy.

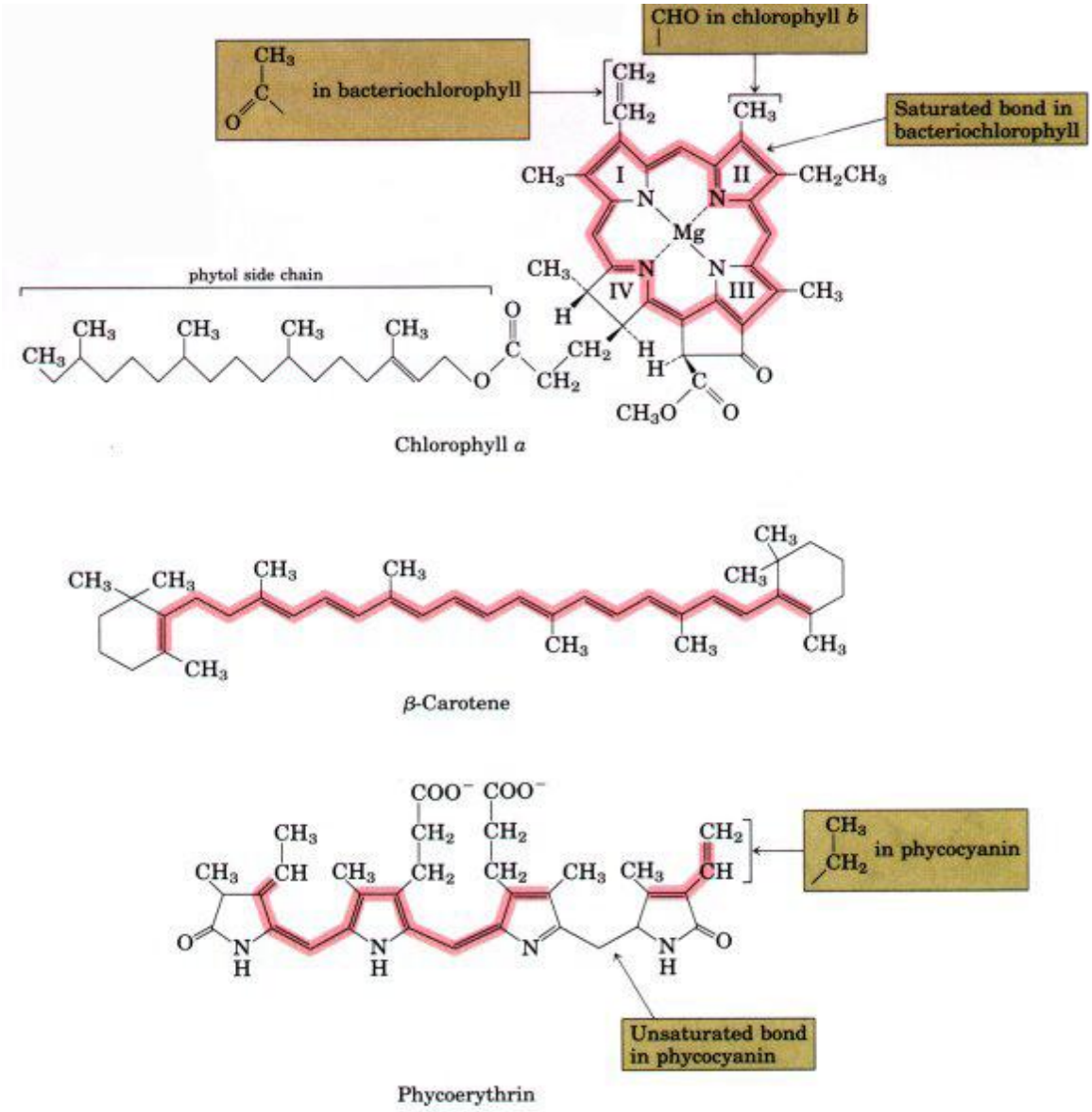


Figure 2.2, Structures of the primary photopigments chlorophylls a and b and bacteriochlorophyll, and of the accessory pigments, β-carotene (a carotenoid) and phycoerythrin and phycocyanin (phycobilins). The areas shaded in pink show structures (alternating single and double bonds) which largely account for the absorption of visible light. Source: (Widjaja et al., 2009)

Photoinhibition caused by excessive light intensity is a major problem in algae biomass productivity (Vonshak, 1997). Photoinhibition is a phenomenon which occurs when the level of light intensity exceeds the saturation level in which the maximum growth rate is

obtained. It has been reported that growth inhibition or reduction in cell population may occur when the light intensity is lower than the critical point (such as complete darkness) (Grobbelaar, 2004, Ogbonna and Tanaka, 2000).

Figure 2.3 shows changes of the specific growth rate with different light intensity levels. The graph indicates the importance of identification of optimum light intensity for obtaining the maximum specific growth rates in microalgae cultivation.

In addition to light intensity, the spectral quality of light and photoperiod (light:dark hours cycle) are important factors to improve the biomass productivity. A study suggested that red and blue wavelengths are associated with photon energy captured by PSI and PSII. The study indicated that microalgal growth under red light (600-700 nm) could promote PSII compared with PSI, while under blue light (400-500 nm) PSI could be induced (Ravelonandro et al., 2008).

Some algae species are capable of altering their photosystem complexes due to environmental changes. For instance some algae species change their light harvesting photosystem I from blue light wavelength range to green light (Ravelonandro et al., 2008).

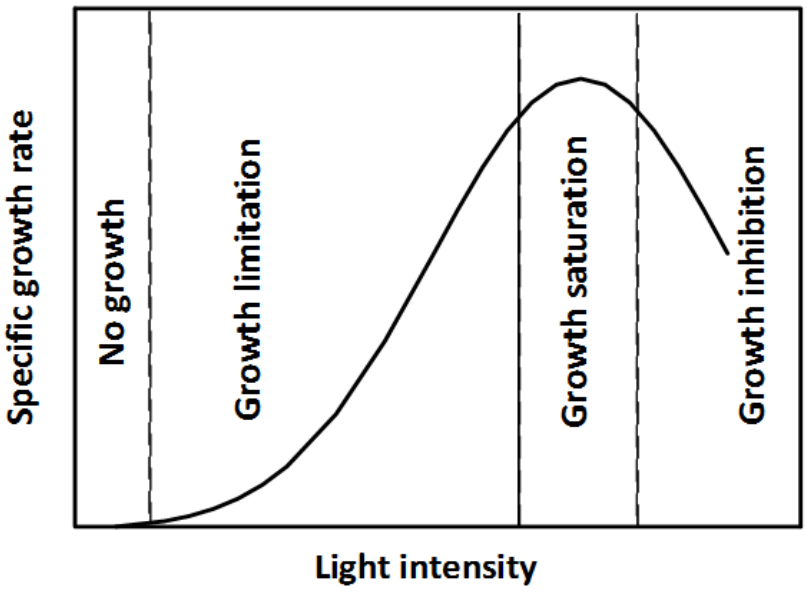


Figure 2.3, Effect of light intensity on photoautotrophic growth of photosynthetic cells. Source: (Ogbonna and Tanaka, 2000).

2.6 Chromatic adaptation

A large number of eukaryotic algae and photosynthetic prokaryotes can adapt their light harvesting ability with fluctuation in the ambient light regime (Bennett and Bogorad, 1973). The relationship between the potential and capability of organisms to receive and capture light energy in various processes such as onset of gene expression, metabolic and biochemical pathways, and assembly and synthesis of building blocks for special sub-cellular structures, have been broadly studied (Bennett and Bogorad, 1973, De Marsac, 1991, Mouget et al., 2004, Postius et al., 2001).

Cyanobacteria in particular have developed a light harvesting mechanism capable of optimisation of the spectral quality of light (Cohen et al., 2006). Some microalgae species are able to adjust their photosynthetic pigment system by changing the constituent of the photosynthetic apparatus and adapting the arrangement of different substances involved in light capture to ultimately transform the absorbed light into chemical energy.

Cyanobacteria also possess phycobilisomes which adjust their light capture capacity by changing the molar ratio of the phycobiliproteins (the pigmented protein within phycobilisomes). Bennet and Bogoard (1973) called this process "complementary chromatic adaptation". The genetic control and complete turnover of the phycobilisome in the chromatic adaptation of cyanobacteria have been studied by molecular biologists. Studying the synthesis of phycobiliproteins will lead to the understanding of genes involved in the mechanism of adaptation to various environmental conditions (Bennett and Bogorad, 1973, Chen et al., 2011).

2.7 Microalgae cultivation techniques

Microalgae cultivation techniques are categorised into two major systems which are open pond systems and closed systems also known as photobioreactors. These systems are typically useful for commercial or research purposes. The following section describes each system in details.

2.7.1 Open pond system

Open ponds are used most commonly for the bulk cultivation of microalgae. Open ponds have been designed in different shapes, sizes, and materials used for construction (Barsanti and Gualtieri, 2006a). Large outdoor ponds can be constructed as unlined with a natural bottom or lined with various inexpensive materials. Clay, cement, or brick are among low cost materials used. However, expensive plastics such as PVC sheets, glass fiber, and polyethylene can be used. There are four major pond design which have been developed among various designs suggested for pond construction (Moheimani, 2005). The main disadvantage of unlined ponds is silt suspension, percolation, and heavy contamination. In addition, unlined ponds can only be used for a limited variety of algal species which are adapted to the soil and environmental condition (Barsanti and Gualtieri, 2006a).

The main designs applied for large scale cultivation of microalgae are:

- Unstirred ponds (natural ponds and lakes)
- Inclined ponds
- Circular ponds with rotating agitator
- Raceway ponds

Unstirred ponds are the most economical designs which have been used for commercial production of β-carotene from some microalgae species (Borowitzka, 1988). The largest natural ponds were built in Australia for commercial production of *Dunaliella salina* (Barsanti and Gualtieri, 2006a). Unstirred ponds are typically shallow uncovered beds of natural water (less than 50 cm in depth) and can be used for cultivation of those species which are capable of growing under suboptimal conditions. Inclined ponds are another type of design with higher productivity. The algae culture suspension flows over a sloping surface from the top to the bottom and is pumped again to the top of the slope.

High turbulent flow, high surface to volume ratio and low culture depth (less than 1 cm) in inclined ponds are the advantages which result in obtaining high cell concentration of up to 10 g L^{-1} (Richmond, 1999). However, the main drawbacks of this design are sedimentation of cells, high evaporating loss and CO_2 desorption as well as high energy consumption for pumping the algal suspension. Circular ponds (as shown in Figures 2.4 and 2,5) with a centrally pivoted agitator have been used widely for wastewater

treatment (Moheimani, 2005). The raceway pond is the most commonly used design, which is a rectangular area divided into a number of channels with oval shapes (Schenk et al., 2008). In order to drive continuously the water flow and suspend the algae in the water, a paddle wheel is used.

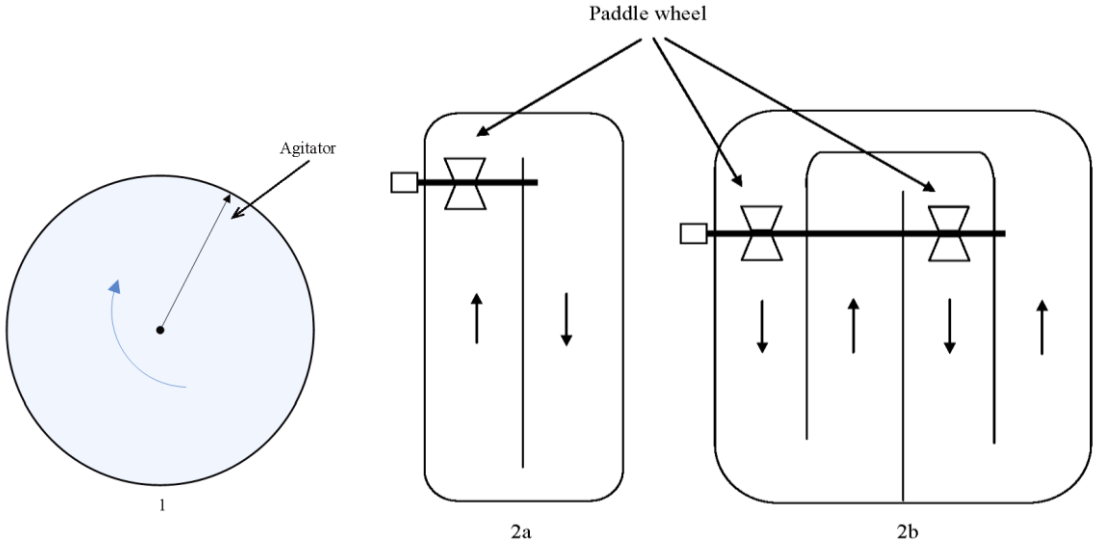


Figure 2.4, Schematic outline of microalgae pond designs. (1) circular pond with rotating agitator; (2a) single pond raceway with paddle wheel; (2b) joined ponds with paddle wheel.

(a)

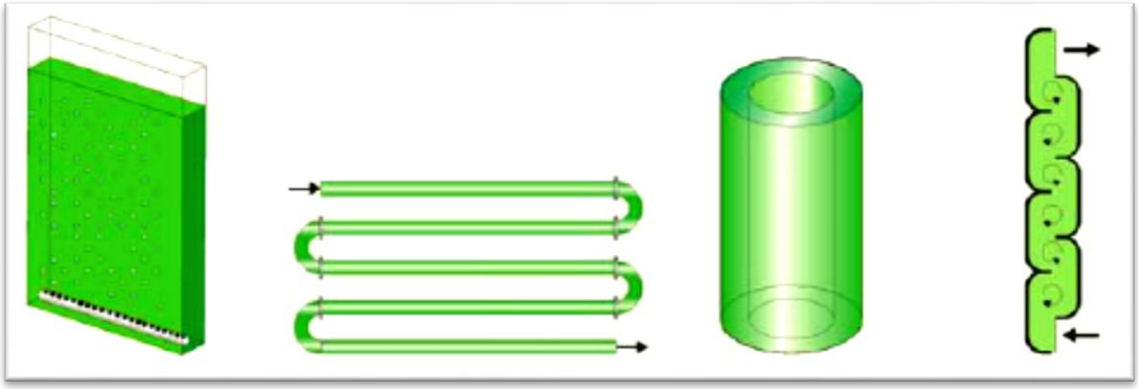
(b)

Figure 2.5, (a) large-scale circular algae pond; (b) large-scale raceway ponds with paddle wheel (Sieg, 2010).

Generally only one mixer should be used per pond to avoid any operational problem such as interference of mixers. To avoid settling and deposition of cells, the required mixing velocity depends on the sinking rate of the cells (Moheimani, 2005).
In order to expose algae to sufficient sunlight the water level in ponds is usually kept shallow. However, open raceways suffer from large amount of water evaporation by being exposed to the open air (Schenk et al., 2008).

2.7.2 Closed systems: Photobioreactors

Photobioreactors (PBR) are closed systems used for cultivation of microalgae. These devices provide a protected environment which isolates the microalgae culture and prevents the exchange of gases or contaminants from the atmosphere. Culture parameters such as temperature, oxygen and carbon dioxide concentration, and pH can be controlled in photobioreactors (Barsanti and Gualtieri, 2006a). Saving energy, water and chemicals as well as their higher productivity (related to bioreactor volume) are the advantages of using photobioreactors (Barbosa et al., 2003). Photobioreactors are mainly designed as tubular, bubble column, plate or flat panel reactors (see Figure 2.6).



A: Plate B: Tubular C: Bubble column D: Plate airlift

Figure 2.6, Different photobioreactors for production of microalgae biomass. A: a classic plate reactor, B: tubular reactor, can be designed in very large size, C: Bubble column reactor, in order to increase surface/volume ratio and to avoid dark parts the inner cylinder is kept empty, D: Plate airlift reactor with baffles, the fluid barrels are controlled to support flashing light effect. Source: (Schenk et al., 2008)

The first photobioreactor design was proposed followed by the investigation on the photosynthesis process in *Chlorella* in the late 1940's (Barsanti and Gualtieri, 2006a). Unlike open ponds, the PBR systems could guarantee optimisation and control of the continuous cultivation process and could be illuminated by both artificial and solar light. However, at the time most of the cultivation scale was conducted in small-scale in indoor laboratory conditions.

In recent years, understanding the growth dynamics of microalgae in large-scale production has supported significant progress in the development of mass cultivation techniques. The largest commercial systems available at present are in Germany and Israel. The German Klotze plant was designed for production of *Chlorella* biomass with a total PBR volume of 700 m^3 and the Algatechnologies plant was constructed for cultivation of *Haematococcus* in Israel (Barsanti and Gualtieri, 2006a, Eriksen, 2008, Ullmann et al., 2007). These plants use tubular photobioreactors and their biomass production has been recognised as economically feasible under Central European conditions. In addition, the Bio-Real Inc. Plant (a subsidiary of Fuji Chemical Industry Co., Ltd.) built in Hawaii (USA) and Astareal in Sweden are producing astaxanthin from the microalgae *Haematococcus* at industrial scale (AstaReal, 2012, FujiChemical, 2012).

Tubular photobioreactors consist of transparent tubes made of glass or plastic with lengths ranging 10-100 m and diameters of 3-6 cm. The tubes can be arranged in vertical, horizontal, vertical helical or straight tubes to obtain higher light penetration into the culture. The culture can be injected mechanically or through airlift pumps into the tubes (Moheimani, 2005).

Using airlift pumps in tubular photobioreactors provides a robust culture system and reduces the risk of contamination. It also avoids cell damage which typically occurs using mechanical pumping. However, mixing intensity in association with the mixing device in this type of photobioreactor should be selected based on the characteristics of the microalgae, as some microalgae species are less tolerant to shear stress and mechanical forces.

Plate (flat panel) photobioreactors are transparent rectangular containers with 1 to 5 cm width. By injecting air through a pierced tube at the bottom of the reactor culture medium circulation can be achieved. Simpler construction and shorter light path as well as better gas exchange compared to the tubular design are the advantages of using plate

photobioreactors. Airlift photobioreactors consist of two divided areas which are interconnected via a baffle or a tube.

In the past few years much research has focused on designing photobioreactors with the previously mentioned principles using more economical materials. For instance, plastic bags (polyethylene) have been used to avoid dark zones in inner part of the bioreactors, or as plate photobioreactor (Richmond, 2004).

One of the latest pieces of research proposed a triangle design (Pulz, 2007). The triangle design (shown in Figure 2.7) was a combination of a bubble column using a principle of mixing by means of an external downcomer. This 3-dimensional photobioreactor has obtained an average aerial productivity of 98 g biomass m^{-2} day^{-1} within 19 days including the condition of sub-optimal lighting (Pulz, 2007, Schenk et al., 2008). Therefore, this design is one of the most productive cultivation systems for microalgae obtaining maximum theoretical average yield of 100 g $m^{-2}day^{-1}$. To maximise biomass productivity and minimise required mixing energy, this photobioreactor with high inner surface areas must be developed to distribute light in all the parts of algal culture.

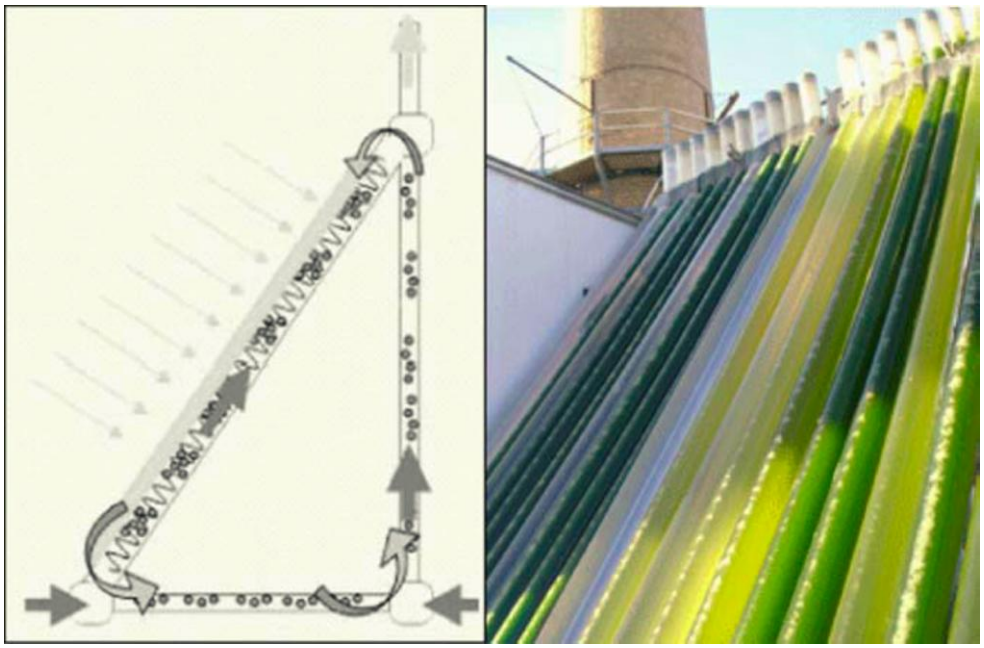


Figure 2.7, GreenFuel's 3D Matrix Algae Growth Engineering Scale Unit, "triangle airlift reactor". On the left there is the drawing from patent US 20050260553, on the right the demonstration plant at the Red Hawk Power Plant, Arizona, USA. Source: (Ecoworld, 2007).

2.8 Culture parameters

Microalgae culture is an artificial environment which in theory must mimic the natural environment for growth of microalgae. Three distinct components can be found in a culture, including the culture medium with essential nutrients, algal cells growing in the medium, and air for mixing the medium and carbon dioxide from the atmosphere. The most important culture parameters are light, temperature, pH, turbulence (mixing), nutrients, and contamination. The importance of each parameter varies depending on the microalgae species as different species have different tolerance towards environmental changes.

2.8.1 *Light (Intensity, spectral quality, photoperiod)*

Light is the main source of energy which drives photosynthesis in microalgae. One of the most important limiting factors for growth of microalgae is light intensity. The requirements for this factor vary with the culture depth and culture density as more concentrated cultures need higher light intensity for better penetration. The level of light intensity in which microalgae reach their maximum growth is typically around 200 to 400 μmol photons $m^{-2}s^{-1}$ for most of microalgae species. The most employed light intensity has been around 200 μmol photons $m^{-2}s^{-1}$ corresponding to about 10% of full daylight (2000 μmol photons $m^{-2}s^{-1}$) (Barsanti and Gualtieri, 2006c).

A number of studies have been focused on the modification of some microalgae strains to obtain higher tolerance to light intensity and avoid the photoinhibition phenomenon. The studies mainly suggested that lowering the number of light harvesting complexes or reducing the size of the chlorophyll antenna can minimise sunlight absorption by each chloroplast (Radakovits, 2010). The suggested strategy may bring two advantages to algal cultures; firstly, higher light penetration can be achieved in high density cultures, and secondly higher photosynthetic efficiency can be obtained as photoinhibition is less likely to occur in cells engaged in light harvesting process (Schenk et al., 2008). In terms of spectral quality red and blue ranges of the spectrum have been widely studied to examine the effect of light wavelengths on growth of microalgae. In addition, photoperiod also varies in different species. Constant illumination is not suitable for many species, although some phytoplanktons can grow naturally under constant lighting.

2.8.2 *Temperature*

The culture temperature should ideally be in the range of the natural habitat of the microalgae species. Most commonly studied species can grow at temperatures between 16 ^{o}C and 27 ^{o}C (Barsanti and Gualtieri, 2006b). Temperatures higher than 35 ^{o}C can be lethal for some species while those lower than 16 ^{o}C can slow down the growth. The microalgae response to changes in temperature of the culture can affect the rate of metabolism, nutrient requirements and cell composition (Richmond, 1999). One of the main problems related to changes of temperature in closed cultivation systems (photobioreactors) is overheating. A simple solution to this problem could be plastic tubes which move the evaporative water out of the system. However, in large scale production cooling systems for the evaporative water are required which are energy consuming and expensive.

2.8.3 *pH*

The optimum pH range for most microalgae species is between 8.2 and 8.7 (Barsanti and Gualtieri, 2006a), although there are species with high tolerance to extreme acid/basic conditions. As the impact of pH on all aspects of media biochemistry is considerable, the maintenance of pH in an optimum range is essential. Metabolic biochemistry of the cell and ionic absorption of the media depend strongly on pH. The effects of these two factors are very significant on algal cultures as they can overcome the neutralizing capacity of exogenous buffering agents. Recently the most economical and practical strategies for pH control in both heterotrophic and photoautotrophic cultures have been reported to be regulated CO_2 dissolution (Schenk et al., 2008). Moreover, metabolic balancing and micro-injection of strong alkalis and acids in heterotrophic cultures have been applied for pH control.

2.8.4 *Mixing*

Mixing models between the injected gas, liquid (media) and solid particles of algal cells are another important factor for the culture maintenance in optimum conditions. Mixing helps circulation of nutrients and the gas through the liquid phase, and helps keeping the uniformity of the culture (Moheimani, 2005).

Any type of closed photobioreactor can create shear stress generated by fluid flow. Increasing the flow rate of the injected gas can have both positive and negative effects on cell growth. Small scale turbulence has negative impacts on algae cell growth

through some mechanisms such as physiological impairment, and mechanical damage on motile cells in particular. While applying air flow for mixing the culture two types of shear could possibly damage the cells. One is localised shear created by bubbles rising and the other is shear stress in the bulk liquid starting by the turbulency in the photobioreactors.

2.8.5 *Nutrient*

Growth media should provide essential nutrients containing elements such as carbon, hydrogen, nitrogen, oxygen, phosphorus, magnesium, copper, iron, zinc, sulphur, potassium, molybdenum, and calcium which are required by all microalgae species. Large quantities of inorganic nutrients like nitrogen and phosphorus consumed by microalgae have been given the term "macro-nutrients" (South and Whittick, 1987). Macro-nutrients must be supplied in significant excess in growth media. Other elements such as copper and molybdenum are required in small quantities. These elements known as micro-nutrients are used as co-factors in enzymatic system of algae or electron transport system. In addition, vitamins such as cyanocobalamin (B_{12}), thiamin, and biotin are essential for growth of many algal species.

The approximate molecular formula of the microalgae biomass is $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Chisti, 2007). This formula can be used as estimation for minimal nutritional requirements (Grobbelaar, 2004).

2.8.6 *Carbon dioxide*

Carbon dioxide is the source of inorganic carbon for growth of microalgae. Some studies on the content of microalgae cells by elemental analysis have revealed that carbon can make up to one-half of cell dry weight (OH-Hama and Miyachi, 1988). Based on that, several aeration techniques have been studied to investigate the effect of carbon dioxide concentration on growth of microalgae.

An aeration technique introduced by Warburg (1919) used enriched air with carbon dioxide in algae suspension. When CO_2 is dissolved in water it is hydrated and appears in three different forms. H_2CO_3, HCO_3^-, CO_3^{2-}, are the different forms of hydrated carbon dioxide (see Figure 2.8) and their concentration can vary depending on the culture pH (Warburg, 1919).

Equations 2.3-2.6 show different stages of the equilibrium in which carbon dioxide dissolves in water.

Dissolution of CO_2 gas:	$CO_2\ (g)$	$\longleftrightarrow$	CO_2	(2.3)
Formation of carbonic acid:	$CO_2 + H_2O$	$\longleftrightarrow$	H_2CO_3	(2.4)
Equilibrium of carbonic acid:	$H_2\ CO_3$	$\longleftrightarrow$	$H^+ + HCO_3^-$	(2.5)
	HCO_3^-	$\longleftrightarrow$	$H^+ + CO_3^{2-}$	(2.6)

Both temperature and pH affect the rate of hydration and solubility of CO_2. Elevation of pH enhances the rate of carbon dioxide transfer into liquid phase. However, this rate may decrease with any increase in temperature.

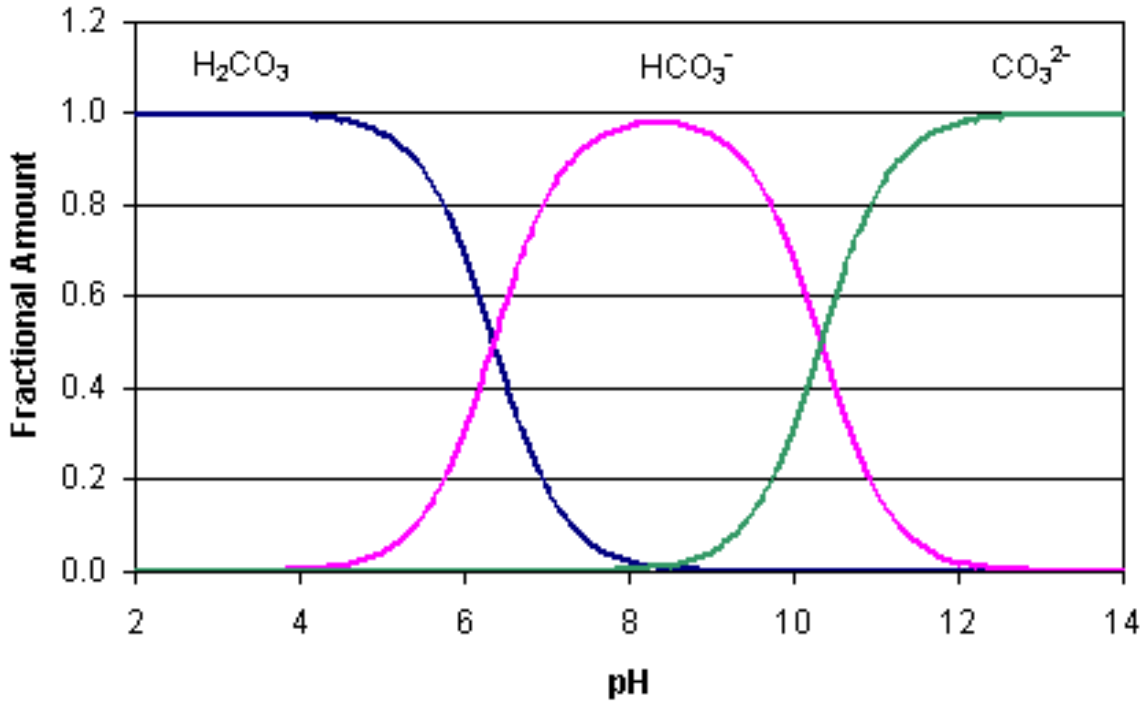


Figure 2.8, Proportions of $H_2\ CO_3$, HCO_3^- ions, CO_3^{2-} ions in fresh water as a function of pH. Source: (Utah-State-University, 2004)

2.8.7 *Contamination*

One of the main challenges in the production of microalgal biomass is the maintenance of a mono-algal culture. While in open ponds the risk of contamination is very high, maintaining one strain of algae in closed cultivation systems is more achievable. However, it is practically impossible to achieve a 100% contaminant free culture in large scales of microalgal mass production. Therefore, regular cleaning of photobioreactors is an important key to avoid significant contamination. Contamination of cultures includes the presence of unwanted algal strains, yeast, mould and bacteria which are the main biological contaminants (Moheimani, 2005).

The contaminants reduce the culture consistency and the productivity yield. One of the contaminants of cyanobacteria cultures are myxobacteria as well as protozoa in open ponds (Richmond, 1986). Myxobacteria are a group of soil-inhabiting bacteria which feed on organic substances and can interfere with the nutrient consumption by microalgae in open ponds.

To overcome this serious problem, selecting algal strains which require specific growth conditions or those capable of survival in extreme conditions is suggested (Borowitzka, 1998). Another solution to reducing contamination is to change the environmental factors such as pH, temperature, and light quality.

2.9 Effects of cultivation conditions on microalgal oil production

The cultivation conditions have significant effects on composition and growth characteristics of microalgae (Chojnacka and Marquez-Rocha, 2004). Growth parameters such as biomass productivity, specific growth rate, and lipid productivity are influenced by the cultivation mode. The cultivation conditions have been categorised into four major types: photoautotrophic, heterotrophic, mixotrophic and photoheterotrophic cultivations. These various types of cultivation conditions are described in the following sections.

2.9.1 *Photoautotrophic cultivation*

In the photoautotrophic cultivation mode, light is the main source of energy and inorganic carbon such as CO_2 is the carbon source (Huang et al., 2010). Under photoautotrophic cultivation the lipid content of microalgae varies between different species in range from 5% to 68%. Generally, the increase in lipid content of microalgae

can be induced by a nutrient limiting or nitrogen limiting factor (Mata et al., 2010). However the results of some studies have shown that higher lipid content leads to lower biomass productivity (Chen et al., 2011). To obtain the optimum cultivation condition it is essential to identify the suitable growth factor. For instance lipid productivity is a more suitable factor to be measured for determination of the potential of microalgae for oil-production. This factor represents the mutual effects of biomass production and lipid content. *Chlorella sp* was reported in the literature to have the highest lipid productivity of about 179 mg L^{-1} d^{-1} under photoautotrophic cultivation (Chiu et al., 2008).

The main advantage of photoautotrophic cultivation is the consumption of CO_2 as the carbon source. Large quantities of carbon dioxide emitted from power plants, breweries, and distilleries for instance can be mitigated by microalgae in this cultivation mode. The other major benefit of using autotrophic cultivation is that in open systems such as open ponds and raceway ponds it has lower contamination risks. For that reason, open systems are typically operated under this type of cultivation (Mata et al., 2010).

2.9.2 *Heterotrophic cultivation*

In the heterotrophic cultivation, organic carbon is used under dark conditions as the sources of both energy and carbon by microalgae (Chojnacka and Marquez-Rocha, 2004). This cultivation condition can be an alternative solution to overcome the problem associated with light limitation in large scale photobioreactors (Huang et al., 2010). Higher lipid content was observed in some microalgae species during heterotrophic growth stage. For instance the lipid content in *Chlorella protothecoides* increased up to 40% when the cultivation condition changed from phototrophic to heterotrophic (Xu et al., 2006). Wide variety of organic carbon sources can be assimilated by microalgae for growth (Liang et al., 2009). Therefore, finding cheaper sources of organic carbon such as corn powder as a replacement for sugars have been studied.

The results reported by Xu et al. (2006) showed higher biomass productivity (2 g $L^{-1}d^{-1}$) and lipid productivity (0.93 g L^{-1} d^{-1}) under heterotrophic condition. The results of another study obtained the highest lipid productivity (3.7 g L^{-1} d^{-1}) by using a 5 L fermentor under fed-batch culture (Xiong et al., 2008). It was reported that heterotrophic cultivation provides lipid productivity 20 times higher than phototrophic cultivation. The disadvantage of Heterotrophic cultivation is that using different types of sugars in the system rises the possibility of bacterial growth in the culture, and hence increases the risk of contamination which becomes a major challenge (Chen et al., 2011).

2.9.3 *Mixotrophic cultivation*

In mixotrophic cultivation, microalgae can use both organic compounds and inorganic carbon (carbon dioxide) as their carbon source to undergo photosynthesis. Microalgae consume organic compounds and carbon dioxide as their carbon source and the CO_2 produced by respiration is utilized under phototrophic condition (Mata et al., 2010). This type of cultivation condition is rarely used in comparison with phototrophic and heterotrophic conditions for microalgae oil production (Chen et al., 2011).

2.9.4 *Photoheterotrophic cultivation*

Photoheterotrophic cultivation occurs when microalgae use light as the source of energy and organic compounds as the carbon source. The main difference between photoheterotrophic and mixotrophic cultivation is that the former needs light as the energy source, whereas the latter assimilate organic compounds. Thus, photoheterotrophic condition requires both light and sugars (Chojnacka and Marquez-Rocha, 2004). Applying this approach for biodiesel production is very rare, however it can be used to enhance the production of some light-regulated metabolites and photosynthetic pigments (Ogbonna et al., 2002).

2.9.5 *Comparison of different cultivation conditions*

Microalgae oil production varies between different species, however heterotrophic growth condition can provide higher oil productivity compared with other types of cultivation. This system has been considered as an interesting option, however it can easily get contaminated particularly in open cultivation systems (Chen et al., 2011). Moreover, the cost of organic carbon source should be taken into account from the commercial aspect. Table 2.2 compares the characteristics of different cultivation conditions.

The most commonly used cultivation system is photoautotrophic which is relatively easy to scale up and is also a promising choice in open ponds systems, as it can use carbon dioxide from the waste streams of industrial sectors. Low cost scale up of photoautotrophic cultivation has made it an attractive method. However, slow cell growth and low biomass productivity, which leads to lower oil productivity compared with heterotrophic cultivation, is the disadvantage of using this system.

Cultivation condition	**Energy source**	**Carbon source**	**Cell density**	**Reactor scale-up**	**Cost**	**Issues associated with scale-up**	**Examples**
Photoautotrophic	Light	Inorganic	Low	Open pond or photobioreactor	Low	Low cell density High condensation cost	Cyanobacteria
Heterotrophic	organic	Organic	High	Conventional fermentor	Medium	Contamination High substrate cost	*E. coli*
Mixotrophic	Light and organic	Inorganic and organic	Medium	Closed photobioreactor	High	Contamination High equipment cost High substrate cost	*Chlorella*
Photoheterotrophic	Light	Organic	Medium	Closed photobioreactor	High	Contamination High equipment cost High substrate cost	Purple and green photosynthetic bacteria

Table 2.2, Comparison of the characteristics of different cultivation conditions. Source:(Chen et al., 2008, Chojnacka and Marquez-Rocha, 2004).

To date, the use of photoheterotrophic and mixotrophic cultivation has not been considerably studied and discussed in literature, as these systems may require a special photobioreactor design for scale up which increases the operation cost (Chen et al., 2011).

2.10 Microalgae illumination system

The light source and light intensity are key factors for phototrophic growth condition (Mata et al., 2010). Sunlight has been used as the main light source for open cultivation systems whereas closed cultivation systems are mainly illuminated by artificial light sources such as optical fibres (OF) and light-emitting diodes (LEDs). Illumination strategies for microalgae cultivation systems can be categorised into three groups: artificial, natural solar and a combination of artificial lighting and solar light.

2.10.1 *Artificial Light sources*

One of the main problems of using artificial illumination systems in photobioreactors is the high cost of installation and operation process. However, many studies have been focused on developing efficient and cost-effective techniques to overcome this problem (Chen et al., 2011). Light intensity levels inside the culture are highly influenced by the cell concentration and the distance of the light source from the culture. The light shading effect which occurs when cell concentration increases or bio-films are formed may cause rapid reduction in light intensity levels (Chen et al., 2008).

Moreover, in theory high light efficiency can be achieved by providing a short light path which means that the light source should be placed close to the microalgae culture. However, in practice conventional light sources generate a significant amount of heat which can change the primary environmental condition provided for the culture growth.

Equation 2.7 shows the exponential reduction of light intensity with distance from a photobioreactor wall as the product and cell concentration increase:

$$I_L/I_o = exp(-\gamma L) \quad (2.7)$$

where I_L is the light intensity at depth L, I_o is the original incident intensity and γ is the turbidity (Chen et al., 2011).

A variety of illumination strategies have been applied in different photobioreactor designs to improve the microalgae oil/lipid content (Ma and Hanna, 1999). Table 2.3 provides the detailed features and electricity consumption for various artificial light sources.

Various studies have proposed photobioreactor designs which can enhance biomass production and improve light supply efficiency although the economic viability has not necessarily been taken into account (Barsanti and Gualtieri, 2006b). Some illumination systems have been proposed in which incandescent lamps were located directly within a photobioreactor composed of three concentric glass cylinders (Tsygankov et al., 1994).

Light source	Feature	Operation stability	Electricity consumption of the light source (kw-h) based on 40L photobioreactor
Conventional artificial light sources	Higher biomass productivity, higher stability, large illumination area, low constructing cost	High	40.32
LED	Lower energy consumption, lower heat generation, longer life-expectancy, higher tolerance frequency of on–off switching, higher stability, low constructing cost	High	20.16
Optical fiber excited by metal–halide lamp (OF-MH)	Higher energy consumption, lower area of land required, good light path, uniform light distribution, lower space requirement, low contamination risk	Moderate	36.0
Optical fiber excited by solar energy (OF-solar)	Low electricity consumption, good light path, uniform light distribution, lower space requirement, low contamination risk, lower cost	Low	1.0
LED/OF-solar combined with wind power/solar panel	No electricity consumption, good light path, uniform light distribution, lower space requirement, low contamination risk	High	0.0

Table 2.3, Features and electricity consumption for various artificial light sources. Source: (Chen et al., 2011)

Another illumination strategy was introduced by El-Shishtawy et al. (1997), in which a light receiving face and reflection sheet were combined to transfer light to the microalgae culture. The improved photobioreactor designs have proved to be efficient however the high operating cost and power consumption is the main barrier to their practical application (El-Shishtawy et al., 1997). Thus, the light sources should be inexpensive, long lasting and reliable to be cost-effective for commercial applications of microalgae cultivation (Chen et al., 2011).

2.10.1.1 *Light emitting diodes (LEDs)*

To improve the overall energy conversion, light sources with narrow spectral outputs can be used. These light sources partly cover the photosynthetic absorption spectrum and eliminate the unusable wavelengths of light. Up to date, the only light sources that are suitable for this purpose are light-emitting diodes (LEDs). Using LEDs as artificial light sources brings a number of advantages in terms of energy consumption and reliability. They come in small sizes which can be installed in any type of photobioreactors.

Moreover, LEDs have a high tolerance for switching on/off, they are long-lasting and efficient, and generate less heat during their operational period. The narrow light emission spectra between 20 and 30 nm in LEDs can be specified for the photosynthetic light spectrum. The emission wavelengths of blue and red LEDs for instance are around 450-470 nm and 645-665 nm, which exploit suitable wavelengths for photosynthesis (Yeh and Chung, 2009). Different LEDs were combined and used in a study for indoor cultivation of *Chlorella pyrenoidosa* as the only light source to improve the biomass production and key products of microalgae (Lee, 1994).

Wang et al. (2007) used different light-emitting diodes with various illumination intensities and wavelengths of light in order to examine the effect of light source on the culture of *Spirulina platensis.* Results showed that in the photoautotrophic condition, the culture of *Spirulina platensis* reached the highest specific growth rate and biomass production under red LED light (Wang et al., 2007) whilst blue LED light exhibited the lowest efficiency in photon conversion into biomass.

Another study suggested that red LED with low light intensity was appropriate for microalgae cell growth, whereas blue LED with short wavelengths would be suitable for inducement of morphological changes in *H.pluvialis* and enhancement of astaxanthin (a feed additive) accumulation (Katsuda et al., 2004). Shorter wavelengths (380-470 nm) of LED light induced astaxanthin production of up to 5-6% on a biomass basis, although growth inhibition also occurred as result of the induction. The results suggested that red LEDs can be used initially for enhanced biomass accumulation followed by high intensity illumination by blue LEDs to induce astaxanthin.

The impact of incident light intensity, flashing frequency and light:dark cycle on the algal cultivation have also been investigated (Katsuda et al., 2006). Katsuda et al.(2006) indicated that using blue LED flashing light was an effective technique to improve algae cell growth and astaxanthin production.

LEDs have also been used in plant research for investigating the effect of light quality on plant morphology and metabolism. Johkan et al. (2011) reported that high intensity green LED light promoted the growth of lettuce leaves. The study showed green light at low densities can poorly penetrate into the plant leaf, however lettuce plants illuminated under high intensity green light developed a normal growth pattern (Johkan et al., 2011).

2.10.1.2 *Optical fibres*

Optical fibres (Earp et al.) are another potential source of artificial light which can be applied for the illumination of microalgae culture systems. Optical fibres were originally plastic-clad with end-light illumination. However, their light conversion efficiency can be improved by means of mechanically polished surfaces which emit the light to the whole surface of the core and create side-light optical fibres (Lee and Kim, 1998).

The advantage of using optical fibers is that they distribute the light consistently within the photobioreactor without heat generation and the high surface to volume ratio makes it possible for them to be directly used in the microalgae cultivation system.

In order to supply light with various wavelength distributions for internal illumination in photobioreactors, artificial light engines can excite side-light optical fibers (Matsunaga et al., 1991). Side-light optical fibers have been used in various photobioreactors designs for microalgae cultivation as well as microbial desulfurization (Henshaw and Zhu, 2001).

2.10.2 *Natural light*

The use of solar energy as the most abundant natural source of light for cultivation of microalgae has been broadly studied (Moheimani and Parlevliet, 2013). General operating problems such as high electricity consumption, operating costs and environmental pollution can be solved by utilizing solar energy efficiently.

Solar panels are one of the technologies widely used for absorbing sunlight and converting it into electricity. In a recent study, Moheimani et al. (2013) introduced and investigated a novel technique in which a combination of solar panels (electrical energy) and microalgae cultivation system (chemical energy) were used. Another study on the use of optical fibres reported a significant reduction in electricity consumption by introducing sunlight driven by optical fibres into the photobioreactors (Chen et al., 2011). However, the light intensity variation during the day remains the major problem of using optical fibre-solar in microalgae illumination systems.

In open cultivation systems, day-night cycles are the main problem as they depend on the season, weather, operating time and solar spectrum. The variation of light intensity can lead to alterations in cellular metabolism mode, biochemical composition and productivity in microalgae.

Solar spectrum includes a range of short wavelength bands with high energy content which can be harmful for photosynthetic organisms. Due to anthropogenic depletion of the ozone layer it is predicted that the levels of ultra violet radiation (UV-B 280-315 nm) will increase. Therefore, some studies have focused on investigating the effect of ultra violet irradiation on various biological matters (Xiong et al., 1997).

As UV irradiation rate depends strongly on the latitude and altitude of the algal growth area, it is important to consider the simulation condition and different aspects of solar irradiation (Holzinger and Lutz, 2006). The real biological effects of UV in experiments are important and should be considered, as neglecting the contribution of only a small amount of UV-B irradiation with short wavelengths can lead to damage the cells and cause severe alterations in genetic properties of microalgae (He and Hader, 2002).

It is well known that UV has direct influences on respiration pathways in microalgae, which can increase the formation of reactive oxygen species (ROS). Increased production of ROS under UV irradiation can lead to DNA damage, degradation of

proteins, peroxidation of lipids, and bleaching of photosynthetic pigments such as chlorophylls and phycobiliproteins (He and Hader, 2002, Vega and Pizarro, 2000).

The irradiation conditions are cited by some researchers as KJ m^{-2} $time^{-1}$ whereas some others use intensity expressed in units like W m^{-2} or μmol photons m^{-2} s^{-1} (photon flux density unit). The units of Watt and Joule can be converted if the time information is available, but this is often missing and therefore makes comparison of the given units often difficult. A correct comparison of irradiation condition can be achieved if the sensors used for irradiation measurements are described and properties of filters including ageing problem which can affect the spectral range are given (Holzinger and Lutz, 2006).

Cyanobacteria are inhabitants of environments such as shallow waters, terrestrial cliffs and rocks and therefore are often highly exposed to UV irradiation. Cyanobacteria can develop various UV tolerance strategies. For instance, the presence of UV screening compounds such as carotenoides, mycosporin-like amino acids (MAAs), and scytonemin in their outer sheath have been reported (Franklin et al., 2003).

In addition, cyanobacteria have developed some repair strategies including DNA damage repair mechanism, efficient repair of the photosynthetic apparatus and synthesis of UV-stress proteins. Freshwater cyanobacteria can be regarded as well adapted organisms to high UV irradiations. Hence, cyanobacteria can be selected for research about UV irradiation tolerance and ultra-structural alterations. To achieve accurate results, harsh UV conditions and UV exclusion experiments must be carried out to distinguish the effects of high photosynthetically active radiation (PAR, 400-700 nm) and UV radiation.

In addition, unicellular freshwater green algae can be exposed to high UV irradiation in their natural habitats. Some species of green algae develop MAAs compounds in response to UV exposure (Xiong et al., 1997).

Cell size alteration by increasing UV-B irradiation has been observed in the freshwater green algae *Selenastrum capricornutum*. However, no severe physiological change in cells was reported (Holzinger and Lutz, 2006).

In general, UV-B (280-315 nm) and UV-A (315-400 nm) have been considered as unfavourable irradiation conditions for photosynthetic organisms. However, different organisms can develop various tolerance strategies to harsh environmental conditions.

The collection depth of algal species is an important factor responsible for the degree of deleterious effects of high PAR and UV irradiation. Therefore, marine algae species which grow in deeper water levels are more sensitive to high UV exposure than freshwater algae. Various biochemical compounds have been synthesised by fresh water algae for screening against harmful UV (Holzinger and Lutz, 2006). These compounds found in many algal species can be summarised as:

- UV absorbing compounds such as mycosporin-like amino acids MAAs (capable of reducing UV-induced photoinhibition).
- Non-photosynthetically active accessory pigments like carotenoids.
- Biopterin glucoside (screening in the range of 320-390 nm).
- Sporopollenin (screening UV-A and UV-B in microalgae cell walls).

Another important factor for developing UV resistance can be the degree of nutrition in microalgae. For instance the presence of nitrogen in growth media of different species of red algae supported the production of UV screening compounds such as MAAs.
It is suggested that for long term studies of UV impact, monitoring the changes in ultra-structure of microalgae cells and effect of screening compounds are necessary. Moreover, experiments with low irradiation enhancement which provide reasonable predictions on patterns of changes in UV levels in nature should be carried out (Holzinger and Lutz, 2006).

