Effects of Light, Co₂ and Reactor Design on Growth of Algae: An Experimental Approach to Increase Biomass Production

by

Sebastián Mejía Rendón

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ABSTRACT

Effects of Light, CO₂ and Reactor Design on Growth of Algae: An Experimental Approach to Increase Biomass Production

Sebastian Mejia Rendon
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Advisor: Paul Voroney

The increase in atmospheric carbon dioxide concentrations and resulting global climate change coupled with the high cost of fossil fuels are encouraging the search for alternative sources of fuels and methods to produce food without using agricultural land. Algal biomass production has the potential to address these problems; algae are rich in fats, proteins and carbohydrates. Algae fix carbon dioxide and are relatively easy to grow. They can be grown in both fresh and marine water. Algal oil can be used as a feedstock to make biodiesel, the omegas and antioxidants can be used as food supplements and components of medicines; the proteins can be used for animal feedstock and food supplements; and the carbohydrates can be used to produce ethanol. Study of the factors affecting growth of microalgae play a critical role for the development of efficient methodologies for harvesting their products. Experiments were conducted with six algae to evaluate effects of light source, CO₂ concentration and reactor design; small bubble column versus flat panel photo-bioreator.
Light treatments that supplemented artificial light with daily solar radiation at 4614 mg L\(^{-1}\) resulted in 15% more biomass production than those treatments that provided 24 h of artificial illumination (4016 mg L\(^{-1}\)) during 49 days of incubation. Treatments with *Chlorella vulgaris* during 15 d that combined continuous exposure from LEDs emitting blue + red radiation (430 and 625 nm) and from white light resulted in greater biomass production, 1003 mg L\(^{-1}\) and 1045 mg L\(^{-1}\), respectively, than those from a single light source (red at 571 mg L\(^{-1}\) and blue at 828 mg L\(^{-1}\)). Growth of *Nannochloris sp* and *Scenedesmus sp* during 13 d of incubation exposed to flashing lights in a bubble column photo-bioreactor resulted in more biomass production (1041 mg L\(^{-1}\) and 1130 mg L\(^{-1}\), respectively) and higher cell weight than those algae growing under continuous light (892 mg L\(^{-1}\) and 1018 mg L\(^{-1}\), respectively. Experiments with bubble column photo-bioreactors and *Chlorella vulgaris* growing under increasing CO\(_2\) concentrations, from 0.035\% to 8.5\%, during a 15 d incubation increased biomass production from 148 mg L\(^{-1}\) to 1592 mg L\(^{-1}\). A flat panel, industrial scale photo-bioreactor resulted in biomass production of 2337 mg L\(^{-1}\) during 15 d, which was significantly more than that obtained in bubble column reactors.

This research has shown that algal biomass production can be increased when grown under illumination that supplemented solar radiation with artificial light during the night time. An increase in the concentration of CO\(_2\) supplied to the algae increased algal biomass production 10 fold. Exposure as light dark cycles, the flashing effect, which utilizes 85-90\% less energy consumption than exposure to continuous light, resulted in greater biomass production. A light path less than 5 cm resulted in increased biomass production by >1.5 fold. Algae growing in a flat panel bioreactor developed the highest biomass production, and the research suggests that this bioreactor design can be a reliable system for growing algae at a large scale.
I would like to thank all of those who have been involved during the course of this research study, especially to Gabriel Jaime Colmenares who assisted in setting up and conducting the algal growth experiments. I have special thanks to my parents who have provided support and encouragement during my studies. I wish to thank the staff in the former Department of Land resource Science (now the School of the Environmental Sciences) for their assistance. I also would like to thank the members of my PhD advisory committee: Drs. Kari Dunfield, Hongde Zhou and Paul Voroney, for their guidance through out my program. I have a very special thanks to my supervisor Paul Voroney who provided me the opportunity to conduct research in the area of photosynthetic microorganisms and guided my PhD program.
# TABLE OF CONTENTS