2.10.3 *Luminescent solar concentrator (LSC)*

A luminescent solar concentrator (LSC) is a device used for absorbing incident solar radiation and emitting fluorescence photons (Wilson et al., 2010) and was introduced more than three decades ago for concentrating sunlight in studies about the use of photovoltaics for electricity generation (Goetzberger and Greube, 1977). An LSC collects sunlight from a large area and concentrates and directs it to a small area of solar cells minimising the amount of silicon material which is typically used in solar cells. In comparison, imaging concentrators use lenses, mirrors or a combination of both to collect and concentrate sunlight, and they are capable of obtaining high concentrations of sunlight (several hundreds of sun).

However, imaging concentrators require tracking to precisely follow the directions of sunlight in the sky and illuminate the solar cells (Wilson, 2010).

An LSC is made of transparent materials which are doped with fluorescent dyes to collect sunlight. When the transparent sheet receives the incident light on the front surface, it absorbs the sunlight by fluorescent dye molecules. The dye emits the captured photons and transports them towards the edges of the sheet by internal reflection. The transmitted photons are then collected by solar cells which are attached to the edges of LSC (see Figure 2.9).

There are different types of fluorophores which have been used in LSC. The three main categories of fluorophores are organic dyes, rare earth materials and quantum dots. Organic dyes have either naphtalimide or perylene structural basis and they can be dissolved in a broad range of organic solvents such as chloroform, acetone, and methyl methacrylate (Wilson, 2010).

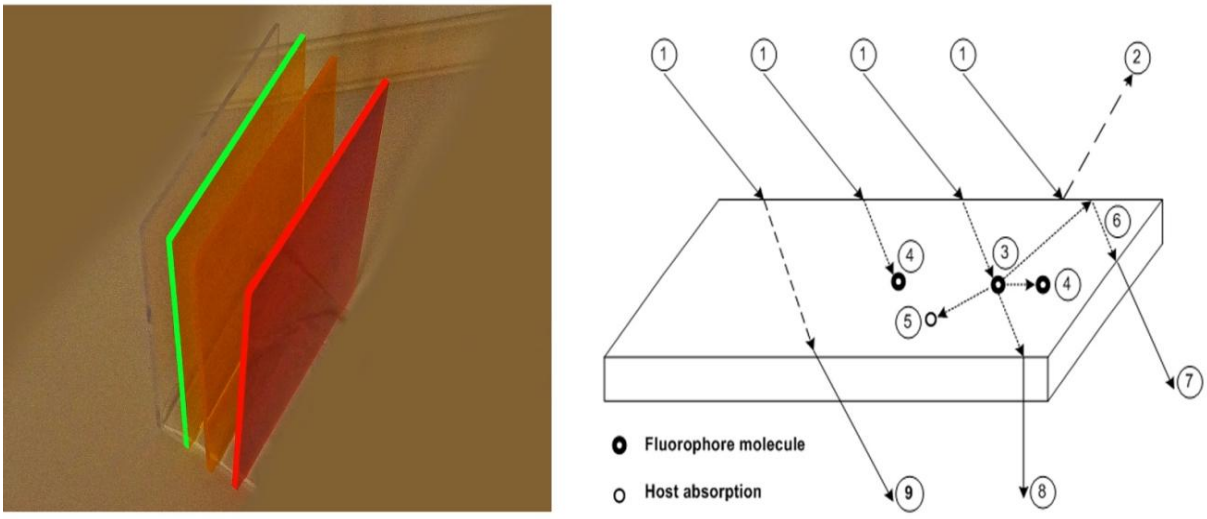


Figure 2.9, Luminescent acrylic sheets (left side), Cross-section of a luminescent sheet showing different paths of light (right side): 1- incident light, 2- surface reflection, 3- light absorption and emission by fluorophore molecule, 4- complete light absorption by fluorophore, 5- light absorption by host material, 6- trapped emission, 7- light emission from the edges, 8- fluorescence emission from the surface, 9- unabsorbed light.

The great solubility of organic dyes makes their incorporation into a various range of polymeric host materials possible. Rare earth fluorophores consist of rare earth ions such as Neodymium (Nd^{3+}) and Ytterbium (Yb^{3+}) ions which show high quantum yields and outstanding photostability (Reisfeld and Kalisky, 1981, Batchelder et al., 1981). Rare earth fluorophores are suitable for doping into host materials such as glass, glass ceramics, and solgels (Wilson, 2010) and their emission spectrum is in the infrared (IR) ranges. Similarly, the emission spectrum of quantum dots (nano-crystals of inorganic compounds) is in IR ranging from 850nm to 1900nm and therefore their spectral and physical properties are not suitable for construction of photobioreactors for microalgae cultivation.

For the purpose of this study, visible- emitting organic fluorescent dyes were selected as they are resistant to temperature and suitable for injection-moulding processes in polymers such as poly methyl methacrylate (PMMA). Photo-stability is an essential factor for the performance of organic dyes. The main problem using original organic dyes was that they had poor photo-stability, capable of performing well for only a few weeks under solar irradiation (Hermann, 1982). New fluorophores with stability of several years under light exposure were later introduced and studied. For instance, the Lumogen F range dyes (BASF, Ludwigshafen, Germany) with great photo-stability and high quantum yields were developed and used for various solar applications including day-lighting system (Sousa et al., 2012).

A number of research studies have focused on the area of photovoltaic greenhouses (Moheimani and Parlevliet, 2013, Parida et al., 2011, Perez-Alonso et al., 2012) and the combination of photovoltaic modules with agricultural production is a well-established concept. Photovoltaic solar energy used in greenhouses has been considered as a potential source of electrical power to pump water, supply fans, and desalinate water for agricultural products (Parida et al., 2011).

A study about optimisation of a three colour luminescent solar concentrator produced a day-lighting system with the ability to transport sunlight to various areas of a building (Earp et al., 2004). The study used PMMA as host material for three LSCs, violet, pink and green. This illumination system which was used in a building, proves the potential of LSC as an alternative lighting system.

2.10.4 *Combination of light sources for microalgae cultivation: Optical Fibre-solar/ multi-LED with solar panel/wind power generator*

Variation of solar light intensity with season, weather, and location makes the use of natural light systems unstable. Chen et al., (2008) monitored the irradiation intensity by installing a light dependent resistor (LDR) on the photobioreactor. During daytime, the Optical Fibre-solar was used for illumination and if a reduction in the levels of light intensity was observed, the artificial light source LED was automatically turned on (Earp et al., 2004).

Therefore, this combination of illumination system ensured a stable and sufficient light supply in the photobioreactor. Solar panels as well as wind power generators were introduced later to provide all the energy required for the artificial light sources and to achieve further decrease in electricity consumption.

Figure 2.10 shows the schematic description of the combined system for microalgae cultivation.

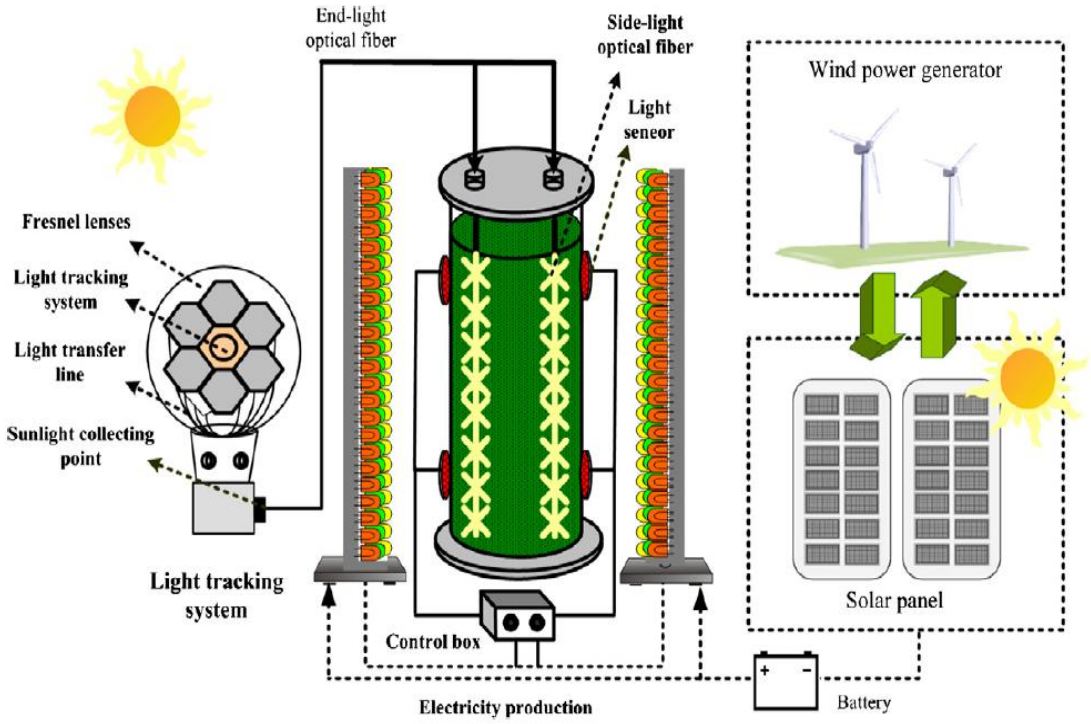


Figure 2.10, Schematic description of the solar-energy-excited optical fibre photobioreactor system with an internal light source (optical fiber excited by sunlight collecting system) and a multi-LED light source using the electricity generated by a solar panel and wind power generator. Source: (Chen et al., 2011).

2.11 Microalgae downstream processing

This section reviews downstream processing of microalgae including harvesting methods, products, environmental applications, and genetic and metabolic engineering.

2.11.1 *Harvesting methods*

Microalgae biomass contains oil and significant quantities of protein and carbohydrates which can be harvested and used for various purposes (Sanchez Miron et al., 2003). Different harvesting methods for microalgae biomass, including chemical flocculation, biological flocculation, filtration and screening, centrifugation, flotation, gravity sedimentation and electrophoresis techniques have been investigated (Li et al., 2008, Uduman et al., 2010). The advantage of using the two flocculation techniques is that they can operate with very low cost, however they require a long processing period (due to the low mass fraction in culture broth) and the risk of decomposition of bio-reactive products is high.

On the other hand, effective methods like centrifugation, filtration and ultrasonic flocculation are expensive to operate. The value of the final product, the size of microalgae cells and biomass concentration play the key roles in selecting the appropriate harvesting technique. Microalgae harvesting can be achieved primarily by bulk harvesting in which some amounts of solid biomass can be separated from the algal suspension by flotation, gravity sedimentation, or flocculation (Brennan and Owende, 2010).

Centrifugation is typically used for algal cells recovery from the liquid broth. Centrifugation is an effective method for producing "extended shelf-life concentrates" for aquaculture in particular (Grima et al., 2003). However it has been reported that due to high gravitational and shear forces induced by centrifugation, microalgae cell structure can be damaged (Knuckey et al., 2006). It is also very likely that large scale continuous centrifuges are costly and they would exert damaging shear forces on algal cells reducing the compatibility for scale up.

The flocculation process causes dispersed particles to aggregate and to create large particles for settling (Ho et al., 2011). There are various types of flocculation such as autoflocculation, chemical coagulation, and combined flocculation. Autoflocculation is a result of CO_2 consumption during the photosynthesis process by microalgae, when the pH of the culture increases and precipitation of carbonate salts with algal cells occurs.

Simulation of autoflocculation which can assist harvesting of algal cells, has been demonstrated by increasing pH values with addition of sodium hydroxide (Sukenik and Shelef, 1984).

Addition of chemicals in different solid-liquid separation processes for inducement of flocculation is a common pre-treatment method in large scale microalgae harvesting (Lee et al., 1998). Due to the chemical composition, flocculants have been classified as inorganic and organic (or polyelectrolyte) coagulants. Inorganic coagulants negatively charge algal cells by adsorption of ions from organic compounds and ionization of surface functional groups (Uduman et al., 2010). An effective way of harvesting can be achieved by disruption of the system stability through inorganic coagulants which can reduce or neutralize the surface charge. However, drawbacks of this method can be the high risk of contamination (of the end product), generation of significant amount of sludge, and high sensitivity to pH variations.

The risk of biomass contamination is low when biodegradable organic flocculants (e.g. chitosan) produced from natural sources are used (Divakaran and Sivasankara Pillai, 2002). The most effective types of organic coagulants recognised for microalgae harvesting are cationic polymers. Anionic and non-ionic polymers were found inappropriate for this purpose due to the inadequate distance to form bonding between particles (Bilanovic et al., 1988).

The efficiency of the flocculation process can be affected by concentration of microalgae biomass, surface functional groups, charge density of molecules, ionic strength and pH of the liquid, polymer molecular weight and mixing patterns of the liquid (Grima et al., 2003). The optimum condition for flocculation can be achieved when biomass concentration and polymer molecular weight are high and the mixing level is low (as it avoids shear stress and brings the cells together).

Combined flocculation can be used as multi-stage process for harvesting freshwater or marine microalgae. For instance, flocculation in sea water can be achieved by using inorganic coagulants such as ferric chloride or alum in combination with polyelectrolytes in the primary stage followed by ozone oxidation in the next stage (Sukenik et al., 1988).

One of the most effective harvesting methods of microalgae is filtration. Various types of this harvesting method can be used such as pressure filtration, micro and ultra filtration, vacuum filtration, tangential flow filtration (TFF) and dead-end filtration

(Chen et al., 2011). The process normally involves running the algal culture continuously through a filter allowing the medium to pass and collecting the accumulated algae paste over the filter. Tangential flow filtration (TFF) has been considered as an energy-efficient dewatering method compared to dead-end filtration methods. It has been reported that using this method of filtration can recover 70-89% of freshwater microalgae while retaining the structure, motility, and properties of the collected algal cells (Petrusevski et al., 1995).

The choice of filtration types varies due to the size of algae species. For instance vacuum and pressure filtrations are appropriate for those strains of microalgae with larger cells such as *Spirulina plantensis*, whereas these filtration methods are much less effective for small cells of *Chlorella* or *dunaliella* (Grima et al., 2003).

Floatation is another separation process which is based on the trapping of algae cells by scattered micro-air bubbles in which the bubbles carry the cells to liquid surface. The flotation process, unlike flocculation, does not require the addition of chemicals (Brennan and Owende, 2010). The application of flotation process due to the bubble sizes can be classified into dispersed flotation, dissolved air flotation (DAF), and electrolytic flotation. In dispersed floatation, an air injection system produces bubbles with diameters of 700-1500 μm formed by high speed mechanical agitators, whilst in dissolved air floatation the size of air bubbles are smaller (10-100 μm). In electrolytic floatation method, an electric field moves and separates the charged algal cells from the solution (Brennan and Owende, 2010).

2.11.2 Products from microalgae

Having similar characteristics to higher plants, microalgae could be more cost-efficient sources for biomass production. Microalgae are potential sources of high-value biochemical components such as natural colorants, polyunsaturated fatty acids, proteins and polysaccharides (Chen et al., 2011) as well as a potential biofuel source or food material. Chisti (2007) reviewed biodiesel sources for meeting 50% of all transport fuel requirements of the United States (Chisti, 2007). It was suggested that using microalgae for biodiesel production can satisfy half of fuel needs of the United States by allocating only up to 3% of the total cropping area of the country. Table 2.4 presents the comparison of different fuel sources, for supplying 50% of the fuel needs.

Crop	Oil yield (L/ha)	Land area needed (M ha)	Percent of existing US cropping area
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil palm	5950	45	24
Microalgae (70% oil by wet)	136,900	2	1.1
Microalgae (30% oil by wet)	58,700	4.5	2.5

Table 2.4, comparison of some sources of biodiesel. Source: (Chisti, 2007)

The potential products from microalgae are covered in the following sections.

2.11.2.1 Biodiesel

Biodiesel is typically made by chemically reacting lipids found in animal fats and vegetable oil. Animal fat and vegetable oil contain chains of fatty acids joined by a glycerol molecule which form long chain alkyl (methyl, ethyl, propyl) esters. In the process of producing biodiesel, glycerol is replaced by methanol and forms fatty acid methyl esters (FAME) called biodiesel (Harun et al., 2009).

The production of biodiesel at industrial scale for meeting the current fuel demands has to have standard specification of fuel quality with ideally lower costs compared to conventional fossil fuel. Sufficient feedstock is required for commercial production of biodiesel. Most of microalgae species contain lipids with low degree of unsaturation. Therefore, they can potentially be used as raw material for biodiesel production. In

addition, they also have high growth rate, offering possibility of rapid and sustainable biodiesel production.

The extraction of lipids and chemical processing of microalgae for biofuel production has received great attention in downstream processing, in which trans-esterification of triacylglyceride occurs as a chemical conversion (Sasso et al., 2012). This chemical conversion depends on the level of TAG and phospholipids which vary between different algae species. Among numerous methods of lipid extraction from microalgae the most common methods are liquid-liquid extraction (with solvents), expeller/oil press, ultrasonication techniques, and supercritical fluid extraction (Harun et al., 2009). For oil extraction microalgae biomass need to be dried first.

In the solvent extraction method, organic solvents such as chloroform, acetone, hexane, and benzene are used. The algal cell wall is destroyed by the solvent and the algal oil is released from the aqueous phase to the organic phase. Oil can be separated in the distillation process. However, solvent extraction is time consuming since it requires multiple steps of extraction and large quantities of organic solvents which are often toxic and expensive (Harun et al., 2009).

Press is another extraction method which uses pressure to break cells. Published data however suggests that it only extracts 75% of oil and is reported to be less efficient compared to other methods (Popoola and Yangomodou, 2006).

Supercritical extraction with carbon dioxide is another extraction method which has been reported to be low cost and time efficient, since it uses non-toxic carbon dioxide which can be found in abundance. In this method, algal cells are ruptured through high pressure in supercritical conditions (Macıas-Sanchez et al., 2005).

Ultrasound is also a promising extraction method. In this method, high intensity ultrasound waves form small cavitation bubbles with liquid around algal cells, and these disrupt cell walls and release oil into the solution. In a study on extraction of pigments and fatty acids from the green alga *Scenedesmus obliquus* (Chlorophyceae) over 90% of the desired compounds were extracted using ultrasonication (Wiltshire et al., 2000).

2.11.2.2 *Biomethane*

The residual algal biomass post-harvest can be used as a potential source for production of methane by anaerobic digestion, which in turn can be used to generate the required electrical power for running the facilities of microalgae production. Biogas is produced during anaerobic digestion via anaerobic microorganisms and typically consists of 55-75% methane and 25-45% CO_2 (Holm-Nielsen et al., 2009). The residual biomass can be reprocessed for making fertilizers. Microalgae lack a lignin and have a lower cellulose content compared with higher plants, ensuring process stability and a high conversion efficiency. Production of methane from microalgae by anaerobic digestion is technically feasible. However, the production costs were reported to be higher than the current sources of biomass such as grass and wood which are found in large quantities (Mata-Alvarez et al., 2000, Raven and Gregersen, 2007).

2.11.2.3 *Bio-butanol*

Fermentation of microalgae biomass by microorganisms can produce valuable chemicals such as bio-butanol (Harun et al., 2009). The potential of bio-butanol as a renewable transport fuel has made this organic solvent a potentially valuable product from biological feedstock. The fermentation process of producing bio-butanol is very similar to bio-ethanol but using bacteria, typically *Clostridium* sp. Since bio-butanol can be used in vehicles engines without modification, it has been considered as an interesting alternative fuel. Moreover, due to lower vapour pressure it can be blended with conventional gasoline. However, the main bottlenecks in bio-butanol production are low final butanol concentration and its toxic effects on microorganism cell membrane. Bio-butanol productivity from fermentation is lower compared with ethanol formation (Melzoch K. et al., 2010). Considering the potential of bio-butanol and acetone produced by microalgae feedstock very little research has been focused on them.

2.11.2.4 *Food and pharmaceuticals*

Microalgae can improve the nutritional content of food as they contain high levels of essential biochemical compounds in their biomass (Spolaore et al., 2006a). High protein

content with suitable amino acid structures for food supplement as well as carbohydrates in forms of starch, glucose, sugars and other polysaccharides are among the main reasons to consider them as food sources. In addition, microalgae biomass contains essential vitamins such as A, group B, C, E, biotin, folic acid and nicotinate (Becker, 2007). Bio-pigments such as chlorophyll, carotenoids, and phycobiliproteins, which can be used in a wide variety of commercial applications, are found in microalgae. Among various species of microalgae *chlorella* has high amounts of chlorophyll (Nakanishi, 2001). The chelating agent activity of chlorophyll makes it suitable for pharmaceutical applications such as liver recovery and ulcer treatment. It also supports cell growth and provides high levels of haemoglobin in blood. In the food industry, chlorophyll is used as a natural pigment. As well as chlorophyll which is typically found in all microalgae species, cyanobacteria contain significant amounts of phycobiliproteins. Antioxidant properties as well as liver-protective and anti-inflammatory behaviour of phycobiliproteins play an important role in tumour and leukaemia treatments (Patel et al., 2005, Vadiraja et al., 1998). Phycoerythrin and phycocyanin are the most commonly used phycobiliproteins for applications such as fluorescent pigments. Fluorescent properties of phycobiliproteins make them suitable for fluorescent labelling of antibodies typically used in diagnostic kits in biomedical research (Sekar and Chandramohan, 2008).

Omega-3 is another high value food supplement found in microalgae which can be obtained by purification of the fatty acids such as eicosa pentanoic acid (EPA) and decosa hexaenoic acid (DHA) (Chrismadha and Borowitzka, 1994). Although the main source of omega-3 fatty acids is the fish oil, oxidative instability, unpleasant taste of fish oil and also the unsustainable process of fish oil production have attracted attention to other alternative sources (Luiten et al., 2003). Microalgae are natural sources of omega-3 and the process of algal omega-3 extraction is more cost-effective compared to fish oil. EPA has been found in mass culture of microalgae and various clinical applications such as treatment for asthma, heart and inflammatory diseases, and arthritis have been reported in a number of papers (Doughman et al., 2007). Decosa hexaenoic acid (Chrismadha and Borowitzka) is another fatty acid present in some microalgae species with beneficial characteristics for medical purposes. For instance, it can be used for treatments against cancer and heart diseases, as well as supporting the immune system, low cholesterol and detoxification in the body. The amount of DHA varies in different microalgae species (Chrismadha and Borowitzka, 1994).

2.11.3 *Environmental applications of microalgae*

Microalgae have been used in a wide variety of environmental applications such as CO_2 sequestration and improvement of degradation by reducing energy demand of oxygen supply for bacteria in aerobic treatment processes (Harun et al., 2009). In fact, in an aerobic treatment stage, algae provide oxygen by photosynthesis for bacteria and bacteria in turn are responsible for degradation of organic material in waste water. Figure 2.11 shows the use of algae in the process.

By using the organic compounds in wastewater microalgae can remove carbon, phosphorus, and nitrogenous industrial waste while they can also contribute to biodiversity (Mata et al., 2010). It was reported that *Chlorella vulgaris* used for nutrient removal from wastewater exhibited an average efficiency of 72% for nitrogen removal and 28% for phosphorus removal (Aslan and Kapdan, 2006).

Phenol removal from wastewater by algal culture was reported to be successfully done (Pinto et al., 2003). The advantage of using microalgae for conventional phenol degradation compared to bacteria is that algae use carbon dioxide as their carbon source while bacteria need an extra carbon source for functioning in the process. However, slow growth rate of microalgae compared to bacteria is the limiting factor for degradation.

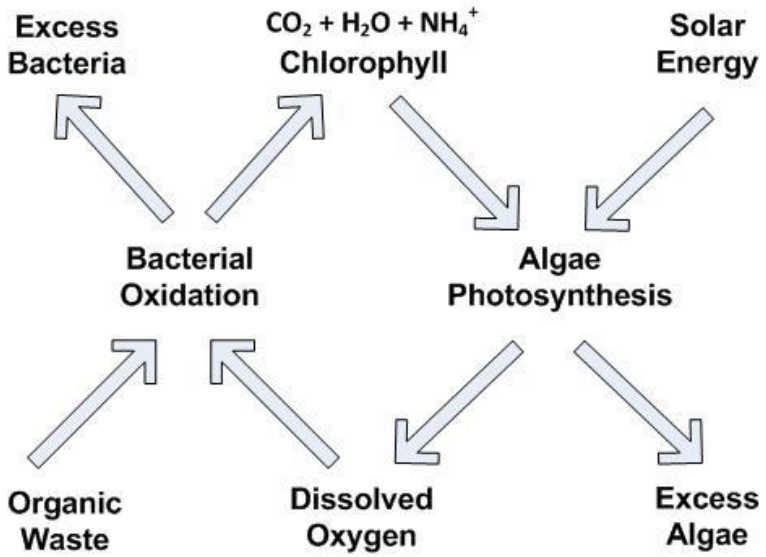


Figure 2.11, use of algae in some environmental applications in a high rate algae pond. Source: (Oswald and Gotaas, 1957).

The use of cyanobacteria for water treatment may not be practical compared to the current chemical or mechanical methods, for challenges such as removal of heavy metal ions. However, cyanobacteria can survive in high concentration of toxic metals including copper, zinc and cadmium (Thajuddin and Subramanian, 2005). On exposure to heavy metals some of the unicellular marine and freshwater strains have the potential to synthesise a low molecular weight metal-binding protein called metalothionin. Some studies at the National Facility for Marine Cyanobacteria (NFMC) have investigated different strains of cyanobacteria which are appropriate for treatment of harmful effluents which contain pesticides, detergents, and antibiotics (Thajuddin and Subramanian, 2005).

2.12 *Genetic and metabolic engineering*

Genetic and metabolic engineering can be applied to enhance the productivity of microalgae and overcome some of the limiting factor for the growth (Roessler et al., 1994). Sequencing of algal DNA is now more accessible and efficient compared to the past as genomes of microalgae species suitable for industrial production are becoming available (Blatti et al., 2013). To date, a bio-informatic analysis of at least 16 algal genome sequences from green and red microalgae have been introduced (Sasso et al., 2012). Applying genetic engineering can potentially enhance photosynthetic efficiency (higher biomass yield on light), increase growth rate and oil content of biomass, improve temperature tolerance and consequently reduce cooling expenses and eliminate photoinhibition phenomenon. Moreover, the harvesting problems can be overcome by introducing trans-genes which can cause auto-flocculation (Gressel, 2008).

Some studies have focused on targeting genes which control the lipid synthesis in microalgae (Zeng et al., 2011). During the exponential phase of growth, when maximum biomass productivity can be achieved, lipid accumulation is relatively low. In contrast, in stress conditions they accumulate various lipids and targeting over-expression of the genes which control the lipid synthesis pathway is one of the options influencing algal oil production (Hu et al., 2008).

However, inducing lipid accumulation by the genes manipulation in the active phase of growth can result in a reduction of cell division. A solution to this challenge is to control the expression of these genes by an inducible promoter that is activated when

algal cells are in stationary phase of growth and the culture reaches high densities (Zeng et al., 2011).

One of the important biochemicals present in microalgae is the protein which contains amino acids (Zeng et al., 2011). The rate of amino acids synthesis can be altered by changing the enzymes involved and the synthesis of these enzymes can be manipulated by changing the activity of the corresponding genes (Merrick, 1992).

Increasing the photosynthetic efficiency to ideally reduce the influence of photoinhibition on algal cell growth is another area which genetic engineering can improve. Most of the attempts have focused on changing the size of the chlorophyll antenna (Radakovits et al., 2010). One of the methods used to tackle this was to introduce random mutagenesis strategies which could generate mutants with smaller photosynthetic pigment antenna. However, some publications reported that an RNA-interference-based strategy which compared to random mutagenesis can be a more efficient and convenient method to be applied in many microalgae species (Mussgnug et al., 2007). The additional benefit of light-harvesting manipulation is the increased biomass productivity in laboratory conditions, although more research needs to be done to demonstrate the performance of the algal mutants in large-scale cultures where various environmental conditions can influence the competition between natural (wild) and mutant algal species.

2.13 Conclusions

Microalgae are a group of photosynthetic organisms with simple structures and generally high growth rates. Several studies have focused on evaluating the potential of these organisms for carbon bioremediation and producing biomass (the upstream stage) and use of their biomass for environmental applications, food supplements and production of biofuel (the downstream stage). The production of biofuel from microalgae is a sustainable process which can be used in the primary stage as a tool for carbon dioxide sequestration and provide the power required for the running facilities. The available methods of microalgae cultivation can be categorized into two main systems: open raceways which can be operated with low cost and closed bioreactors which are costly but more efficient. New engineering techniques have been proposed recently to overcome the problems of large scale production of microalgae biomass. One of the key factors in microalgae cultivation is the choice of illumination strategies.

Development of selective and flexible artificial light sources such as LED and optical fibres can significantly contribute to improvements in growth and productivity rates. Research studies on the use of natural illumination for enhanced growth of microalgae have recently focused on the use of combined systems in which sunlight can be collected by solar panels and an artificial source can recover the light losses during daylight variations. Luminescent photobioreactors are one of the promising designs which can use either natural or artificial illumination sources and tailor the spectral quality of light based on the commercial applications.

Studies on the metabolism of microalgae and the limiting factors on their growth rate can provide information for engineers to design photobioreactors which can control these factors and obtain higher efficiency.

The downstream stage involves harvesting techniques such as filtration, flocculation and centrifugation for algal biomass recovery in which the selection of the appropriate technique depends on the target product. It also covers biomass processing methods (chemical, biological or thermolytic) for biofuel production and other applications, such as biomass by-products for animal feed.

Chapter 3: Materials and methods

3.1 Introduction

This chapter outlines the materials, theories, and analytical methods used in this study. Microalgae strains, their growth requirements and their growth kinetic model are initially illustrated. This is followed by a description of illumination sources, measurements of the light quality (intensity, spectrum), and the luminescent photobioreactors (PBR). The chapter then finishes by illustrating the methods of measuring the carbon dioxide fixation rates, bio-pigments content, lipid content, and elemental analysis. The analytical methods described in this chapter have been applied in a broad range of experiments. Some of these methods have been repeated in different cultivation modes in order to provide consistent and comparative results.

3.2 Microalgae strains

In this section the microalgae strains and the culture media compositions used in this study are described.

3.2.1 Chlorella vulgaris

The green microalgae *C.vulgaris* (CCAP 211/79) was cultivated in a bold basal medium with 3-fold nitrogen and vitamin (3N-BBM+V).

The medium 3N-BBM+V contained the following components:

25.0g L^{-1} $NaNO_3$; 2.5g L^{-1} $CaCl_2.2H_2O$; 7.5g L^{-1} $MgSO_4.7H_2O$; 7.5g L^{-1} $K_2HPO_4.3H_2O$; 17.5g L^{-1} KH_2PO_4; 2.5g L^{-1} NaCl; trace element solution ($FeCl_3.6H_2O$, 97.0 mg L^{-1}; $MnCl_2.4H_2O$, 41.0 mg L^{-1}; $ZnCl_2$, 5.0 mg L^{-1}; $CoCl_2.6H_2O$, 2.0 mg L^{-1}; $Na_2MoO_4.2H_2O$, 4.0 mg L^{-1}); Vitamin B_1 (0.12 g Thiaminhydrochloride in 100 ml distilled water. Filter sterile), Vitamin B_{12} (0.1 g Cyanocobalamin in 100 ml distilled water, take 1 ml of this solution and add 99 ml distilled water. This solution was then filter sterilised). The culture media was autoclaved and stored in a container.

3.2.2 *Gloeothece membranacea*

The cyanobacteria microalgae *G.membranacea* (CCAP 1430/3) was cultivated in a blue green algae medium (BG 11).

The medium BG 11 contained the following components:

15g L^{-1} $NaNO_3$; 4.0g L^{-1} K_2HPO_4; 7.5g L^{-1} $MgSO_4.7H_2O$; 3.6g L^{-1} $CaCl_2.2H_2O$; 0.6g L^{-1} Citric acid; 6g L^{-1} Ammonium ferric citrate green; 0.1g L^{-1} $EDTANa_2$; 2.0g L^{-1} Na_2CO_3; Trace metal solution (2.86g L^{-1} H_3BO_3; 1.81g L^{-1} $MnCl_2.4H_2O$; 0.22g L^{-1} $ZnSO_4.7H_2O$; 0.39g L^{-1} $Na_2MoO_4.2H_2O$; 0.08g L^{-1} $CuSO_4.5H_2O$; 0.05g L^{-1} $Co(NO_3)_2.6H_2O$). Medium BG 11 was then autoclaved and the pH was adjusted to 7.1.

3.3 Culture method

Indoor cultures allow control over temperature, nutrients, contamination and illumination. The most commonly used algae culture methods are batch cultures. Batch cultures are closed systems with no input or output of materials and due to their simplicity and cost effectiveness, these have been widely used in laboratory studies.

Since the aim of this study is to investigate the effect of illumination on microalgae growth and their products, batch cultures were of particular interest (Barsanti and Gualtieri, 2006b). Batch culture systems are highly dynamic and the biomass concentration in these systems increases continuously until the limiting factors such as lack of nutrients are reached. In addition, microalgae cells produce some biochemical components and these cell products increase constantly in batch cultures. The population of cells in a dynamic system show a typical growth pattern similar to a sigmoid curve which includes six phases of growth. After the inoculation, growth may not start immediately. Although cells are viable, they may not start to divide in a new culture environment. Depending on the age of the seed culture, lag phases can vary from few hours to days. To reduce the time for up-scaling and to minimise the length of the lag phase, it is important to carry out inoculation from exponentially growing seed cultures.

The seed cultures of both microalgae strains were incubated at 20^{o}C under cool white luminescent light with an intensity of approximately 20 μmol photons m^{-2} s^{-1} on 12:12 hours light: dark period for a week. After one week, new cultures were inoculated at different concentrations.

3.4 Biomass production

The gravimetric estimation of microalgae cell dry weight is typically used for direct measurements of growth. This method, also known as total suspended solids, was used for the determination of biomass density (Eaton et al., 1995a)`as follows:

Whatman GF/C (2.5 cm) filters were placed on the filtration apparatus including air vacuum (Fisher Scientific, Loughborough, England), suction flask and filter holder. The filters were washed with three successive 20 ml portions of deionised water. Once the maximum amount of liquid was removed by suction, the filters were removed and dried in an oven at 70^{o}C for 24 hours and stored in a vacuum desiccator over silica gel until required. The filters were then weighed to four decimal places on an analytical balance. 10 ml of algae culture were then filtered and after completion of filtration they were transferred to an oven at 90^{o}C for 4 hours to get dried. The filters were then placed in a desiccator and weighed when needed. The biomass concentration was calculated from the equation (3.1).

$$Biomass\ (mg/L) = (A - B) \times 1000/\ (sample\ volume,\ ml) \quad (3.1)$$

where, *A* is the weight of filter plus algae dried residue (mg) and *B* is the weight of filter (mg) (Eaton et al., 1995b).

3.5 Calibration curves

The relationship between the biomass density and optical density of the microalgae samples was determined by a spectrophotometric method in which the culture absorbance was measured at the wavelength of 680 nm. Calibration curves (Figures 3.1 and 3.2) were obtained, showing the relationship between the cell dry biomass density (X_b, g L^{-1}) and optical density (OD_{680}):

$$X_b\ (C.vulgaris) = 0.5942 \times OD_{680} + 0.0024 \quad (3.2)$$

$$X_b\ (G.membranacea) = 0.6922 \times OD_{680} + 0.0021 \quad (3.3)$$

In addition, cell concentration (number of cells per litre of culture) was determined using a haemocytometer and optical microscope (CH2 Olympus optical Co. Ltd, Tokyo, Japan).

The dependence between cell concentration (X_c, g L^{-1}) and the optical density (OD_{680}) were established from the calibration curves:

$$X_c\ (C.vulgaris) = 17.776 \times OD_{680} + 0.4499 \quad (3.4)$$

$$X_c\ (G.membranacea) = 13.53 \times OD_{680} + 0.1283 \quad (3.5)$$

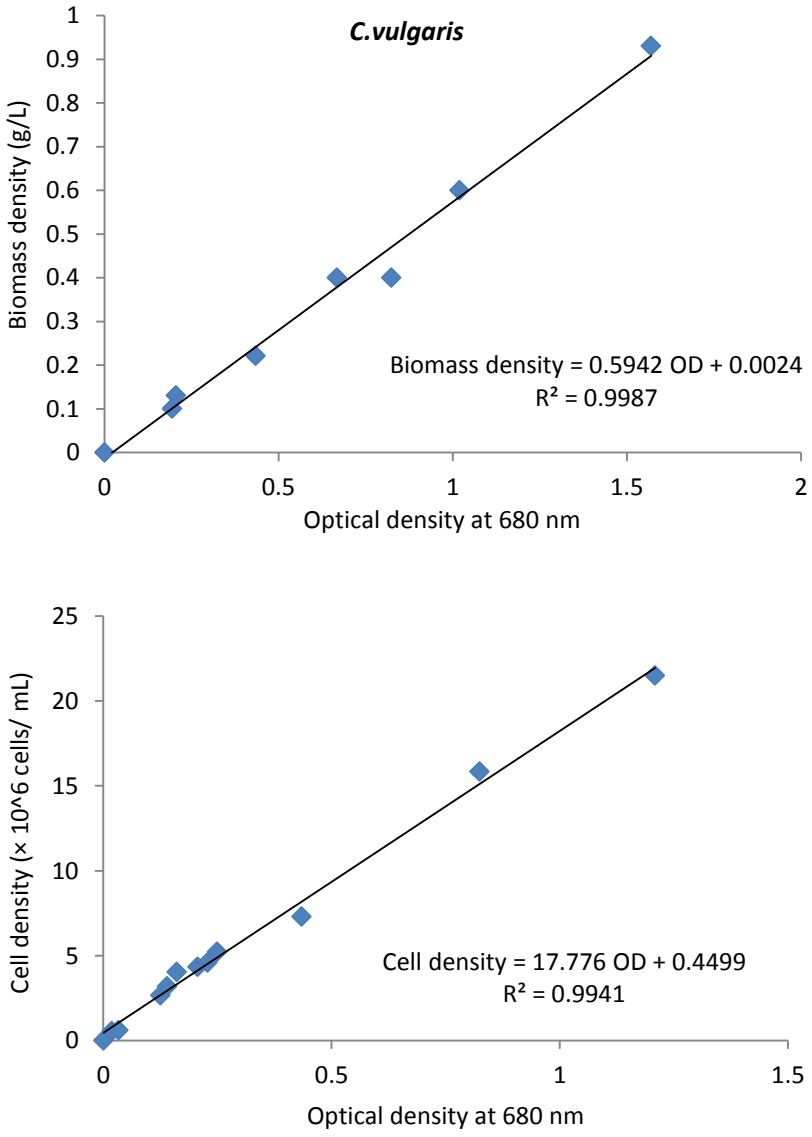
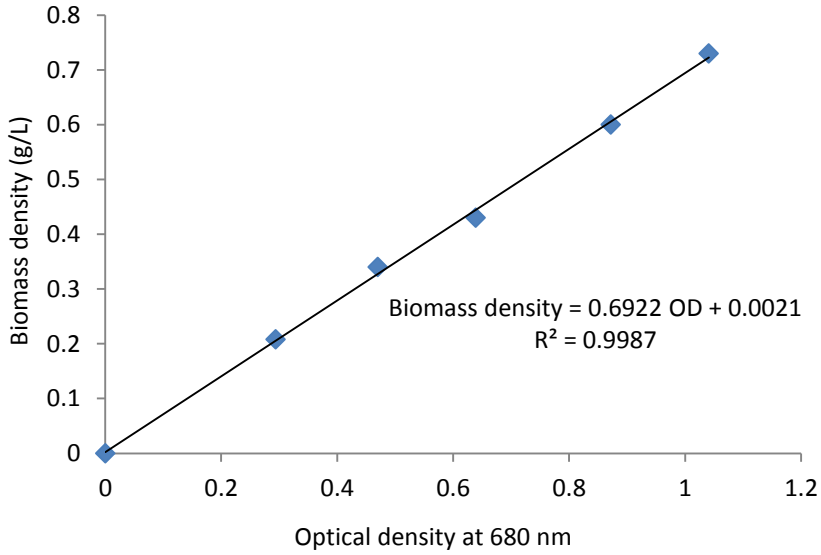


Figure 3.1, Calibration curves of (a) biomass density vs. optical density, (b) cell concentration vs. optical density for *C.vulgaris.*



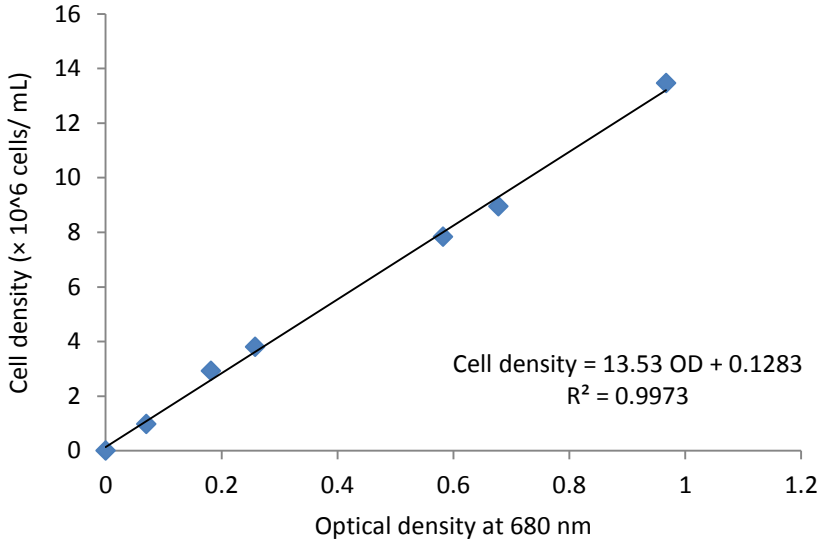


Figure 3.2, Calibration curves of (a) biomass density vs. optical density, (b) cell density vs. optical density for *G.membranacea*.

3.6 Kinetic model

Growth curves can be obtained from the biomass density or cell concentration data at intervals such as once per day. Once the plot is made, the specific growth rate and biomass productivity are calculated.

Microalgae growth can be modelled by a first order dynamic equation:

$$dX/dt = \mu X \tag{3.6}$$

where, μ is the specific growth rate (day^{-1}), X is the biomass concentration ($g\ L^{-1}$) and t is the number of days respectively. The specific growth rates of the cultures can be calculated using Equation (3.7):

$$\mu = [ln\ (X_t/X_0)]/(t - t_0) \quad (3.7)$$

The biomass productivity rate ($g\ L^{-1}\ d^{-1}$) (also known as linear growth rate) can be estimated using Equation (3.8):

$$P = (X_t - X_0)/\ (t - t_0) \quad (3.8)$$

Where P is the biomass productivity rate and is measured during the exponential phase of growth.

3.7 Illumination sources

In order to simulate outdoor illumination conditions for microalgae, 150 W xenon arc lamps (Ceralux, Luxtel) and 300W xenon arc lamps (Cermax, Perkin Elmer) were used. The lamps were driven by 300 W power supplies (PS300-12, Perkin Elmer). Xenon arc lamps are widely used in solar simulators by the photovoltaic industry, as they are capable of producing a broad illumination spectrum including short wavelengths of ultra violet (UV), visible ranges of the photosynthetic active radiation (PAR) and long wavelengths of infra-red (IR) (Gouveia et al., 1996). The lamps were mounted in light boxes equipped with fans to provide ventilation, cooling, and to minimise local temperature variations. Figure 3.3 shows the light box and xenon lamps used in this study.

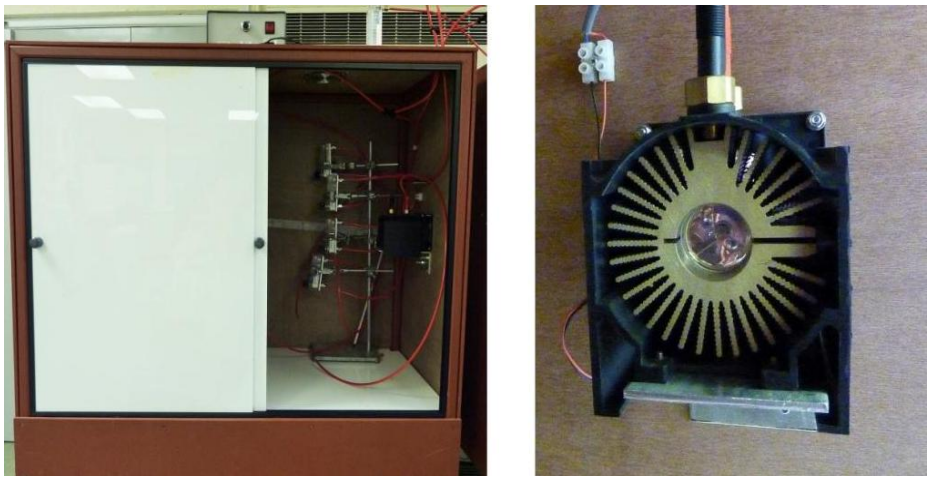


Figure 3.3, Light box (left side) equipped with xenon lamp (right side) used in this study for illumination of cultures either in static or aerated modes.

3.8 Light intensity

Light intensity in form of photon flux provides the number of photons that are available for photosynthesis. The photon energy of wavelength 680 nm can typically provide the energy level required for chlorophyll-a to start photosynthesis. Shorter wavelengths of visible spectrum contain higher energy levels. If the photons are absorbed by microalgae at these wavelengths, due to their high energy levels, they have to be released as heat by the cells in order to initiate photosynthesis. Therefore, for the study of microalgae growth it is important to measure the light intensity in terms of photon flux within the PAR range. In order to mimic the natural environmental condition for growth of the two freshwater species local to Scotland, the light intensity in a cloudy day (in October) in Edinburgh was measured. The light intensity was about 200-250 μmol photons m^{-2} s^{-1} and based on that the experiments were set.

A PAR light meter is an irradiance detector which is sensitive to visible light and insensitive to light outside PAR range. The internationally accepted irradiance unit is now expressed as moles of photons per unit area per unit time μmol photons m^{-2} s^{-1} (previously known as μEinsten m^{-2} s^{-1}). 1 μmol of photon is equal to 6.02×10^{17} photons at a corresponding wavelength. Therefore, in this study the light intensity was measured in unit of photon flux using a PAR light meter (Skye Instruments, Quantum sensor, Powys, Wales).

3.9 Luminescent acrylic filters

The fluorescent dyes or fluorophores used in this study were obtained from the Lumogen F dyes series (BASF Aktiengesellschaft, Ludwigshafen, Germany). Visible-emitting organic dyes were selected for this study (see Figure 3.4). The following colorants were tested and their emission spectrum was measured:

- Lumogen F Violet 570 (naphthalimide)
- Lumogen F Yellow 083 (perylene)
- Lumogen F Orange 240 (perylene)
- Lumogen F Red 305 (perylene)
- Clear (no colourant; used as control growth conditions in all cases)

Luminescent acrylic sheets were made of transparent poly methyl methacrylate (PMMA) doped with the fuorophores mentioned above. Luminescent acrylic sheets

containing different dyes were placed as filters directly between the xenon lamps and the culture flasks, such that the light transmitted and emitted through the face of the sheets became incident on the culture flasks. Figure 3.4 illustrates cross section of a luminescent sheet showing different light paths when illuminated by a light source.

Photons of light can be absorbed by the host material (PMMA) and the fluorophores. Small amount of photons can also be reflected through the surface. However, the majority of photons are transmitted through the surface and the edges of the luminescent sheet. Depending on the wavelength range of fluorophores, the photons of light absorbed by fluorophores were emitted at a different wavelength and received by the algae culture.

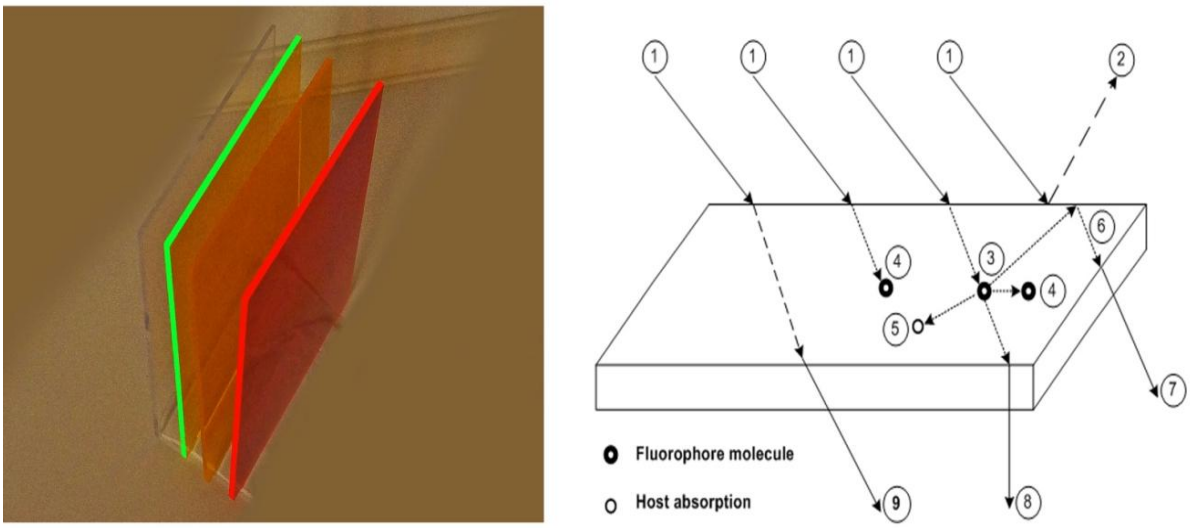


Figure 3.4, Luminescent acrylic sheets (left side), Cross-section of a luminescent sheet showing different paths of light (right side): 1- incident light, 2- surface reflection, 3- light absorption and emission by fluorophore molecule, 4- complete light absorption by fluorophore, 5- light absorption by host material, 6- trapped emission, 7- light emission from the edges, 8- fluorescence emission from the surface, 9- unabsorbed light.