## CHAPTER 1  INTRODUCTION

1. **BACKGROUND**
   - ALGAL GROWTH FOR PHARMACEUTICAL PRODUCTION AND FOOD SUPPLEMENTS
   - OIL SYNTHESIS
   - CO₂ FIXATION
   - PHOTOBIOREACTORS
   - ILLUMINATION
     - FLASHING LIGHT AND DARK PERIOD
     - PHOTOINHIBITION IN ALGAE
     - WEIGHT LOSS DURING NIGHT PERIOD
   - ALGAE GROWTH UNDER ARTIFICIAL AND OUTDOOR CONDITIONS
     - GROWTH UNDER INDOOR ARTIFICIAL CONDITIONS
     - GROWTH UNDER OUTDOOR CONDITIONS
   - COMMERCIAL PRODUCTION OF ALGAE
   - ALGAE USED DURING THE COURSE OF THIS RESEARCH

2. **FORMAT OF THE THESIS**

3. **REFERENCES**

## CHAPTER 2  RESEARCH OBJECTIVES

1. **GENERAL GOAL**
2. **SPECIFIC RESEARCH OBJECTIVES**

## CHAPTER 3  THE PHOTOSYNTHETIC MACHINERY: A DETAILED DESCRIPTION OF THE LIGHT REACTION; PSI, PSII, ATP SYNTHASE, STATE OF TRANSITION, PHOTO-DAMAGE, REGENERATION, AND MACHINERY ARCHITECTURE

1. **ABSTRACT**
2. **INTRODUCTION**
3. **EVOLUTION OF PHOTOSYNTHESIS: ANOXYGENIC AND OXYGENIC**
   - ANOXYGENIC PHOTOSYNTHESIS
     - THE PURPLE NON-SULFUR BACTERIA
     - THE GREEN AND PURPLE SULFUR BACTERIA
CHAPTER 4  GROWTH OF TWO MARINE ALGAE AND TWO FRESH WATER ALGAE UNDER SOLAR AND LED ILLUMINATION