3.9.1 Water bath casting of luminescent filters

Water bath casting was first invented in 1937 (Rohm, 1937). Water bath casting is one of the most common techniques for casting PMMA using a monomer/polymer mixture. The mould used for casting consists of two glass plates which are separated by a resilient gasket. Monomer and polymer powders were obtained from Lucite and the monomer contained 5 ppm w/w of Topanol inhibitor to prevent polymerisation by itself in storage (Wilson, 2010). Monomer and polymer were weighed in the ratio 9:1 (monomer : polymer). The dyes were dissolved into the bulk of the monomer at this stage, as the lower viscosity of the pure monomer makes the addition of the dyes easier. The monomer was heated to 60^{o}C on a magnetic stirrer and the polymer powder was added gradually. The mixture was stirred for about an hour, making a clear syrupy liquid. The syrup was then removed from the heater and left to cool to the room temperature. The casting syrup was later poured down the mould in a continuous stream to prevent bubbles. Clips were then used at the top edge of the mould to keep it closed. The mould containing syrup, was heated in a 60^{o}C water bath initiating the polymerisation. The mould was kept in the bath for about 18 hours to complete the polymerisation and was then removed the next morning. The concentrations of the dyes used, with the same optical density in a 0.3 m thick PMMA sheet, were 313 ppm for violet/ blue, 164 ppm for yellow/green, 134 ppm for orange, and 377 ppm for red. The price of the Lumogen dyes was around £22 per gram.

3.10 Wavelength spectra

The output spectrum of the lamps was measured using a high resolution spectrometer (HR2000 Ocean Optics, USA). The output spectrum of the lamps using the described luminescent filters is presented in Figure 3.5. The graph shows the light intensity (counts) vs. light wavelength (nm) for different luminescent-filtered xenon light as provided for algal growth.

The unfiltered spectrum shows the output of xenon lamps without any filter. The measurements showed that the lamps contain some UV and IR radiation which is out of the visible range. The control (clear PMMA), violet, green, orange and red filters show different wavelength spectra and they are all in the photosynthetic active radiation range.

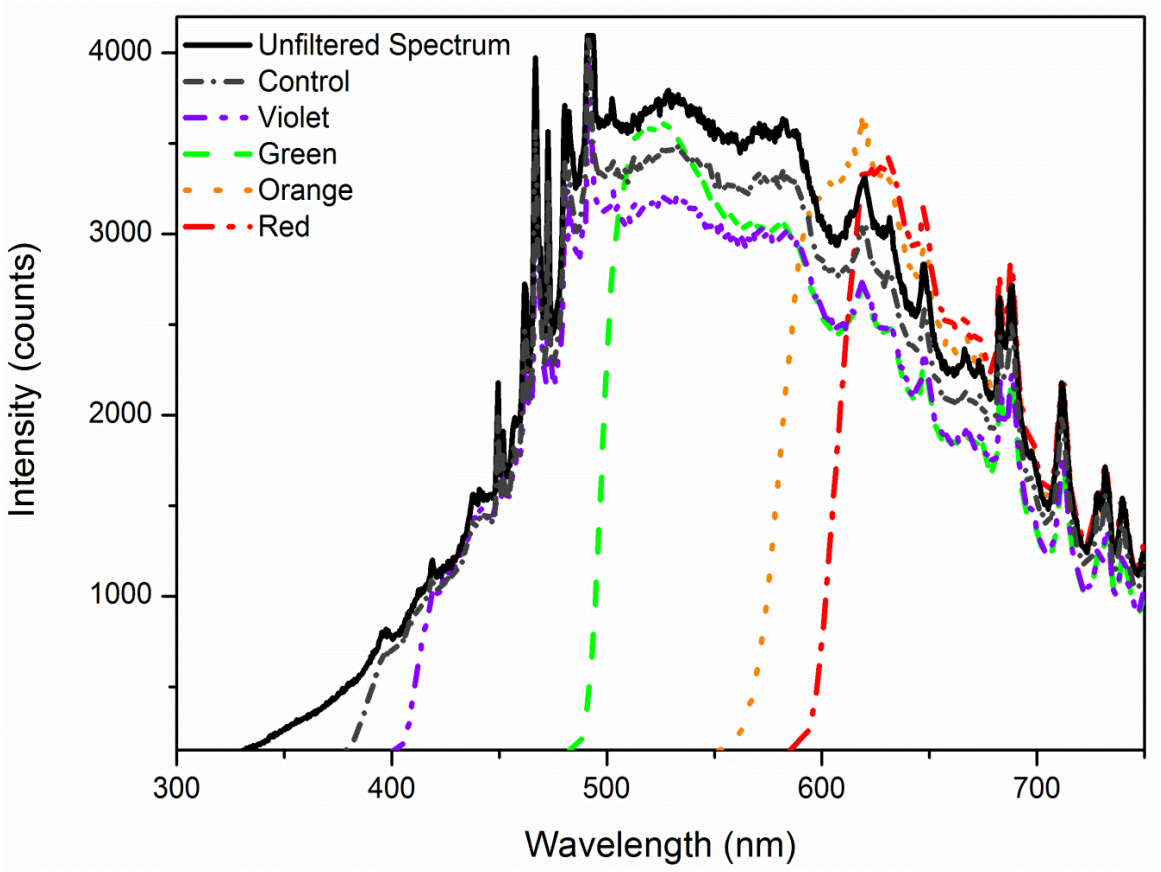


Figure 3.5, Graph of light intensity (counts) vs. light wavelength (nm) for different luminescent-filtered xenon light as provided for algal growth. Unfiltered, control (clear PMMA), violet, green, orange and red filters show different wavelength spectra.

3.11 Luminescent photobioreactors

Bubble column photobioreactors constructed from luminescent acrylic polymer were used in this study. Bubble column photobioreactors are commonly used for microalgae cultivation, however to date no bioreactor design has been constructed using luminescent dyes. The main body of the photobioreactor was capable of spectral conversion of light inside the photobioreactor. The luminescent pigments used absorb light at specific wavelengths and emit them at longer wavelengths. A schematic diagram of the photobioreactor and details of the luminescent PBR are presented in Figures 3.7 and 3.7. The photosynthetic active radiance inside the reactor was measured in unit of photon flux (μmol m^{-2} s^{-1}) using a PAR light meter (Skye Instruments, Quantum sensor, Powys, Wales). The emission spectrum of the light inside the vessel is shown in Figure 3.8. The vessel was 50.8 mm in diameter (47 mm internal diameter) and 260 mm in height, giving the working volume of 450 ml.

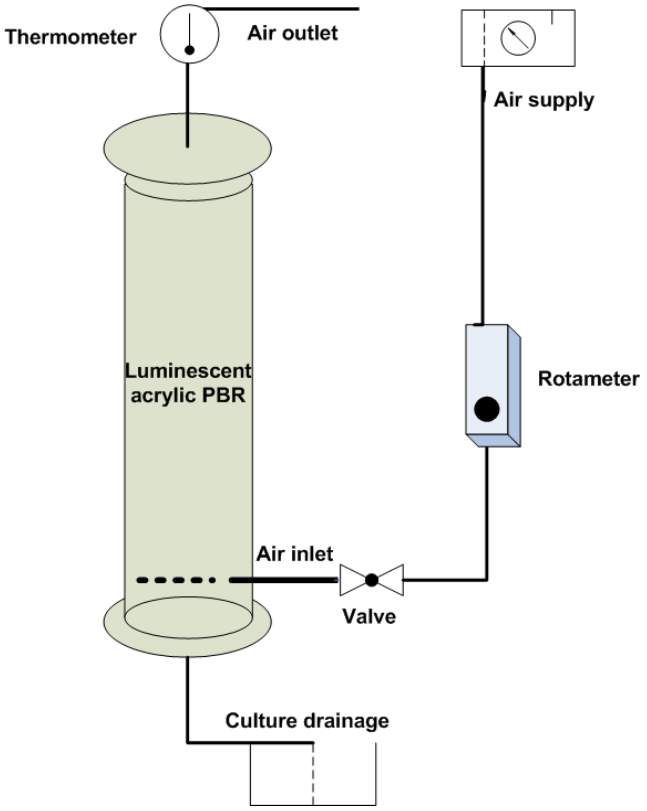


Figure 3.6, Schematic diagram of the luminescent acrylic bubble column photobioreactors.

Figure 3.7, Left to right clockwise: Luminescent PBR in six different ranges, PBR in operation inside the light box, top view of the PBR, side views of the PBR.

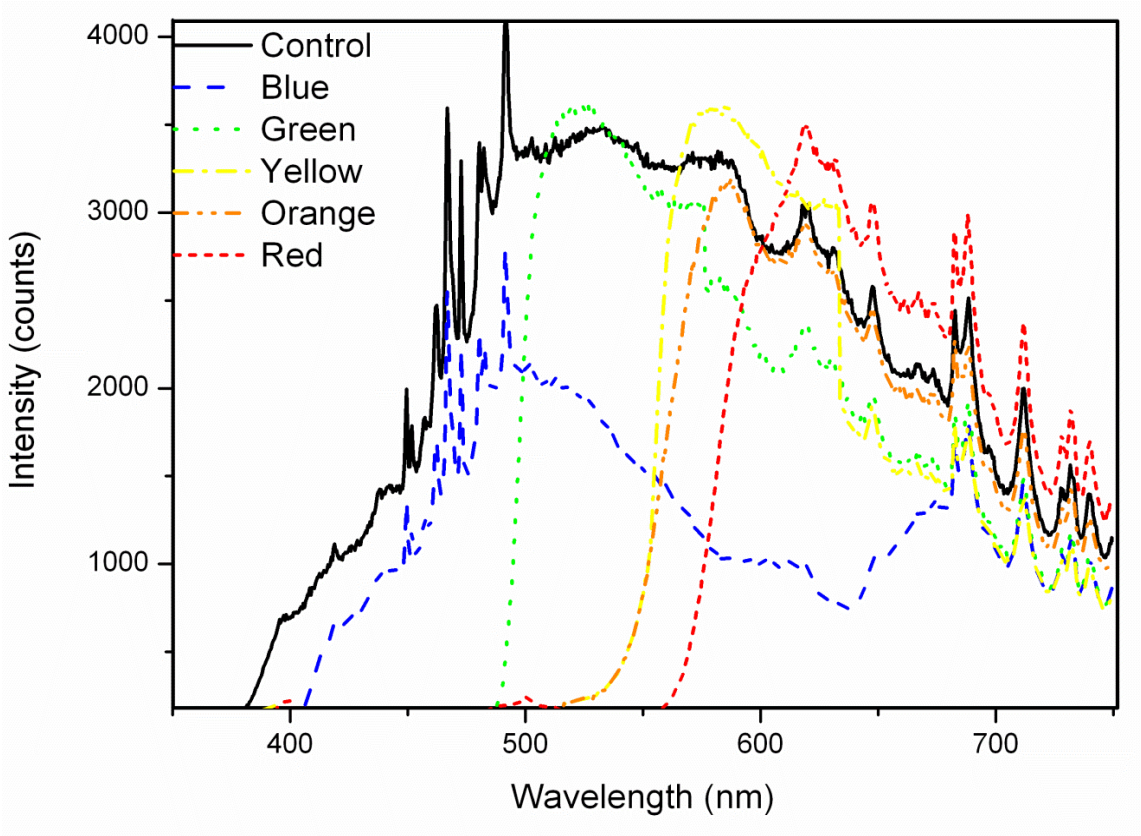


Figure 3.8, Graph of light intensity (AU) vs. light wavelength (nm) for different luminescent filtered light inside the photobioreactors provided for algal growth. Control, blue, green, yellow, orange and red filters show different wavelength spectra.

The culture was mixed by sparging with compressed air injected at the flow rate of 0.2LPM from the bottom of the photobioreactor, through a perforated stainless steel sparger (10 holes of 1 mm diameter) located 3 cm from the bottom of the reactor. The air flow was controlled by a rotameter (FMA3306, Omega Engineering Limited, Manchester, UK). The main advantages of using bubble column photobioreactors are the high surface to volume ratio, low capital cost, well mixed and homogenous culture and efficient mixing of air and carbon dioxide (Singh and Sharma, 2012).

3.12 CO_2 fixation rate

According to the mass balance of microalgae, the fixation rate of carbon dioxide can be calculated. Microalgae biomass contains approximately 50% carbon based on dry weight (Sanchez Miron et al., 2003). This carbon is mainly derived from carbon dioxide which is available from the air or from waste streams of factories. A mole of CO_2 has a mass of 44 grams including 12 grams of carbon.

Based on these premises theoretical carbon dioxide consumption by each gram of microalgae can be obtained from equation (9) as below:

$$\frac{44\ (g\ CO_2/mol)}{12\ (g\ Carbon/mol)} \times \frac{0.50\ (\text{g Carbon})}{(g\ algae\ biomass)} = 1.83\ (g\ CO_2/\ g\ algae\ biomass) \qquad (3.9)$$

Equation (3.9) shows that in theory one gram of algal dry cell weight can fix approximately 1.83 grams of carbon dioxide. However, in experimental works carbon content of microalgae changes depending on the species and culture conditions. Biomass productivity and experimental carbon content obtained from elemental analysis influence the rate of carbon dioxide consumption. Equation (10) shows the relationship between these factors:

$$CO_2\ fixation\ rate\ (mg\ L^{-1}\ day^{-1}) = P \times C_{carbon} \times (M_{CO2}/M_C) \qquad (3.10)$$

Where P is the biomass productivity (mg), C_{carbon} is the experimental carbon content and M_{CO2} and M_C are the molar mass of CO_2 and carbon respectively.

3.13 Bio-pigments

All microalgae contain at least one type of chlorophyll. Chlorophyll-a is the primary pigment responsible for photosynthesis. Generally, each microalgae strain has its own particular combination of pigments providing an individual colour and photosynthetic character. This photosynthetic character reflects the absorption peak of the pigments in the PAR spectrum. Identification of the optimum wavelength ranges for synthesis of these pigments and examination of the influence of spectral conversion of light on them is crucial. Therefore, the presence of chlorophylls in both strains of microalgae and their variations under different conditions has been studied. In addition, phycobiliprotein content of cyanobacteria as accessory pigments has been determined.

3.13.1 Chlorophylls

A modified method of Jeffery and Humphrey (1975) for spectroscopic determination of chlorophyll concentration was applied (Cheirsilp and Torpee, 2012). Due to cell density, 5 - 10 ml of the cultures were filtered onto glass fibre Whatman filters GF/F

type, 25 mm in diameter. The filters were then folded once with the algae inside and stored at -20^{o}C. Chlorophyll extraction was carried out by grinding the filters in a few millilitres of 90% acetone in a glass homogenizer in an ice bath under low light conditions for 1 minute. After grinding, the chlorophyll extracts were transferred to a graduated and stoppered centrifuge tube, rinsed with exactly 10 ml of 90% acetone (i.e. 10 ml+ dead volume of filter).

The extract was centrifuged (Multifuge 3 S-R, Heraeus, Hanau, Germany) for 10 minutes at 500×g (where g is the gravitational acceleration 9.81 m s^{-2}). After completion of centrifugation the absorbance of the supernatant (OD) was measured at 750, 664, 647, and 630 nm against a 90% acetone blank.

The concentration of chlorophyll a and b was calculated according to the equations below (Jeffrey and Humphrey, 1975):

$$Chlorophyll\text{-}a = (11.85*(OD_{664} - OD_{750}) - 1.54*(OD_{647} - OD_{750}) - 0.08\ (OD_{630} - OD_{750}))*V_e/L*V_f \quad (3.11)$$

$$Chlorophyll\text{-}b = (-5.43*(OD_{664} - OD_{750}) + 21.03*(OD_{647} - OD_{750}) - 2.66\ (OD_{630} - OD_{750}))*V_e/L*V_f \quad (3.12)$$

Where: L = Cuvette light-path (cm), V_e = Extraction volume (ml), V_f = Filtered volume (L). Concentrations are given in unit mg m^{-3}. The OD reading at 750 nm is a correction for turbidity. Because the optical density of the extract is very sensitive to changes in the acetone to water proportions it was important to use exactly 90% acetone.

3.13.2 Phycobiliproteins

The water soluble luminescent proteins found in cyanobacteria strains perform as accessory pigments to capture light in portions of visible spectrum which are hardly used by chlorophylls. The phycobiliprotein content was determined using a modified method of Bennet and Bogorad (1973) in which the cyanobacteria culture was centrifuged at 4000×g for 10 minutes. The harvested cell mass was washed with distilled water and suspended in 2 ml of sodium phosphate buffer (0.1 mM, pH 7.0). The suspension was then placed in an ultrasound bath (Elmasonic S10, Elma GmbH & Co. KG, Singen, Germany) for cell disruption followed by freezing and thawing several times in the dark for further extraction.

The disrupted cells were later centrifuged at 4300×g for 15 minutes at 4^{o}C. The clear supernatant containing phycobiliproteins was collected for spectroscopy measurements. The concentrations of phycobiliproteins were calculated according to the equations below (Bennett and Bogorad, 1973):

$$Allophycocyanin\ (APC)(\ mg\ ml^{-1}) = [A_{652} - 0.208(A_{620})]/5.09 \quad (3.13)$$

$$C\text{-}phycocyanin\ (C\text{-}PC)(mg\ ml^{-1}) = [A_{620} - 0.474(A_{652})]/5.34 \quad (3.14)$$

$$C\text{-}phycoerythrin\ (C\text{-}PE)(mg\ ml^{-1}) = [(A_{562} - 2.41(PC) - 0.849(APC)]/9.62 \quad (3.15)$$

3.14 Lipid content

The purpose of this study was to achieve comparative results in which general aspects of biochemical composition of microalgae were investigated. Variations of the total lipids content of microalgae in different cultivation conditions can provide an overall view of the performance of luminescent photobioreactors for enhancement of oil production in microalgae.

Lipids are essential components in microalgae where they function as storage products, membrane components, and more importantly as sources of energy (Becker, 1994a). Lipids extracted by lipophilic organic solvents such as chloroform or petroleum ether are commonly known as total lipids. Lipid accumulation is typically induced by fluctuation of growth conditions or various types of stress and it can be expressed as variation in fatty acids compositions or total lipids. As lipid synthesis occurs with reduction of photosynthesis activity Becker (1994) recommended fast algae growth under optimum conditions followed by a specific stress factor, such as nitrogen starvation, to obtain maximum lipid content.

Lipid content was determined using a modified chloroform – methanol solvent based method (Bligh and Dyer, 1959). The optimised analytical method for extraction of lipids used a chloroform – methanol 1:1 solvent system in which there was no need for pre-treatment of biomass or addition of antioxidants (Ryckebosch et al., 2012).

400 mg of algae paste or 100 mg of lyophilized algae was weighed and placed in solvent resistant tubes and 4 ml methanol was added. After adding 2 ml of chloroform

and 0.4 ml of distilled water, the mixture was vortexed for 30 seconds and placed in an ultrasound bath for 10 minutes. 2 ml water and 2 ml chloroform were then added, and the mixture was centrifuged at 3300×g for 10 minutes. After centrifugation the upper layer containing methanol and water was removed and the lower layer was transferred to a clear tube and passed through a layer of anhydrous sodium sulphate using a Whatman no.1 filter in a funnel. The remained solids were re-extracted using 4ml of a chloroform – methanol 1:1 solution. The solvents were removed by a rotary evaporator (Rotavapor R-114, BUCHI, Flawil, Switzerland) and a green layer of algae lipid was obtained at the end of extraction.

The total lipid content was calculated gravimetrically according to equation 16:

$$\textit{Total lipid content \%} = \textit{algae lipid (g)/ algae biomass (g)} \times 100 \quad (3.16)$$

3.15 Elemental analysis

Similar to lipid determination, this study examined the influence of several cultivation conditions on elemental carbon, hydrogen, and nitrogen content of microalgae. Carbon is the most important element in microalgae cells and can make up to 50% of the dry biomass. Determination of elemental carbon shows the cell's response to specific growth conditions. Furthermore, it is an indication of CO_2 sequestration rate.

Nitrogen content is another essential element in microalgae cells. Nitrogen content can be related to the lipid accumulation by the cells. Moreover, extreme cultivation conditions can induce nitrogen storage in the cells as an environmental response. For instance, nitrogen starvation in the culture media can lead to lipid accumulation as storage compounds for the cells. In this study, the media composition was maintained constant to observe only the influence of light quality variations. The data related to the elemental content of the cells could further assist the overall view of the performance of luminescent photobioreactors.

Total carbon, nitrogen and hydrogen content of the freeze-dried microalgae biomass was measured by elemental analysis (Exeter CE440 CHN-O/S, Control Equipment Corporation, Lowell, MA, USA). The biomass samples were weighed accurately to one millionth of a gram inside a small tin capsule. The capsule was placed in a combustion tube at 950^{o}C with pure oxygen to form tin oxide. The temperature in the tube was increased well above 1800^{o}C as a result of this combustion and samples were

vapourised to complete combustion. At this stage, samples in a gas form went through a reduction tube to remove any unused oxygen. Different gases from the sample were then mixed at a precise pressure, temperature and volume, and small portion of this mixture went through a series of thermal conductivity cells where the quantity of each gas was recorded. From these readings the percentages of carbon, nitrogen and hydrogen in the sample were calculated.

The data obtained was used to determine the carbon content of the cells for calculation of carbon dioxide absorption. In addition, nitrogen content was related to the lipid amounts produced by microalgae cells.

3.16 Conclusions

This chapter illustrated the materials and methods used in this study. The chapter described the microalgae species and their culture media composition. The description of the illumination sources used in this study along with the light quality measurements provided an insight into one of the most important cultivation parameters (light) and its effects on the microalgae. The analytical methods clearly illustrated the theory behind the experimental procedures for determination of biomass production, pigments content, and lipid contents. The chapter also described the theory behind the carbon dioxide fixation by microalgae, and the elemental analysis which provides the data related to carbon uptake and lipid content.

Chapter 4: Static cultivation

4.1 Introduction

In this chapter the effect of static or non-aerated cultivation mode on growth of microalgae is examined. Growth factors such as the specific growth rate and biomass productivity have been determined to provide comparative data to those of other cultivation modes. In the photoautotrophic cultivation mode, light is the main source of energy and inorganic carbon (such as CO_2) is used as the carbon source (Huang et al., 2010). Photons can be absorbed as nutrient by microalgae cells, thus the quality of light in terms of intensity and wavelength is critical for cell growth (Wang et al., 2007).

The chapter starts with a description of the cultivation conditions, followed by biomass determination. It provides the corresponding growth profiles and compares different growth patterns. In addition, it gives an insight into chlorophyll production under the static condition.

Previously, a study focused on optimisation of light quality and quantity for plant growth, used fluorescent films with different fluorescent pigments (Chrismadha and Borowitzka, 1994). The results of the study showed that blue fluorescent films enhanced the growth of strawberry fruit whereas red fluorescent films delayed the fruit production considerably. The study suggested that using blue fluorescent films in greenhouses could potentially promote the production of strawberry fruit. Another study reported that red light increased the number of blossoms on rose flowers and was also the favourable light for growth of the tomato fruit, due to the morphogenetic reaction of the photosynthetic plants to changes of light conditions (Sukenik, 1991).

In the present chapter the influence of light source on two strains of microalgae *Chlorella vulgaris* (green algae) and *Gloeothece membranacea* (cyanobacteria or blue-green algae) has been studied by modifying light wavelength range using luminescent sheets. The strains were chosen as geographically local representatives of both prokaryotic and eukaryotic microalgae. The choice of local species minimises any environmental impact in the event of accidental release from large-scale commercial culture. The study investigated the effects of the selected light source on biomass content and photosynthetic pigment production. All aspects of the experiments were

designed to mimic local natural growth conditions, to reproduce as best as possible a potential local commercial operation.

4.2 Cultivation condition

The seed cultures of both microalgae strains were prepared by cultivation in 250 ml flasks containing 100 ml of the media (3N-BBM+V for green algae and BG 11 for cyanobacteria). The cultures were incubated in a light box at 20^{o}C for a week under cool white fluorescent light with an intensity of approximately 20 μmol photons m^{-2} s^{-1} on 12:12 hours light: dark period. 20 ml of the seed cultures were then used for inoculation of the main growth media.

Optical densities (at 680 nm) of the new cultures of *C.vulgaris* and *G.membranacea* after inoculations were 1.6 and 1.0 respectively. The cultures were then transferred to light boxes. The light boxes were designed as rectangular chambers illuminated by full spectrum xenon lamps using luminescent sheets as light filters, and a ventilation system to prevent overheating inside the light box. The temperature inside the light box was maintained at 23±2^{o}C and recorded twice a day using a thermometer located near the culture flasks. Light intensity of the sunlight measured in a cloudy day in Edinburgh was about 200 – 250 μmol photons m^{-2} s^{-1}. To obtain a similar outdoor light intensity of a typical cloudy day in Scotland, the cultures of *C.vulgaris* were illuminated under the intensity of 250 μmol photons m^{-2} s^{-1}.

Some cyanobacteria species adapted to the Scottish climate showed photo-inhibition even at low levels of light intensity thus *G.membranacea* was illuminated under the intensity of 150 μmol photons m^{-2} s^{-1}. The light intensity was measured at the same point between the luminescent sheet and the growth culture for consistency.

4.3 Biomass production

This section describes the biomass production of *C.vulgaris* and *G.membranacea*.

4.3.1 *Chlorella vulgaris*

Four different colours of luminescent acrylic sheets, as described in Chapter 3, were used as light filters against 150W xenon lamps with intensity of 250 μmol m^{-2} s^{-1}, along with a reference condition of a clear acrylic sheet with no added fluorophore. The light emission in PAR by violet light (peak wavelength 400-450 nm) produced the most efficient wavelength bands required for growth of microalgae cells. The highest biomass

accumulation was achieved under this light colour. However, a maximum specific growth rate of 0.13 day^{-1} was obtained under orange light (peak wavelength 585-620 nm) at the first day of cultivation.

The biomass density versus time profiles in Figure 4.1 shows the growth of *C.vulgaris* in five different light conditions. The curves of Figure 4.1 were fitted using a standard sigmoid Weibull growth model. The population of cells in a dynamic system, as described by Barsanti et al. (2006), show a typical growth pattern similar to a sigmoid curve which includes different phases of growth.

The *C.vulgaris* biomass concentration increased rapidly under orange and green light conditions at the first day, with specific growth rates of 0.13 day^{-1} and 0.10 day^{-1} respectively, however cells under violet and control light stayed in the lag phase for two days. When exposed to red light, *chlorella* cells entered the exponential growth phase after 4 days.

Overall, growth patterns were similar under green, orange and violet light with slightly higher average biomass production under green light. In addition, maximum biomass productivity of 0.03 g L^{-1} day^{-1} was achieved under these three light conditions, suggesting these favour biomass production. However, significantly lower specific growth rates of 0.07 day^{-1} and 0.05 day^{-1} were obtained under red light and control (full spectrum) light. Red light with a narrow spectrum of 600 – 700 nm did not promote rapid growth of *C.vulgaris* cells. The results show that the absence of violet and green wavelength bands had a high impact on cells productivity.

On the other hand, data obtained from the control light condition showed low biomass productivity and linear growth phase for 13 days which suggest that shorter wavelength radiation was a growth inhibition factor. Therefore, two light conditions which were unfavourable for *C.vulgaris* cultivation affected the growth in different ways. Lack of essential wavelength bands in PAR range in the former, resulted in slow growth and low productivity, whereas in the latter, presence of shorter wavelength radiation had inhibition effects on *C.vulgaris* cells.

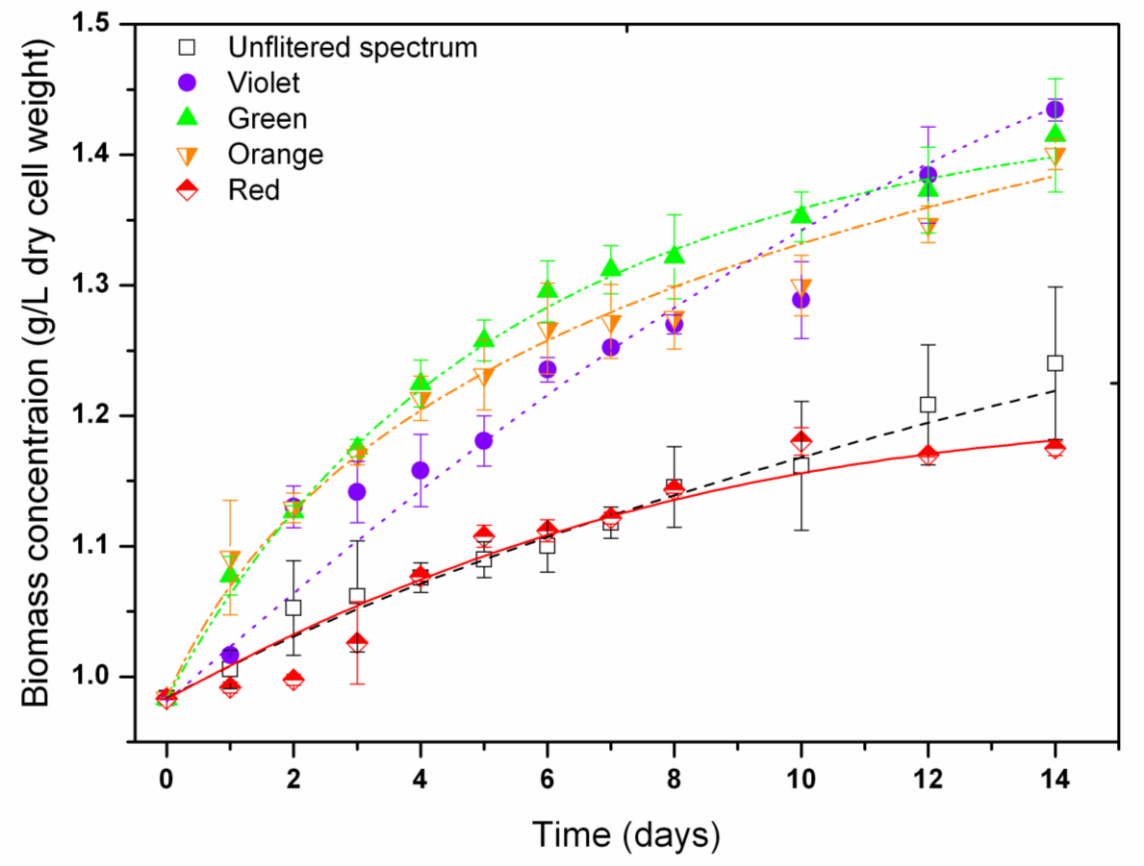


Figure 4.1, Biomass density (cell dry weight per litre of culture) of *C.vulgaris* under violet, green, orange, red and control light recorded over a 14 day period. The curves are fitted using a standard sigmoid Weibull growth model. Data are the means ± SD of 5 replicates.

4.3.2 *Gloeothece membranacea*

The biomass density versus time profiles in Figure 4.2 shows the growth of *G.membranacea* under five different light conditions with the intensity of 150 µmol m^{-2} s^{-1}. Exponential growth in cyanobacteria cells started at the first day of inoculation. Similar growth patterns were obtained under all light conditions except red light with violet light being the most efficient light. Slightly lower biomass content was obtained under orange, green and control light conditions.

A maximum specific growth rate of 0.16 day^{-1} was achieved at the first day under the violet light condition. Violet light resulted in the highest biomass production of 1.41 g L^{-1} and productivity of 0.05 g L^{-1} day^{-1}, while red light showed the least efficiency in converting light energy into biomass.

Lack of essential shorter wavelength bands in the red light condition highly influenced the growth of *G.membranacea*. However, chromatic adaptation in cyanobacteria cells resulted in almost similar growth patterns under the other four light conditions, with violet being the most efficient. High tolerance of fresh water species of cyanobacteria cells to UV and shorter wavelength radiation (Holzinger and Lutz, 2006) can justify its comparable growth under control light to green and orange light. The illumination of cyanobacteria cells by violet light could potentially be the most effective way to produce maximum biomass content.

The results show the great potential of using specific and modified wavelengths of light for improving biomass content. The luminescent dyes used do not simply remove light of certain wavelength ranges, but rather absorb light at a shorter wavelength and re-emit at longer ones. Whilst the layout used here does not maximise the use of re-emitted photons, the overall loss of luminous energy is very low, and a significant fraction of absorbed energy is being re-emitted at a longer wavelength. The growth parameters are presented in Table 4.1.

A study on the growth and oil accumulation of marine microalgae *T.suecica* (Go et al., 2012) showed that the microalgae cells remained in their exponential growth phase longer under the lower light intensity of 108.9 μmol m^{-2} s^{-1}, and increasing light intensities did not lead to higher biomass production due to the photo-inhibition.

Since all photosynthetic pigments (such as chlorophyll-a) have optimal absorption ranges, it seems likely that the modification of wavelength conditions to move "unwanted" wavelength regions into the absorbance regions of chlorophyll could improve photosynthetic performance, and hence growth, without requiring increased light intensity. The results presented in this chapter validate that hypothesis.

Since high levels of UV radiation are harmful to microalgae (Holzinger and Lutz, 2006) then the use of violet filter to absorb UV radiation and re-emit the energy in the PAR range has a double benefit of enhancing visible spectrum energy whilst limiting harmful UV exposure.

This is also an advantage for photobioreactor design, as glass or plastic used for construction would normally absorb the UV energy and convert it to heat. By adding the dye, this energy can pass through the photobioreactor wall as visible light. The benefits of the violet dye in this regard are demonstrated in the improved growth of both species of microalgae when compared to full-spectrum daylight at similar luminous intensity.

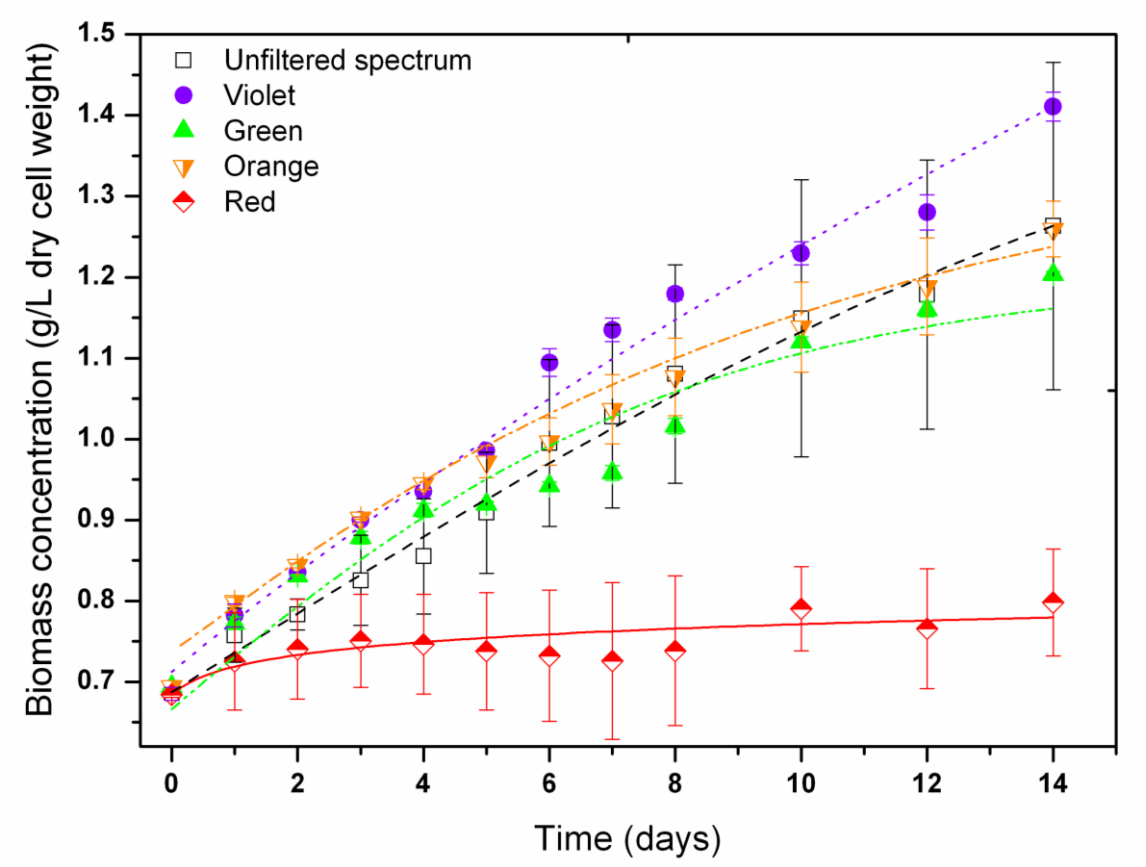


Figure 4.2, Biomass density (cell dry weight per litre of culture) of *G.membranacea* under violet, green, orange, red and control light recorded over a 14 day period. The curves are fitted using a standard sigmoid Weibull growth model. Data are the means ± SD of 5 replicates.

Light condition	*C.vulgaris*			*G.membranacea*		
	C_{14day} (10^6 cell ml^{-1})	μ_{max} (day^{-1})	Productivity (g L^{-1} day^{-1})	C_{14day} (10^6 cells ml^{-1})	μ_{max} (day^{-1})	Productivity (g L^{-1} day^{-1})
Control	37.48	0.06	0.02	24.78	0.15	0.04
Violet	43.29	0.11	0.03	27.66	0.16	0.05
Green	42.71	0.10	0.03	23.61	0.11	0.03
Orange	42.27	0.13	0.03	24.71	0.15	0.04
Red	35.53	0.07	0.01	15.69	0.06	0.01

Table 4.1, Growth parameters of *C.vulgaris* and *G.membranacea* under different light conditions.

The experiments carried out using other dyes demonstrated the variation in growth according to the photosynthetic requirements of the two species. *C.vulgaris* performed well under green and orange light. *G.membranacea* also grew well under green and orange light, but showed no real improvement in growth from the daylight equivalent in these cases. Whilst *G.membranacea* showed considerably reduced growth under red light, *C.vulgaris* produced an approximately similar growth pattern under red light to that observed under the daylight equivalent. The effect of the proposed illumination method was a combination of reduced UV inhibition and increased levels of re-emitted photons. This is clearly not down to UV inhibition alone, or the clear PMMA (as control test) would demonstrate equivalent growth levels.

4.4 Photosynthetic pigment production

This section illustrates the effect of different light conditions on photosynthetic pigment production.

4.4.1 *Chlorella vulgaris*

There were three different patterns of photosynthetic pigment production in *C.vulgaris* under the light conditions provided. Pigmentation increased considerably under the red light over a period of 14 days, with the highest percentages of chlorophylls per gram of biomass. Red light induced chlorophyll-a production, in particular with a specific photosynthetic pigment production of 1.29%. In addition, red light favoured the pigmentation of chlorophyll-b and c by 0.38%, and 0.27% (g Chlorophyll / g dry biomass) respectively.

The time course of chlorophyll-a content of *C.vulgaris* biomass is presented in Figure 4.3.The results show that providing red wavelength bands (650-700 nm), which is the absorption spectrum for chlorophyll-a, could potentially promote pigmentation in *C.vulgaris*. Chlorophyll-a production under violet and full-spectrum light followed similar profiles, with linear increase for the first five days followed by a steady trend in the next days. However, the levels of pigmentation under these two light conditions were significantly lower than those under orange and green light at the beginning of the cultivation. High concentrations of chlorophyll-a in the biomass under orange and green light is attributable to the high corresponding specific growth rates μ and exponential growth phase in the first two days.

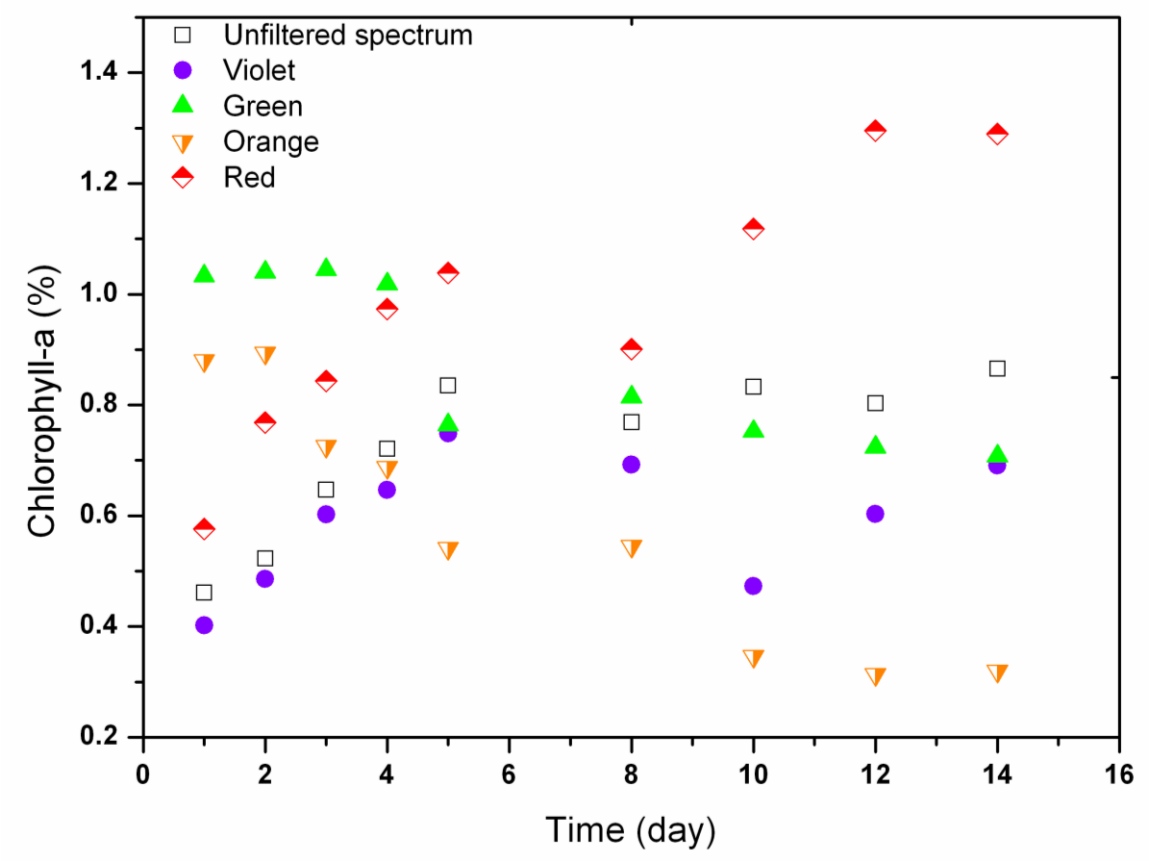


Figure 4.3, Variations of chlorophyll-a content (% g-chlorophyll/g-biomass) in *C.vulgaris* biomass over time under violet, green, orange, red and control light. Data correspond to the biomass profiles of Figure 4.1.

4.4.2 *Gloeothece membranacea*

Profiles of the photosynthetic pigment content of *G.membranacea* biomass showed an increase in chlorophyll-a content for 3 days under all light conditions with slightly higher percentages under green and orange light (Figure 4.4).

The highest chlorophyll-a (1.23% g Chl/g biomass) was obtained under green light while pigmentation under red light remained relatively low. The results indicate that there was a dynamic pattern of pigmentation in cyanobacteria when cells where in exponential growth phase during the first 5 days. This means that although red light in the static condition did not support growth of *G.membranacea* photosynthetic pigments produced chlorophyll-a as much as possible to sustain the growth in the active phase.

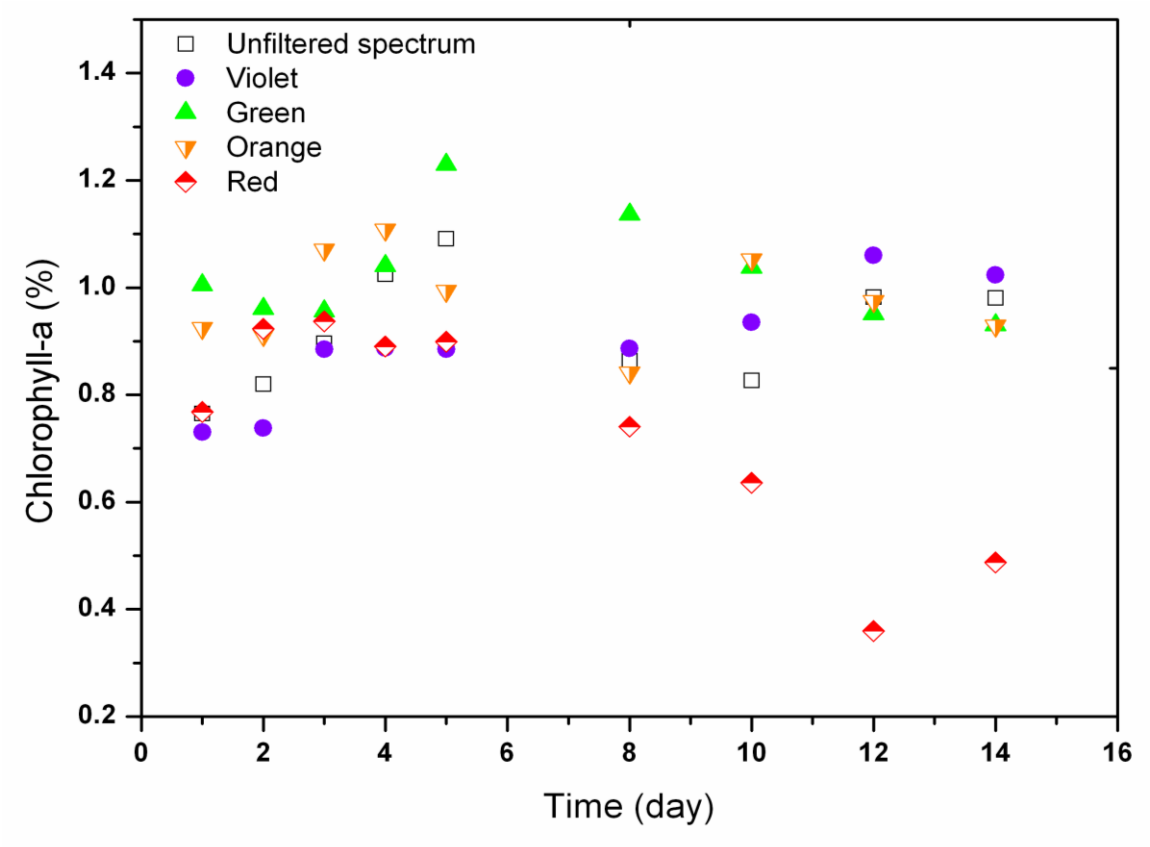


Figure 4.4, Variations of chlorophyll-a content (% g-chlorophyll/g-biomass) in *G.membranacea* biomass over time under violet, green, orange, red and control light. Data correspond to the biomass profiles in Figure 4.2.

Previous work in a related area has shown that red LED illumination gives the highest growth rates and chlorophyll-a production for *S.platensis* (Chen et al., 2010, Wang et al., 2007). This work expands from this by using light wavelength ranges rather than specific colours and clearly shows a species variance in terms of optimal light wavelength ranges. We hypothesise that this variance is at least partially due to the variation in photosynthetic pigmentation between the species under varying light conditions.

4.5 Discussion

Photosynthetic pigmentation under different light conditions exhibited the major differences between the green algae strain *C.vulgaris* and the cyanobacteria *G.membranacea*. Firstly, *C.vulgaris* cells showed completely different behaviour when illuminated with different light wavelengths compared to their previous light

environment. This suggests that for the first few days of cultivation, *C.vulgaris* cells were responding to the environmental alterations by adapting their photosynthetic antenna. This resulted in high chlorophyll production under green, orange, and red light conditions which contained specific wavelength bands of PAR. In comparison, full-spectrum daylight and violet light which modified natural daylight to PAR, produced average chlorophyll contents.

On the other hand, *G.membranacea* cells had a relatively similar pigmentation production under all light conditions. This was likely to be due to their environmental adaption abilities enabling them to survive with only green light or a combination of green, yellow and orange portions of the light spectrum (500-650 nm) barely used by other microalgae species (Mur et al., 1999).

However, their average pigment concentrations were higher than the *C.vulgaris* cells. In both strains of microalgae, the control light condition (combination of different wavelengths of light) resulted in an average pigmentation value between the other light conditions.

Furthermore, there was no linear relationship between the biomass concentration and chlorophyll content in both of the microalgae strains, a finding also observed from earlier work in the area (Sanchez Miron et al., 2002). This means that illumination of microalgae providing specific wavelengths does not necessarily improve both pigmentation and biomass production.