4.1 ABSTRACT

4.2 INTRODUCTION

4.3 MATERIALS AND METHODS

4.3.1 MICROORGANISM

4.3.2 LIGHT SOURCE

4.3.2.1 EXPERIMENT 1 (DAILY SOLAR RADIATION)

4.3.2.2 EXPERIMENT 2 (DAILY SOLAR PLUS 12 H WHITE LIGHT)

4.3.2.3 EXPERIMENT 3 (24 H BLUE LEDS)

4.3.2.4 EXPERIMENT 4 (24 H RED + BLUE LEDS)

4.3.2.5 EXPERIMENT 5 (DAILY SOLAR PLUS 12H RED AND BLUE LEDS)

4.3.3 LIGHT DISPERSION

4.3.4 MIXING, QUANTIFYING CARBON SOURCE UNDER AUTOTROPHIC GROWTH AND TEMPERATURE MEASUREMENTS

4.3.5 GROWTH MEDIA PREPARATION

4.3.6 CELL DENSITY ANALYSIS

4.3.7 GROWTH RATE METHODOLOGY
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3.8</td>
<td>BIOMASS AND CELL WEIGHT METHODOLOGY</td>
<td>66</td>
</tr>
<tr>
<td>4.3.9</td>
<td>EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS</td>
<td>67</td>
</tr>
<tr>
<td>4.4</td>
<td>RESULTS AND DISCUSSION</td>
<td>67</td>
</tr>
<tr>
<td>4.4.1</td>
<td>ALGAL GROWTH UNDER DAILY SOLAR RADIATION</td>
<td>68</td>
</tr>
<tr>
<td>4.4.2</td>
<td>ALGAL GROWTH UNDER DAILY SOLAR RADIATION + 12 H WHITE LIGHT</td>
<td>68</td>
</tr>
<tr>
<td>4.4.4</td>
<td>ALGAL GROWTH UNDER 24 H BLUE + RED LEDS</td>
<td>69</td>
</tr>
<tr>
<td>4.4.5</td>
<td>ALGAL GROWTH UNDER DAILY SOLAR RADIATION + 12 H BLUE + RED LEDS</td>
<td>70</td>
</tr>
<tr>
<td>4.4.6</td>
<td>FINAL POPULATION DENSITY FOR FOUR ALGAL SPECIES GROWING UNDER 5 LIGHT TREATMENT</td>
<td>70</td>
</tr>
<tr>
<td>4.4.7</td>
<td>SPECIFIC GROWTH RATE FOR VARIOUS LIGHT TREATMENTS</td>
<td>72</td>
</tr>
<tr>
<td>4.4.8</td>
<td>BIOMASS PRODUCTION</td>
<td>75</td>
</tr>
<tr>
<td>4.4.9</td>
<td>CELL WEIGHT</td>
<td>76</td>
</tr>
<tr>
<td>4.5</td>
<td>CONCLUSIONS</td>
<td>77</td>
</tr>
<tr>
<td>4.6</td>
<td>REFERENCES</td>
<td>79</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 5</th>
<th>BIOMASS PRODUCTION OF SIX ALGA SPECIES GROWING UNDER LED AND SOLAR RADIATION</th>
<th>85</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>ABSTRACT</td>
<td>85</td>
</tr>
<tr>
<td>5.2</td>
<td>INTRODUCTION</td>
<td>85</td>
</tr>
<tr>
<td>5.3</td>
<td>MATERIALS AND METHODS</td>
<td>88</td>
</tr>
<tr>
<td>5.3.1</td>
<td>MICROORGANISM</td>
<td>88</td>
</tr>
<tr>
<td>5.3.2</td>
<td>BIOREACTOR SYSTEM</td>
<td>88</td>
</tr>
<tr>
<td>5.3.3</td>
<td>LIGHT SOURCE</td>
<td>88</td>
</tr>
<tr>
<td>5.3.4</td>
<td>LIGHT DISPERSION</td>
<td>89</td>
</tr>
<tr>
<td>5.3.5</td>
<td>EXPERIMENT 1: 24 H RED + BLUE LEDS</td>
<td>89</td>
</tr>
<tr>
<td>5.3.6</td>
<td>EXPERIMENT 2: DAILY SOLAR + 12 H LEDS</td>
<td>89</td>
</tr>
<tr>
<td>5.3.7</td>
<td>MIXING, QUANTIFYING CARBON SOURCE UNDER AUTOTROPHIC GROWTH AND TEMPERATURE MEASUREMENTS</td>
<td>90</td>
</tr>
<tr>
<td>5.3.8</td>
<td>CULTURE MEDIUM</td>
<td>90</td>
</tr>
<tr>
<td>5.3.9</td>
<td>BIOMASS DETERMINATION</td>
<td>90</td>
</tr>
<tr>
<td>5.3.10</td>
<td>EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS</td>
<td>91</td>
</tr>
<tr>
<td>5.4</td>
<td>RESULTS</td>
<td>91</td>
</tr>
<tr>
<td>5.4.1</td>
<td>BIOMASS PRODUCTION FOR FRESH WATER ALGAE GROWING UNDER TWO DIFFERENT LIGHT TREATMENTS</td>
<td>91</td>
</tr>
<tr>
<td>5.4.2</td>
<td>MEAN AND TOTAL BIOMASS PRODUCTION</td>
<td>94</td>
</tr>
<tr>
<td>5.4.3</td>
<td>BIOMASS VARIANCE</td>
<td>96</td>
</tr>
<tr>
<td>5.5</td>
<td>DISCUSSION AND CONCLUSIONS</td>
<td>97</td>
</tr>
<tr>
<td>5.6</td>
<td>REFERENCES</td>
<td>100</td>
</tr>
</tbody>
</table>
# CHAPTER 6  EFFECT OF USING DIFFERENT LEDS LIGHTS ON THE GROWTH AND BIOMASS PRODUCTION OF CHLORELLA VULGARIS  

6.1  ABSTRACT ............................................................................................................... 105  

6.2  INTRODUCTION ....................................................................................................... 105  

6.3  MATERIALS AND METHODS .................................................................................. 107  

6.3.1  MICROORGANISM ............................................................................................... 107  

6.3.2  LIGHT SOURCE .................................................................................................... 107  