The results obtained from the experiments carried out showed that although violet light promoted biomass production in both species of microalgae, it was not the most efficient light condition for inducing pigmentation. Green light improved chlorophyll production in the *G.membranacea,* whereas red light was the sole light condition with significant effect on pigmentation in *C.vulgaris*. Therefore, the effect of light wavelengths on pigmentation was species specific.

Chlorophyll-a was the major light harvesting pigment in both strains. However, *C.vulgaris* contained significant amounts of chlorophyll-b while *G.membranacea* lacked chlorophyll-b. The lack of chlorophyll-b is likely to explain the fact that, unlike green algae, cyanobacteria contain phycobiliproteins as light harvesting pigments in a wavelength range of 500-650 nm (Satoh et al., 2001). High pigmentation production under green and orange light in particular shows that these light conditions provided the suitable wavelengths for *G.membranacea* (see Table 4.2).

Light condition	*C.vulgaris*		*G.membranacea*
	Chl-a	**Chl-b**	**Chl-a**
Control	0.86	0.28	1.09
Violet	0.74	0.24	1.06
Green	1.04	0.39	1.23
Orange	0.89	0.37	1.11
Red	1.29	0.38	0.94

Table 4.2, Maximum photosynthetic pigment content (% g Chl/g biomass) of *C.vulgaris* and *G.membranacea* under different light conditions.

In both species, the growth rates and cell densities obtained are lower than many currently used algal species. A feature of this work has been the use of naturally-growing local species and the mimicking of local culture conditions. Under Scottish conditions, for which algal growth is generally slower, this can be achieved under optimised growth conditions.

Growth rate could likely be increased by increased illumination intensity or by the provision of increased concentrations of CO_2 but these are not considered as part of this specific study which focuses on illumination wavelength-related growth behaviour. The increased growth demonstrated is due to a combination of reduced UV inhibition and increased levels of re-emitted photons as previously pointed out. It is clearly not down to UV inhibition alone or the clear PMMA control would demonstrate equivalent growth levels to that shown under the fastest growing light conditions for each species.

The implications of this work are very significant in the area of microalgae culture, carbon capture and photobioreactor design. Whilst much work has been done on artificial lighting of algal culture (Ravelonandro et al., 2008), a photobioreactor utilising natural light would present the most environmentally suitable solution. In addition, it is important to mention that the irradiance supply has a direct impact on the biochemical

composition of microalgae cells including lipids (Chrismadha and Borowitzka, 1994) and it can influence the metabolism of fatty acid synthesis (Sukenik, 1991).

However, the effect of light on lipid production in microalgae is species specific (Ho et al., 2012). Low light intensity has been reported to be more suitable for lipid accumulation in two species of microalgae (Cheirsilp and Torpee, 2012) and, as a result, optimal light conditions can be different for cell growth and lipid production.

Cheirsilp and Torpee (2012) argued that the lower efficiency of oil extraction and the higher cost of downstream processing were the result of low lipid production in microalgae under high light intensity. The contrast between the results of the study mentioned and other reports prove that those species adapted to lower light intensities, are therefore worthy of investigation in terms of their valuable biochemical compositions.

In areas such as Northern Europe, where sunlight intensity is lower and more varied throughout the year, a solution such as this to improve the light energy to biomass conversion efficiency of local microalgae species represents an important piece of progress.

4.5 Conclusions

This chapter reviewed the effect of light quality on static microalgae cultures. Biomass and pigment production of two species of microalgae under static cultivation condition using wavelength selective illumination techniques was investigated. Luminescent acrylic sheets provided light of modified wavelength ranges to *C.vulgaris* and *G.membranacea*. Growth and chlorophyll-a production was significantly promoted under different illumination spectra.

From the data obtained, highest specific growth rates of 0.131 day^{-1} were observed for *C.vulgaris* using the orange range, whilst the violet range supported growth of *G.membranacea* with the specific growth rate of 0.160 day^{-1}. Red light had a significant effect on the pigmentation of *C.vulgaris*, however it was the least efficient light condition for the cultivation of *G.membranacea*.

The results obtained in this chapter indicate the importance of identifying suitable light conditions for various applications. For instance, the influence of light wavelengths on

pigment production can vary in different microalgae species. Some wavelengths are particularly efficient for inducing the production of a target pigment whilst others are in general suitable for the enhancement of biomass productivity, which is an important factor in industrial scales. Modified illumination strategies such as these could allow enhanced cultivation of microalgae under natural rather than artificial light.

Chapter 5:
Cultivation in luminescent photobioreactors

5.1 Introduction

In this chapter the results of the experiments carried out for determining the biomass production in photobioreactors are presented. The experiments included the cultivation of microalgae under two different culture densities in order to identify the effect of initial culture density on growth, and the penetration path of various wavelengths of light into the culture. In addition, the second set of experiments focused on cultivation in luminescent photobioreactors at different light intensities. The aim was to obtain results which could identify the differences between various wavelengths of light at certain light intensities and their influence on cell growth.

5.2 Cultivation with different culture densities

Five different luminescent acrylic bubble column photobioreactors (PBR), as described in Chapter 3, were illuminated by 150W and 300W xenon lamps inside the light box, along with a reference condition of a clear acrylic photobioreactor. Luminescent PBR in blue, green, yellow, orange, and red, provided different spectral qualities for the investigation of various culture conditions.

The cultures were inoculated at two different seed culture densities. Inoculation densities were chosen to ensure growth in the photobioreactors and examine the effect of the initial culture density on overall growth. These two main groups of culture concentration, tested under six different light conditions, provided an insight into the combined effect of wavelength and density on the growth of each microalgae strain.

5.2.1 *Chlorella vulgaris*

5.2.1.1 *Biomass production*

C.vulgaris was cultivated in two different densities, with a low concentration seed culture of 0.32 g L^{-1} and a high concentration seed culture of 0.9 g L^{-1}. Growth curves

of *C.vulgaris* in six different photobioreactors and two different culture densities over a period of two weeks are presented in Figure 5.1. The curves were fitted using a standard sigmoid Weibull growth model as described by Barsanti et al. (2006). The population of cells in a dynamic system show a typical growth pattern similar to a sigmoid curve which includes different phases of growth.

The most efficient spectral quality of light for conversion of light energy into biomass in both culture densities was that found within the red light-emitting PBR. A maximum biomass accumulation of 1.49 g L^{-1} and 1.92 g L^{-1} for low and high density cultures were achieved within the red PBR. Red light has a narrow spectrum of 600 – 700 nm, with the lowest level of light energy and longest wavelength band which means this wavelength range of PAR cannot penetrate in deep or highly dense cultures.

Therefore, exposure to this light condition needs either low density or well mixed cultivation. *C.vulgaris* cells illuminated with the red, orange or yellow PBRs (lower energy and longer wavelengths of light) stayed in the lag phase for one day however cells growing within blue and green PBRs (shorter wavelengths of light) entered their exponential phase two days after cultivation. Overall, the culture density influenced the growth parameters such as the specific growth rate and biomass productivity in the exponential phase of growth. However, its effect on the final biomass production was minimal. The highest biomass productivities of 135 mg L^{-1} day^{-1} and 142 mg L^{-1} day^{-1} during the first five days of cultivation (exponential phase) were achieved in red and green PBRs, suggesting these light conditions are best suited for the support of biomass production.

Although the highest biomass productivities were obtained in the red and green PBRs in both high and low density cultures, this parameter was higher in low density cultures (see Table 5.1). Particularly, in the green PBR the productivity of the low density group was three times greater than for the high density group. The results support the fact that microalgae cells illuminated with green wavelength ranges can be more influenced by the shadowing effect, as green light penetration into the culture is weaker than other wavelength ranges.

Both red and green light have been reported to induce pigmentation in *C.vulgaris* during the exponential growth phase. Given the small cultivation scale of this local strain of microalgae in the UK, the biomass productivities achieved in this study (shown in Table

5.1) were higher than those under similar phototrophic conditions (Illman et al., 2000, Scragg et al., 2002).

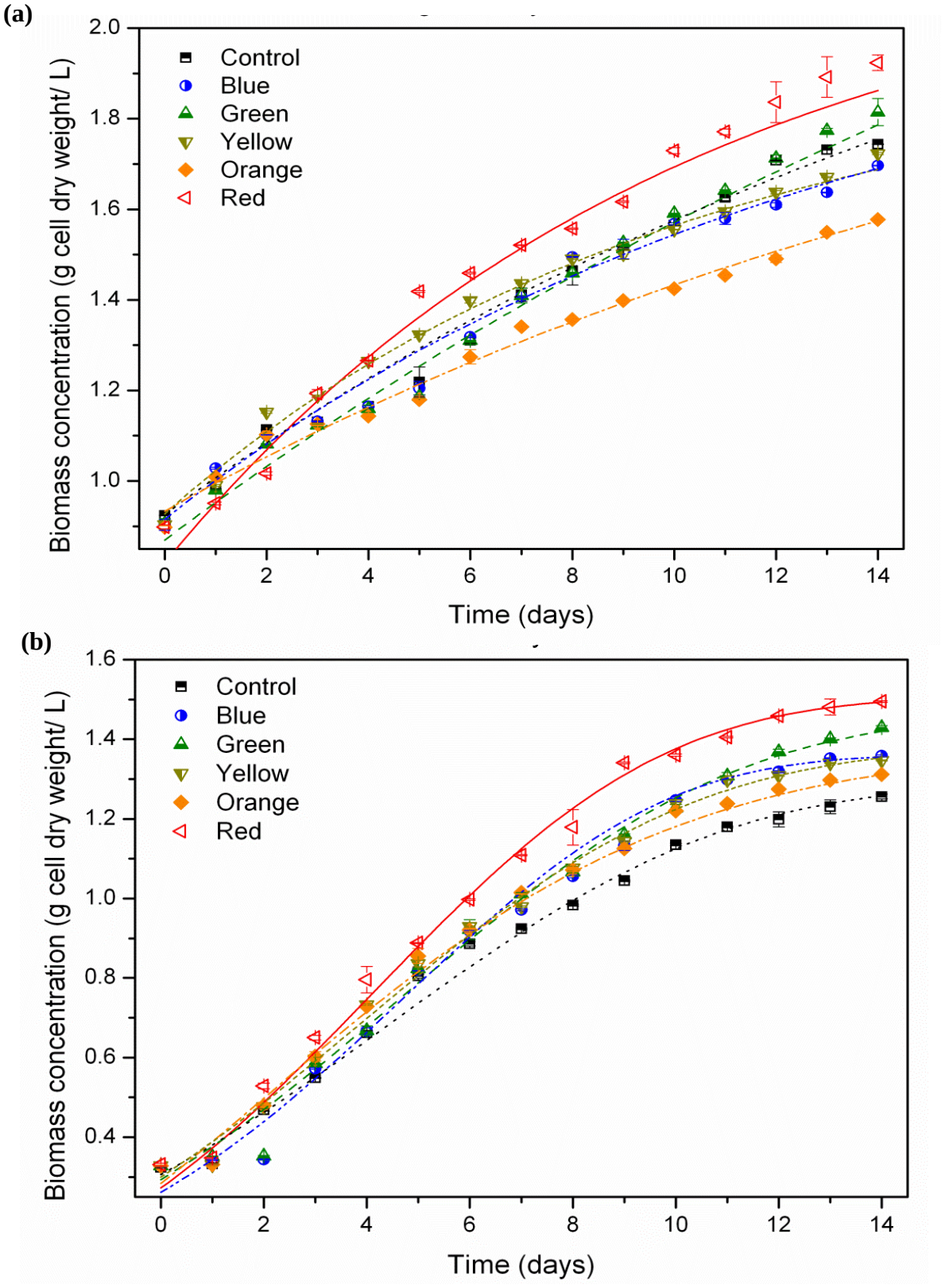


Figure 5.1, Growth curves of *C.vulgaris* culture, (a) high density (b) low density culture, under blue, green, yellow, orange, red and full-spectrum light recorded over a 14 day period. The curves are fitted using a standard sigmoid Weibull growth model.

Although the relationship between biomass accumulation and chlorophyll production is not linear, the inducement of pigmentation by these light conditions can affect biomass productivity in the first few days of cultivation where the exponential growth occurs.

The other growth parameter highly affected by culture density was the specific growth rate μ. For instance, in low density cultures in the green PBR, the maximum specific growth rate obtained in the exponential phase was 0.51 day^{-1}. However, only 20% of this value was obtained in the high density culture. This effect was more pronounced in the green and blue PBRs. The culture density affects the light path and penetration into the microalgae cells (Vasumathi et al., 2012) and for this reason, in low density cultures the chance of each microalgae cell receiving light at specific wavelengths was greater than those cells within high density cultures.

In the previous chapter we demonstrated that green light was the most favourable light condition for growth of *C.vulgaris*, and whilst red light produced highly pigmented cultures, the biomass productivity was poor (Mohsenpour et al., 2012). Compared with the results presented here, the cultivation mode clearly has a great impact on the way *C.vulgaris* cells respond to light conditions.

Light condition	***C.vulgaris***					
	Low density culture			**High density culture**		
	C_{14day} (10^6 cell ml^{-1})	μ_{max} (day^{-1})	Productivity (mg L^{-1} day^{-1})	C_{14day} (10^6 cell ml^{-1})	μ_{max} (day^{-1})	Productivity (mg L^{-1} day^{-1})
Control	37.95	0.34	118.12	52.54	0.25	59.01
Blue	41.01	0.50	119.02	51.13	0.26	44.00
Green	43.14	0.51	142.05	54.66	0.19	52.09
Yellow	40.66	0.36	126.00	51.92	0.39	83.32
Orange	39.61	0.37	118.02	47.57	0.39	42.32
Red	45.09	0.42	135.03	57.92	0.51	117.14

Table 5.1. Effect of culture density and light condition on growth parameters of *C.vulgaris*

The photobioreactors bubbled with air provide culture mixing and mass transfer, and thus reduce the damaging effects of lack of light penetration at the dark zone (Posten, 2009) at the centre of the photobioreactor. Due to their lower energy levels, the longer wavelengths of light such as red may underperform in static cultures (as discussed in Chapter 4) and can be highly influenced by cell's shadowing effects. Therefore, mixing through air bubbles provides a suitable condition for the cells to absorb light at longer wavelengths which are unable to penetrate into deep or highly dense cultures.

5.2.1.2 *Lipid production and CHN analysis*

Carbon, Hydrogen and Nitrogen (CHN) analysis were carried out in order to investigate the relationship between lipid accumulation and carbon, hydrogen and nitrogen content of microalgae cells under different cultivation conditions (Roessler et al.). The data obtained not only demonstrated the relationship between total lipids and nitrogen contents of the cells produced in different luminescent photobioreactors, but it also compared the effect of the initial culture density. Figure 5.2 shows the effect of initial culture density on the CHN content of *C.vulgaris*. It has been reported that nitrogen content of microalgae is a function of nitrogen supply (available in the culture media) and irradiance quality (Edwards et al., 2006).

In general, the carbon content of *C.vulgaris* was higher in low density cultures. The highest difference in carbon content observed was 27% in the red PBR at low density cultures. By contrast, the nitrogen content of cells increased in high density groups in all photobioreactors. The highest nitrogen content of 7.2% was observed in the blue PBR in the high density culture. Since the media compositions in all cultivation conditions were maintained constant, the observed changes in elemental content of the cells were noticeable. A study focused on nitrogen requirements and nitrogen storage among phytoplankton and algae indicated that increased nitrogen content can be a reflection of storage which maintains algae productivity during periods of nutrient shortage (Pedersen and Borum, 1996). The data obtained from the nitrogen content supports the hypothesis that in high density cultures the condition for growth becomes more competitive. Consequently, higher nitrogen content of cells in the high density group reflects the cultivation condition in which cells lose more carbon and store more nitrogen.

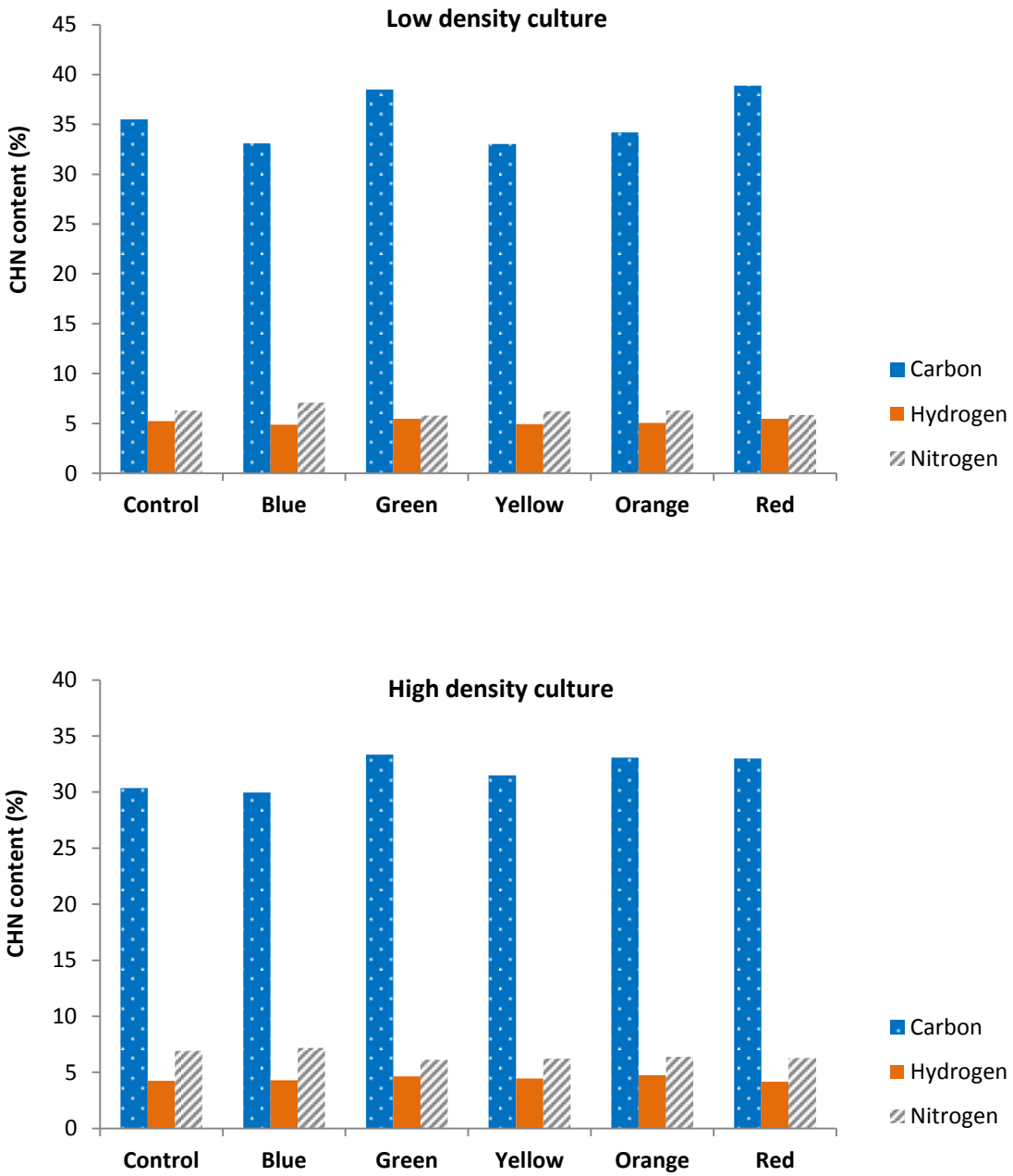


Figure 5.2, Effect of culture density on CHN content of *C.vulgaris* obtained from elemental analysis in six different luminescent photobioreactors.

In addition, several studies have reported that the nitrogen limiting conditions can considerably enhance lipid accumulation in microalgae (Converti et al., 2009, Ho et al., 2012, Illman et al., 2000, Rodolfi et al., 2009). Rodolfi et al. (2009) reported that the biomass productivity was reduced while nitrogen-deprived condition stimulated lipid

accumulation. Enhanced lipid accumulation in microalgae can be related to the environmental stress received by severe conditions (Guedes et al., 2010). Illman et al. (2000) showed that with a nitrogen starvation condition, the lipid content of *C.vulgaris* was doubled compared to the control condition.

One of the aims of this study was to observe the changes in biochemical compositions of microalgae when cultivated in a luminescent PBR. The results obtained from the lipid extraction showed that more lipids were accumulated in high density cultures. The highest lipid content in low density cultures achieved in the green PBR was 10.6%. Cultivation in the green PBR induced the highest lipid accumulation in both culture density groups.

When the cell population increases the nutrient requirement and competition for receiving light as a source of energy can increase. The way that photons of light penetrate into the highly dense cultures depends strongly on the wavelength ranges.

Therefore, lipid accumulation can vary in those photobioreactors in which the effect of light quality is more significant. For instance, comparing the two culture density groups there was a significant difference in the lipid content of *C.vulgaris* in green, yellow and red PBRs.

Cells cultivated in the blue PBR contained the highest nitrogen levels of approximately 7.2%, although lipid production was relatively low in this photobioreactor. The results obtained were inconclusive in terms of reaching a distinguished correlation between the elemental and lipid analysis.

Figure 5.3 shows that the total lipids increased up to two times in high density cultures. This amplified lipid accumulation can be due to the environmental stress induced by the highly populated microalgae culture. The results obtained can also be related to the use of nitrogen in the media by highly populated cultures. In the high density culture group, nutrient removal occurs faster than the low density group. This may be related to the fact that in highly populated cultures, the number of cells consuming the nitrogen and other essential nutrients increases and as a result, the productivity decreases and the accumulation of storage products such as lipids is induced.

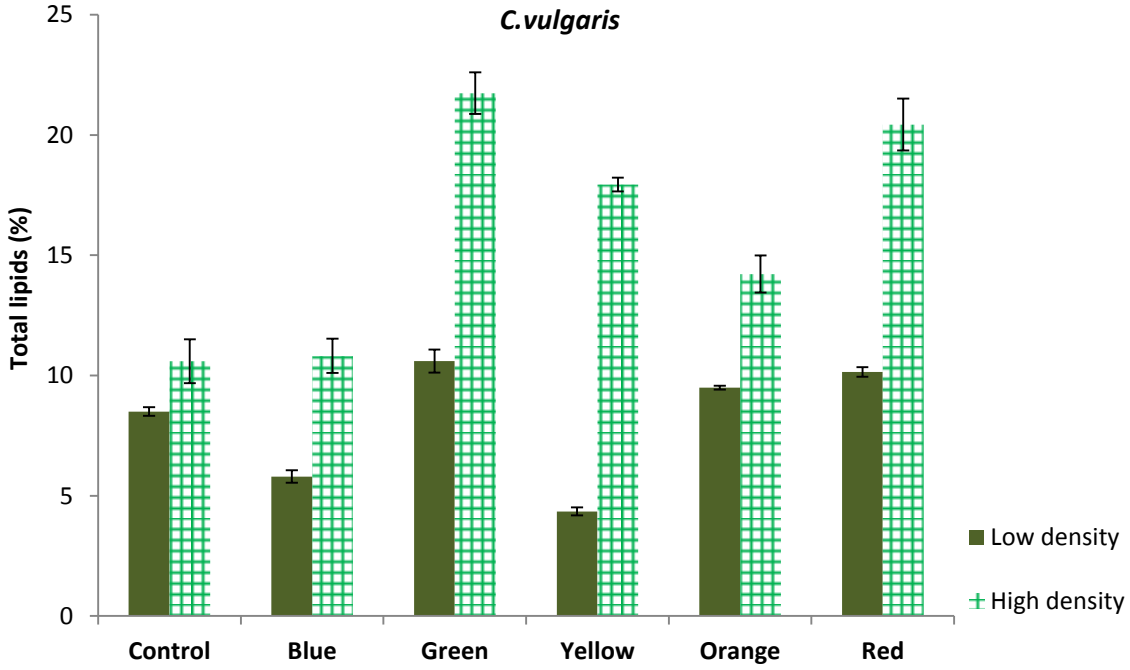


Figure 5.3, Effect of culture density on total lipids of *C.vulgaris* in six different luminescent photobioreactors.

5.2.2 *Gloeothece membranacea*

5.2.2.1 *Biomass production*

G.membranacea was cultivated in six different photobioreactors (as described in Chapter 3) and at two different densities with a low concentration seed culture of 0.46g L^{-1} and a high concentration seed culture of 0.91g L^{-1}. Luminescent PBRs in blue, green, yellow, orange, and red, provided different spectral qualities for the investigation of various culture conditions.

The growth curves of *G.membranacea* under different light conditions with the intensity of 150 µmol m^{-2} s^{-1} inside the photobioreactor are presented in Figure 5.4.

Exponential growth in cyanobacteria cells started one day after inoculation. Similar growth patterns were obtained under all light conditions. Similar to the green algae *C.vulgaris*, the growth of *G.membranacea* was highly promoted by red light with a maximum biomass production of 2.27 g L^{-1}.

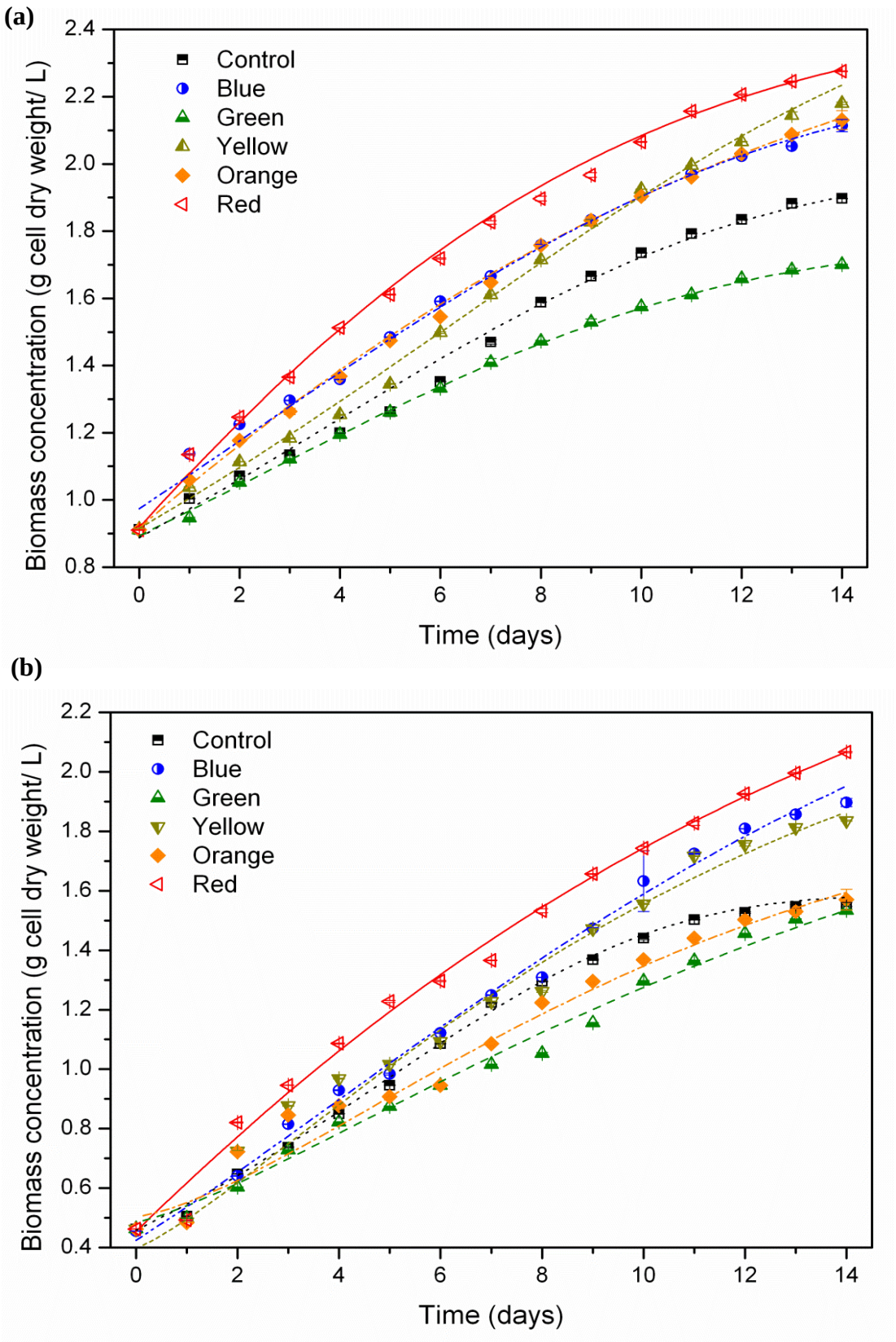


Figure 5.4, Growth curves of *G.membranacea*, (a) high density (b) low density culture under blue, green, yellow, orange, red and full-spectrum light recorded over a 14 day period. The curves are fitted using a standard sigmoid Weibull growth model.

It was reported previously in Chapter 4 that the red light emitted by luminescent acrylic filters could not properly support cyanobacterial growth in a static culture (Mohsenpour et al., 2012). However, the improved mixing achieved within the photobioreactors reduces the depth of light penetration necessary, and this allows for improved growth under these light conditions.

Growth parameters were highly influenced by the culture density. This effect was more obvious in the maximum specific growth rates of cultures illuminated by red, orange and yellow light, where the values of μ were almost three times greater in low density cultures.

The maximum μ achieved under red light at an intensity of 150 μmol m^{-2} s^{-1} was 0.51 day^{-1}, while the same parameter of the modified Monod model was 0.44 day^{-1} for red LED at higher intensities (Wang et al., 2007).

The highest biomass productivities of 184 mg L^{-1} day^{-1} and 132 mg L^{-1} day^{-1} during the first five days, where the exponential phase occurred were achieved in red and yellow PBRs, suggest that the longer wavelengths of light are best suited for the support of biomass production.

In addition, in high density cultures, red and orange light induced the highest productivity, which supports the results related to other growth factors. As it can be seen in Table 5.2 productivity in the red PBR was enhanced by 53% in the low density culture group.

Studying the variations in growth parameters of two different strains of microalgae indicates the importance of considering the strain's characteristics. While culture density affected considerably the growth of *C.vulgaris* in shorter wavelength bands (Blue-Green light) in *C.vulgaris*, this impact was more pronounced in *G.membranacea* with longer wavelengths (Yellow-Red light).

Light condition	*G.membranacea*					
	Low density culture			**High density culture**		
	C_{14day} (10^6 cell ml^{-1})	μ_{max} day^{-1}	Productivity (mg L^{-1} day^{-1})	C_{14day} (10^6 cells ml^{-1})	μ_{max} (day^{-1})	Productivity (g L^{-1} day^{-1})
Control	30.16	0.34	110.13	36.80	0.09	60.52
Blue	36.79	0.50	123.00	40.98	0.22	87.09
Green	29.80	0.51	94.15	33.00	0.10	78.58
Yellow	35.61	0.36	132.36	42.26	0.127	78.07
Orange	30.48	0.37	105.73	41.30	0.15	103.37
Red	40.08	0.42	184.00	44.11	0.22	120.00

Table 5.2. Effect of culture density and light condition on growth parameters of G.*membranacea.*

5.2.2.2 *Lipid production and CHN analysis*

Similar to *C.vulgaris*, the elemental analysis of *G.membrancea* showed that increasing culture density resulted in lower carbon and higher nitrogen content in cells. As shown in Figure 5.5, the results confirm that the initial culture density had some influences on the CHN results. Whilst the carbon content in all luminescent PBR at the high density was slightly lower than the low density cultures, there was a reverse correlation with the nitrogen content (with the exception of green and orange PBR).

Increasing the initial culture density reduced the carbon content significantly in the red PBR. The carbon content in the red PBR was 31.5% and was almost halved in the high density group. Cyanobacterial cells grown in the green PBR had the lowest carbon content in both density groups. This supports the results from biomass production which indicated that the green PBR was the least efficient for cultivation of *G.membranacea*.

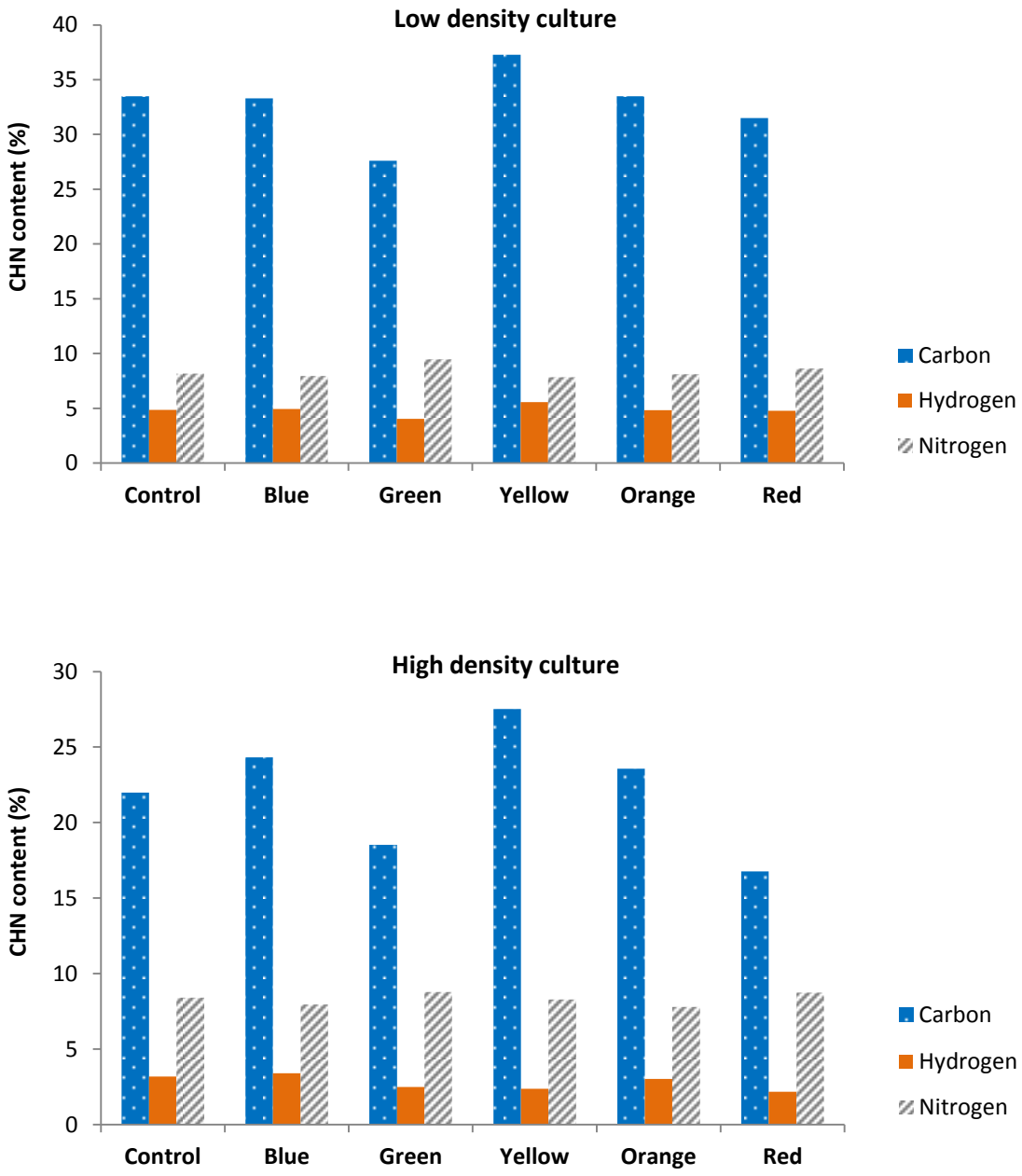


Figure 5.5, Effect of culture density on CHN content of *C.vulgaris* obtained from elemental analysis in six different luminescent photobioreactors.

Under stress conditions, lipid accumulation occurs as photosynthetic activities of microalgae cells decrease (Becker, 1994a) . To obtain maximum lipid production, Becker suggested to provide the optimum conditions for fast growth (high specific growth rate and productivity), and impose nitrogen starvation or other stress factors. The data obtained from the nitrogen content supports the hypothesis that in high density cultures the condition for growth becomes more competitive. Consequently, a higher nitrogen content of cells in the high density group reflects the cultivation condition in

which cells lose more carbon and store more nitrogen. Figure 5.6 shows the effect of culture density on total lipids.

This effect can be compared to those studies in which N-depletion in media leads to the formation of more storage biochemical compounds such as lipids. There was a significant difference in the lipid contents between low and high density cultures. Whilst the highest lipid accumulation occurred in the yellow PBR in both culture density groups, the green PBR was the least efficient. The most significant impact of increasing the culture density was observed in the blue PBR, where lipid accumulation was enhanced up to three times whilst the nitrogen levels remained unchanged. The results obtained for *G.membranacea* show different responses compared to *C.vulgaris*. Overall, the results from culture density groups were inconclusive and unable to provide a clear correlation between elemental contents and total lipids. However, it seemed that the blue PBR most influenced the cell's nitrogen or lipid content at different culture densities.

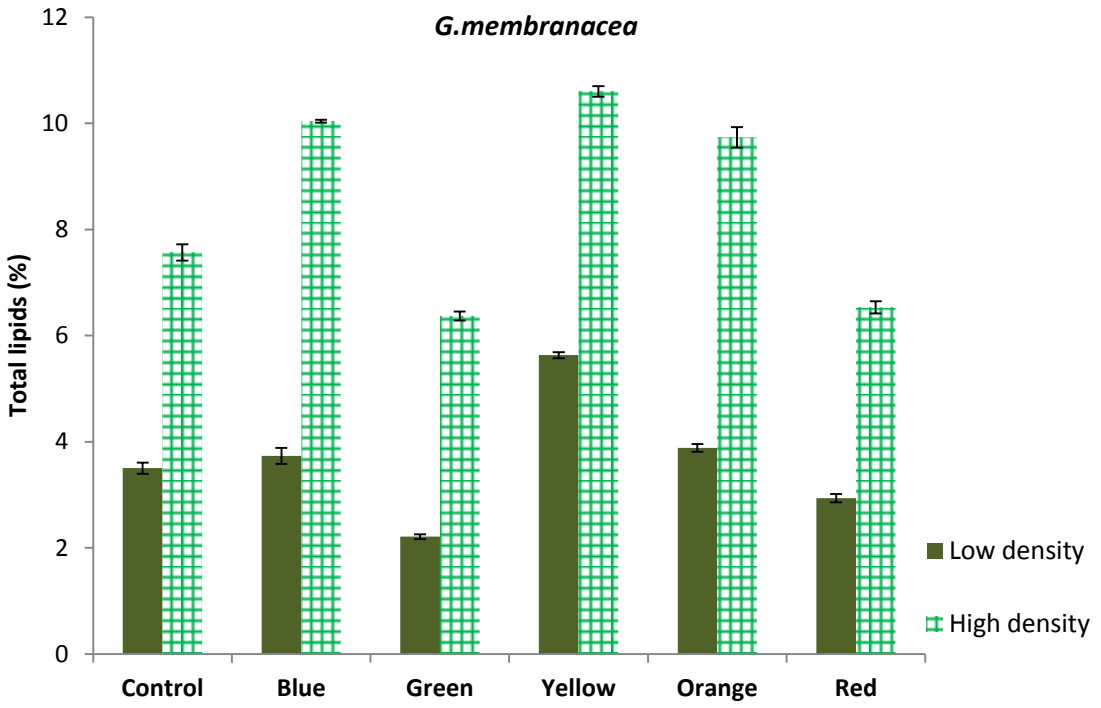


Figure 5.6, Effect of culture density on total lipids of *G.membranacea* in six different luminescent photobioreactors.

5.3 Cultivation under different light intensities

Light intensity can influence the growth in different stages through light limitation, light saturation, and light inhibition conditions (Spolaore et al., 2006b). At lower light levels, microalgae growth is linear and biomass productivity is enhanced with increased irradiance indicating first-order kinetics (Ravelonandro et al., 2008). During the light saturation stage, growth follows zero-order kinetics meaning that increasing light intensity does not affect the growth anymore. This stage indicates the optimum light intensity for a specific microalgae strain. Finally, light inhibition occurs when high illumination intensity damages microalgae cells and growth is inhibited. The microalgae cells at this stage may lose their colour (bleached). The tolerance limit of microalgae is highly depended on the species.

Wang et al. (2007) demonstrated the relationship between the light intensity and the specific growth rate (μ) at various wavelengths of light produced by LED lamps. The study suggested that increasing the light intensity enhanced μ up to a certain intensity level. This level varies with different microalgae species. Therefore, obtaining results from cultivation under different light intensities can indicate the intensity threshold for a specific strain and the optimum irradiance condition.

Three different illumination intensities of 50, 100 and 300 μmol m^{-2} s^{-1} were chosen to establish comparative results for the effect of photon flux of selected wavelengths on growth parameters.

5.3.1 *Chlorella vulgaris*

5.3.1.1 *Low light intensity 50 μmol photon m^{-2} s^{-1}*

A short lag phase in *C.vulgaris* culture in the red PBR was followed by an exponential growth phase in which the highest productivity of 144.19 mg L^{-1} day^{-1} was obtained. Similar to the results obtained from different culture densities, red and green light enhanced biomass production. However, growth in other photobioreactors was linear.

The results suggested that at the low intensity light condition, the blue wavelength range was the least efficient light condition and the lowest specific growth rate of 0.21 day^{-1} supported this conclusion. Figure 5.7 shows the growth profiles at low light intensity.

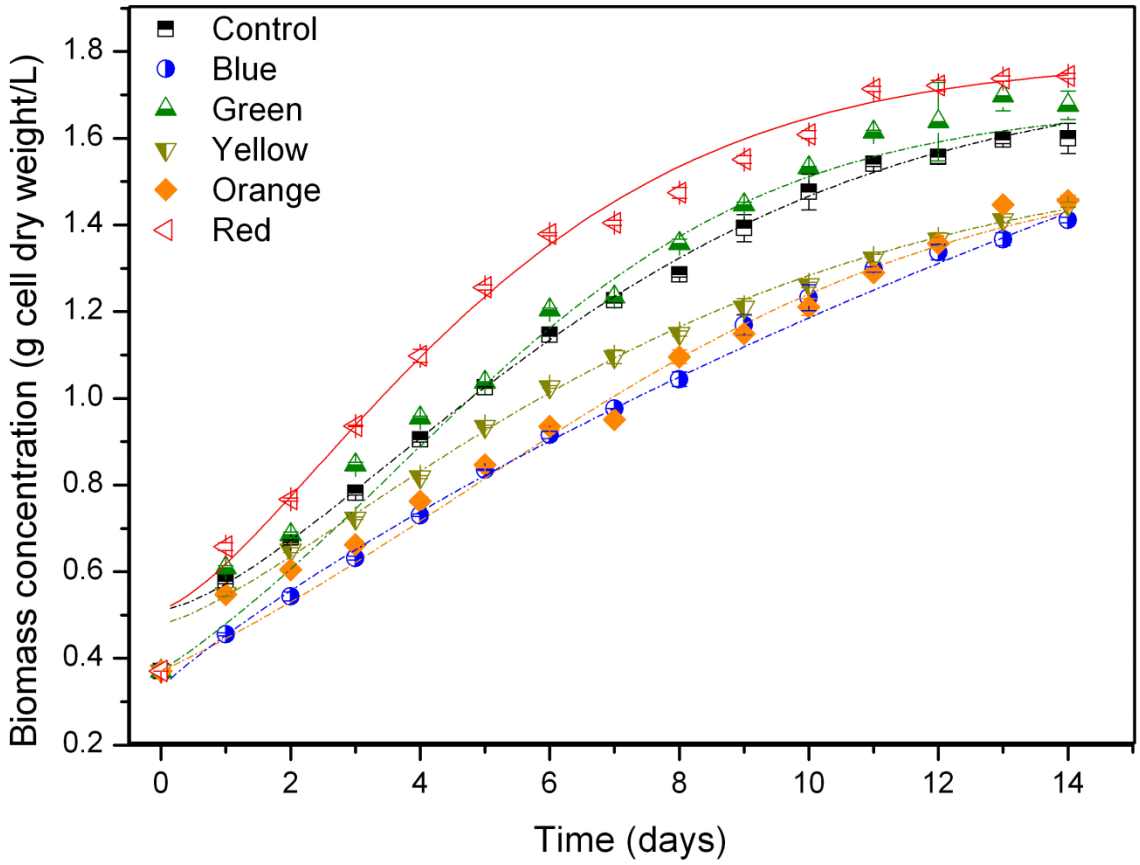


Figure 5.7, Growth curves of *C.vulgaris* illuminated at 50 µmol photon m^{-2} s^{-1} intensity under blue, green, yellow, orange, red and full-spectrum light recorded over a 14 day period. The curves are fitted using a standard sigmoid Weibull growth model.

Chen et al. (2010) reported that the lowest µ was obtained when a blue LED was used for cultivation of *Spirulina platensis*. The study suggested that at low light intensities the microalgae cells illuminated by blue LED consumed more chlorophyll to prevent growth inhibition. However, the biomass productivity achieved at low intensity light was higher compared to the high density culture groups, where cells have similar competitive conditions for absorbing photons of light. This means that even though insufficient light intensity may slow down the growth, cultures with lower initial cell population are more dynamic.

5.3.1.2 Medium intensity light 100 µmol photon m^{-2} s^{-1}

Increasing light intensity to medium levels enhanced biomass production in all photobioreactors with the exception of the blue PBR. However, the maximum specific growth rates were lower than the low intensity condition and biomass productivity was unaffected (see Figure 5.8). Overall, no significant alteration in growth patterns was observed by increasing intensity from low to medium levels. The highest biomass accumulation in the red PBR was 1.87 g L^{-1} and the lowest in the blue PBR was 1.4 g L^{-1}. The results obtained suggested that the intensity levels between 50 and 100 µmol photon m^{-2} s^{-1} were in the range of light-limitation stage of growth for *C.vulgaris*.

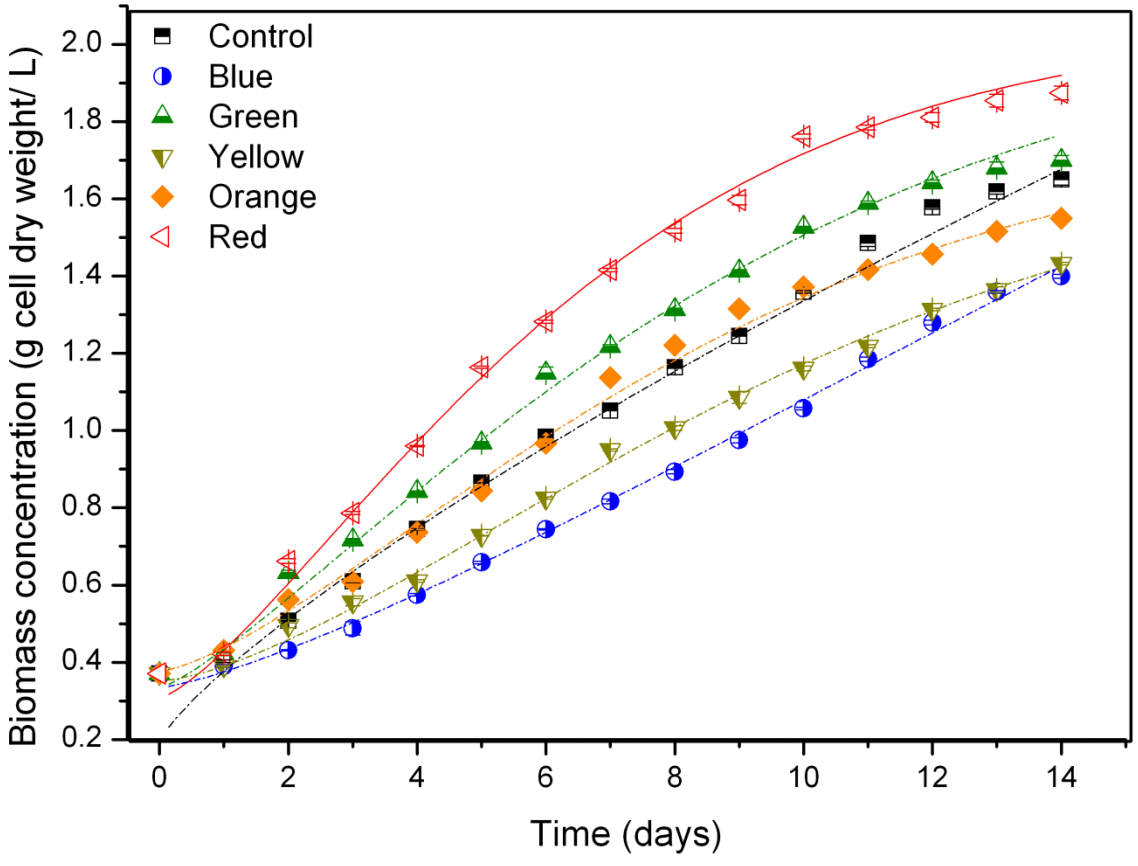


Figure 5.8, Growth curves of *C.vulgaris* illuminated at 100 µmol photon m^{-2} s^{-1} intensity under blue, green, yellow, orange, red and full-spectrum light recorded over a 14 day period. The curves are fitted using a standard sigmoid Weibull growth model.