6.3.3  LIGHT EMISSION INTENSITY .............................................................................. 107  

6.3.4  REACTOR MANAGEMENT ................................................................................... 109  

6.3.5  CULTURE MEDIUM ........................................................................................... 109  

6.3.6  EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS .................................. 109  

6.3.7  ALGAL POPULATION, LIGHT ABSORPTION PEAKS AND BIOMASS ANALYSIS .... 110  

6.4. RESULTS AND DISCUSSION .................................................................................. 112  

6.4.1  THE EFFECT LIGHT IN THE ALGAL ABSORPTION SPECTRA .............................. 112  

6.4.2. POPULATION GROWTH OF C. VULGARIS EXPOSED TO DIFFERENT LIGHT SOURCES 113  

6.4.3  STANDING BIOMASS PRODUCTION UNDER DIFFERENT LIGHT SOURCES ........ 115  

6.4.3.1  STANDING BIOMASS ....................................................................................... 115  

6.4.3.4  EFFECT OF LIGHT SOURCE ON ALGAL CELL WEIGHT ................................. 117  

6.5  CONCLUSIONS ......................................................................................................... 118  

6.6  REFERENCES ........................................................................................................... 119  

# CHAPTER 7  BIOMASS PRODUCTION OF MARINE AND FRESH WATER ALGA GROWING UNDER FLASHING AND CONTINUOUS LED LIGHT  

7.1  ABSTRACT ............................................................................................................... 125  

7.2  INTRODUCTION ....................................................................................................... 125  

7.3  MATERIALS AND METHODS .................................................................................. 127  

7.3.1  MICROORGANISM ............................................................................................... 127  

7.3.2  PHOTO REACTOR ............................................................................................... 127  

7.3.3  LIGHT SOURCE AND FLASHING DEVICE .......................................................... 128  

7.3.4  LIGHT DISPERSION ............................................................................................ 128  

7.3.5  MIXING, QUANTIFYING CARBON SOURCE UNDER AUTOTROPHIC GROWTH AND TEMPERATURE MEASUREMENTS ................................................. 129  

7.4.5  CULTURE MEDIUM ........................................................................................... 129  

7.4.6  BIOMASS DETERMINATION .............................................................................. 129  

7.4.7  EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS .................................. 130  

7.5  RESULTS AND DISCUSSION .................................................................................. 131
9.3.3 ILLUMINATION AND TEMPERATURE ............................................... 160
9.3.4 CARBON DIOXIDE SUPPLY ......................................................... 161
9.3.5 CULTURE MEDIUM ................................................................. 161
9.3.6 BIOMASS DETERMINATION ....................................................... 162
9.3.7 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS .......... 162

9.4 RESULTS .................................................................................. 163
  9.4.1 CHANGES IN ALGAL CELL DENSITY UNDER AIR SUPPLY (380-410 PPM OF CO₂) ..................... 163
  9.4.2 BIOMASS PRODUCTION WITH INCREASING CO₂ TREATMENTS .............................................. 164
  9.4.3 BIOMASS COMPARISON OF ALGAE GROWING AT DIFFERENT CO₂ CONCENTRATIONS .......... 165

9.5 DISCUSSION AND CONCLUSION ............................................. 166

9.6 REFERENCES ........................................................................... 167

CHAPTER 10: GENERAL CONCLUSIONS ...................................... 169
LIST OF TABLES

Table 1.1. Algae biomass production in different growing systems ______________________ 4

Table 1.2. Studies with algae under flashing lights ________________________________ 6

Table 1.3. Optimal light intensity for growing algae ______________________________ 7

Table 1.4. Quantum yield in photon absorption by biomass under different photon flux _____ 8

Table 1.5. Taxonomy of the six algal species grown during the course of this research ______ 11

Table 4.1. Population densities (cells mL⁻¹) after 18 days of incubation for four different algae under five different light treatments ____________________________________________ 71

Table 4.2. Growth rate (d⁻¹) under different lights treatments after 18 d of incubation _____ 73

Table 5.1. Biomass production for 6 different alga species___________________________ 87

Table 5.2. Biomass production for three fresh water algae under two light sorces ________ 93

Table 5.3. Biomass production for three marine algae under two light sources__________ 93

Table 5.4. ANOVA test for total biomass production_______________________________ 96

Table 6.1. Effect of distance from the LED light source on light power__________________ 108

Table 6.2. Standing biomass of C. vulgaris growing under white, blue, red and red + blue light ______________________________________________________________ 115
Table 6.4. Cell weight of \textit{C. vulgaris} grown under 4 different light sources  