5.3.1.3 *High intensity light 300 µmol photon m^{-2} s^{-1}*

Increasing the light intensity from medium to high improved all the growth parameters in the exopnential phase. The active phase of growth occurred in the early hours of inoculation (see Figure 5.9). The highest biomass density of 2.12 g L^{-1}, specific growth rate of 0.74 day^{-1}, and productivity of 205.98 mg L^{-1} day^{-1} were achieved under red light. Figure 5.10 shows that increasing the light intensity enhanced biomass productivity in the active growth phase considerably. Compared to the low light intensity, the specific growth rate was enhanced up to 86% at the high light intensity. Table 5.3 presents various growth parameters at different light intensity levels.

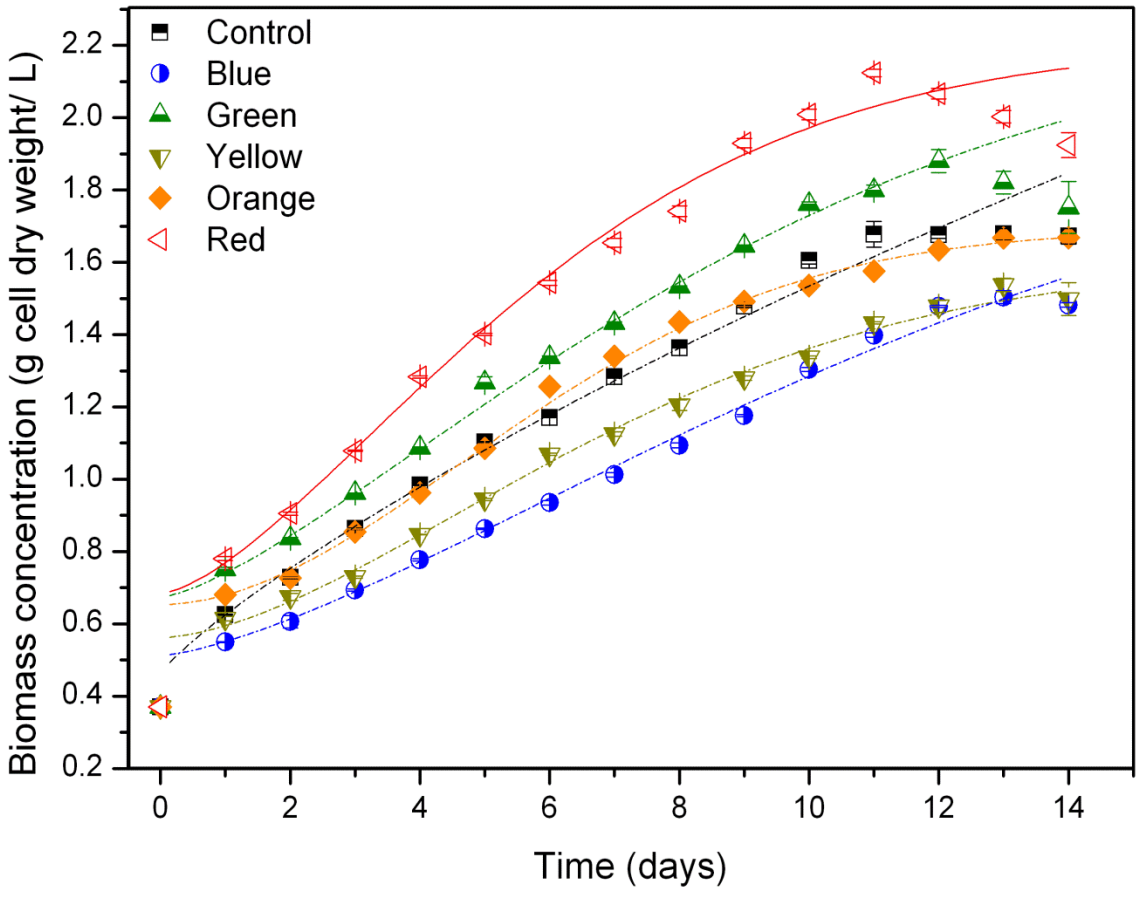


Figure 5.9, Growth curves of *C.vulgaris* illuminated at 300 µmol photon m^{-2} s^{-1} intensity under blue, green, yellow, orange, red and full-spectrum light recorded over a 14 day period. The curves are fitted using a standard sigmoid Weibull growth model.

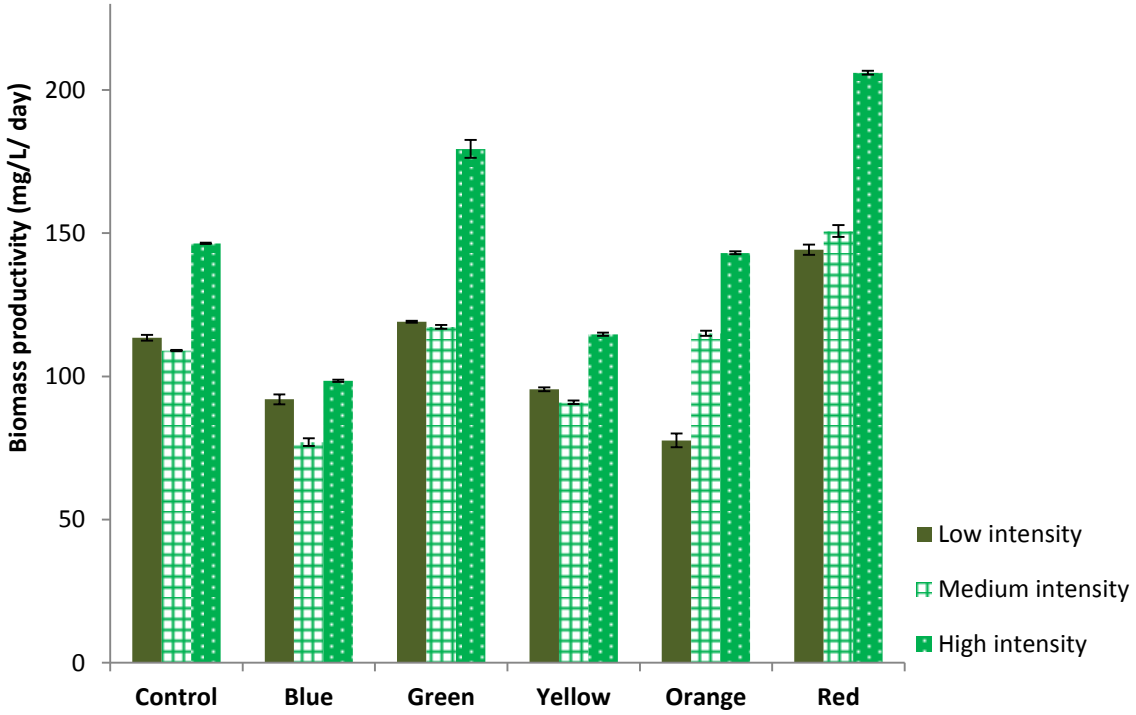


Figure 5.10, Biomass productivity *of C.vulgaris* at different light intensities in luminescent photobioreactors.

Light condition	*C.vulgaris*					
	50µmol photon m^{-2} s^{-1}		100µmol photon m^{-2} s^{-1}		300µmol photon m^{-2} s^{-1}	
	C_{14day} (10^6 cell ml^{-1})	μ_{max} (day^{-1})	C_{14day} (10^6 cell ml^{-1})	μ_{max} (day^{-1})	C_{14day} (10^6 cell ml^{-1})	μ_{max} (day^{-1})
Control	47.89	0.44	49.41	0.23	50.06	0.52
Blue	42.26	0.21	41.91	0.16	44.37	0.39
Green	50.18	0.50	50.89	0.41	52.43	0.70
Yellow	43.30	0.39	42.89	0.24	44.85	0.50
Orange	43.60	0.39	46.39	0.27	49.94	0.61
Red	52.19	0.57	56.10	0.45	57.58	0.74

Table 5.3, Effect of light intensity on growth parameters of *C.vulgaris*.

This change in the active phase of growth compared to lower intensity levels identifies the light limitation stage up to 250 μmol photon m^{-2} s^{-1} and light saturation between 250 and 300 μmol photon m^{-2} s^{-1}. The stationary phase occured 10 days after inoculation. This can be due to the high growth rates at the exponetial phase, when cells tend to consume the nutrients availble in the media. Therefore, growth can be inhibited due to the lack of essential nutrients. Ho et al. (2012) investigated the effect of light intensity on the microalgae *S.obliquus* CNW-N using LEDs as the light source. It was reported that the specific growth rate increased dramatically by rising intensity at the beginning of the cultivation, when cells are in active phase of growth. Eventually, it levelled off by increasing the light intensity to 540 μmol photon $m^{-2}s^{-1}$. At this intensity, a significant decrease in biomass productivity was reported.

5.3.1.4 *Lipid production and CHN analysis*

Figure 5.11 shows the effect of light intensity on CHN elements of *C.vulgaris*. The low light intensity condition appeared to have caused major elemental changes in *C.vulgaris* compared to the other conditions. The carbon content of *C.vulgaris* was relatively low at the low intensity light condition and increased up to 46% by enhancing the intensity to medium levels, followed by a slight decrease at the high intensity condition. The same trend was applied for the nitrogen content of cells, although the nitrogen stored at high intensity condition was relatively lower than the others.

As discussed before, the carbon content can be associated to the optimum growth condition and it can vary at different growth phases. The low carbon content can be justified for the cultures illuminated at low intensity (light-limitation condition). However, increasing the intensity to medium levels led to higher carbon storage, and the *C.vulgaris* cells consumed the accumulated carbon with further increase of light intensity.

Edwards et al. (2006) reported that changes in the irradiance condition can influence the nitrogen content of microalgae, depending on the species and reaction of photosynthesis machinery. It was suggested that any light condition which decreased the amount of photosynthesis led to lower nitrogen demand thus greater nitrogen storage.

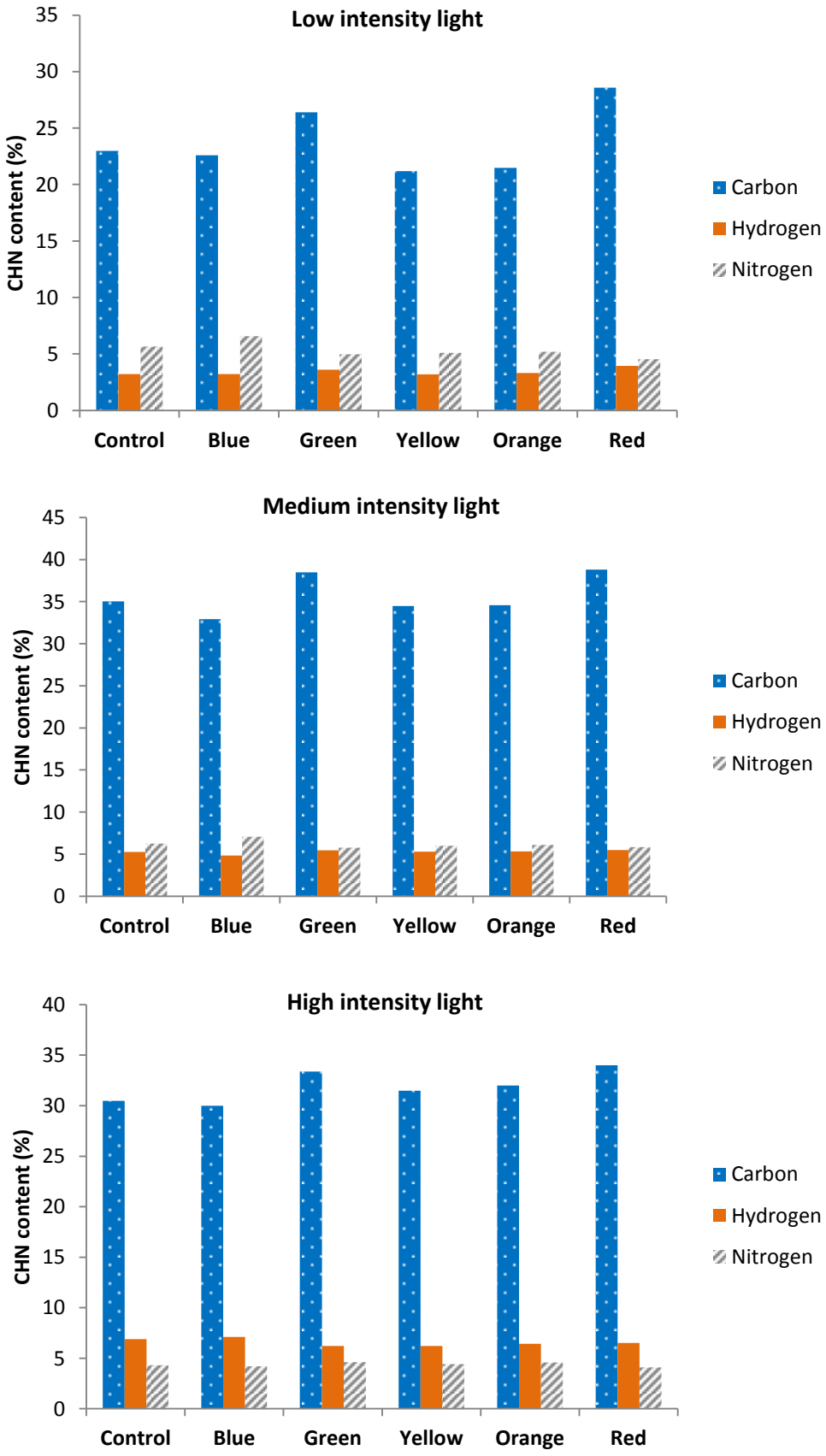


Figure 5.11, Effect of light intensity on CHN content of *C.vulgaris* obtained from elemental analysis in six different luminescent photobioreactors

The same trend applied to the lipid data obtained. The highest lipid accumulation occurred at the low intensity condition, where the blue PBR induced the maximum lipid content of 21.25% (see Figure 5.12).

A recent piece of study compared the effect of blue LED light on growth and lipid accumulation of *C.vulgaris* with that of white fluorescent light (Atta et al., 2013). It was reported that blue light at the intensity of 200 μmol photons m^{-2} s^{-1} and photoperiod of 12:12 (light: dark) produced the highest amount of lipids (23.5%). The amount of lipid accumulated in the blue light condition was reported to be 27% higher than that of white fluorescent light.

In addition, another study focused on the effect of light wavelengths on lipid production of *Chlorella.sp* by comparing the blue and red light. The study reported that the blue light induced a maximum lipid content of 0.778 g L^{-1}, which was 20% higher than that of red light (Perez-Pazoz and Fernandaz-Izquierdo, 2011). It has also been suggested that the activity of some enzymes, such as carbonic anhydrase, is influenced by the blue light (Roscher and Zetsche, 1986).

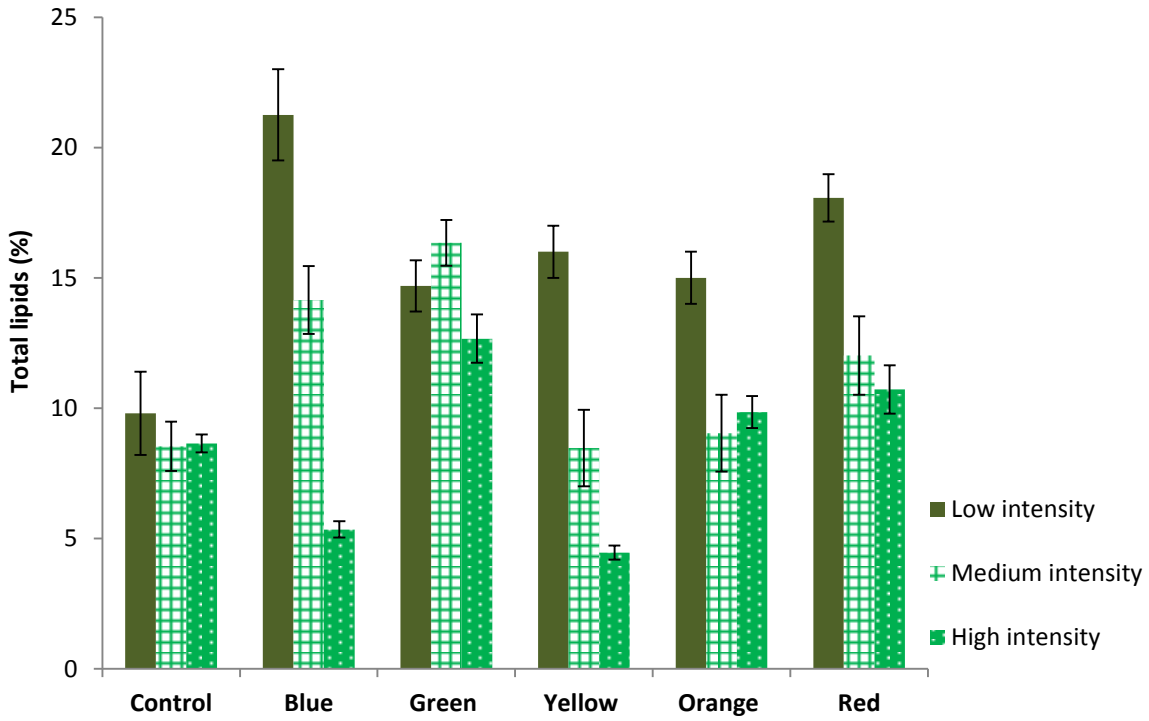


Figure 5.12, Effect of light intensity on total lipids content (g lipid/g biomass %) of *C.vulgaris* in six different luminescent photobioreactors.

The elevation of light intensity to medium levels reduced the lipid accumulation up to 50% in the blue PBR. Lipid production was reduced by further increasing the light intensity and comparing the performance of all luminescent PBRs, the green PBR seemed to enhance lipid production the most. At high intensity levels there was a rapid reduction of lipid content, particularly in the blue and yellow PBRs.

A research about the changes of lipids and fatty acids in microalgae in response to light intensity indicated that increasing irradiance levels decreased the lipid content (Guedes et al., 2010). Since lipids are major components of chloroplast in microalgae, elevation of light intensity decreased the chloroplast activity for photosynthesis hence reduced the lipid content.

5.3.2 *Gloeothece membranacea*

5.3.2.1 *Low light intensity 50 µmol photon m^{-2} s^{-1}*

G.membranacea was cultivated with a low concentration seed culture of 0.46 g L^{-1}, as the results of the previous section indicated that the growth was enhanced when a diluted initial culture density was used. Growth profiles of the six cultures in the luminescent photobioreactors under low light intensity are shown in Figure 5.13.

The highest biomass densities of 1.61 and 1.54 g L^{-1} were obtained in the red and control PBRs respectively. Cell growth stayed in a constant phase (zero-order kinetics) until the second day of inoculation, following an exponential phase in the red and control PBRs and a linear stage (first-order kinetics) as indicated by Ravelonandro et al. (2008) in other photobioreactors.

The maximum specific growth rate was in similar value ranges in all different PBRs, with the exception of the green PBR which was lower, at around 0.16 day^{-1}. Overall, the biomass productivity was relatively low, particularly under the green light which was (58.62 mg L^{-1} day^{-1}). Red light enhanced the biomass productivity to a highest level of 114.7 mg L^{-1} day^{-1}. The biomass productivity was calculated in the exponential phase (between days 2-7), when the maximum specific growth rate was obtained. In general, biomass production under low light intensity was relatively slow and linear, and cell growth profiles were smooth.

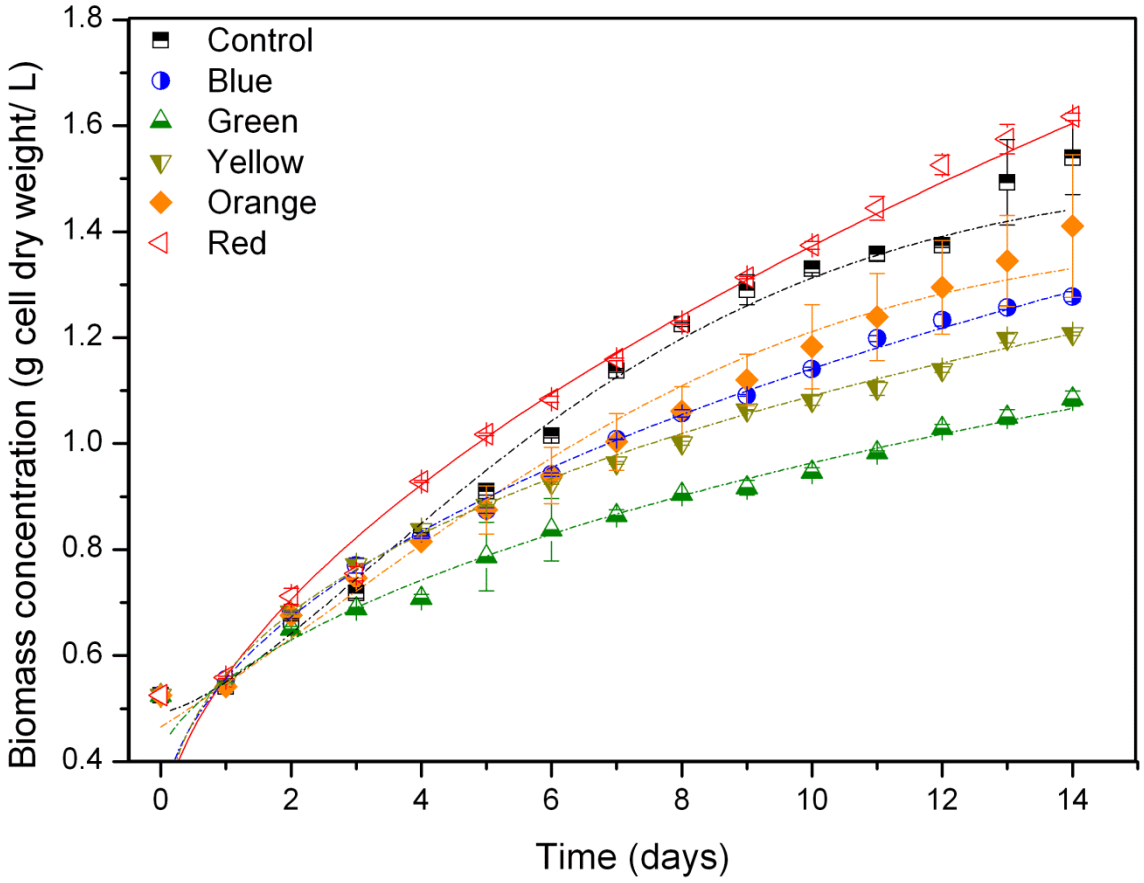


Figure 5.13, Growth curves of *G.membranacea* illuminated at 50 µmol photon m^{-2} s^{-1} intensity under blue, green, yellow, orange, red and full-spectrum light recorded over a 14 day period. The curves are fitted using a standard sigmoid Weibull growth model.

5.3.2.2 *Medium intensity light 100 µmol photon m^{-2} s^{-1}*

Unlike growth profiles at low light intensity, biomass production at medium intensity was non-linear (see Figure 5.14). However, growth in the yellow and green PBRs was improved by increasing irradiance compared to the low light condition. The highest biomass density of 2.0 g L^{-1} was achieved in the red and blue PBRs. The specific growth rates were almost identical in the control, blue, yellow and orange PBRs whilst this growth factor was halved (0.13 day^{-1}) in the green PBR compared to the red PBR (0.26 day^{-1}). Overall, biomass productivity was slightly improved under medium light compared to low light intensity particularly in the yellow PBR with 70% increase. The results indicated that, compared to *C.vulgaris*, the elevation of the intensity from 50 to 100 µmol photon m^{-2} s^{-1} produced considerable alterations in growth parameters of cultures in various luminescent PBRs. This effect was more pronounced in the blue, yellow and orange PBRs with improved biomass productivity.

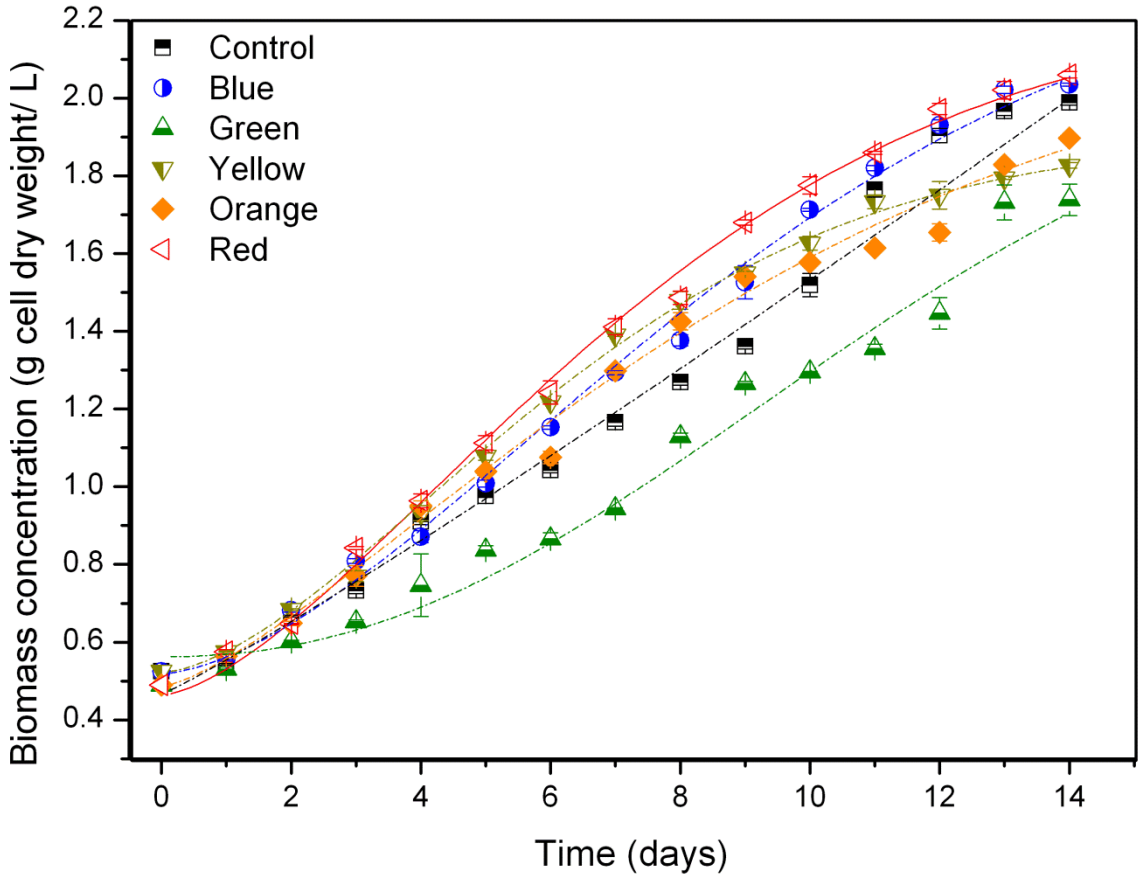


Figure 5.14, Growth curves of *G.membranacea* illuminated at 100 µmol photon m^{-2} s^{-1} intensity under blue, green, yellow, orange, red and full-spectrum light recorded over a 14 day period. The curves are fitted using a standard sigmoid Weibull growth model.

5.3.2.3 High intensity light 300 µmol photon m^{-2} s^{-1}

Under the high light intensity all the cultures remained in the lag phase for one day after the inoculation. Red and control light conditions entered an exponential phase following a linear and stationary phase. However, the other light conditions followed a linear pattern and from day 10, biomass density of all the cultures remained constant (see Figure 5.15) and cell loss occurred by the end of two weeks. When the intensity was raised, the red light performed better in the exponential growth phase compared to lower light intensities.

The highest biomass density of 2.52 g L^{-1}, productivity of 180.8 mg L^{-1} day^{-1}, and specific growth rate of 0.45 day^{-1} were achieved in the red PBR. The results indicated that the growth was enhanced by increasing light intensity up to 150 µmol photon $m^{-2}s^{-1}$ irradiance, where cells remain in light limitation stage. Elevation of the light intensity

up to 300 µmol photon m^{-2} s^{-1} promoted the growth, although after 9 days cells entered the stationary stage. This means that increasing the intensity levels above this threshold led to the high consumption of nutrients which caused cell loss. Figure 5.16 shows the biomass productivity and Table 5.4 presents the growth parameters of *G.membranacea* at different light intensity levels.

The results showed that the elevation of the irradiation intensity enhanced productivity in all photobioreactors. The red PBR was particularly influenced by these changes and it induced the highest productivity.

In the previous chapter it was suggested to cultivate the cyanobacteria cultures under 150 µmol photon m^{-2} s^{-1} intensity levels. However, it must be noted that cultivation in photobioreactors and sub-culturing under higher light intensities can improve the tolerance of the selected species into light conditions. Therefore, the response of cells to the light intensity alterations can be influenced by the condition in which seed cultures were maintained.

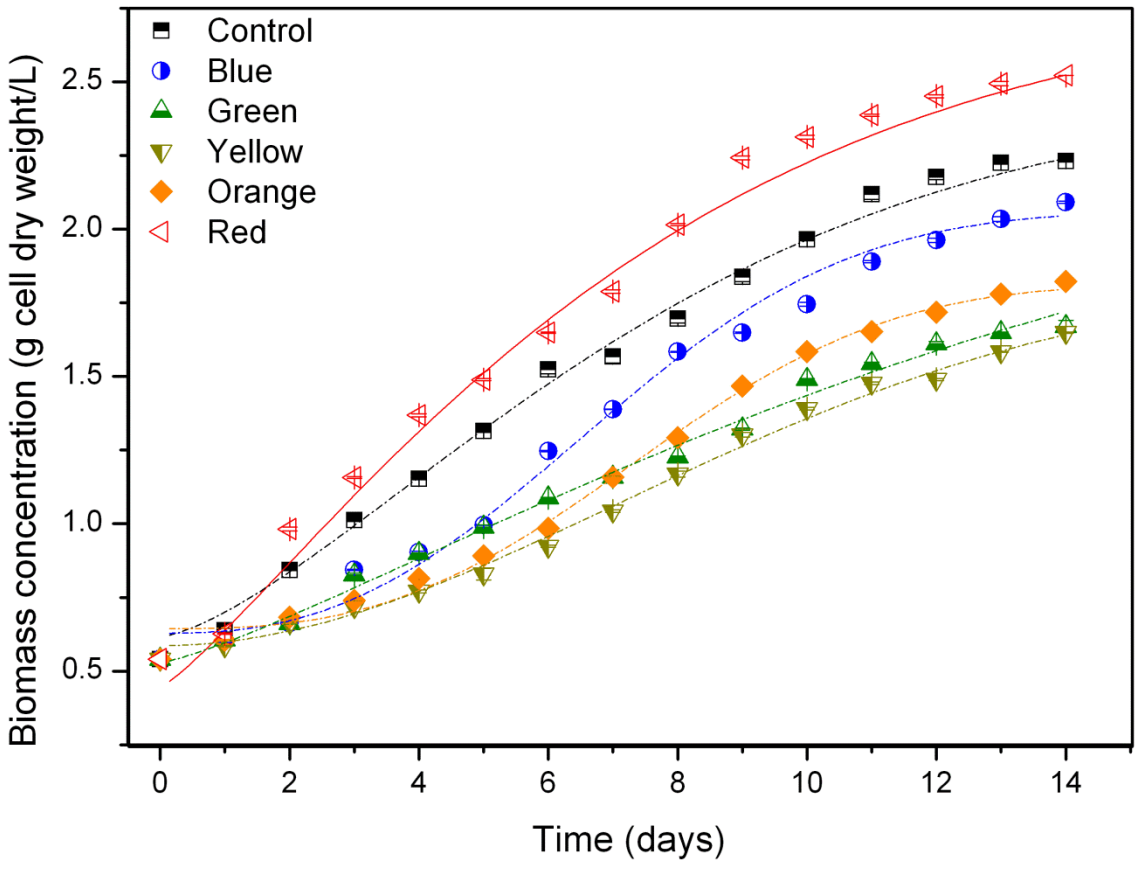


Figure 5.15, Growth curves of *G.membranacea* illuminated at 300 µmol photon m^{-2} s^{-1} intensity under blue, green, yellow, orange, red and full-spectrum light recorded over a 14 day period. The curves are fitted using a standard sigmoid Weibull growth model.

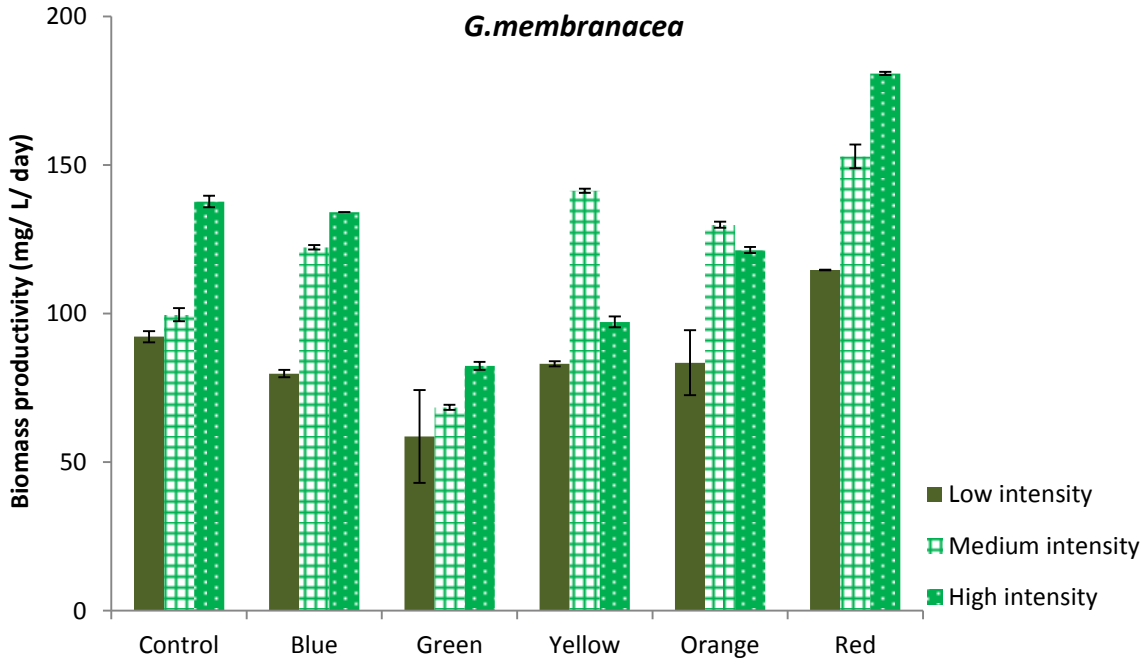


Figure 5.16, Biomass productivity of G.*membranacea* at different light intensities in luminescent photobioreactors.

Light condition	*G.membranacea*					
	50µmol photon m^{-2} s^{-1}		100µmol photon m^{-2} s^{-1}		250µmol photon m^{-2} s^{-1}	
	C_{14day} (10^6 cell ml^{-1})	μ_{max} (day^{-1})	C_{14day} (10^6 cell ml^{-1})	μ_{max} (day^{-1})	C_{14day} (10^6 cell ml^{-1})	μ_{max} (day^{-1})
Control	29.89	0.21	38.57	0.21	43.20	0.28
Blue	24.82	0.21	39.45	0.21	40.51	0.24
Green	21.10	0.16	35.44	0.19	32.37	0.22
Yellow	23.46	0.21	33.72	0.13	31.95	0.13
Orange	27.39	0.22	38.55	0.21	35.30	0.12
Red	31.38	0.24	39.95	0.26	48.83	0.45

Table 5.4, Effect of light intensity on growth parameters of G.*membranacea.*

5.3.2.4 *Lipid production and CHN analysis*

The effect of light intensity on CHN elements of *G.membranacea* was more pronounced compared to *C.vulgaris*. At low intensity levels the carbon content in some of the PBRs was particularly low. The lowest carbon content obtained in the yellow PBR was 10.9%. In addition, the highest nitrogen content of 11.5% was induced in the yellow PBR. The same trend was observed in the blue PBR.

Overall, illumination with low intensity provided more extreme conditions for the cells and increasing the intensity to medium levels enhanced carbon storage considerably. A 70% increase of the carbon content, particularly in the red PBR, showed the sensitivity of cyanobacterial cells to the irradiation conditions. Lower nitrogen content observed at the medium intensity showed that the cyanobacterial cells were in a better growth condition. Further increases of the light intensity led to slightly lower carbon and nitrogen contents.

However, the difference in nitrogen storage between the medium and high intensity conditions was insignificant. Figure 5.17 shows the variation of CHN elements in *G.membranacea* under different irradiation conditions.

The lipid data obtained also supported this hypothesis. High lipid accumulation at the low intensity condition was followed by a reduction when the irradiation intensity increased. The lipid content can be correlated to the carbon and nitrogen content (see Figure 5.18). As seen previously, the major difference in lipid accumulation occurred in the blue and yellow PBRs.

A comparison between the performances of different luminescent PBRs demonstrated that the most suitable condition for biomass production was not necessarily associated with maximum lipid production. Microalgae cells respond to different environmental conditions by consumption or storage of various biochemicals.

Accumulation of lipids is generally associated with a condition in which cells have to adapt to the extreme or even unsuitable conditions such as nutritional changes, starvation of essential growth compounds, or pH variations. *G.membranacea* exhibited a more prominent response to the changes of light availability both in culture density and light intensity groups.

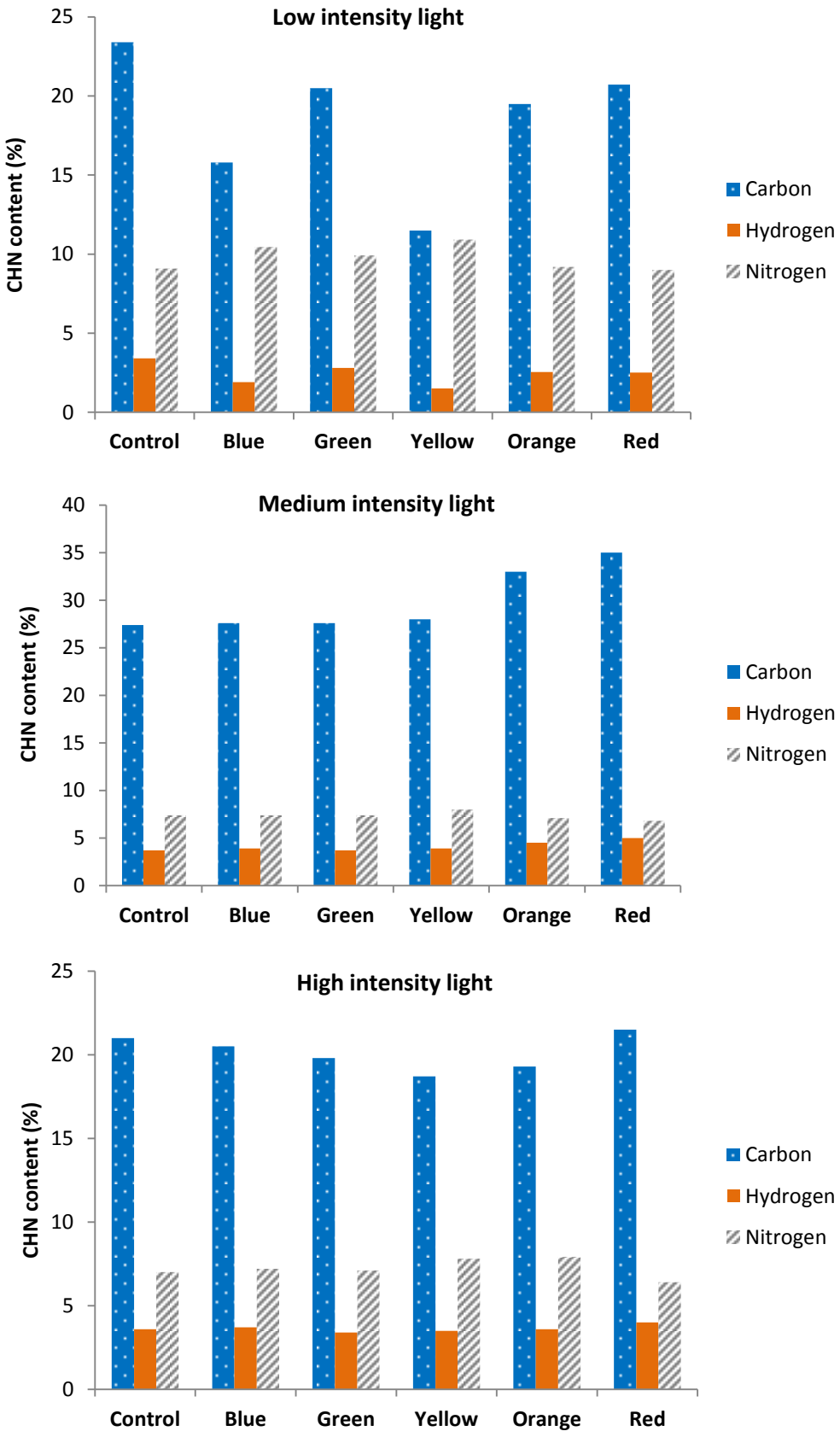


Figure 5.17, Effect of light intensity on CHN content of *G.membranacea* obtained from elemental analysis in six different luminescent photobioreactors.

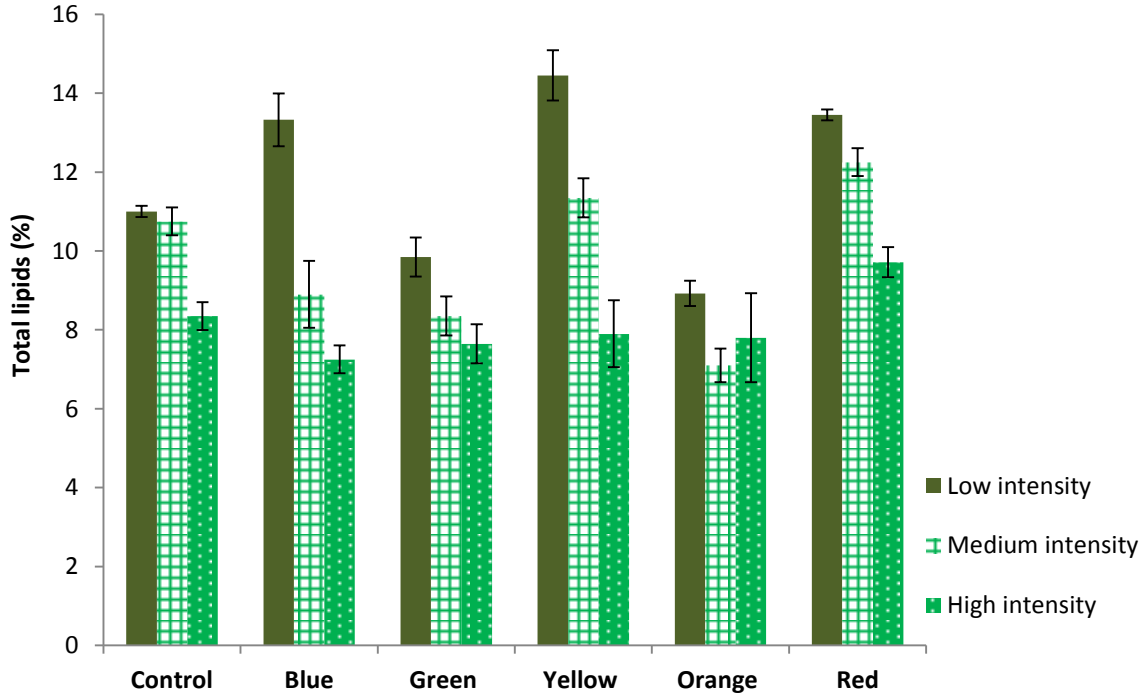


Figure 5.18, Effect of light intensity on total lipids content (g lipid/g biomass %) of *G.membranacea* in six different luminescent photobioreactors.

5.4 Conclusions

This chapter reviewed the cultivation of microalgae in luminescent photobioreactors and investigated the effects of culture density and spectral quality of light on microalgae. By incorporating luminescent dyes into materials for bioreactor construction, the tailoring of light conditions was combined with improved mixing and gas transfer. The effect of improved mixing on the utilisation of light was evidently demonstrated by the increased relative growth of both tested species in the red luminescent PBR.

The effect of the initial culture density and cell shadowing showed that lower densities are favourable for higher biomass productivity and growth rates. The results also indicated that the blue and yellow PBRs induced lipid accumulation at various cultivation conditions. In addition, three different light intensity levels were examined for the illumination of microalgae. It was demonstrated that at low light intensities the dynamics of the microalgae cells varied depending on the wavelength ranges provided. Moreover, carbon and nitrogen, the two major compounds found in the cells, changed in more extreme conditions. From the results obtained it was clear that whilst low intensity

irradiation could cause slow growth, it induced lipid accumulation in both strains. When the intensity levels were increased, cells recovered the carbon which they consumed and there was relatively higher carbon storage.

In Chapter 4 it was reported that static cultures of *G.membranacea* showed very limited growth under this spectral range. However, this would appear to be due to the very limited penetration of this wavelength range into static cultures, rather than a more direct effect on the organisms themselves. By improving the mixing, the light penetration issue is resolved and growth is improved. However, it should be noted that this reduced penetration is very likely to present a further issue as photobioreactors are scaled up, requiring more selective optimisation of lighting conditions as scales increase.

The results obtained in this chapter demonstrated that, within well-mixed photobioreactor culture, modified light in the red spectral region promoted the biomass production of *C.vulgaris* and *G.membranacea*. However, cultivation of microalgae under red light with a narrow wavelength band and lower energy content for penetration into the culture has a critical requirement of a well-mixed culture. This work clearly indicates that luminescent photobioreactors can be used to increase the efficiency of outdoor cultivation systems in areas of sub-optimal sunlight exposure, without the need for artificial lighting systems. This offers a cheaper and more energy-efficient route to large-scale algal culture and CO_2 mitigation.

Chapter 6:
Carbon dioxide uptake in photobioreactors

6.1 Introduction to carbon dioxide uptake by microalgae

This chapter reviews the effect of carbon dioxide aeration on the cultivation of microalgae in luminescent photobioreactors. The aim of this chapter is to review the influence of CO_2 on growth characteristics, biomass productivity, total lipids, elemental compositions, pH variation, and the rate of CO_2 fixation. The experiments presented were set in three different aeration qualities including pure air (consisting of 0.03% CO_2), 5% CO_2 enriched air, and 15% CO_2 enriched air.

Both temperature and pH affect the rate of hydration and solubility of CO_2. Elevation of pH enhances the rate of carbon dioxide transfer into liquid phase. However, this rate may decrease with any increase in temperature. The temperature was maintained constant in all experiments, as the aim was to investigate the effect of different culture methods in relation to luminescent illumination by photobioreactors. Consequently, all the growth factors were measured on this basis. In addition, in order to examine different CO_2 aeration rates, which naturally change the pH of cultures, no other methods such as modification of media or addition of acids were used for pH alteration. As large scale production of microalgae is not realistically compatible with pH control, the experiments presented in this chapter were set to resemble such conditions.

Some studies on the content of microalgae cells by elemental analysis have revealed that carbon can make up to one-half of cell dry weight (OH-Hama and Miyachi, 1988). The rate of carbon dioxide fixation depends on the carbon content and biomass productivity during cultivation, particularly at the exponential growth phase. Although there was no media alteration in these sets of experiments, carbon content of microalgae cells can vary in each luminescent photobioreactor.

6.2 Effect of aeration with CO_2 enriched air on biomass production

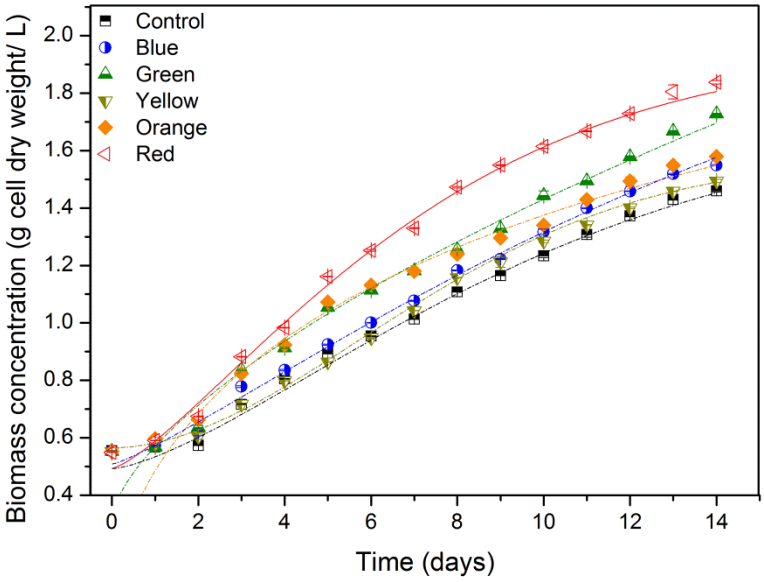
6.2.1 *Chlorella vulgaris*

Biomass production at the exponential growth phase was enhanced with 5% CO_2 aeration compared to pure air at the control condition. Although the final biomass densities obtained were relatively similar, the maximum biomass production, at 5% CO_2 aeration, was achieved in half of the cultivation period required for the pure air condition. Figure 6.1 shows the biomass profiles of *C.vulgaris* in six different luminescent photobioreactors during two weeks of cultivation. Red and green PBRs as well as the control PBR enhanced biomass production. However, after five days, growth was limited and cells remained in the stationary phase for a week until growth was inhibited and cell loss occurred. The highest specific growth rate of 0.38 day^{-1} was achieved in the red PBR. In addition, the maximum biomass productivity of 296.6 and 264.5 mg L^{-1} $day^{-1}$was obtained under red and control light condition (see Table 6.1).