Table 7.1. Cell weight of two algae species under two light treatments  

Table 8.1. LED light dispersion and light reducing intensity through distance  

Table 8.2. Analysis of variance of the standing biomass for four different CO\textsubscript{2} concentration under constant light sources  

Table 8.3. Analyses of the standing biomass if the light regime changes and the CO\textsubscript{2} concentration remains constant  

Table 8.4. Mean standing biomass production under increasing CO\textsubscript{2} concentrations and exposed to four different light sources  
LIST OF FIGURES

**Figure 1.1.** Features of the six algal species studied during the course of this research 12

**Figure 3.1.** Chloroplast structure 34

**Figure 3.2.** Thylakoid disk including PSI, PSII and ATP synthase 35

**Figure 3.3.** Organization of the thylakoid disk including the stroma lamellae 36

**Figure 3.4.** Three-dimensional structure of the PSII antennae and reaction center 38

**Figure 3.5.** Proposed structure of PSII based on X-ray images 40

**Figure 3.6.** Photosystem I structure 49

**Figure 3.7.** Photosynthetic apparatus in algae 53

**Figure 4.1.** Algal growth under daily solar radiation 68

**Figure 4.2.** Algal growth under daily solar radiation + 12 h white light 68

**Figure 4.3.** Algal growth under to 24 h blue LEDs in 4-L reactors 69

**Figure 4.4.** Algal growth under 24 h red + blue LEDs 70

**Figure 4.5.** Algal growth under daily solar radiation + 12 h blue + red LEDs 70

**Figure 5.1.** Mean algal biomass production under two light treatments 94

**Figure 5.2.** Total algal biomass production under two light treatments 95

**Figure 5.3.** Biomass variance during the experiment period 97
Figure 6.1. Experiment set up, including 24 replicates  

Figure 6.2. Algal absorption spectra after 15-d incubation exposed to blue, red, red+blue and white LED lights  

Figure 6.3. Growth curves of the four light treatments with 6 replicates of each treatment  

Figure 7.1. Standing biomass production under two light treatments for a marine algal Nannochloris sp and a fresh water alga Scenedesmus sp  

Figure 7.2. Population density of two algae under two light treatments  

Figure 7.3. Photograph analysis of Nannochloris sp and Chlorella sp include size and some organelles  

Figure 8.1. Changes in standing biomass under different concentration of CO₂ and different wavelength illumination  

Figure 9.1. Reactor unit from experimental set up  

Figure 9.2. Experimental set up showing the night illumination  

Figure 9.3. Population density of four alga species growing in a flat panel industrial scale reactor  

Figure 9.4. Biomass production of two fresh water algae C. vulgaris, Scenedesmus sp and two marine algae Tetraselmis sp, Nannochloris sp exposed to increasing CO₂ concentrations in an FPB  

xv
CHAPTER 1 INTRODUCTION

1.1 BACKGROUND

GLOSSARY

Light intensity: \(1 \mu \text{mol m}^{-2} \text{s}^{-1} = 1 \mu \text{E m}^{-2} \text{s}^{-1} = 0.342 \text{ W m}^{-2} = 54 \text{ Lux}\)

Standing biomass: The weight of the algae population in a volume unit (mg L\(^{-1}\) or g L\(^{-1}\))

Biomass production: It is the weight of the algae that is been produce in a period of time in a volume unit, sometimes the standing biomass is the biomass production over time (mg L\(^{-1}\) d\(^{-1}\))

Flashing light: Periods of illumination and periods of darkness in milliseconds

1.1.1 ALGAL GROWTH FOR PHARMACEUTICAL PRODUCTION AND FOOD SUPPLEMENTS

Algal biomass production has great potential as a source of pharmaceutical metabolites and biochemical compounds such as antibiotics, nutraceuticals and other natural drugs, for treating diseases in humans and animals (Fenical, 1997; Wright et al., 1999), and for use in different food supplements (Fan et al., 2008; Lucumi and Posten, 2006; Hu et al., 1996a). For example, the marine algae *Nannochloropsis* sp is known for the production of astaxanthin, zeaxanthin and canthaxanthin as well as for production of useful lipids and biomass with high nutritional value for human consumption (Rocha et al., 2003). The company Quantum Nutrition Inc located in Ontario Canada produces a food supplement called Schinoussa Sea Vegetables, which is a mixture of algal species suchs as *Spirulina* sp, *Chlorella* sp AFA and red algae species. The use of photosynthetic microorganisms for the production of pharmaceutical compounds has been called molecular farming (Lucumi and Posten, 2006).

The algal *Haematococcus pluvialis* has been grown for the production of astaxanthin (3,3-dihydroxy-\(\beta\), \(\beta\)-carotene-4, 4-dione) a pharmaceutical compound and a food supplement found in
stores under different brands such as Now Foods a company located in Bloomingdale Illinois USA. For the production of astaxanthin *H. pluvialis* is grown under lumostatic conditions (light intensity 40 µmol m⁻² s⁻¹ and air containing 5% CO₂) a standing biomass of 5 g L⁻¹, a cell population 6*10⁶ cells mL⁻¹ and a astaxanthin production of 130 mg L⁻¹ was obtained (Lee et al., 2006). Using a flat panel photobioreactor with superficial mixing velocity of 0.4 cm s⁻¹ and light intensity of 20 µmol m⁻² s⁻¹, Issarapayup et al. (2009) obtained a population of 4.1 *10⁵ cells mL⁻¹ and a population doubling time of 46 h.

### 1.1.2 OIL SYNTHESIS

The growth of algal species for lipid production is an alternative technology for use as a renewable energy source. However, to achieve this goal it is essential that current algal lipid contents be increased. A technique for increasing the lipid content of alga is growth of under low nitrogen supply. Under nitrogen limitation, *Chlorella vulgaris* increased lipid synthesis from 17% to 58%, but the biomass production was reduced from 680 mg L⁻¹ d⁻¹ to 24 mg L⁻¹ d⁻¹ (Doucha and Livansky, 2009; Scragg et al., 2002; Watanabe and Saiki, 1997). While algae store carbohydrates synthesized during the period of photosynthesis (exposure to light) and consume those carbohydrates during the dark period with respiration, fatty acid biosynthesis and consumption occur during the light period (Rebollos Fuentes et al., 1999). The composition of algal fatty acids has been shown to be affected by the conditions under which they are grown, and for the algal species *Pavlova lutheri* the fatty acids ranged in size from 14 to 22 carbon chains (Meireles et al., 2008).

### 1.1.3 CO₂ FIXATION

Algae cells contain 45-50% organic carbon (on dry weight basis), therefore it is the highest metabolic requirement constraining growth after water. The carbon is required in the form of
CO₂ for photosynthesis reactions, and theoretically the production of 1 kg of algal biomass requires the uptake of 1.83 kg CO₂ (Doucha et al., 2005). In reality, algal cells also produce various carbon compounds as sub-products that are released from the cell during growth. Thus, the amount of CO₂ required for the production of 1 kg of algae biomass is 4.4 kg, taking into consideration a net CO₂ absorption of 38.7% growing under reactor conditions (Doucha et al., 2005).

1.1.4 PHOTOBIOREACTORS

Horizontal tubular photobioreactors are the most practical and scalable systems for algal production and are commonly used for pharmaceutical and industrial purposes. For a tubular photobioreactor it is recommended that it be 2.4-10 cm in diameter and have a maximum length of 80-84 m, and have a nutrient media flow velocity of 30-50 cm s⁻¹ (Acién Fernández et al., 2001; Molina Grima et al., 1999; Baquerisse et al., 1999). Bubble-column systems are also a common design for photobioreactors, with recommended dimensions being 20 cm in diameter and 4 m column height (Sanchez Miron et al., 1999). Both systems can provide sustainable conditions for growing algal species. In a bubble column photobioreactor the marine algal Tetraselmis suecica attained a biomass production of 560 mg L⁻¹ d⁻¹ and a doubling time that ranged from 26-28 h (Chini Zitelli et al., 2006). In experiments using the horizontal tubular bioreactor for growth of Phaeodactylum tricornutum, biomass production was 1200 mg L⁻¹ d⁻¹ (Acién Fernandez et al., 2001) which doubles the biomass production of the bubble column photobioreactor.