C.vulgaris cells had a full growth cycle, as shown by the sigmoid growth models. These growth cycles comprised a more dynamic pattern in the red, green and control PBRs. The results indicated that 5% CO_2 promoted the active phase of growth, which generally occurs in the first week of cultivation. Hence, it may be favourable for short term cultivation. In addition, the highest biomass content achieved at this aeration mode was improved compared to aeration with pure air.

The growth profiles with 15% CO_2 aeration were similar to 5% although the growth was inhibited five days after cultivation. The maximum specific growth rate was enhanced in the red PBR to 0.49 day^{-1}, whilst biomass productivity decreased slightly at this light condition. Overall, the results showed that 15% CO_2 aeration inhibited the growth of *C.vulgaris* at early stages. This may be related to the role of carbon dioxide in reducing the pH and making the culture media acidic. To investigate the influence of other culture parameters such as nutrient media or elemental carbon present in the media, it is recommended to keep the pH within the neutral ranges. This can be achieved either by adding buffers to the media or adjusting the rate of CO aeration to the levels suitable. Similar conclusions were obtained in previous studies (Chiu et al., 2008, Edwards et al., 2006, Pedersen and Borum, 1996). Zheng et al. (2012) reported that 5% CO_2 was the optimum aeration condition for *C.vulgaris* and when the culture was aerated with 15% CO_2 the growth parameters such as C_{max}, P_{max}, μ_{max} considerably decreased compared to 5% CO_2 aeration.

(a)



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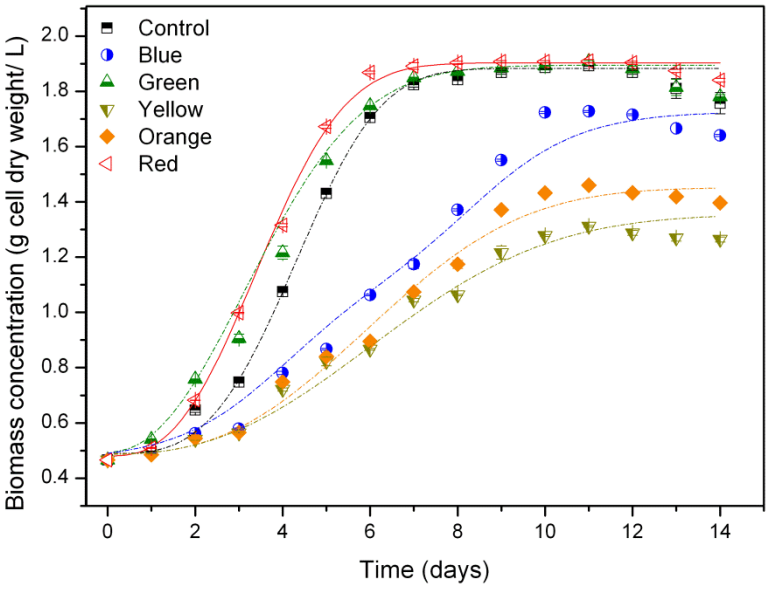

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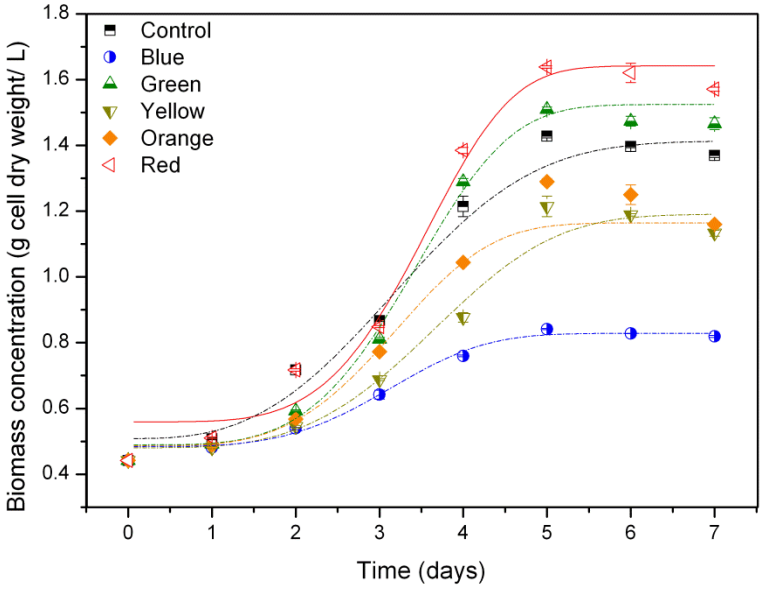


Figure 6.1, Growth curves of *C.vulgaris* aerated with (a) pure air, (b) 5% CO_2 enriched air, (c) 15% CO_2 enriched air, under blue, green, yellow, orange, red and full-spectrum light recorded over 14 and 7 days. The curves are fitted using a standard sigmoid Weibull growth model.

However, the results of previous studies can only be compared to those related to the control PBR, as interference of various wavelength ranges can have different impacts on growth.

The biomass productivity may increase with the elevation of CO_2% (v/v) in the gas mixture up to a certain level, and identification of this level depends greatly on different species. It must be noted that adaption of *C.vulgaris* to lower concentrations of CO_2 can assist further tolerance to higher CO_2 concentrations (Yun and Park, 1997). Yun et al. (1997) cultivated *C.vulgaris* in waste water and flue gas, with the aim of obtaining an economically feasible system. They reported that pre-adaptation to 5% CO_2 considerably improved tolerance of *C.vulgaris* to 15% CO_2, which is typically found in industrial flue gas.

A recent study on optimisation of CO_2 bio-mitigation reported that the growth parameters of *C.vulgaris* were enhanced up to 45% when CO_2 concentration increased from 2% to 6% (Anjos et al., 2013). It was suggested that the effect of CO_2 on culture parameters could be linked to the availability of carbon in the culture media. Moreover, high concentrations of CO_2 could reduce the pH of the culture media which consequently influences the activities of some important enzymes assisting photosynthesis process. Increasing concentrations of CO_2 can influence key enzymes in carbon metabolism including carbonic anhydrase and Rubisco (Yang and Gao, 2003).

Aeration condition / **Light condition**	***C.vulgaris***					
	Pure air (0.03% CO_2)		**5% CO_2**		**15% CO_2**	
	μ_{max} (day^{-1})	**Productivity (mg L^{-1} day^{-1})**	**μ_{max} (day^{-1})**	**Productivity (mg L^{-1} day^{-1})**	**μ_{max} (day^{-1})**	**Productivity (mg L^{-1} day^{-1})**
Control	0.22	89.13	0.36	264.52	0.34	230.25
Blue	0.23	94.51	0.30	125.08	0.17	90.12
Green	0.27	103.32	0.34	247.48	0.46	254.56
Yellow	0.17	92.30	0.24	81.70	0.24	183.46
Orange	0.21	96.09	0.28	87.84	0.30	200.24
Red	0.27	132.97	0.38	296.65	0.49	282.00

Table 6.1, Effect of CO_2 aeration on growth parameters of *C.vulgaris*.

6.2.2 *Gloeothece membranacea*

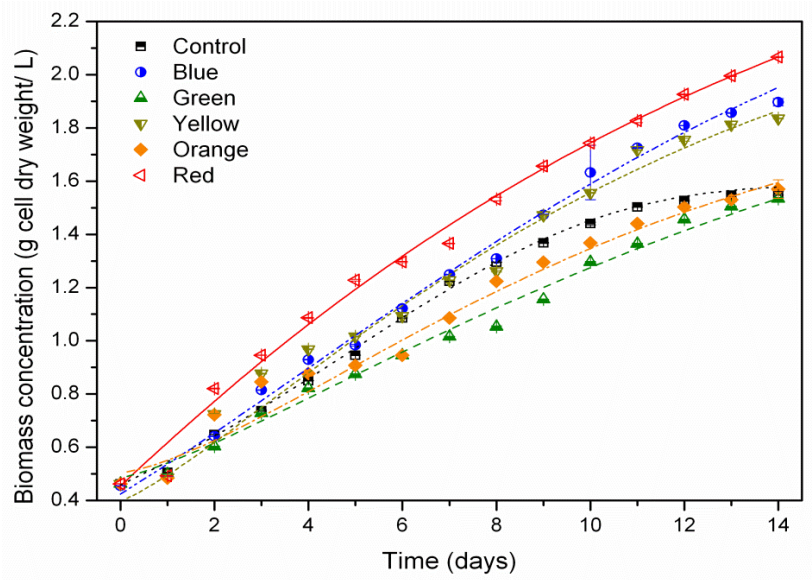
The effect of 5% CO_2 aeration on biomass production of *G.membranacea* was significantly different than that of *C.vulgaris*. Figure 6.2 shows the biomass profiles of *G.membranacea* over a period of 14 days at different CO_2 concentrations. The biomass profiles under all light conditions showed linear growth during two weeks of cultivation. As a result, biomass productivity in all photobioreactors was halved compared to the condition in which only pure air (control aeration) was used (see Figure 6.2). The P_{max}, μ_{max} obtained were lower than those achieved with pure air. Compared to pure air experiments the biomass productivity decreased at 5% CO_2 aeration and remained unchanged at 15% CO_2.

Compared to *C.vulgaris*, *G.membranacea* had higher tolerance to 15% CO_2 aeration by growing in this condition over a period of two weeks. Cyanobacteria cells showed significant tolerance to higher carbon dioxide concentrations and maximum biomass production of 2.25 g L^{-1} day^{-1} was achieved in the control photobioreactor. Thomas et al.,(2005) investigated the tolerance of various cyanobacterial species to high concentrations of CO_2. The study reported that *Anabaena* and *Synechococcus* survived at high pressures of 100% CO_2. Sensitivity of the strains to high concentrations of CO_2 was found to be linked to initial low pH levels (pH 5-6), although it was not solely dependent on that (Thomas et al., 2005). Table 6.2 presents the effect of CO_2 aeration on maximum specific growth rate (μ_{max}) and biomass productivity of *G.membranacea*.

Aeration condition / Light condition	***G.membranacea***					
	Pure air (0.03% CO_2)		**5% CO_2**		**15% CO_2**	
	μ_{max} (day^{-1})	**Productivity (mg L^{-1} day^{-1})**	**μ_{max} (day^{-1})**	**Productivity (mg L^{-1} day^{-1})**	**μ_{max} (day^{-1})**	**Productivity (mg L^{-1} day^{-1})**
Control	0.34	110.13	0.13	63.87	0.28	177.5
Blue	0.50	123.00	0.15	64.43	0.22	120.9
Green	0.51	94.15	0.15	80.96	0.16	101.6
Yellow	0.36	132.36	0.13	50.63	0.16	84.01
Orange	0.37	105.73	0.14	53.78	0.22	94.20
Red	0.42	184.00	0.18	86.33	0.33	189.70

Table 6.2, Effect of CO_2 aeration on growth parameters of *G.membranacea*.

(a)



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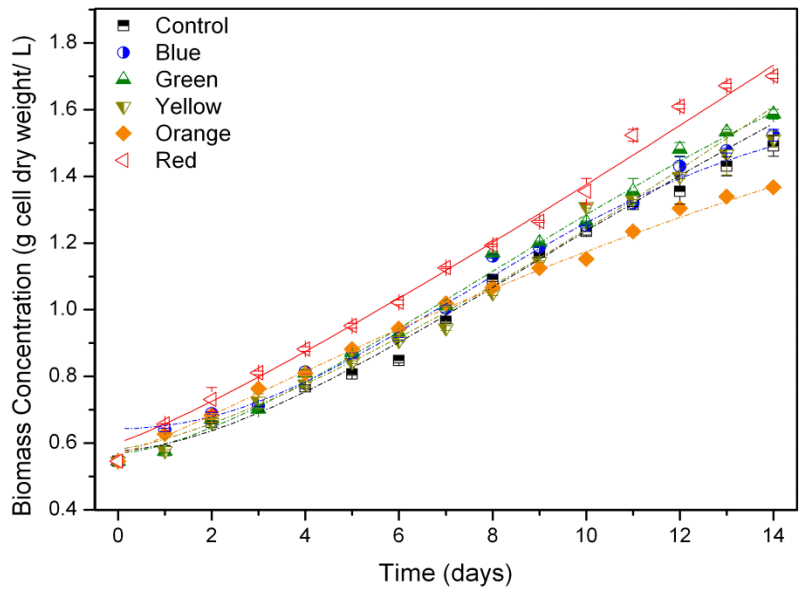

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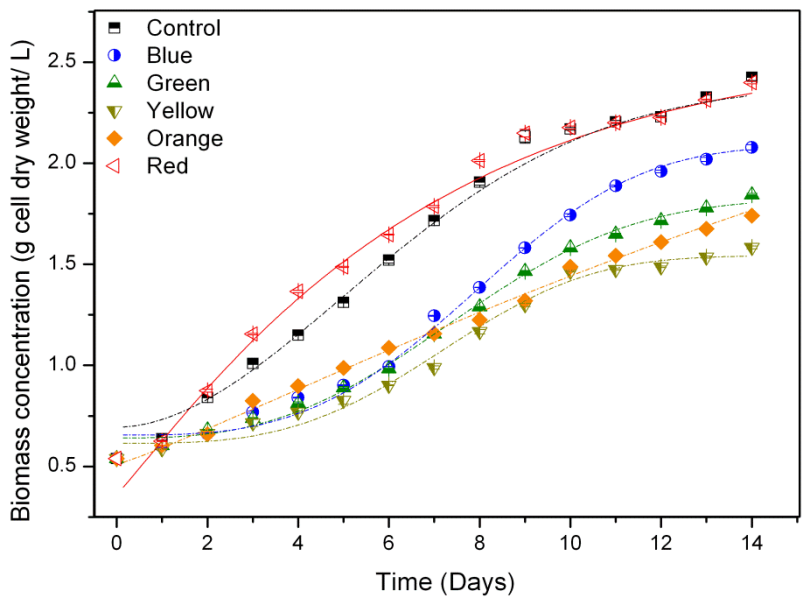

Figure 6.2, Growth curves of *G.membranacea* aerated with (a) pure air, (b) 5% CO_2 enriched air, (c) 15% CO_2 enriched air, under blue, green, yellow, orange, red and full-spectrum light recorded over a 14 day period. The curves are fitted using a standard sigmoid Weibull growth model.

6.3 Effect of aeration with CO_2 enriched air on CO_2 fixation rate and CHN content

Various studies have focused on the ability of microalgae in carbon dioxide fixation (Anjos et al., 2013, Chiu et al., 2008, Diao et al., 2004, Ho et al., 2011, Kumar et al., 2011, Edwards et al., 2006). In this study, the rate of CO_2 fixation was measured according to the carbon content of cells obtained from elemental analysis (CHN analysis). Although some research papers suggest the calculation of theoretical carbon content, the aim of this study was to compare the effects of a specific culture condition in different luminescent photobioreactors. Therefore, the experimental carbon content is entirely suitable to identify the influence of various aeration and spectral conditions on elemental compositions and CO_2 fixation rates. In addition, the CO_2 fixation rate is significantly dependent on biomass productivity. Consequently, an increase in P_{max} can improve carbon uptake by microalgae. It must be noted that this rate only represents the active phase of growth when growth is exponential and P_{max} is achieved.

According to mass balance of microalgae, the fixation rate of carbon dioxide can be calculated. Microalgae biomass contains approximately 50% carbon based on dry weight (Sanchez Miron et al., 2003). This carbon is mainly derived from carbon dioxide which is available from the air or from waste streams of factories. A mole of CO_2 has a mass of 44 grams including 12 grams of carbon. Based on these premises, the theoretical carbon dioxide consumption by each gram of microalgae can be obtained from:

$$\frac{44\ (g\ CO_2/mol)}{12\ (g\ Carbon/mol)} \times \frac{0.50\ (\text{g Carbon})}{(g\ algae\ biomass)} = 1.83\ (g\ CO_2/\ g\ algae\ biomass) \qquad (6.1)$$

Equation (6.1) shows that in theory one gram of algal dry cell weight can fix approximately 1.83 grams of carbon dioxide. However, in experimental works carbon content of microalgae changes depending on the species and culture conditions. Biomass productivity and experimental carbon content obtained from elemental analysis influence the rate of carbon dioxide consumption. Equation (6.2) shows the relationship between these factors:

$$CO_2\ fixation\ rate\ (mg\ L^{-1}\ day^{-1}) = P \times C_{carbon} \times (M_{CO2}/M_C) \qquad (6.2)$$

Where P is the biomass productivity (mg), C_{carbon} is the experimental carbon content obtained from elemental analysis and M_{CO2} and M_C are the molar mass of CO_2 and carbon respectively.

6.3.1 *Chlorella vulgaris*

The carbon content of *C.vulgaris* varied in different PBRs with the changes of carbon dioxide concentration in the air stream. In the control PBR, the carbon content was solely influenced by the aeration quality rather than the light condition, and it was enhanced up to 25% by increasing CO_2 concentrations to 5%. A further increase of CO_2 from 5% to 15% improved the carbon storage in all photobioreactors. Although the quality of the enriched air may have influenced the carbon content of *C.vulgaris*, there are other factors which must be taken into account. For instance, the growth stage in which the elemental analysis takes place has a significant effect on measurements. In all the measurements, elemental analysis was carried out at the end of cultivation period. However, when *C.vulgaris* was aerated with 15% CO_2 the growth phase was relatively short and in less than a week cells entered the stationary phase. Therefore, the cultivation period was shorter than usual, and the cells were harvested and freeze dried for the analysis. Overall, the results obtained indicated that some wavelength ranges such as blue can influence various elements in the microalgae cells.

Figure 6.3 shows that by increasing the concentrations of carbon dioxide in the air supply, the carbon content of *C.vulgaris* in the control, red and green PBRs increased up to 24%, 15% and 8% respectively.

In addition, analysis of the cell's nitrogen content showed that increasing CO_2 aeration to 15% induced nitrogen storage in *C.vulgaris*. High nitrogen content of the cells is usually associated with the cell's response to unsuitable environmental conditions. This effect was particularly pronounced in the red PBR with 60% increase in the nitrogen content.

The carbon dioxide fixation rate was improved by aeration with 5% CO_2. The red PBR provided the most suitable condition in which 337 mg L^{-1} day^{-1} CO_2 was fixed. Overall, increasing CO_2 concentration in the media enhanced the rate of CO_2 fixation. Figure 6.4 illustrates the rate of CO_2 fixation of *C.vulgaris* in six different luminescent photobioreactors aerated with air, 5% and 15% CO_2 enriched air.

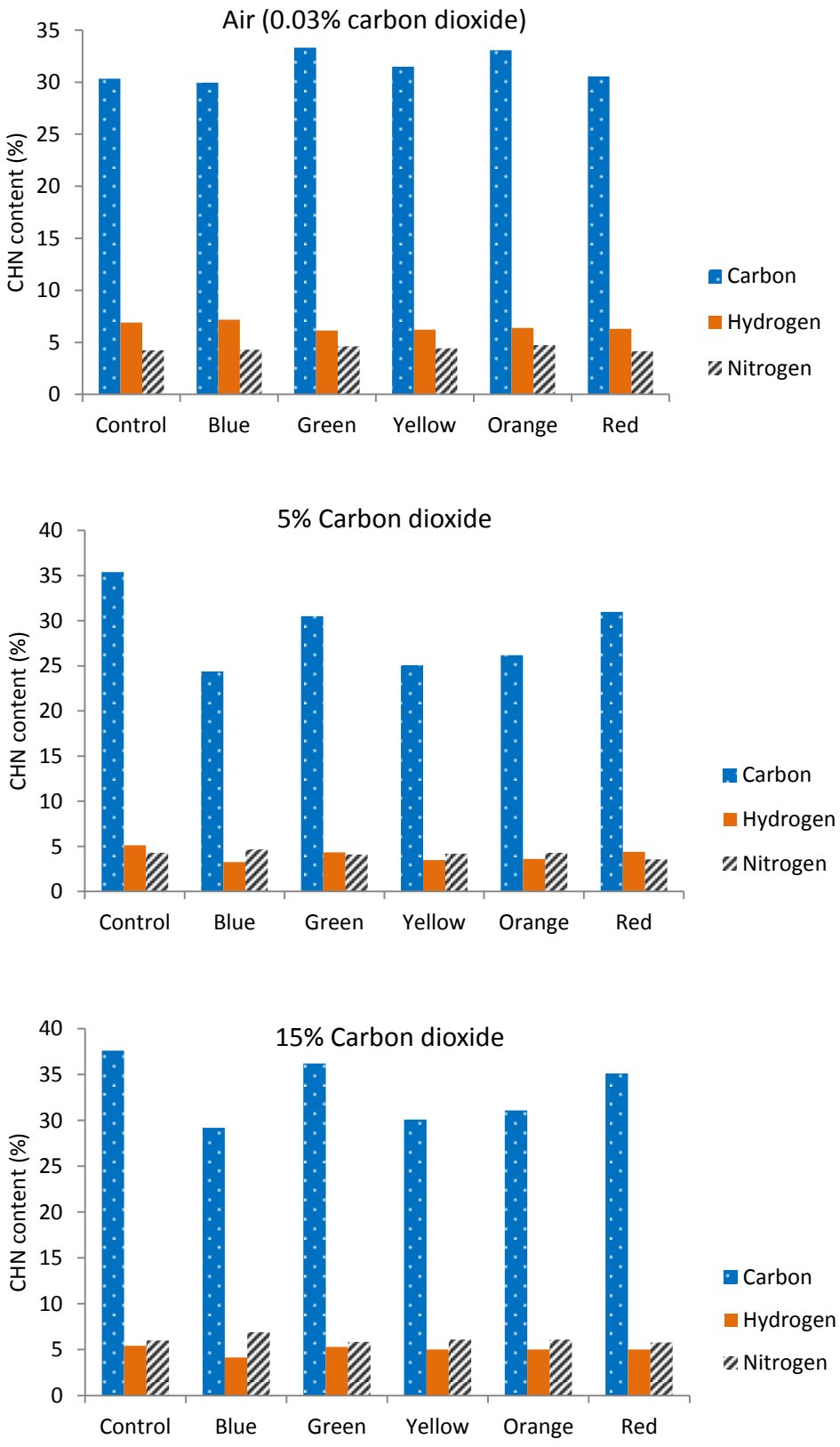

Figure 6.3, Effect of various CO_2 supply conditions on CHN content of *C.vulgaris* obtained from elemental analysis in six different luminescent photobioreactors.

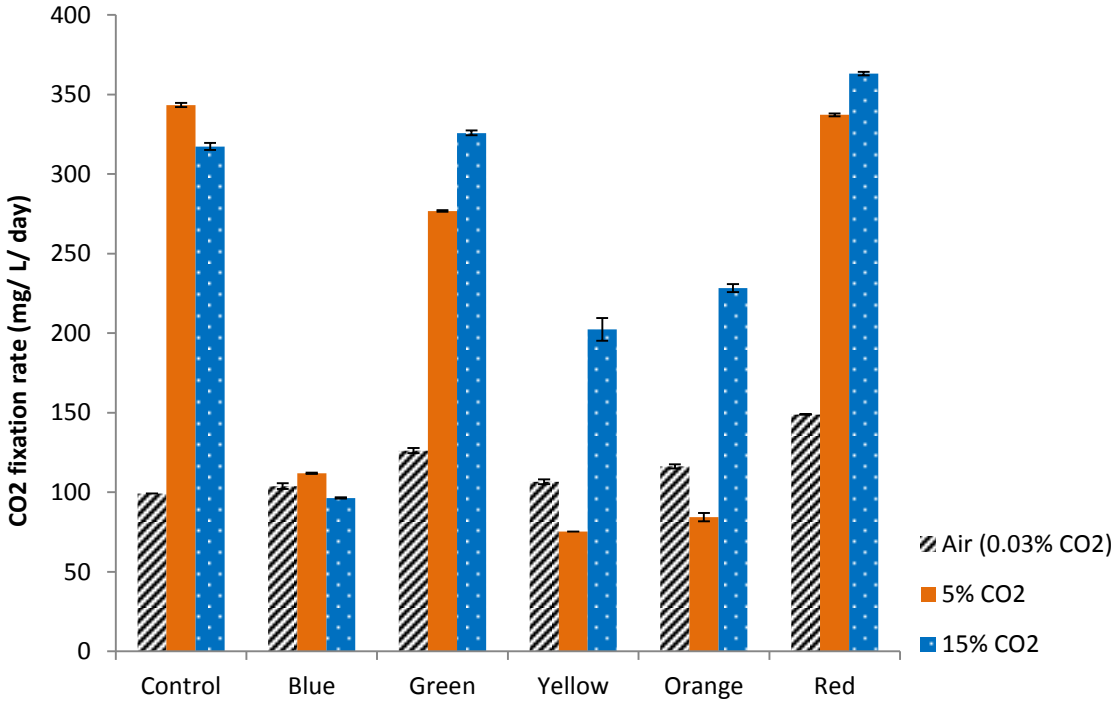

Figure 6.4, Effect of various CO_2 supply conditions on CO_2 fixation rate of *C.vulgaris* in six different luminescent photobioreactors.

The cells of *C.vulgaris* in control, green and red photobioreactors responded to changes in CO_2 concentrations more significantly than others. This can be due to the active role of these wavelength ranges in producing the highest biomass productivity and carbon content as previously described in Chapter 5.

The blue PBR was the least efficient in up-taking carbon dioxide compared to the other photobioreactors at all aeration concentrations. Carbon content and productivity of *C.vulgaris* in the blue PBR was particularly low when aerated with 15% CO_2.

Overall, there was no substantial difference in the CO_2 fixation rate in all photobioreactors when aerated with pure air comprising 0.03% CO_2 (v/v). However, in all luminescent photobioreactors, with the exception of the blue PBR, increasing the concentration of CO_2 in the gas mixture had significant impacts on CO_2 sequestration.

6.3.2 *Gloeothece membranacea*

The carbon content of *G.membranacea* reduced with increasing carbon dioxide concentrations. However, when the elemental analysis was compared between different PBRs at a given CO_2 concentration, there was no major difference between the carbon or nitrogen contents.

In contrast, nitrogen storage of the cells was enhanced by increasing the CO_2 concentration from 5% to 15%. The yellow PBR induced the highest nitrogen content. Figure 6.5 illustrates that by increasing the concentrations of carbon dioxide in the air supply, the carbon content of *G.membranacea* decreased.

G.membranacea grew over a period of two weeks, at 15% CO_2 aeration condition. However, due to their lower carbon content and biomass productivity, the former was less efficient in sequestrating carbon dioxide. Although the lowest biomass productivity was obtained with 5% CO_2 aeration, it increased up to the values achieved with pure air. The highest CO_2 up-take at the rate of 212.43 mg $L^{-1}day^{-1}$ was achieved in the red photobioreactor aerated with pure air. *G.membranacea* sequestrated more carbon dioxide compared to *C.vulgaris*, when aerated with air containing 0.03% CO_2, whilst the fixation rate by *C.vulgaris* was considerably higher with 5% and 15% CO_2 enriched air.

Figure 6.6 shows the rate of CO_2 fixation of *G.membrancea* in six different luminescent photobioreactors aerated with air, 5% and 15% CO_2 enriched air.

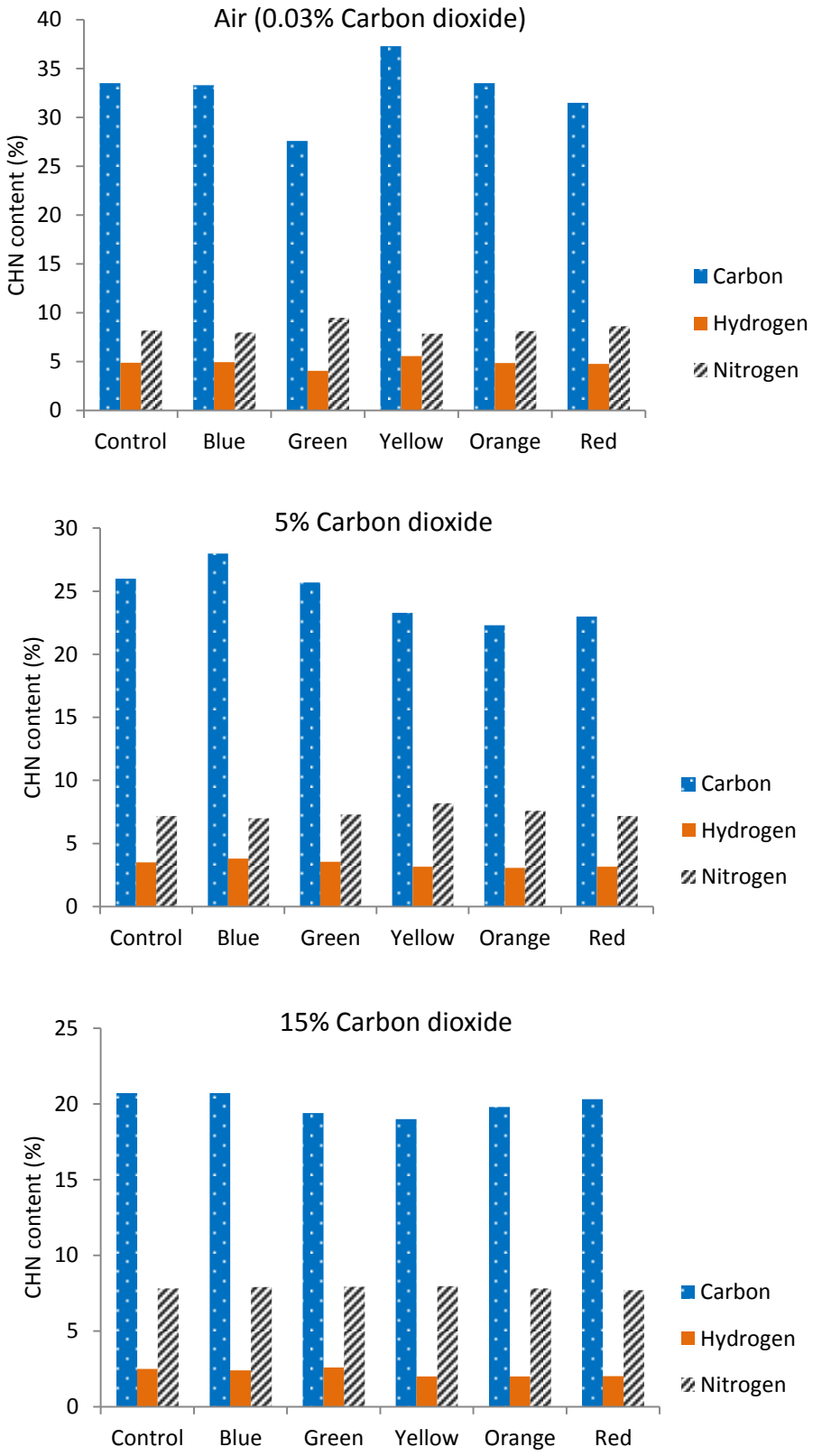

Figure 6.5, Effect of various CO_2 supply conditions on CHN content of *G.membranacea* obtained from elemental analysis in six different luminescent photobioreactors.

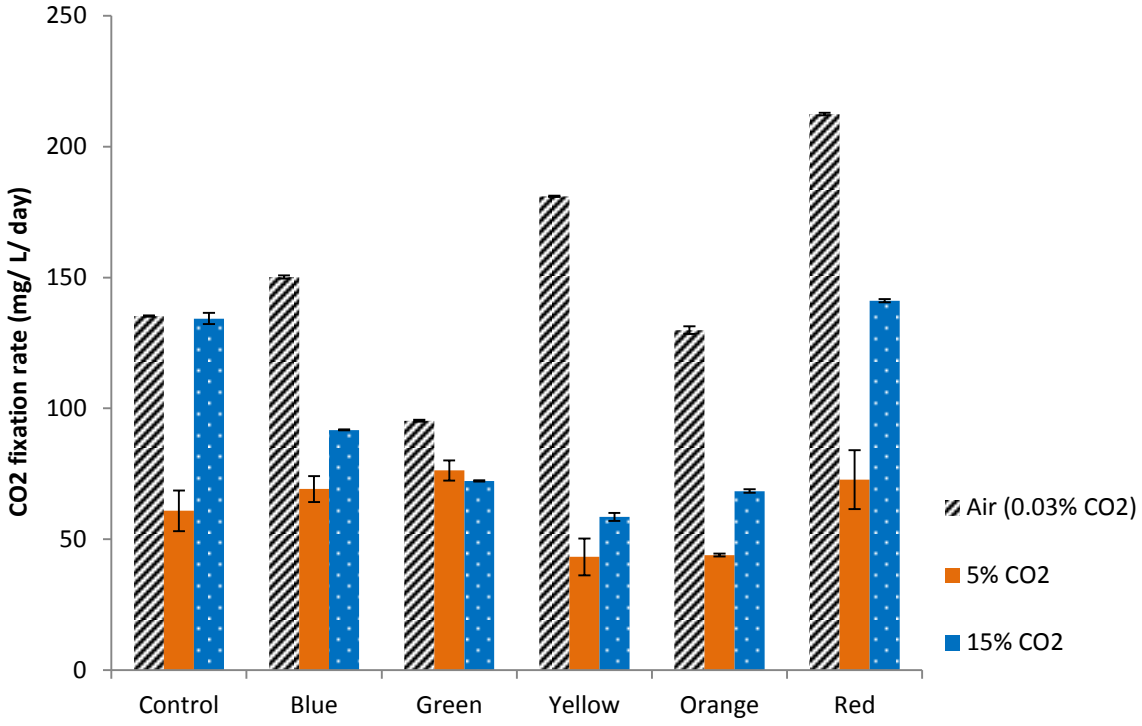

Figure 6.6, Effect of various CO_2 supply conditions on CO_2 fixation rate of *G.membrancea* in six different luminescent photobioreactors.

6.4 Effect of aeration with CO_2 enriched air on total lipids

6.4.1 *Chlorella vulgaris*

Lipid production varied greatly at different CO_2 concentrations. When 5% CO_2 was used, the lipid content of *C.vulgaris* reduced compared to the pure air condition with the exception of the control and blue PBRs. Increasing CO_2 to 15% improved lipid accumulation, particularly in the control and blue PBRs (see Figure 6.7).

The blue PBR induced the highest lipid content of 25.6% at 15% CO_2, which was three times greater than 5% CO_2 aeration. However, in relation to the level of lipid accumulation at 5% and 15% CO_2, pure air favoured lipid production in the green, yellow, orange and red (longer wavelength ranges) PBRs.

It must be noted that the response to extreme conditions such as this was observed more in the blue PBR, which supports the findings discussed previously. The elemental analysis results indicated that the nitrogen content in the blue PBR increased to 6.92% at high concentrations of carbon dioxide. Similarly, lower carbon content signalled the consumption of carbon in extreme conditions.

Roscher et al. (1986) suggested that the activity of some enzymes responsible for carbon metabolism and lipid accumulation is controled blue light. This hypothesis was supported by a study in which the effect of blue and red light on lipid content of *Chlorella.sp* was investigated (Perez-Pazoz and Fernandaz-Izquierdo, 2011). The study concluded that the concentration of lipids and fatty acids can be manipulated by light wavelengths, as the lipid profile achieved under blue light was different from that obtained with red light.

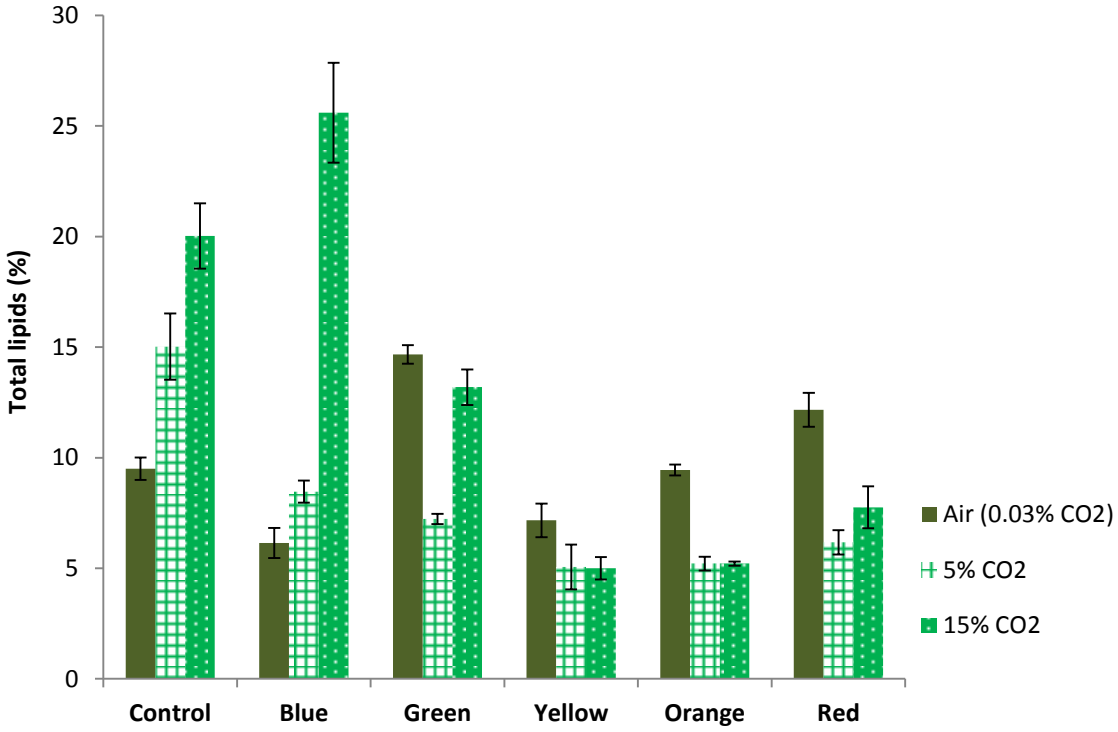

Figure 6.7, Effect of various CO_2 supply conditions on total lipid content of *C.vulgaris* in six different luminescent photobioreactors.

6.4.2 *Gloeothece membranacea*

The lipid data obtained from *G.membranacea* exhibited a clear trend at various aeration conditions. Increasing carbon dioxide concentrations enhanced lipid accumulation. Aeration with 15% CO_2 enhanced lipid production at least twice the amount produced at 5% CO_2 in all photobioreactors. It was clear that aeration with carbon dioxide had a significant and direct influence on lipid accumulation in *G.membranacea*. For instance, the most significant difference was observed in the yellow PBR in which lipid content was exponentially enhanced up to six times when 15% CO_2 aeration was applied. The highest lipid content of 36% (total lipids per gram of biomass) was achieved in the blue PBR at 15% CO_2. The lowest lipid accumulation was observed in the orange and red PBRs with longer wavelengths. However, there are various factors affecting the elemental content and lipids in microalgae, which make it difficult to predict the direct effect of a particular wavelength range. Furthermore, the lipid data supports the results of elemental analysis, showing that more carbon was consumed and more nitrogen stored when cultures were aerated with 15% CO_2.

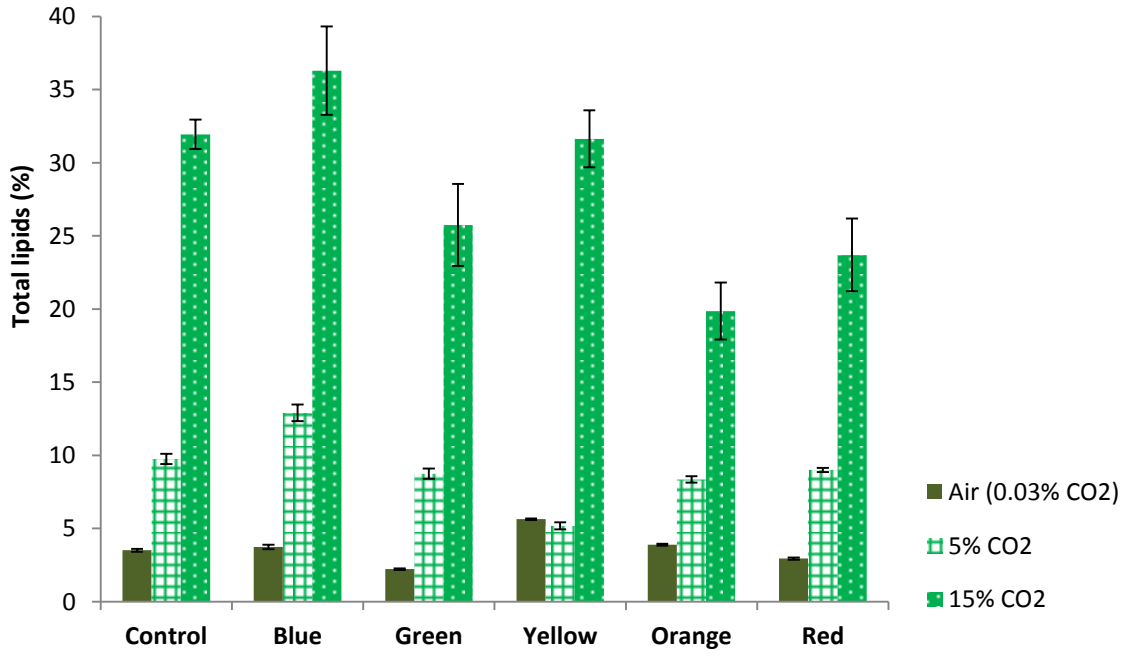

Figure 6.8, Effect of various CO_2 supply conditions on total lipid content of *G.membranacea* in six different luminescent photobioreactors.

6.5 Effect of aeration with CO_2 enriched air on pH

In the cultivation of microalgae, the impact of the pH on all aspects of media biochemistry is considerable. Therefore, maintenance of the pH in an optimum range is essential. Metabolic biochemistry of the cell and ionic absorption of the media depend strongly on the pH. The results presented in this section illustrate the pH variations during the cultivation period as an indication of the culture dynamics. As microalgae grow and produce a basic environment in the culture, the pH increases naturally. However, addition of carbon dioxide and the growth dynamics in different luminescent photobioreactors can affect the pH of the culture.

6.5.1 *Chlorella vulgaris*

Figure 6.9 shows the pH variation in *C.vulgaris* at different aeration conditions. The pH of *C.vulgaris* cultures increased rapidly after the first day of cultivation when aerated with pure air. Typically, in microalgae cultures the pH increases rapidly during the exponential growth phase. The rise in pH of up to 24% was more pronounced in the red and green PBRs, whilst it increased slowly in the blue PBR, with only 2% increase in the second day. Overall, the pH levels increased up to 11 in the active phase of growth and slightly decreased towards the end of the cultivation period.

When 5% CO_2 was used in the air supply, the pH of all the cultures remained almost neutral for two days and increased slightly afterwards. The pH levels remained constant with minor variations between 7 and 7.7 towards the end of the cultivation and decreased slightly on the last day. The minor variations in the pH showed that the growth was maintained by the cells although carbon dioxide was injected into the medium. In other words, the neutral pH ranges at 5% CO_2 indicated a survival phase at the threshold in which carbon dioxide was tolerated by *C.vulgaris*.

However, when cultures were aerated with 15% CO_2 the pH levels dropped to 6 at the first day of cultivation. Using higher concentrations of carbon dioxide can reduce the pH by converting the unutilised CO_2 to H_2CO_3 in the culture media (Widjaja et al., 2009). The pH of the media is a major determinant of the availability of carbon for microalgae photosynthesis.

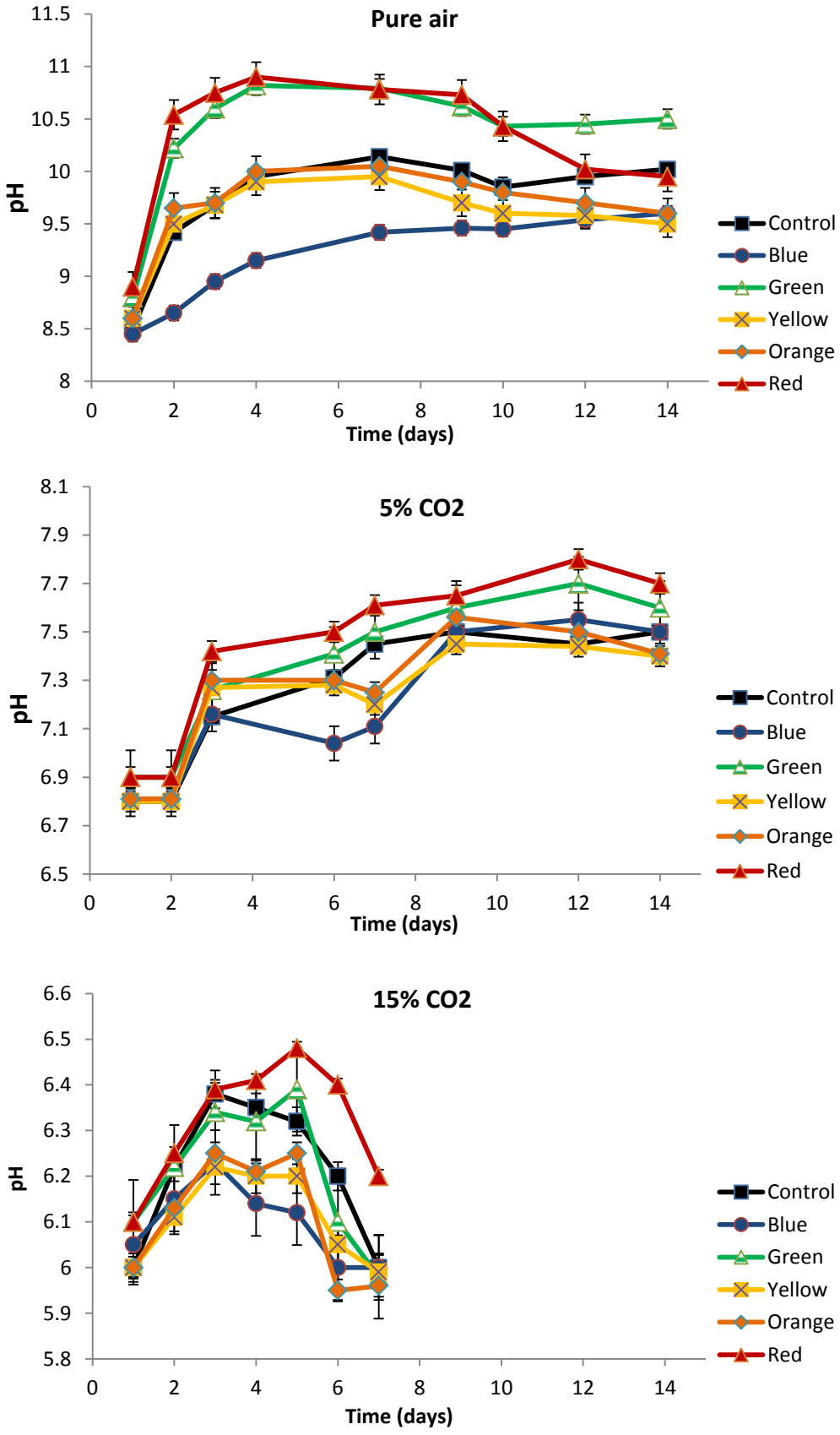

Figure 6.9, Effect of various CO_2 supply conditions on pH of *C.vulgaris* in six different luminescent photobioreactors.

The use of high CO_2 concentrations can also reduce the pH in the media and can decrease the activity of extracellular enzymes such as carbonic anhydrase (Tang et al., 2011). Reduced levels of these enzymes decrease the photosynthetic activity and thus inhibit the growth.

Due to the exponential cell growth, there was a minor increase in pH levels for 4 days. The highest pH obtained was 6.5 in the red PBR. The pH levels reduced when growth was inhibited by high concentrations of carbon dioxide after the fifth day. Eventually the media became acidic and no growth was observed.

Overall, the pH variations indicated the optimum levels in which growth occurs. The most significant exponential growth occurred between day 2 and 6 at 5% CO_2 aeration in which the pH was within neutral ranges.

6.5.2 *Gloeothece membranacea*

Figure 6.10 shows the effect of various CO_2 supply conditions on the pH of *G.membranacea* in six different luminescent photobioreactors. The pH ranges in *G.membranacea* were similar to *C.vulgaris* when aerated with pure air. The highest pH was observed in the red PBR where the highest biomass was produced. The pH rise was associated with the growth.

When aerated with 5% CO_2 the pH remained in neutral ranges for three days after the cultivation started and rose to around 9 in the yellow, orange, and red PBRs. On the other hand, the pH was relatively lower in the control, blue, and green PBRs, and it only increased slightly to 8 for the rest of the cultivation period. In addition, changes of pH from 7.5 to 9.5 showed the tolerance of *G.membranacea* to 5% CO_2 compared to *C.vulgaris*.

Increasing the carbon dioxide concentration to 15% reduced the pH of the media considerably. The lowest pH observed at this condition was around 5.5, which gave more acidic characteristics to the cultures. However, the growth was maintained even in acidic conditions and *G.membranacea* recovered the pH levels up to 6.5. Overall, the data obtained seems to indicate the higher tolerance of *G.membranacea* to lower pH levels in comparison with *C.vulgaris*. Therefore, for applications in which more acidic media is applied, the cyanobacterial strain seemed to be a more suitable option.