The horizontal tubular and bubble column photobioreactors provide different physical conditions for growing algae and they include internal light and gas dispersion, mass transfer, surface volume ratio, mixing, turbulence and nature of the turbulence. These factor are important because they control availability of CO₂ and light inside the reactor (Sanchez Miron et al., 1999; Garcia Camacho et al., 1999).
The flat panel photobioreactor is another type of reactor system with chambers that are 2.4-10 cm thick and a nutrient flow rate of 50 cm s\(^{-1}\). Its main advantage is less electrical energy consumption than the tubular horizontal photobioreactor (Sierra et al., 2008). A modification of the flat panel photobioreactor is the thin-layer reactor which has a reduced thickness to 0.6-1 cm (Doucha and Livansky, 2009; Doucha et al., 2005; Livansky and Doucha, 1997). Table 1.1 summarizes photobioreactors systems.

Table 1.1. Algae biomass production in different growing systems.

<table>
<thead>
<tr>
<th>System</th>
<th>Algal specie</th>
<th>Biomass Production (mg L(^{-1}) day(^{-1}))</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airlift tubular</td>
<td><em>Porphyridium cruentum</em></td>
<td>1500</td>
<td>Camacho Rubio et al., 1999</td>
</tr>
<tr>
<td>Airlift tubular</td>
<td><em>Phaeodactylum tricornutum</em></td>
<td>1200</td>
<td>Acién Fernández et al., 2001</td>
</tr>
<tr>
<td>Airlift tubular</td>
<td><em>Phaeodactylum tricornutum</em></td>
<td>1900</td>
<td>Molina Grima et al., 2001</td>
</tr>
<tr>
<td>Inclined tubular</td>
<td><em>Chlorella sorokiniana</em></td>
<td>1470</td>
<td>Ugwu et al., 2002</td>
</tr>
<tr>
<td>Undular row tubular</td>
<td><em>Arthrospira platensis</em></td>
<td>2700</td>
<td>Carlozzi. 2003</td>
</tr>
<tr>
<td>Outdoor helical tubular</td>
<td><em>Phaeodactylum tricornutum</em></td>
<td>1400</td>
<td>Hall et al., 2003</td>
</tr>
<tr>
<td>Parallel tubular (AGM)</td>
<td><em>Haematococcus pluvialis</em></td>
<td>50</td>
<td>Olaizola. 2000</td>
</tr>
<tr>
<td>Bubble column</td>
<td><em>Haematococcus pluvialis</em></td>
<td>60</td>
<td>Garcia-Malea López et al., 2006</td>
</tr>
<tr>
<td>Flat plate</td>
<td><em>Nannochloropsis sp</em></td>
<td>270</td>
<td>Cheng-Wu et al., 2001</td>
</tr>
<tr>
<td>Semicontinuous system</td>
<td><em>Nannochloropsis oculata</em></td>
<td>497</td>
<td>Chiu et al., 2009</td>
</tr>
<tr>
<td>Semicontinuous system</td>
<td><em>Nannochloropsis oculata</em></td>
<td>296</td>
<td>Chiu et al., 2010</td>
</tr>
<tr>
<td>Outdoor photobioreactor</td>
<td><em>Phaeodactylum tricornutum</em></td>
<td>300</td>
<td>Sánchez Mirón et al., 2003</td>
</tr>
<tr>
<td>Inclined tubular</td>
<td><em>Chlorella sorokiniana</em></td>
<td>1470</td>
<td>Ugwu et al., 2002</td>
</tr>
</tbody>
</table>

1.1.5 ILLUMINATION

1.1.5.1 FLASHING LIGHT AND DARK PERIOD

Photobioreactor can be illuminated either internally or externally from outside the reactor. However, all illumination sources provide excessive light in areas close to the light source and insufficient light for photosynthesis at distances away from the light source. Light dispersion is not uniform (Molina et al., 1999) so that cells near to the light source receive more light than
those farther away (Frohlich et al., 1983). The cells themselves physically limit light dispersion, producing shadows which block light transmission to other cells in the reactors and this reduces photosynthesis (Ree and Gotham, 1981; Laws, 1980; Bannister, 1979; Tamiya et al., 1953). A mathematical model that calculates the proportions of light vs dark zones in an algal reactor culture concluded that the intensity of stirring does not significantly affect the proportion of non-illuminated zones (Livasnsky, 1979). Therefore biomass production would not likely be significantly enhanced by stirring under light limiting conditions.