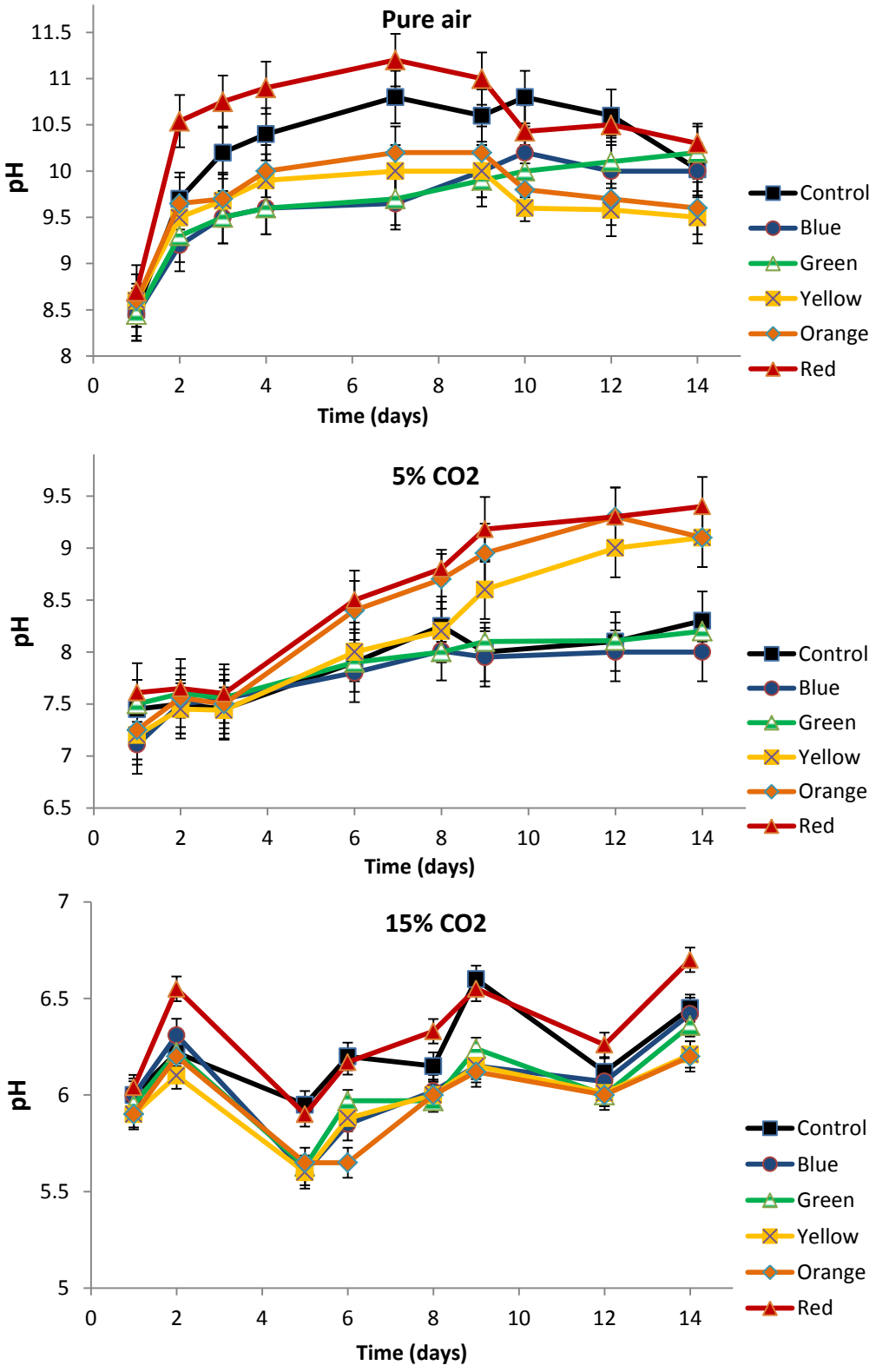

Figure 6.10, Effect of various CO_2 supply conditions on pH of *G.membranacea* in six different luminescent photobioreactors.

6.6 Conclusions

This chapter examined the influence of CO_2 aeration on biomass production, carbon dioxide fixation rate, cell's essential elements (carbon, nitrogen, and hydrogen) and lipid content as well as the pH variations in the culture media. The biomass productivity may increase with elevation of CO_2% (v/v) in the gas mixture up to a certain level and identification of this level depended prominently on different species.

Biomass production of *C.vulgaris* at exponential growth phase was enhanced with 5% CO_2 aeration compared to pure air at the control condition. Red and green light enhanced biomass production. However, after five days growth was limited and cells remained the stationary phase for a week until growth was inhibited and cell loss occurred. The growth profiles with 15% CO_2 aeration were similar to 5% although the growth was inhibited five days after cultivation. Overall, there was no substantial difference in the CO_2 fixation rate in all photobioreactors when aerated with pure air comprising 0.03% CO_2 (v/v). However, in all luminescent photobioreactors with the exception of the blue PBR, increasing the concentration of CO_2 in the gas mixture had significant impacts on CO_2 sequestration. Different aeration conditions led to some changes in the pH media of *C.vulgaris*. The culture aerated with pure air became more basic as a result of cell growth and natural changes in the ionic interactions in the media. Using 5% CO_2 maintained the pH in neutral ranges, particularly in the active growth phase. However, 15% CO_2 made the culture too acidic which consequently influenced the growth inhibition.

The effect of 5% CO_2 aeration on biomass production of *G.membranacea* was significantly different compared to *C.vulgaris*. The biomass profiles under all light conditions showed linear growth during two weeks of cultivation. Unlike *C.vulgaris*, growth inhibition did not occur in *G.membranacea* with 15% CO_2 aeration. Cyanobacteria cells showed significant tolerance to higher carbon dioxide concentrations and maximum biomass production of 2.25 g L^{-1} was achieved in the control photobioreactor. However, due to their lower carbon content and biomass productivity, *G.membranacea* was less efficient in sequestrating carbon dioxide. The lipid data obtained from *G.membranacea* exhibited a clear trend at various aeration conditions. Increasing carbon dioxide concentrations enhanced lipid accumulation. Aeration with 15% CO_2 enhanced lipid production at least twice the amount produced at 5% CO_2 in all photobioreactors. When pure air was used, the pH variation was similar

to *C.vulgaris* cultures with a basic nature. 5% CO_2 reduced the pH to neutral ranges for the first 4 days and increased to around 9 afterwards.

Chapter 7:
Bio-pigment production in photobioreactors

7.1 Introduction to pigment categories and their function

Photosynthetic pigments are biochemical compounds responsible for absorbing specific portions of solar spectrum to perform photosynthesis. Since the pigments react with specific wavelengths of light, they appear in different colours and forms. Due to the phylo-genetic age of microalgae, they have developed various pigments that give them peculiar characteristics when dealing with different light conditions (Becker, 1994b). This chapter reviews the effect of light condition, culture mode and different aeration conditions on bio-pigments in microalgae. The chapter precisely examines these effects at specific wavelengths of light and compares the results corresponding to other growth parameters under the above conditions. The analytical methods of measuring these pigments have been described in details in Chapter 3 (materials and methods).

7.2 Chlorophylls

Chlorophylls are the most common pigments present in different forms in photosynthetic organisms. All microalgae species contain chlorophyll-a which is responsible for converting sunlight into chemical energy. Other pigments in various concentrations absorb light at specific wavelengths and transfer the absorbed photons to chlorophyll-a molecules for photosynthesis. The function of these pigments and their portions in selected microalgae species is an indication of their tolerance to different environmental conditions and sensitivity to the quality of light.

Both microalgae strains used in this study contained chlorophylls. *C.vulgaris* contains chlorophyll a and b but the cyanobacterial strain *G.membranacea* comprises only the former. The following sections demonstrate the effect of various culture conditions on the chlorophyll content of microalgae.

7.2.1 Effect of culture density

This section investigates the effect of initial culture density on bio-pigment production in microalgae.

7.2.1.1 *Chlorella vulgaris*

Overall, the average chlorophyll contents (percentage of chlorophylls per gram of dry biomass) in all luminescent photobioreactors were similar. Chlorophyll concentrations were more variable in low density cultures, supporting the conclusions drawn from the biomass production data (Chapter 5) regarding the more pronounced effects of light quality where lower cell densities minimise masking effects.

Figures 7.1 and 7.2 that illustrate within high density cultures, chlorophyll contents showed reduced variation in different luminescent photobioreactors. Total chlorophylls in low density cultures increased at the second day of cultivation when the exponential phase occurred. The highest chlorophyll content produced in the green PBR was 1.94%, with a corresponding biomass density of 0.35 g L^{-1}. It seemed the chlorophyll content in the green PBR was more influenced by the initial culture density, although the overall effect of culture density on average chlorophyll production was minimal.

It must be noted that the red PBR also induced pigmentation in *C.vulgaris*, but relative to its biomass production this effect was insignificant. For instance, the highest chlorophyll content observed in red PBR was 1.07% corresponding to the chlorophyll density of 16 mg L^{-1} which was the maximum value obtained in all photobioreactors.

Similarly, in high density cultures the values obtained for chlorophyll density were twice as much of low density cultures, but the portions of pigments per gram of dry biomass were relatively lower than those in low density cultures. For instance, green light enhanced total chlorophyll production up to a density of 16.26 mg L^{-1}, corresponding to only 0.99% of biomass in high density cultures.

One assumption is that green light may provide a condition in which photosynthetic organisms produce more pigments as a compensation for poor growth conditions. This hypothesis is supported by evidence from some studies which reported that green light is considered to be a wavelength portion of PAR which has limited use in photosynthesis and poorly supports the growth (Johkan et al., 2011). An investigation on the effect of light quality on the accumulation of photosynthetic pigments in the red algae *P.leucosticta* (Rhodophyta) reported that the highest chlorophyll-a content was

obtained under green and white light (Korbee et al., 2005). Studies on chlorophyll a and b and a variety of carotenoids have reported that the percentage absorption of red or blue light by photosynthetic organisms is about 90%, whilst that of green light is about 70-80% (Johkan et al., 2011, Terashima et al., 2009). In a study on sunflower leaves, Terashima et al. (2009) reported that compared to red light, the combination of green and white light can drive photosynthesis more efficiently. Another piece of study indicated that green light can penetrate into the plant canopy more effectively and as a result the addition of green light to red and blue light promotes the growth (Kim et al., 2004).

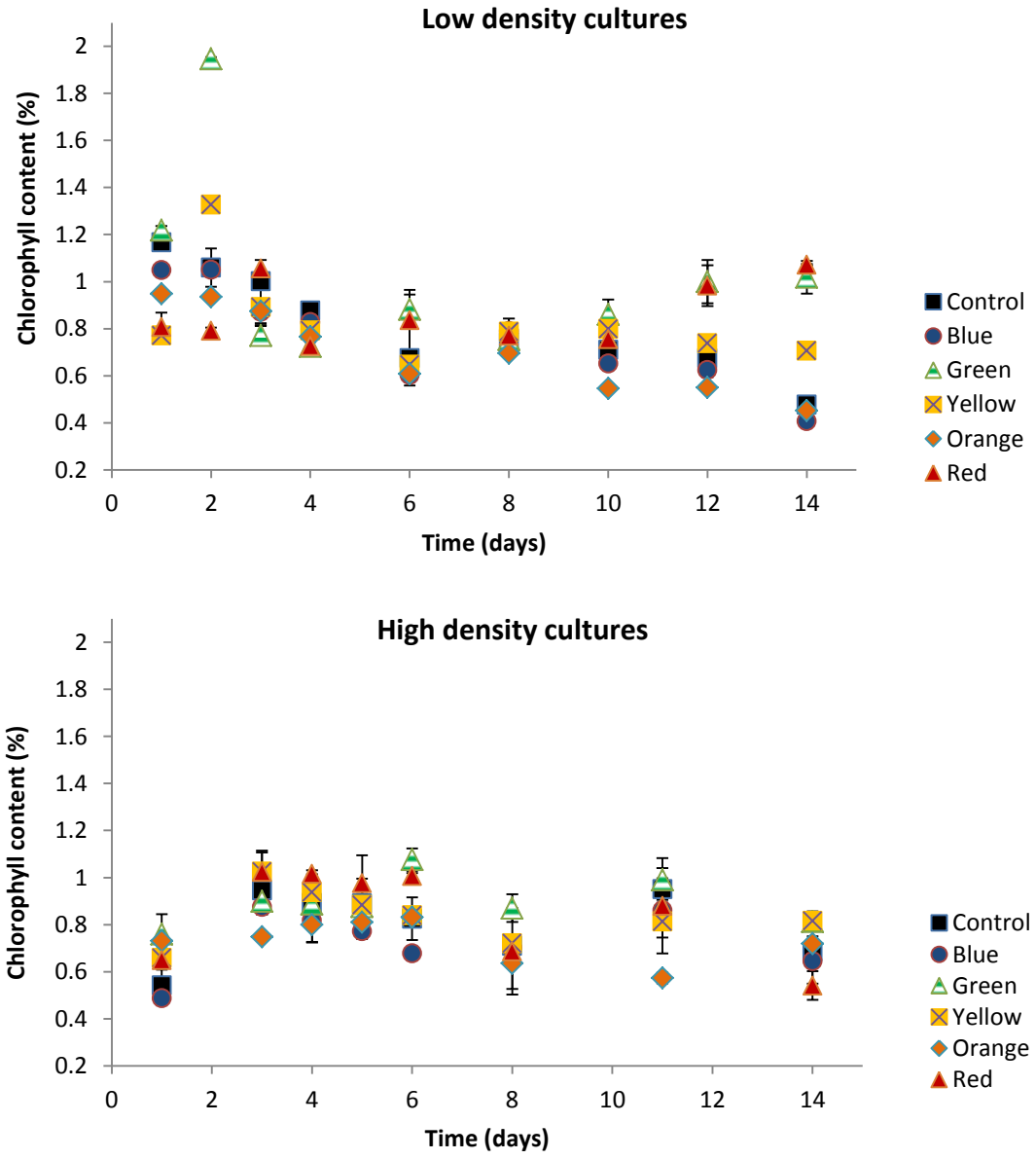

Figure 7.1, Effect of culture density on total chlorophyll content of *C.vulgaris* over a 14 days period in luminescent photobioreactors.

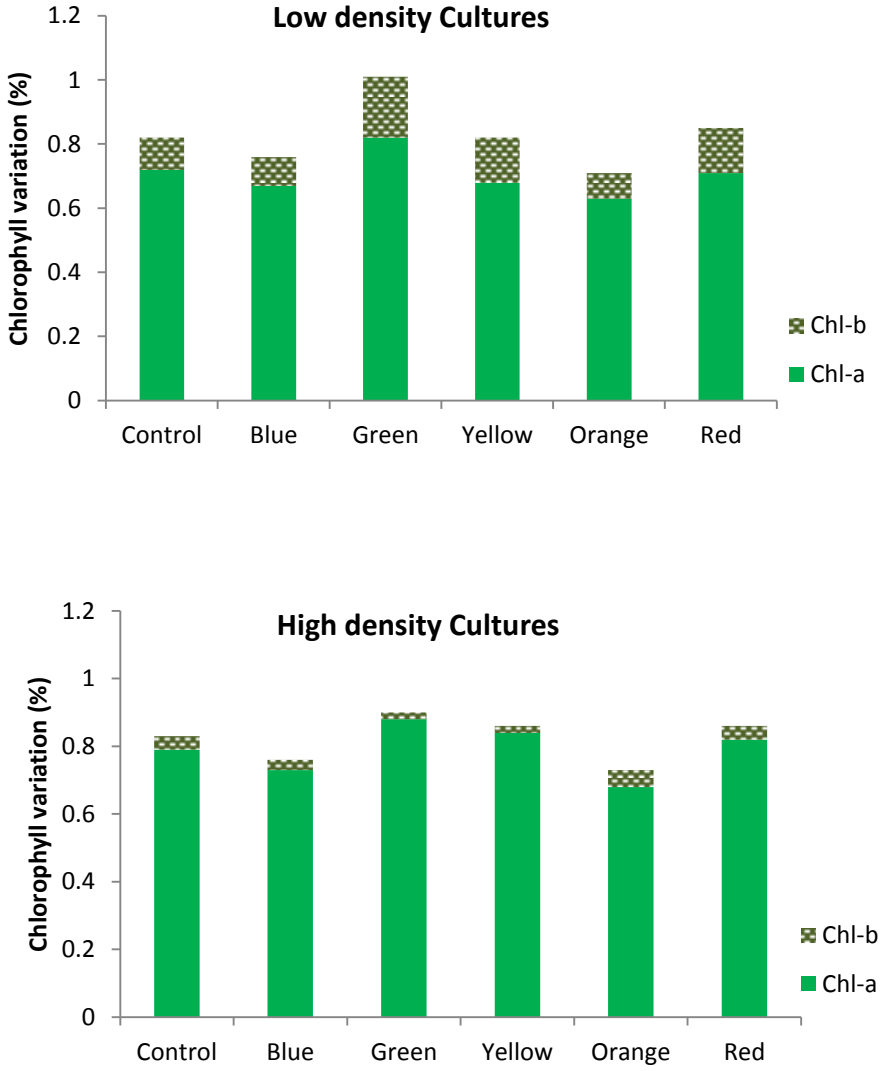

Figure 7.2, Chlorophyll a and b variation in *C.vulgaris* in different culture densities. Values represent average chl % (g chl/g biomass) over a 14 days period in luminescent photobioreactors.

7.2.1.2 *Gloeothece membranacea*

Profiles of chlorophyll content of *G.membranacea* followed two different trends through the cultivation period. In low density cultures, the chlorophyll content was high at the beginning of the cultivation and slightly declined until the end of two weeks, whilst in high density cultures an opposite trend was observed (see Figure 7.3). The different pigmentation patterns can be justified by the culture condition. The exponential growth in low density cultures occurred at the first day of cultivation and

pigment production was induced whilst it slightly decreased when biomass production was supported and further pigmentation was unnecessary.

In contrast, in high density cultures with increasing cell population and cell shadowing effects, the competition to drive photosynthesis induced further pigmentation. Red light enhanced the pigmentation with the highest chlorophyll content of 0.75% corresponding to a chlorophyll density of 17 mg L^{-1}. Red light was the most effective portion of the spectrum to induce chlorophyll production in *G.membranacea*. In general, chlorophyll production in all photobioreactors followed the same pattern in each culture density group.

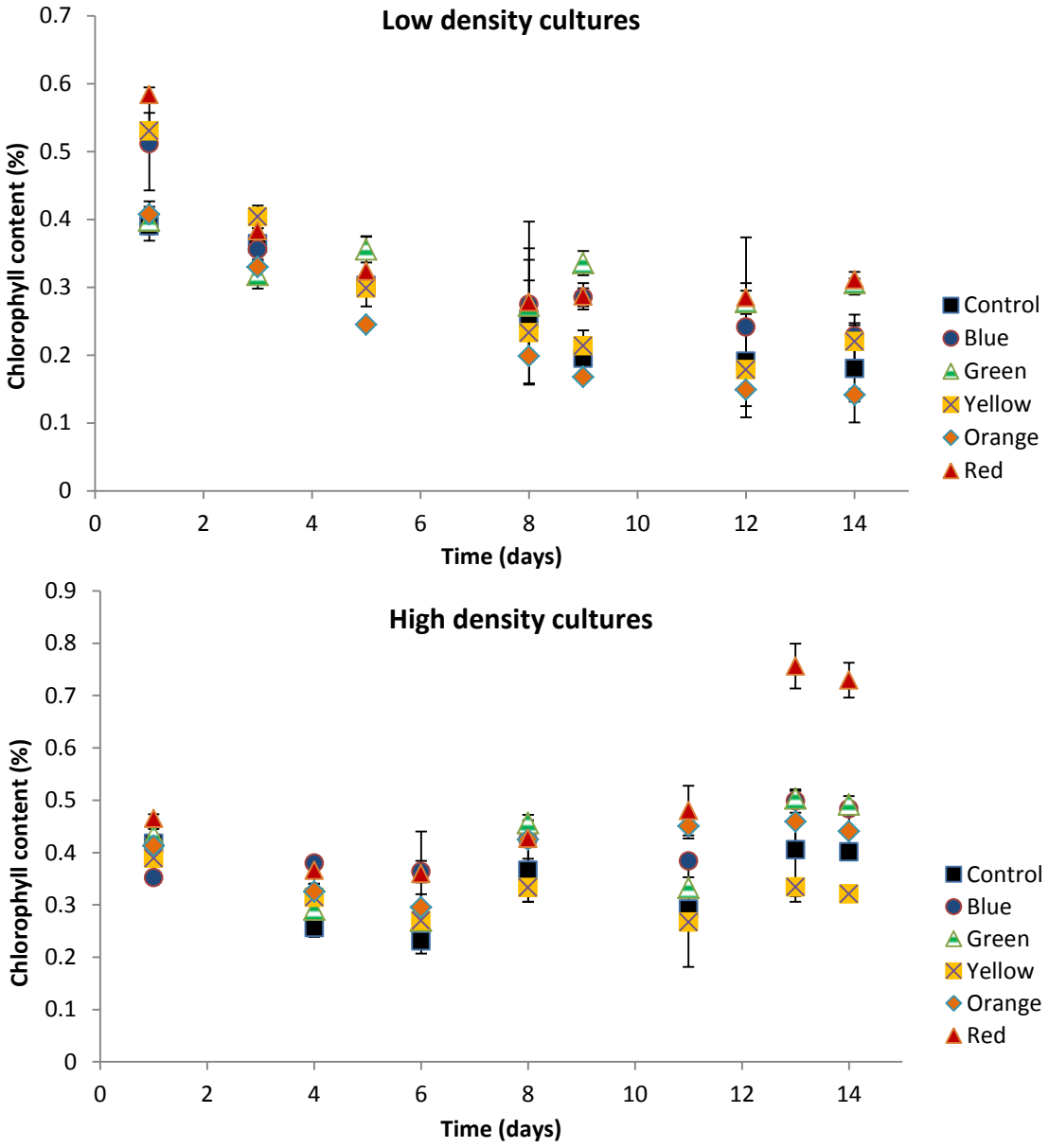

Figure 7.3, Effect of culture density on total chlorophyll content (%) of *G.membranacea* over a 14 days period in luminescent photobioreactors.

However, the average values obtained showed that the chlorophyll content of *G.membranacea* was considerably lower than *C.vulgaris* and there was no significant difference in average pigmentation between the two culture density groups (see Figure 7.4). Whilst a maximum chlorophyll content of 1.94% was obtained in the green PBR in *C.vulgaris*, it was only 0.50% under the same light condition in the *G.membranacea* culture. Therefore, the chlorophyll content can vary depending on the culture condition as chlorophylls adjust the microalgae capability to absorb as much light as possible.

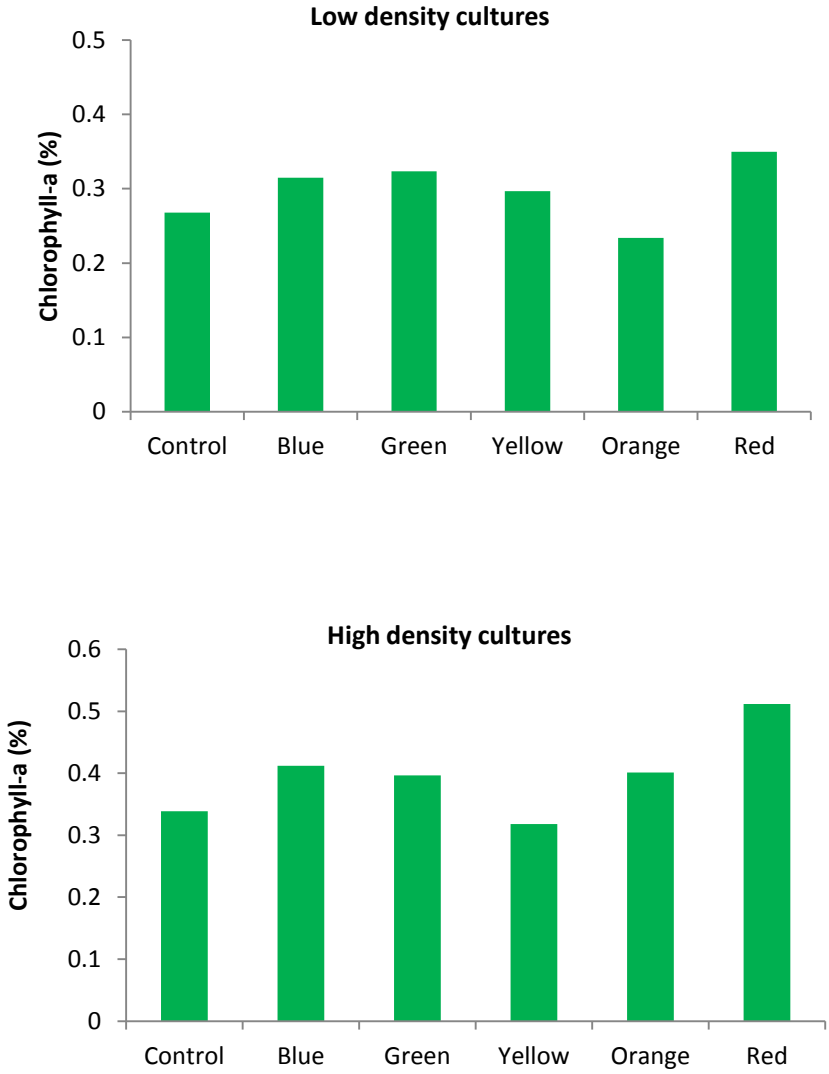

Figure 7.4, Chlorophyll-a variation in *G.membranacea* in different culture densities. Values represent average chl % (g chl/g biomass) over a 14 days period in luminescent photobioreactors.

7.2.2 *Effect of light intensity*

The quality of light in terms of intensity and its effect on biomass production was previously described in Chapter 5. Different culture densities had some influences on growth patterns and the effect of light availability became an important factor. In this section, the optimum density of seed cultures for growth was chosen to examine the effect of light intensity. Light intensity defined and measured as photon flux indicates the performance of each luminescent photobioreactor in transferring light at a given wavelength range.

7.2.2.1 *Chlorella vulgaris*

Increasing the light intensity from low to medium levels enhanced the total chlorophyll content of *C.vulgaris* in all photobioreactors. At low light intensity, the highest chlorophyll content of 0.70% was obtained in the green PBR whilst increasing the intensity to a medium level enhanced chlorophyll production in the blue PBR. At medium intensity, profiles of chlorophyll content were almost constant during the two weeks period, with the exception of day 4 in which the blue PBR induced a maximum chlorophyll content of 1.33% (see Figure 7.5).

There are two reasons behind these different patterns of pigmentation. Firstly, lower numbers of photons were emitted to the cultures at the low intensity, and after a week of cultivation when microalgae cells have passed the active phase of growth and the cell population has increased, they start to shadow each other. Secondly, the shadowing effect is common in all microalgae cultures, although when photon flux density is lower, this effect can be more pronounced. In addition, it was previously demonstrated that increasing the culture density could cause the shadowing effect and the way in which light penetrates into the culture.

On the other hand, there was no significant change on the average chlorophyll content by elevation of light intensity from medium to high, although the pigmentation profiles under the high light intensity were more variable (see Figure 7.6). The yellow PBR favoured chlorophyll production at this condition and it produced the maximum chlorophylls between the other photobioreactors. The highest chlorophyll content of 1.20% in the yellow PBR decreased to a third of this amount by the end of the cultivation period.

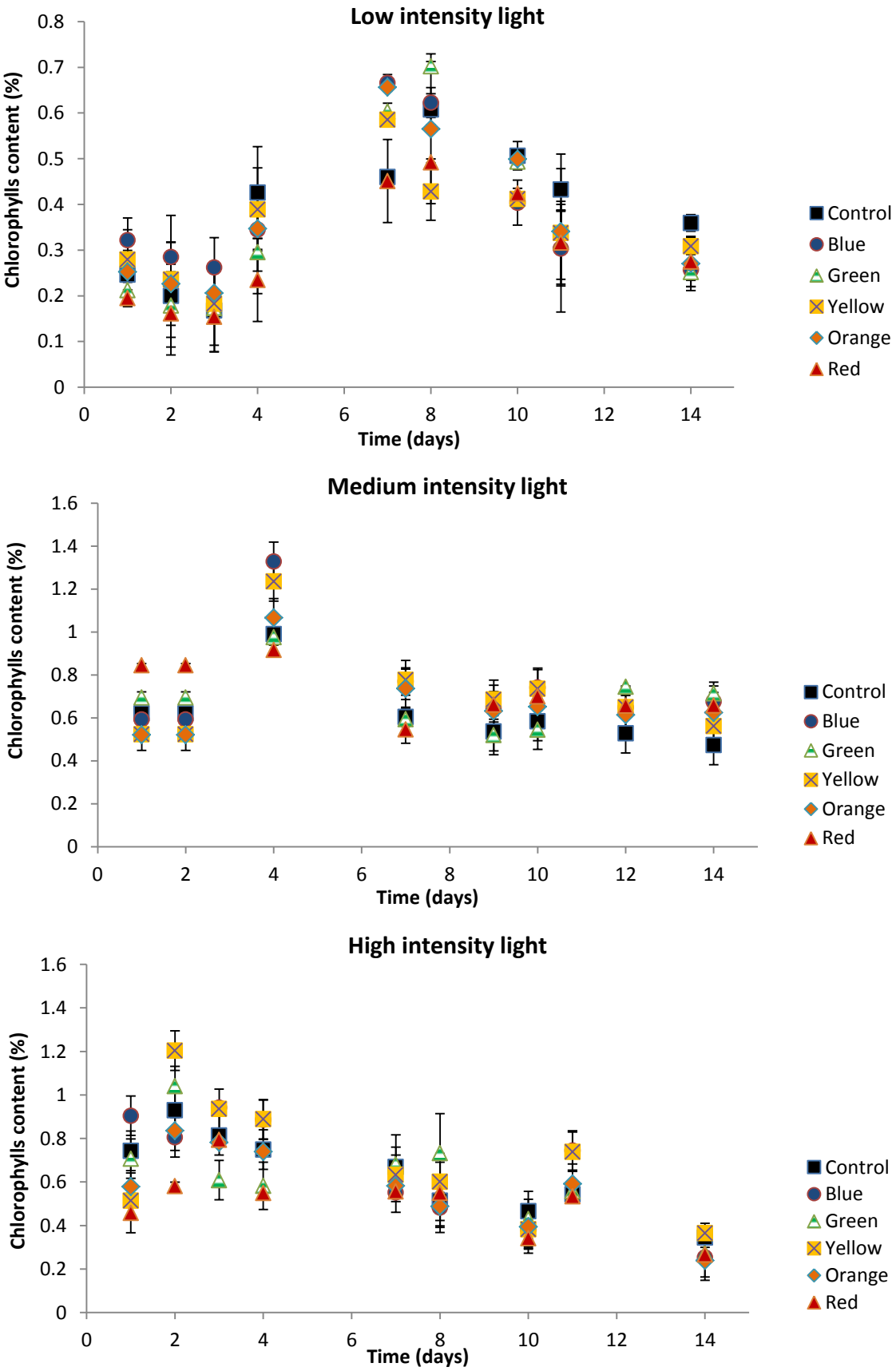

Figure 7.5, Effect of light intensity on total chlorophyll content of *C.vulgaris* over a 14 days period in luminescent photobioreactors.

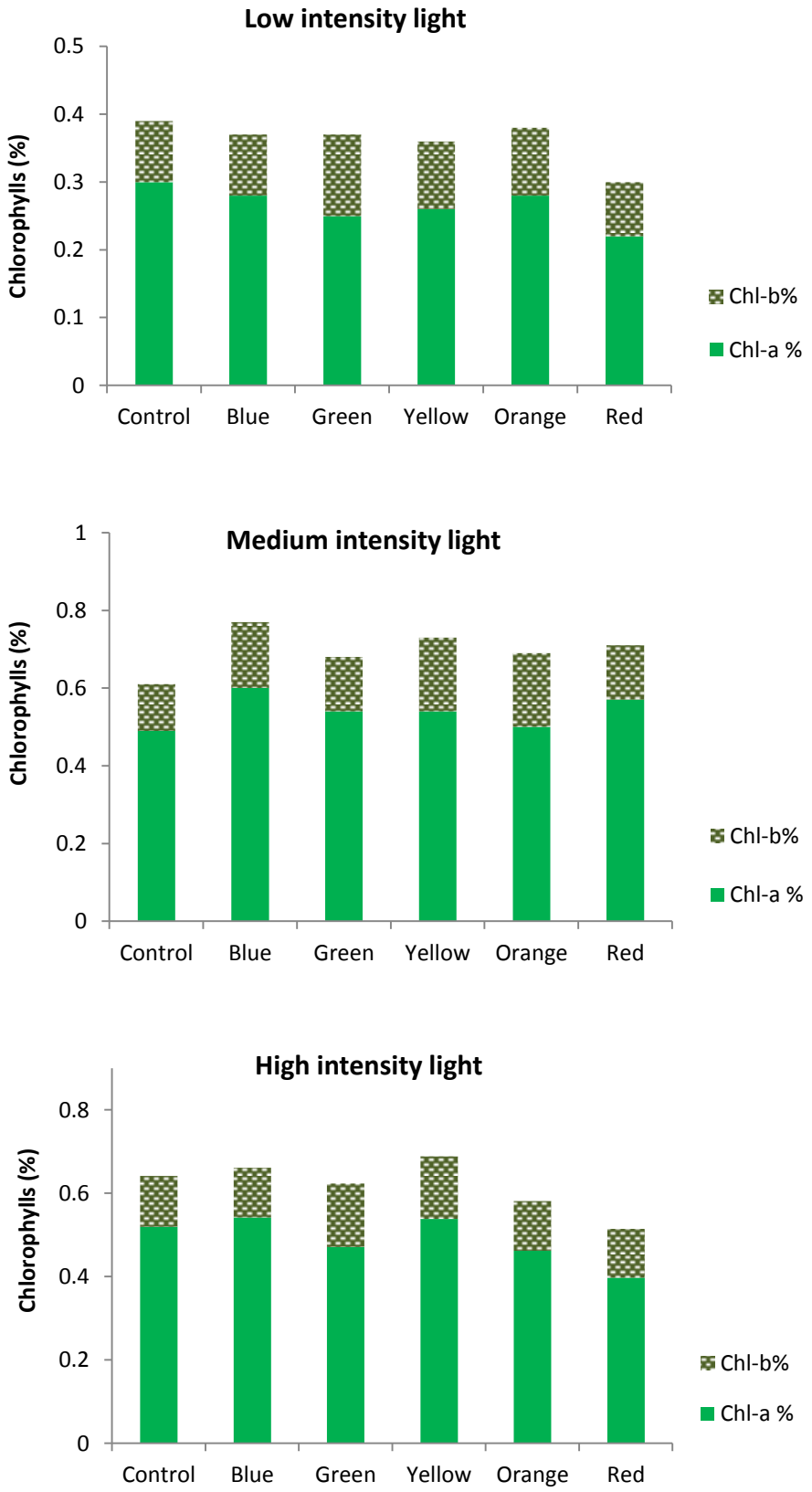

Figure 7.6, Effect of light intensity on chlorophyll a and b content (%) of *C.vulgaris* over a 14 days period in luminescent photobioreactors.

Overall, chlorophyll a and b content of cells varied in different luminescent photobioreactors. However, the highest pigment contents (g chl/ g biomass %) were obtained at medium intensity levels. The highest percentages of chlorophylls at low, medium and high light intensity conditions were achieved in the green, blue and yellow PBRs respectively.

7.2.2.2 *Gloeothece membranacea*

In general, the elevation of light intensity decreased the chlorophyll production in *G.membranacea*. The results showed that in all luminescent PBRs the lowest light intensity of 50 μmol photons m^{-2} s^{-1} produced the highest chlorophyll levels (see Figure 7.7). In addition, various wavelength ranges exhibited different responses to intensity alterations. Whilst the blue and green PBRs favoured pigmentation at low light intensity, the red PBR produced 0.43% chlorophyll-a at medium intensity levels and the yellow PBR performed best at a high light intensity condition. At the lowest light intensity, the blue and green PBRs induced the highest average chlorophyll content of 0.51%. However, this value dropped to 0.25% at the intensity of 100 μmol photons m^{-2} s^{-1} and 0.18% at the intensity of 250 μmol photons m^{-2} s^{-1}, making the blue light the least efficient at the highest intensity after the control condition (see Figure 7.8).

The results can be justified by the fact that at low light conditions, microalgae cells produce more pigments to compensate for the extreme conditions. Similar to high cell density cultures of cyanobacteria, the levels of pigmentation were higher at the lowest light intensity. It is commonly accepted that reducing irradiance intensity can enhance photosynthetic pigmentation, mainly chlorophyll production, as the cell's response to facilitate increased light harvesting (Givnish, 1988).

In a study on the effect of low light intensity and shading on *Euglena gracilis*, it was reported that increasing light intensity reduced the chlorophyll-a content (Beneragama and Goto, 2011). The study showed that at the light intensity of 28 μmol photons m^{-2} s^{-1} chlorophyll-a content of unicellular *Euglena* (at the exponential phase) was more than twice that of the same parameter obtained at 210 μmol photons m^{-2} s^{-1}. However, at the stationary phase only 53% decrease in chlorophyll-a content was observed. The results support the previous studies found in the literature, which focused on the effect of light intensity at a given wavelength on photon absorption by photosynthetic organisms.

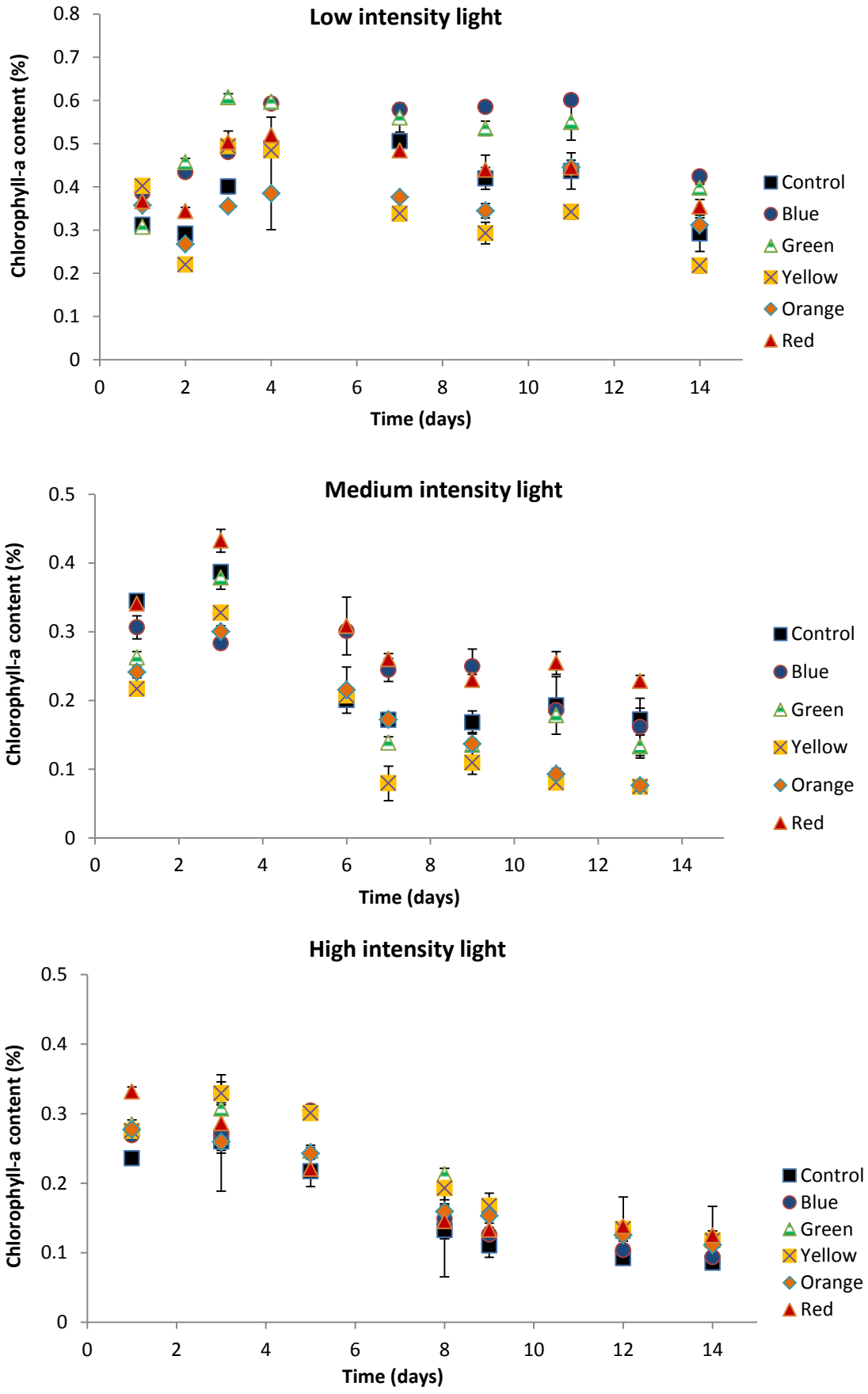

Figure 7.7, Effect of light intensity on total chlorophyll density of *G.membranacea* over a 14 days period in luminescent photobioreactors.

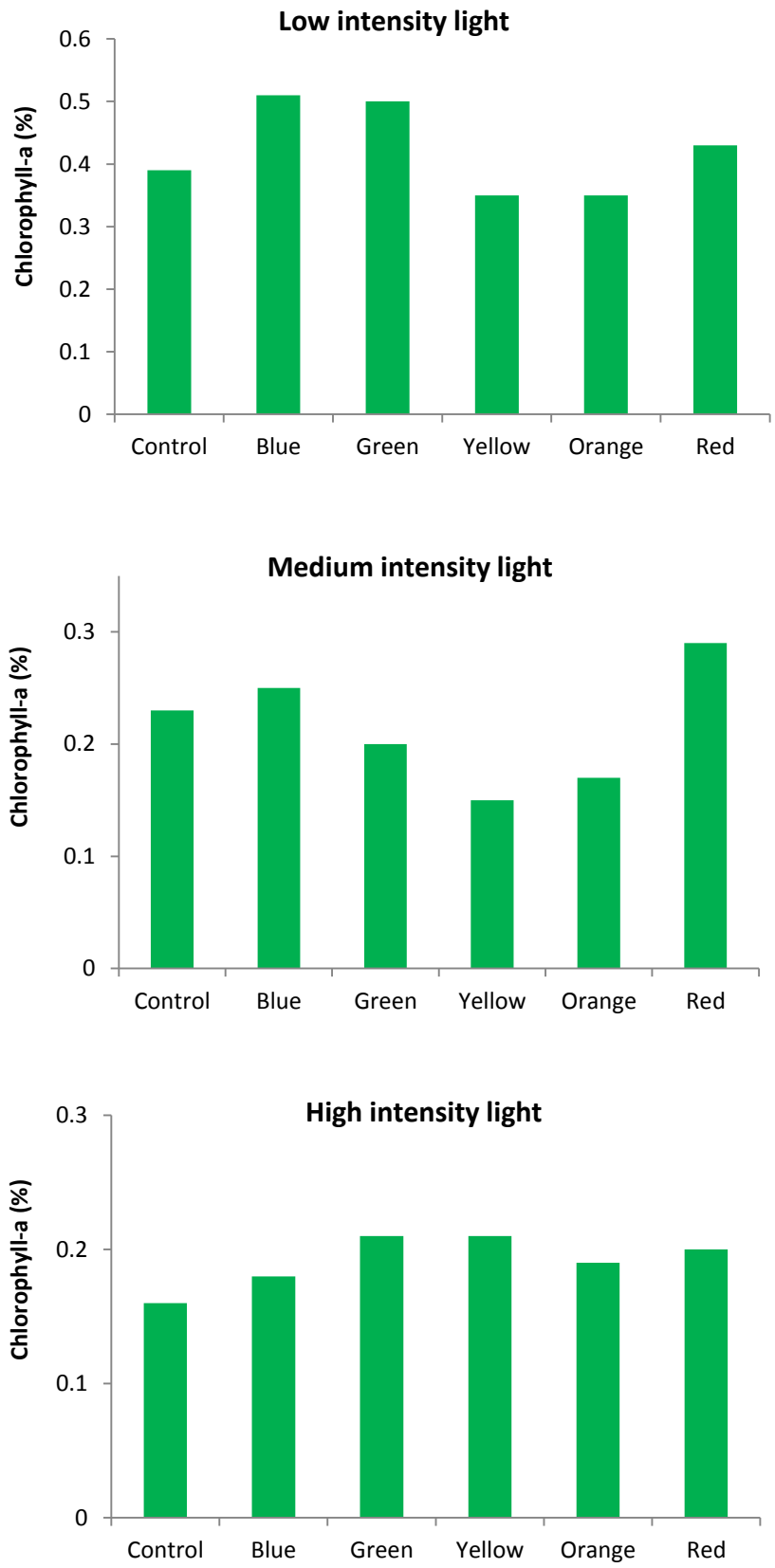

Figure 7.8, Chlorophyll-a variation in *G.membranacea* at different light intensities. Values represent average chl % (g chl/g biomass) over a 14 days period in luminescent photobioreactors.

7.2.3 *Effect of aeration with carbon dioxide*

As mentioned in Chapter 6, different aeration conditions with carbon dioxide can have pronounced effects on cell growth as well as pigment production. The effect of CO_2 aeration on pigmentation was examined under different CO_2 concentrations in air at a constant light intensity for all experiments. The same light intensity of 250 μmol photons m^{-2} s^{-1} was used in order to obtain consistent results with the sole influence of aeration quality.

7.2.3.1 *Chlorella vulgaris*

Aeration with carbon dioxide decreased the chlorophyll content of the cells. The results obtained indicated that increasing CO_2 concentrations had an inverse effect on chlorophyll production in *C.vulgaris*. Figures 7.9 and 7.10 show the profiles of total chlorophylls content vs. time and average chlorophyll a and b portions in each gram of biomass during the cultivation period.

The percentage of total chlorophylls at 5% CO_2 aeration condition was almost double in all different luminescent photobioreactors compared to those at the 15% CO_2 aeration condition. The highest total chlorophylls contents of 0.91% and 0.85% (Figure 7.9) were obtained at the 5% CO_2 aeration condition in the green and red PBRs respectively. Although the concentration of carbon dioxide in enriched air affected chlorophylls production significantly the influence of photobioreactors between different wavelength ranges was minimal. This means that at a given aeration condition there was no significant difference in the total chlorophylls contents in different luminescent photobioreactors.

However, red light enhanced pigmentation slightly more than other light conditions, and 15% CO_2 concentrations lowered average chlorophyll contents to one third of the values obtained with pure air. The effect of carbon dioxide aeration on chlorophylls in algae and other plants have been previously investigated (Atta et al., 2013, Tang et al., 2011, Yang and Gao, 2003).

A study on the effect of carbon dioxide on cyanobacterium *Spirulina platensis* reported that increasing CO_2 concentrations decreased the production of pigments such as chlorophyll, phycocyanin, and carotenoids to around 50% (Yang and Gao, 2003). It was suggested that carbon dioxide can assist degradation of pigments which are excessively produced in normal conditions and are not solely responsible for light harvesting.

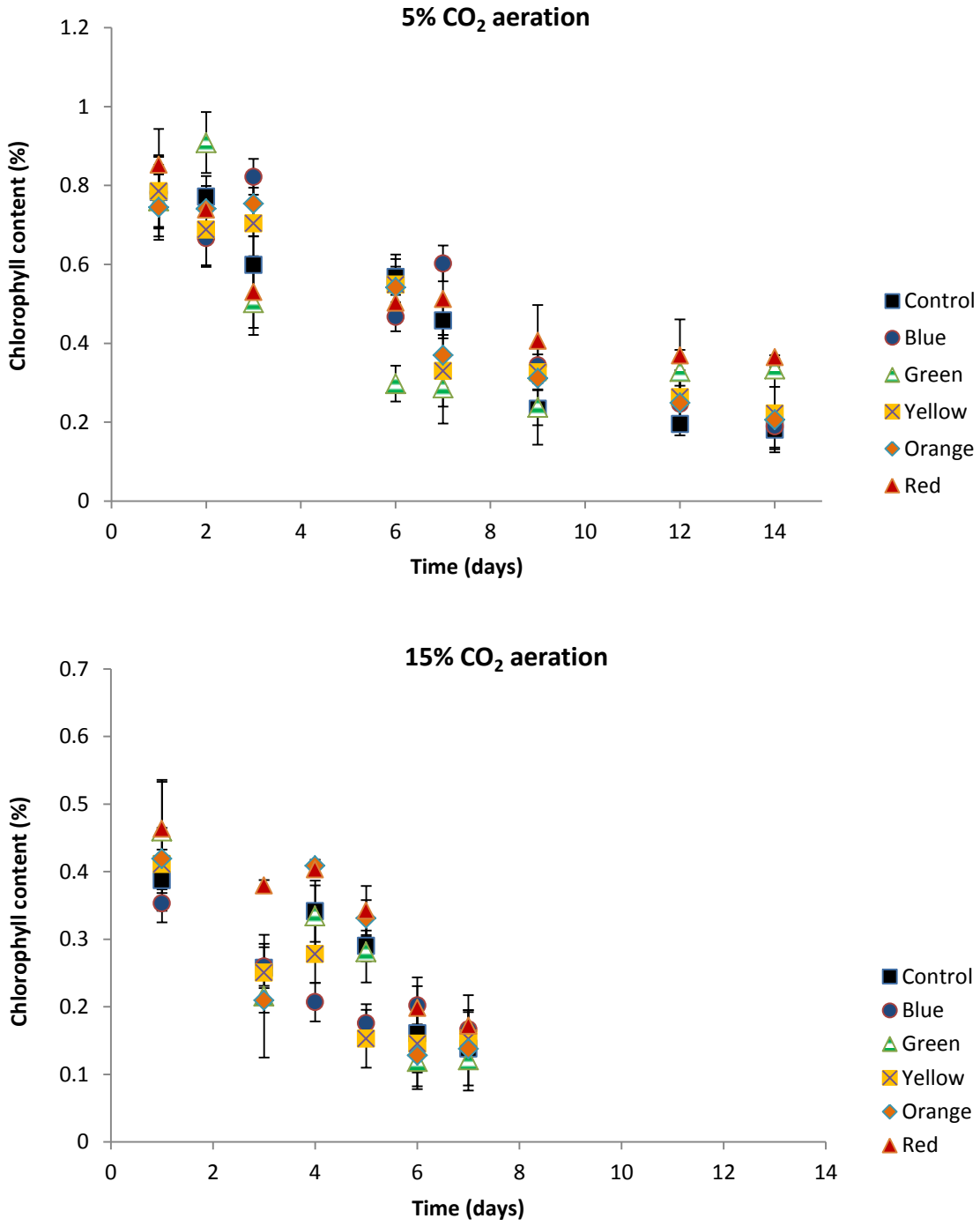

Figure 7.9, Effect of carbon dioxide aeration on total chlorophyll content of *C.vulgaris* over a 14 days period in luminescent photobioreactors.

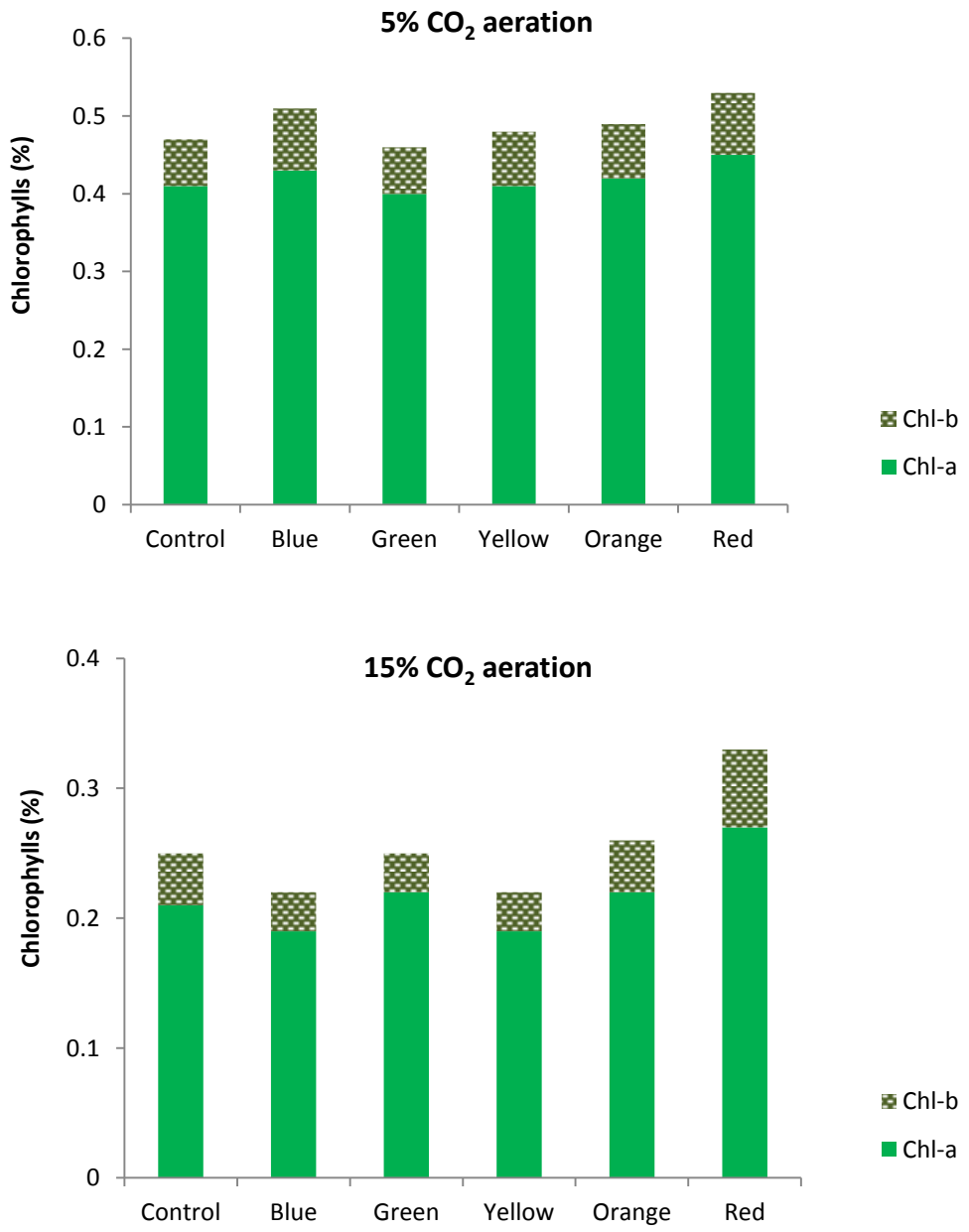

Figure 7.10, Chlorophyll a and b variation in *C.vulgaris* at different carbon dioxide aeration rates. Values represent average chl % (g chl/g biomass) over a 14 days period in luminescent photobioreactors.

7.2.3.1 *Gloeothece membranacea*

Similar trends as seen in *C.vulgaris* apply to *G.membranacea* cultures at different CO_2 aeration rates. In presence of carbon dioxide, the highest chlorophyll content (0.36%) was obtained in the green PBR. However, elevation of CO_2 concentrations led to a major decrease in chlorophyll contents of the cultures illuminated with longer

wavelengths of light. According to the results this means that average chlorophyll production under the red, orange and yellow light was halved with alteration of the CO_2 concentration, increasing from 5% to 15%. Figures 7.11 and 7.12 show the profiles of chlorophyll-a content vs. time and average chlorophyll-a portions in each gram of biomass during the cultivation period. Compared to *C.vulgaris*, where all cultures were affected, it was found that the impact of CO_2 aeration was more pronounced on longer wavelength (warmer colour) ranges in *G.membranacea*.

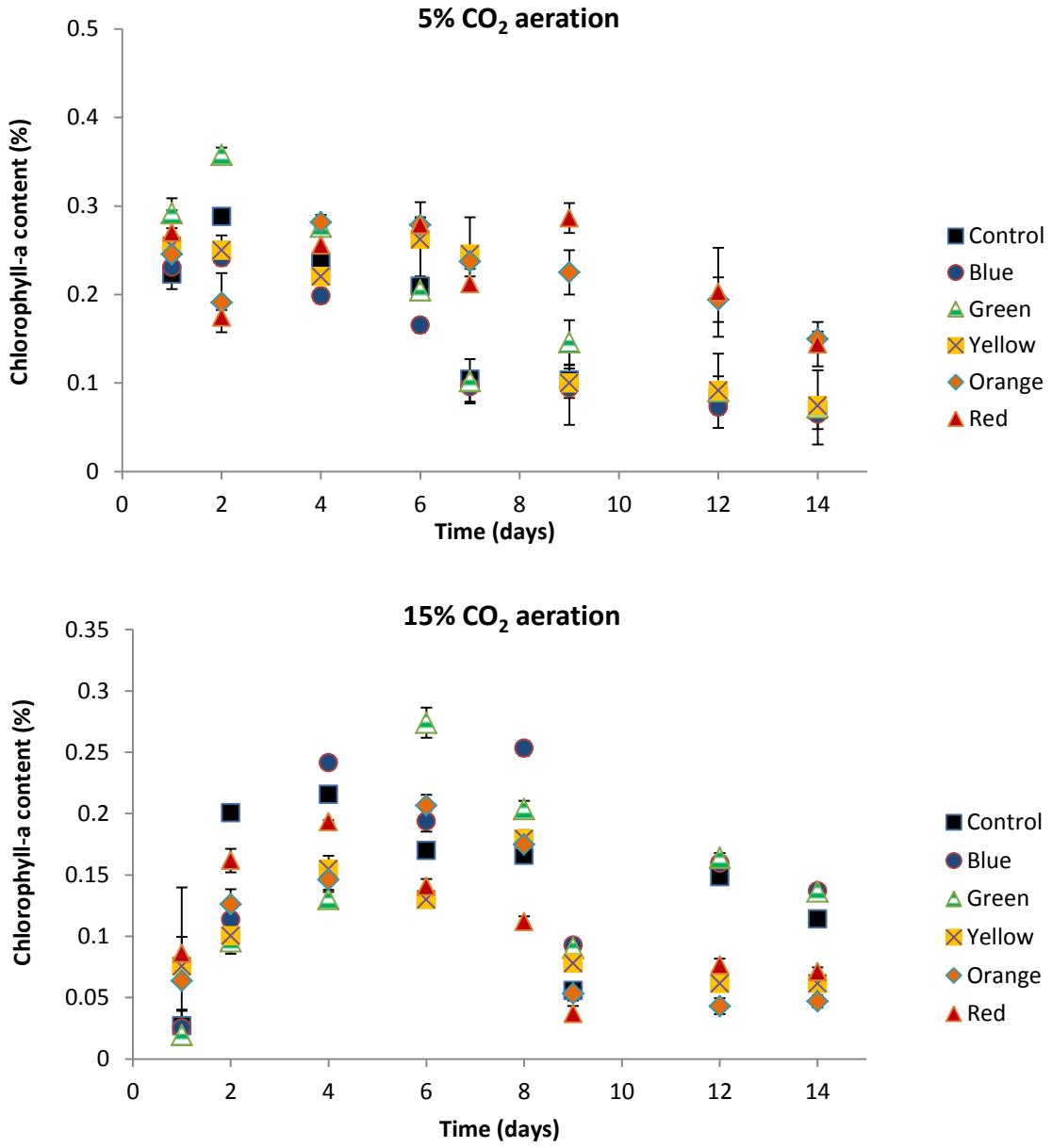

Figure 7.11, Effect of CO_2 aeration on total chlorophyll content (%) of *G.membranacea* over a 14 days period in luminescent photobioreactors.

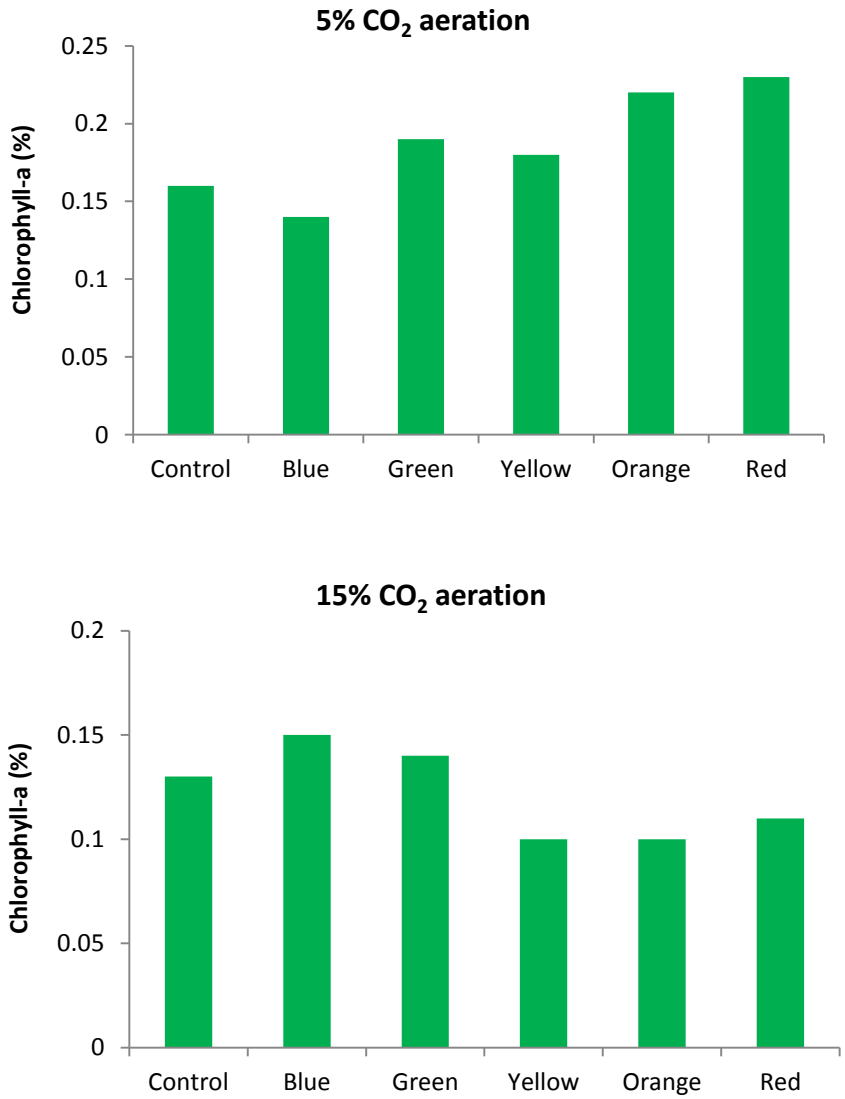

Figure 7.12, Chlorophyll-a variation in *G.membranacea* at different CO_2 aeration rates. Values represent average chl % (g chl/g biomass) over a 14 days period in luminescent photobioreactors.

7.3 Phycobiliproteins

Phycobiliproteins are deep-coloured water soluble accessory pigments with proteinaceous properties. They are the major components of a complex assemblage known as phycobilisomes. Phycobiliproteins are classified into two main groups based on their colour, the phycoerythrins (PE) and the phycocyanins (Fabregas et al.). Phycobiliproteins absorb portions of light energy in the PAR range that are poorly utilized by chlorophylls (Mishra et al., 2012, Sidler, 2004). The existence of these

pigments gives cyanobacteria their unique light harvesting capabilities. They are of major importance, as they almost fill the gap of the light energy unused by chlorophyll-a and carotenoids.

Based on their absorption spectra, three different forms of phycoerythrins can be distinguished. However, there is only one type of phycoerythrin which is available in cyanobacteria and it is knows as C-phycoerythrin (CPE). In addition, there are two types of phycocyanin present in cyanobacteria, known as C-phycocyanin (CPC) and allophycocyanin (APC). Absorption maxima for phycocyanins are between 610 and 665 nm (red region) whilst absorption maxima of phycoerythrins are between 490 and 570 nm (blue-green region).

In addition to chlorophyll content, phycobiliproteins available in cyanobacteria have been determined and discussed in the following section. Observing the changes of these accessory pigments under different culture conditions provided an insight into the chromatic adaptation ability of cyanobacteria in extreme conditions.

7.3.1 *Effect of culture density*

The determination of phycobiliproteins showed that they are the dominant bio-pigments in *G.membranacea*. Figure 7.13 shows the phycobiliproteins variation at different culture densities. In both culture density groups, the red and green PBRs enhanced the levels of phycobiliprotein production. This effect was more pronounced in low density cultures, with highest phycobiliprotein contents of 2.13% and 1.62% obtained under red and green light respectively.

The lowest levels of pigmentation were obtained under the control and blue light. It must be noted that the effect of culture density was almost insignificant on the average phycobiliproteins per gram of biomass. Similar to chlorophylls, by increasing the culture density the phycobiliproteins concentration elevated, but the proportion in relation to the biomass data remained similar.

The results achieved show that red and green light induce increased levels of pigmentation in both microalgae strains, particularly in cyanobacteria in which chlorophylls are not the dominant pigments. The optimum light absorption spectra for phycoerythrins is between 490-570 nm (representing green light) and for phycocyanins is between 610-665 nm (representing red light). These light ranges were provided by the

luminescent acrylic filters as the main body of the photobioreactors used in this study. The expression of phycobiliproteins was more influenced by changes in the wavelength of light used in the experimentations than the expression of chlorophyll in general, suggesting that cyanobacteria may be capable of more rapid and robust adaption to varying light conditions. Modified light in the green spectral region induced higher production levels of both chlorophyll and phycobiliproteins.

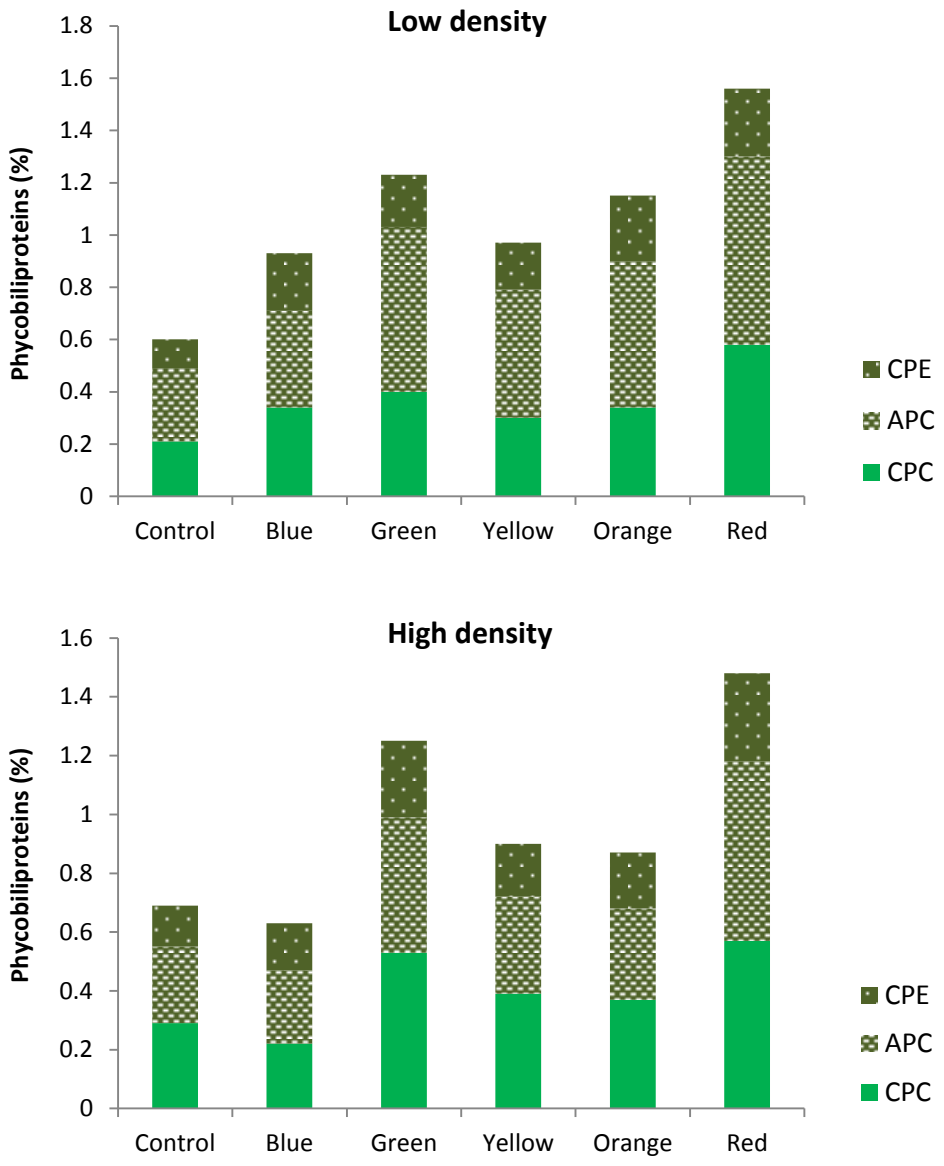

Figure 7.13, Phycobiliproteins variation in *G.membranacea* in different culture densities. Values represent average phycobiliproteins % (g phyco/g biomass) over a 14 days period in luminescent photobioreactors. CPC (C-phycocyanin), APC (Allophycocyanin), CPE (C-phycoerythrin).

The density of the seed culture highly affected the specific growth rate and biomass productivity in both microalgae strains. The cultivation with low density seed culture resulted higher biomass productivity.

Further investigation of the characteristics of the species grown under these modified light conditions showed that chlorophylls a and b were the dominant biopigments in *C.vulgaris,* while phycobiliproteins were the main biopigments in *G.membranacea.*

7.3.2 *Effect of light intensity*

Phycobiliproteins contents were decreased when the light intensity increased at the control condition. The control condition was used for examining the influence of intensity on pigmentation at the full-spectrum daylight. In all luminescent photobioreactors there was a clear pattern (see Figure 7.14). Highest phycobiliproteins contents were achieved at 50 μmol photons m^{-2} s^{-1} light intensity, with a major reduction obtained when increasing the intensity to 100 μmol photons m^{-2} s^{-1} and slightly enhanced levels at 300 μmol photons m^{-2} s^{-1}. The effect of light intensity was evidently observed in the control PBR, where the phycobiliprotein content at the high light intensity condition decreased to a quarter of that of low light intensity. Chen et al. (2010) reported that by increasing biomass production in the cyanobacteria *S.platensis* at high light intensity levels, phycobiliprotein content of cells decreased. The study suggested that when fast growth occurred, cyanobacteria cells required more nitrogen sources and, as a compensation for the limited nitrogen availability in the medium, they consumed phycobiliproteins (Chen et al., 2010).

The red and green PBRs induced pigmentation more than other wavelength ranges at all light intensity groups, with a total phycobiliprotein content of 2.6% and 2.53% respectively, whilst the red PBR was most efficient for the synthesis of phycocyanin (1.15%), and the green PBR for synthesis of phycoerythrin (0.52%).

It was previously observed that there was no significant difference in the total phycobiliprotein content of different culture density groups. However, the results obtained in this section demonstrated that when the photon flux density increased slightly from the low to medium levels, the cyanbacterial cells responded with producing less than half the amount of phycobiliproteins. Therefore, *G.membranacea* responded considerably to the change of light quality rather than availability.

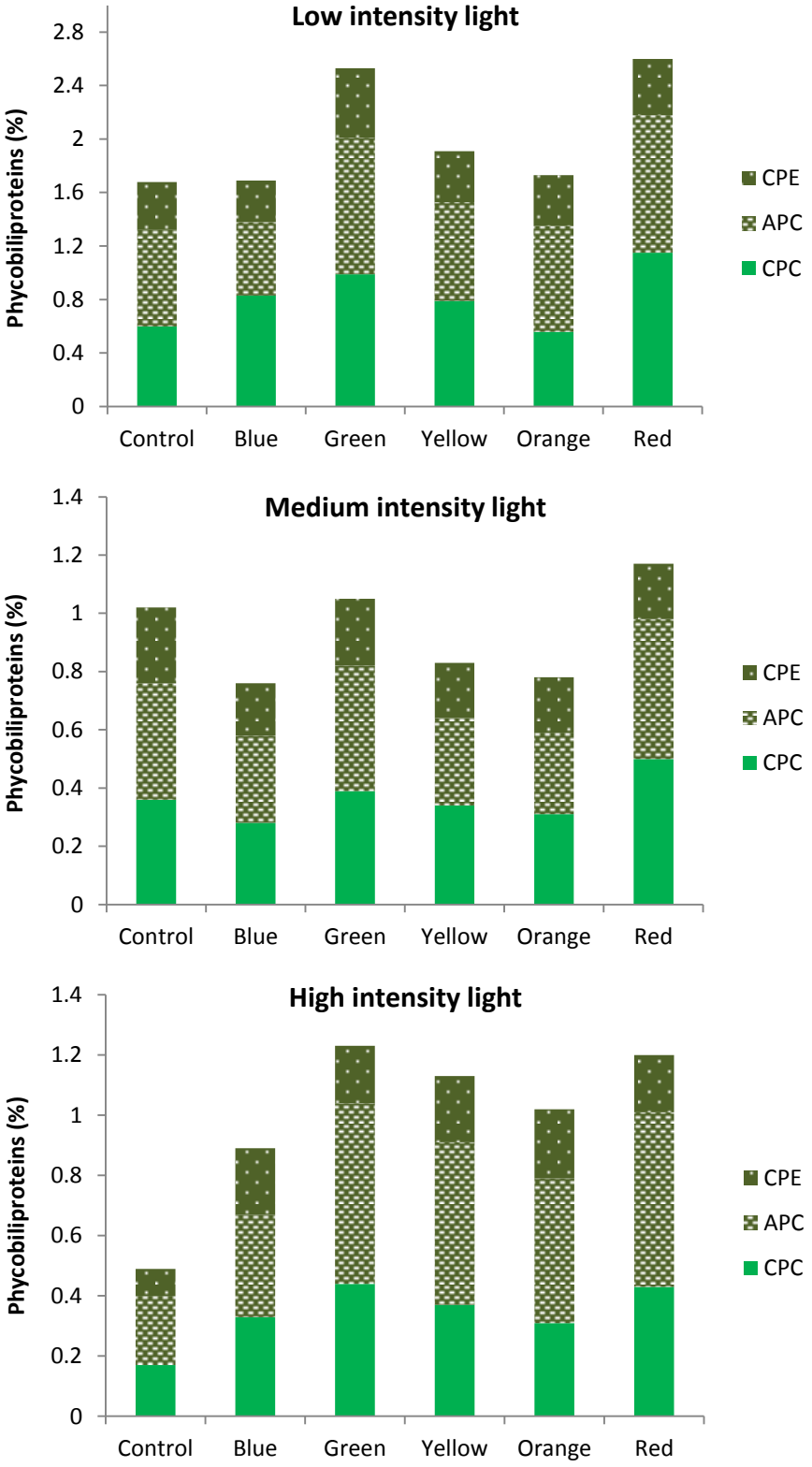

Figure 7.14, Phycobiliproteins variation in *G.membranacea* at different light intensities. Values represent average phycobiliproteins% (g phyco/g biomass) over a 14 days period in luminescent photobioreactors. CPC (C-phycocyanin), APC (Allophycocyanin), CPE (C-phycoerythrin).

7.3.3 *Effect of aeration with carbon dioxide*

Figure 7.15 shows the phycobiliproteins variation at different CO_2 aeration rates. Increasing concentrations of carbon dioxide to 15% decreased the total phycobiliprotein contents of cells, particularly in the green and red PBR. It seemed that the 5% CO_2 aeration condition favoured the production of phycobiliproteins. Phycocyanin content at the 5% CO_2 aeration condition was highest in the green and red PBRs. By increasing CO_2 concentration from 5% to 15% in the green PBR, the phycocyanin content reduced from 1.4% to 0.85% respectively and the phycoerythrin content remained unchanged.

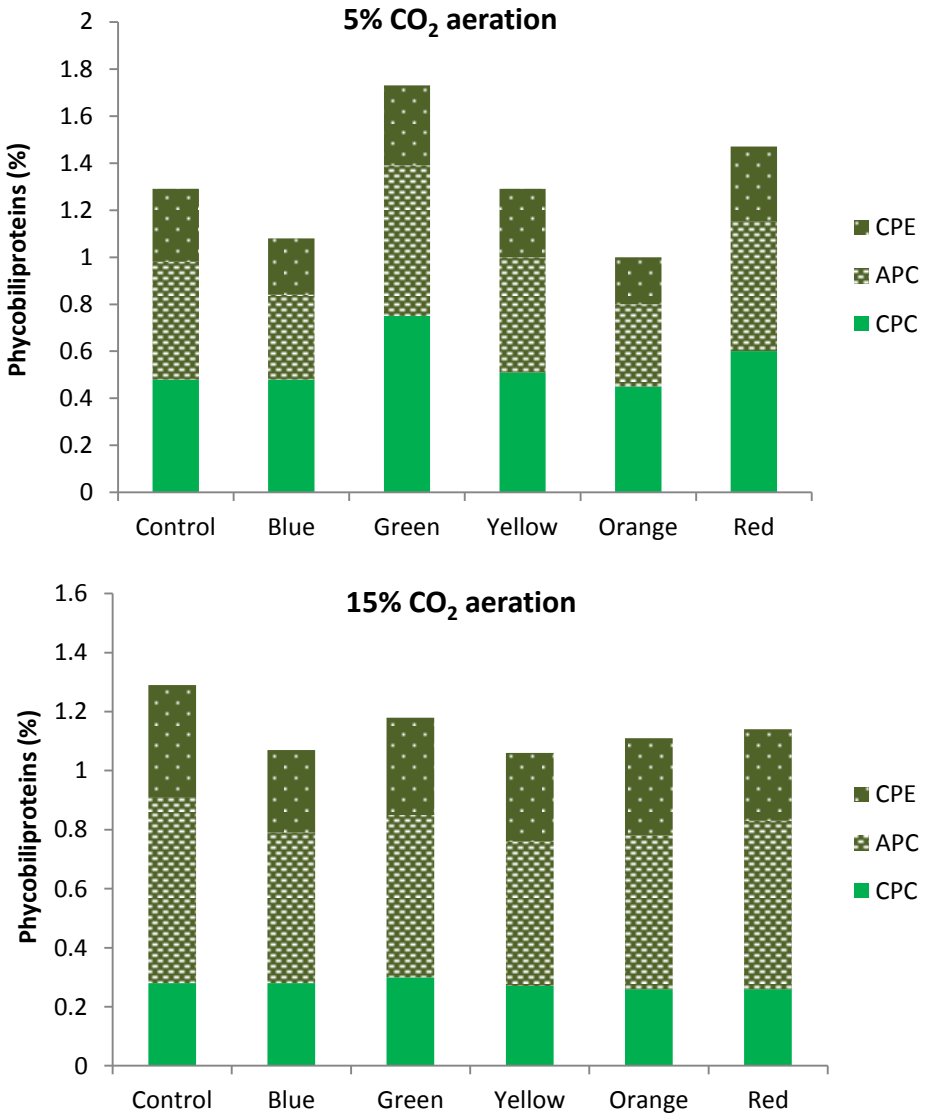

Figure 7.15, Phycobiliproteins variation in *G.membranacea* at different CO_2 aeration rates. Values represent average phycobiliproteins% (g phyco/g biomass) over a 14 days period in luminescent photobioreactors. CPC (C-phycocyanin), APC (Allophycocyanin), CPE (C-phycoerythrin).

Similarly, by increasing CO_2 concentrations, the phycocyanin content in the red PBR reduced by 40%. Eley (1971) reported that increasing carbon dioxide concentration can mainly decrease phycocyanin content of the cells.

It was suggested that phycocyanin is responsible for the balance of light absorption in the photosystems of cyanobacteria and CO_2 aeration can be used as an effective method of regulating the dark reactions with the light reactions (Tang et al., 2011). The results are also supported by another study which compared 1% CO_2 aeration with pure air in which CO_2 aeration reduced phycocyanin content by up to 50% (Yang and Gao, 2003).

7.4 Conclusions

This chapter reviewed the effect of various cultivation conditions on bio-pigment production in the microalgae *C.vulgaris* and *G.membranacea*. By alterations of the environmental factors, particularly the light quality, the production of photosynthetic pigments changed. The response to these changes varied depending on the microalgae species. In general, the effect of the luminescent photobioreactors on pigmentation was more pronounced in low density cultures.

The green and red PBRs favoured chlorophyll production in *C.vulgaris,* whilst the latter also induced pigmentation in *G.membranacea*. Prokaryotic cyanobacterium *G.membranacea* contained chlorophyll-a, as well as phycobiliproteins, although its average chlorophyll production was lower than *C.vulgaris*. In the low density cultures, the chlorophyll production was induced mainly at the beginning of the cultivation period, while in high density cultures pigmentation increased towards the end of the cultivation. The results suggested that as a result of growth competition in highly dense cultures, microalgae cells produced more pigments to compensate the lack of light availability. Phycobiliproteins were the dominant pigments in *G.membranacea* and the red and green PBR improved the synthesis of these pigments.

The response to the changes of photon flux density (light intensity) was different between the two selected species. Increasing light intensity to medium levels enhanced the chlorophyll production in *C.vulgaris* whilst low light intensity improved the pigmentation in *G.membranacea* and further increase of irradiance intensity reduced the chlorophyll content. In addition, when the light intensity increased slightly from low to

medium levels, the cyanbacterial cells responded by producing less than half the amount of phycobiliproteins.

Aeration with increased carbon dioxide concentrations decreased the chlorophyll content of the cells. The results obtained indicated that increasing CO_2 concentrations had an inverse effect on the chlorophyll production in *C.vulgaris*. The red PBR enhanced pigmentation slightly more than other light conditions, and the highest CO_2 concentrations lowered average chlorophyll contents to one third of the values obtained with pure air. Compared to *C.vulgaris*, where all cultures were affected, it seemed the impact of CO_2 aeration was more pronounced on longer wavelength (warmer colour) ranges in *G.membranacea*. Looking at the chlorophyll data obtained, it is clear that the influence of aeration was less significant on phycobiliproteins compared to chlorophylls.

Chapter 8: Conclusions

8.1 General conclusions

This study investigated the effects of various cultivation conditions combined with the use of luminescent material for the cultivation of the microalgae *Chlorella vulgaris* and *Gloeothece membranacea*. The main aim of the study was to determine the influence of light quality at different culture modes on growth of the selected species. In all the experiments carried out, luminescent acrylic polymers were used in different forms and ranges to achieve spectral conversion of light. The cultivation modes were categorised into two main groups: static and aerated cultivation. The main findings obtained for each of these cultivation modes are illustrated below, followed by a summary of the findings obtained from the analysis of the carbon dioxide fixation rates and photosynthesis pigments. The chapter also contains a description of the impact of the work and suggestions for further research.

8.2 Static cultivation

For the static cultivation mode, luminescent acrylic sheets in form of flat panels were used as wavelength shifting filters against the light sources. From the data obtained biomass production was promoted by violet luminescent sheets which emitted the photosynthetic active radiation ranges. However, the specific growth rate was relatively low in both strains, with the highest specific growth rates of 0.13 day^{-1} for *C.vulgaris* and 0.16 day^{-1} for *G.membranacea*. Red light had a significant effect on the pigmentation of *C.vulgaris,* although it was the least efficient light condition for cultivation of *G.membranacea*.

Photosynthetic pigmentation under different light conditions exhibited the major differences between the green algae strain *C.vulgaris* and the cyanobacteria *G.membranacea*. Firstly, *C.vulgaris* cells showed completely different behaviour when illuminated with different light wavelengths compared to their previous light environment. This strongly suggests that for the first few days of cultivation *C.vulgaris* cells were responding to the environmental alterations by adapting their photosynthetic antenna. This resulted in high chlorophyll production under green, orange, and red light conditions which contained specific wavelength bands of the photosynthetic active

radiation (PAR) range. In comparison, full-spectrum daylight and violet light which modified natural daylight to PAR, produced average chlorophyll contents.
It was concluded from the results that red light was particularly inefficient to support the growth in static cultures.

8.3 Aerated cultivation

Aerated cultivation in luminescent bubble column photobioreactors examined the effect of light quality and carbon dioxide aeration on microalgae growth. Mixing cultures provided conditions in which photons of light in the red region of spectrum could penetrate more effectively into the culture.

8.3.1 *Culture density*

Different seed culture density groups were used and the results indicated that highly dense cultures limited the growth, even though the nutrients may have remained available. The results showed that the performance of different wavelengths of light can be dependent on the culture density. The higher populated cultures prevented photons of light from further penetration into the central zone of the photobioreactors. The specific growth rate and productivity in highly dense cultures were lower than the diluted cultures. Overall, the results indicated that the red luminescent photobioreactor enhanced biomass production in both strains of microalgae, whilst blue and yellow photobioreactors (PBRs) induced lipid accumulation at various cultivation conditions.

8.3.2 *Light intensity*

Three different light intensity levels were examined for the illumination of microalgae. It was demonstrated that at low light intensity, the dynamics of the microalgae cells can vary depending on the wavelength ranges provided. Moreover, carbon and nitrogen, the two major elements of the algal cells, changed in more extreme or stress conditions. The highest biomass density of 2.12 and 2.52 g L^{-1} were achieved at high light intensity in red PBR for *C.vulgaris* and *G.membranacea* respectively. From the results obtained it was clear that whilst low intensity irradiation could cause slow growth, it induced lipid accumulation in both strains. When the intensity levels were increased, cells recovered the carbon which they consumed and there was relatively higher elemental carbon content.

8.3.3 *CO_2 enriched air*

Tests were carried out to investigate the influence of CO_2 aeration on biomass production, carbon dioxide fixation rate, cell's essential elements (carbon, nitrogen, and hydrogen), lipid content, as well as the pH variations in the culture media. There was no substantial difference in the CO_2 fixation rate in all photobioreactors when aerated with pure air comprising 0.03% CO_2 (v/v). However, in all luminescent photobioreactors with the exception of the blue PBR, increasing the concentration of CO_2 in the gas mixture had significant impacts on CO_2 sequestration. Cyanobacteria cells showed significant tolerance to higher carbon dioxide concentrations, although due to their lower carbon content and biomass productivity, *G.membranacea* was less efficient than *C.vulgaris* in sequestrating carbon dioxide. *C.vulgaris* sequestrated carbon dioxide up to 363 mg L^{-1} day^{-1} in the red PBR when aerated with 15% CO_2.

The lipid data obtained from *G.membranacea* exhibited a clear trend at various aeration conditions. Increasing carbon dioxide concentrations enhanced lipid accumulation. The highest lipid content of 36.6% was achieved at 15% CO_2 aeration in the blue PBR. Aeration with 15% CO_2 enhanced lipid production of *G.membranacea*, to at least twice the amount produced at 5% CO_2 in all photobioreactors. The most significant difference between the 5% and 15% CO_2 aeration conditions was observed in the yellow PBR, in which the lipid content was enhanced up to six times. The blue PBR induced the highest lipid accumulation of 25.6% in *C.vulgaris*, at 15% CO_2 aeration, which was threefold greater than 5% CO_2 aeration. However, pure air favoured lipid production in the yellow, orange and red PBRs (longer wavelength ranges) with *C.vulgaris*.

It must be noted that lipid storage was noticeably induced at low intensity light and high concentration carbon dioxide and the response to such extreme conditions was mainly observed in the blue PBR. The elemental analysis results indicated that the nitrogen content in the blue PBR increased to 6.92% at high concentrations of carbon dioxide. Similarly, lower carbon content signalled the consumption of carbon in extreme conditions.

8.4 Photosynthetic pigments

The production of photosynthetic pigments can change by alterations in the environmental factors and light quality in particular. The response to these changes can vary depending on the microalgae species. Prokaryotic cyanobacteria *G.membranacea*

contained chlorophylls as well as phycobiliproteins, although the average chlorophyll production was lower than *C.vulgaris*. When cultivated in two different culture density groups, green and red light promoted chlorophyll production in *C.vulgaris*. Phycobiliproteins were the dominant pigments in *G.membranacea* and red light favoured synthesis of these pigments.

In addition, the pigment data obtained demonstrated the sensitivity of *G.membranacea* to the changes of photon flux density (light intensity). When the photon flux density increased slightly from the low to medium levels, the cyanbacterial cells responded with producing less than half the amount of phycobiliproteins. Therefore, *G.membranacea* responded considerably to the change of light intensity rather than quality.

Aeration with increasing concentrations of carbon dioxide decreased the chlorophyll content of the cells. The results obtained indicated that increasing CO_2 concentrations had an inverse effect on chlorophyll production in *C.vulgaris*. The red PBR enhanced pigmentation slightly more than other light conditions and the highest CO_2 concentrations lowered average chlorophyll contents to one third of the values obtained with pure air. Compared to *C.vulgaris*, where all cultures were affected, it seemed that the impact of CO_2 aeration was more pronounced on longer wavelength (warmer colour) ranges in *G.membranacea*. Looking at the chlorophyll data obtained it is clear that the influence of aeration was less significant on phycobiliproteins compared to chlorophylls.

8.5 Summary of main findings

A detailed investigation of the growth characteristics of two microalgae species, by examining various cultivation conditions, illustrated some similar responses from two different major algal groups. Both microalgae had higher productivities in aerated cultures than that of the static mode. Luminescent violet ranges enhanced biomass production in the static mode, as they were the only wavelength ranges which emitted almost all the PAR. As in the static mode there was no culture mixing or circulation, those wavelength ranges with lower energy content were unable to penetrate into the central parts of the culture. The highest biomass productivities achieved under the violet light condition was justified in this way. The results also offered support for the hypothesis that the algal cells produce more pigments under the red and green light conditions in the static mode, to compensate the slow growth as the cell population increased.

Another similar response of both microalgae species was to the red photobioreactors in the aerated cultivation mode. Mixing and aeration assisted the distribution of red light (which has low energy content) into the PBR and both microalgae responded to this condition by producing the highest biomass. The major conclusion, for which consistent results were achieved, was the key advantage of mixing by aeration to enhance productivity in microalgae. The influence of the red light condition on algal growth was more pronounced with the increase of light intensity. The highest light intensity examined in this study improved the specific growth rate and biomass density more than any other cultivation condition. Therefore, these similar responses illustrated the advantage of higher intensities when using red light.

Using different concentrations of carbon dioxide (in the air stream) revealed the major differences between the selected microalgae. The highest biomass productivity was achieved at 5% CO_2 aeration condition in *C.vulgaris*, whilst 15% CO_2 concentration favoured *G.membranacea*. The higher carbon content and biomass productivity in *C.vulgaris* revealed the ability of this algal species to achieve higher CO_2 fixation rates than that of *G.membranacea*. However, there was a similar response to the conditions in which the highest lipids were produced. It is well accepted that stress conditions induce lipid storage in algal cells. In both microalgae, 15% CO_2 aeration condition enhance oil production and this effect was significantly improved in the blue PBR. The combination of blue light and carbon dioxide was distinctively suitable for induction of oil production in algal cells.

In general, red, green, and blue light had some influences on pigment production. Whilst chlorophyll production varied at different culture conditions between these three PBRs, phycobiliproteins were mainly influenced by green and red PBRs. The light condition produced by these PBRs was essential for the induction of photosynthetic pigments. It was also demonstrated that the blue PBR was suitable for higher lipid production compared to the other photobioreactors.

A summary of the most suitable cultivation conditions which can target different aspects of algal growth, biomass productivity, pigments and oil production are presented in Table 8.1.

Culture mode	***Cultivation condition***	***Target / Product***	***Luminescent Filter/ PBR***	***Microalgae***
Static	Static	Biomass [a]	Violet	Both
		Chlorophyll [b]	Red	*C.vulgaris*
			Green	*G.membranacea*
Aerated	Low density culture	Biomass Productivity [c]	Red	Both
		Chlorophyll	Green/ Red	*C.vulgaris*
		Phycobiliprotein [d]	Green/ Red	*G.membranacea*
	High density culture	Chlorophyll	Red	*G.membranacea*
		Lipid [e]	Green	*C.vulgaris*
			Blue/ Yellow	*G.membranacea*
	Low light intensity	Lipid	Blue	*C.vulgaris*
			Blue/ Yellow	*G.membranacea*
		Chlorophyll	Blue/ Green	*C.vulgaris*
		Phycobiliprotein	Green/ Red	*G.membranacea*
	Medium light intensity	Chlorophyll	Blue/ Green	*C.vulgaris*
	High light intensity	Biomass	Red	Both
	5% CO_2	Biomass productivity	Red	*C.vulgaris*
		Chlorophyll	Red	Both
		Phycobiliprotein	Green/ Red	*G.membranacea*
	15% CO_2	Biomass productivity	Red	*G.membranacea*
		CO_2 fixation rate [f]	Red	*C.vulgaris*
		Lipid	Blue	Both

Table 8.1 Summary of main findings. The *target/product* column in all cases refers to the optimal condition in which a culture parameter or a target product of each microalgae was significantly improved. The units of the target parameters are: [a] *g* L^{-1}, [b] *% (g chl g biomass*$^{-1}$*)*, [c] *mg* L^{-1} day^{-1}, [d] *% (g phycobiliprotein g biomass*$^{-1}$*)*, [e] *% (g oil g biomass*$^{-1}$*)*, [f] *mg* CO_2 L^{-1} day^{-1}.

8.6 Impact of the research study

Mass production of microalgae for various industrial and environmental applications is highly dependent on the illumination quality. Using sunlight (free and abundant source of light) for manufacturing wavelength selective light could potentially offer an efficient illumination strategy. Manipulation of microalgae cells through alteration of the light condition can enhance the accumulation of high-value biochemicals. Biochemical compounds such as lipids (fatty acids), chlorophylls, carotenes, and phycobiliproteins can be widely used in food, pharmaceuticals, and more importantly renewable energy industry. The results obtained in this study showed that some of the luminescent PBRs are particularly suitable for the production of a particular target product. For instance, red and green PBRs were suitable for production of bio-pigments such as chlorophyll-a and phycobiliproteins. Whilst the blue PBR enhanced accumulation of lipids in both algal species.

Findings obtained from this study are potentially relevant for countries in Northern Europe, where light intensity is lower and where there are considerably lower rates of mass cultivation of microalgae compared to the areas near the equator. The results obtained from the proposed strategy demonstrated higher growth for equivalent illumination density. Using local species adapted to this climate and studying their capabilities for production of targeted products manipulated by light are then of particular interest.

These results have considerable implications for improved cultivation of microalgae under natural light and development of luminescent photobioreactor design. Using variation in light, the proposed photobioreactor design can tailor and improve the composition of lipids and photosynthetic compounds. The results also demonstrated that the culture pH and mixing through aeration could influence various growth parameters. Most commercial photobioreactor designs are focused on the optimisation of light availability. However, if this can be overcome, mixing and pH control could be more critical for optimised cultivation of microalgae in photobioreactors.

8.7 Suggestion for future work

In order to achieve a comprehensive study of the development of luminescent photobioreactors, it is recommended to use a variety of local microalgae species in future studies. Due to the time and scale of this study, the development of luminescent

photobioreactor design in pilot scale was limited. Design of the pilot scale photobioreactors in tubular and flat panel shapes requires manufacturing luminescent plastics in large dimensions. For simulating outdoor light conditions, a room illuminated with full-spectrum light is needed where photobioreactors are placed and a comparative study of different shapes of luminescent material (cylindrical or flat panel) could offer a better insight to the most efficient method of cultivation.

The potential of luminescent acrylic photobioreactors in industrial scale could be examined by some experimental and economic plans carried out at a pilot scale. Luminescent PBR are potentially suitable for some urban design applications where photobioreactors are integrated in green buildings to generate power and capture the CO_2 emissions of the building. Integration of photobioreactors inside and outside the building where culture circulation is possible could potentially solve the problem of temperature control during the night or during colder seasons. The main findings of this study provided an insight into the optimised cultivation conditions. However, the effect of temperature variations, which is one of the important culture parameters, was not investigated. It is highly recommended that future studies examine the growth and oil production of microalgae at different temperatures, to obtain the optimum conditions for outdoor cultivation.

The performance of the luminescent PBR for biofuel production could be further investigated through various methods. For instance, the influence of different nutrient regimes in the media, on lipid production, could be combined with the effect of light quality. Light quality can also influence the composition of fatty acids in microalgae oil and it is advised to screen and analyse the changes of different fatty acids during the cultivation time. Analysis of the fatty acids profiles by chromatography techniques could provide an insight into the suitable wavelength ranges for targeting both pharmaceuticals and biodiesel production.

As it was demonstrated in Chapter 7, light quality significantly influenced the production of chlorophylls and phycobiliproteins. Other commercially valuable accessory pigments such as β-carotene and astaxanthin can be produced and manipulated by different wavelengths of light. An overall examination of these pigments, under blue, green, and red light which are influential portions of PAR spectrum, could target various pharmaceutical applications. These pigments can also be

used as antioxidants, food colouring, and natural fluorophores in construction of fluorescent materials.

Furthermore, microalgae contain significant amounts of proteins. Their protein content can be tested and manipulated for various industrial purposes. As the elemental analysis of microalgae was demonstrated in Chapters 5 and 6, the nitrogen content of cells was affected by the luminescent PBR. The elemental nitrogen content can be linked to the environmental conditions imposed and also be representative of the cell's protein content. Protein is one of the most essential sources of nutrition which is in high demand in the global market. Unicellular microalgae are potential sources of proteins and they are considered to be an excellent source of lysine and threonine (essential amino acids in the food chain).

It is recommended to set up a detailed investigation into the cultivation of unicellular microalgae for production of proteins, under the culture conditions which indicated the highest nitrogen contents in this study. The investigation must then involve an analysis of the amino acids present in algal proteins. The procedure includes hydrolysis (by acids), separation and detection (chromatography) and quantification.

Although the genetic engineering of microalgae is a research area beyond the scope of this study, it is worthy to mention the expression of recombinant proteins in eukaryotic microalgae. Algal recombinant protein expression could be a low-cost and safe method for the production of therapeutic proteins or chemicals for the treatment of infections and diseases. As microalgae are continuously becoming an attractive source of high-value bio-chemicals, the research about their genetic engineering is also expanding. Where possible, it is recommended to consider their genetic manipulation for such research purposes.

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