Since the reactions of light and dark photosynthesis occur continually, light can interfere with the dark process of photosynthesis. Because of this, a more favourable growing condition is possible when there is an alternation between periods of light and dark, and optimal light-dark frequencies between 3 and 25 Hz have been reported (Perner-Nocha and Posten, 2007). Several studies of altered light-dark frequencies have shown increased algal growth (Richmond et al., 2003; Jannsen et al., 1999; Merchuck et al., 1998; Terry, 1986; Laws et al., 1983; Lee and Pirt, 1981).

Solar irradiation intensity can be as high as 2800 µmol m\(^{-2}\) s\(^{-1}\) which greatly exceeds the light saturation point for algae photosynthesis of 50-300 µmol m\(^{-2}\) s\(^{-1}\) (Kim et al., 2006; Degen et al., 2001; Merchuk et al., 1998). The flashing light technique has been suggested to improve light utilization for outdoor algae culture (Terry, 1986). Under photoautotrophic growth the flashing light frequency should be as high as possible for increased cell growth (Hu et al., 1996b).

Light utilization in flashes has been studied for algal photosynthesis with special emphasis on flashing periods, the time interval between flashes, of less than 1 s and 100 ms (Terry, 1986; Phillips and Myers, 1954). Flashing light periods at high frequencies (0.034 Hz or more) increase the efficiency of the photosynthetic process (Sato et al., 2006). At these light flashing frequencies and under optimal illumination intensities of 300 µmol m\(^{-2}\) s\(^{-1}\) to 1200 µmol m\(^{-2}\) s\(^{-1}\), Sato et al. (2010) reported biomass production with *Chaetoceros calcitrans* and *Chlorococum*...
littorale of 266 mg L⁻¹ d⁻¹ and 146 mg L⁻¹ d⁻¹, respectively. Flashing lights at frequencies of 0.001-200 Hz and light intensities of 12-18 µmol m² s⁻¹ were used for *Haematococcus pluvialis* production and the results show that astaxanthin concentrations were higher in cultures exposed to flashing light frequencies higher than 1 Hz compared to algae growing under continuous light (Katsuda et al., 2008). See Table 1.2 for studies conducted with algae growing under flashing light treatments.

Table 1.2. Studies with algae under flashing lights.

<table>
<thead>
<tr>
<th>Algal specie</th>
<th>Min (µmol m⁻² s⁻¹)</th>
<th>Max (µmol m⁻² s⁻¹)</th>
<th>Flash light (Hz)</th>
<th>Light/Dark ratio</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chlorella sp</em></td>
<td>0</td>
<td>750</td>
<td>0.3-0.0074</td>
<td>1/1.0</td>
<td>Grobbelaar. 1991</td>
</tr>
<tr>
<td><em>Scenedesmus sp</em></td>
<td>0</td>
<td>750</td>
<td>0.8-0.0038</td>
<td>1/1.0</td>
<td>Grobbelaar. 1991</td>
</tr>
<tr>
<td><em>Chlamydomonas reinhardtii</em></td>
<td>100</td>
<td>240</td>
<td>0.01</td>
<td>1/3/8.7</td>
<td>Janssen et al., 1999</td>
</tr>
<tr>
<td><em>Chlorella sorokiniana</em></td>
<td>240</td>
<td>630</td>
<td>0.025</td>
<td>3/1.0</td>
<td>Janssen et al., 1999</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>2</td>
<td>12</td>
<td>25-200</td>
<td>1/1.0</td>
<td>Katsuda et al., 2006</td>
</tr>
<tr>
<td><em>Phaeodactylum tricornutum</em></td>
<td>22</td>
<td>700</td>
<td>0.5-1</td>
<td>1/10.0</td>
<td>Laws et al., 1983</td>
</tr>
<tr>
<td><em>Chlorella pyrenoidosa</em></td>
<td>80</td>
<td>168</td>
<td>2.5-25 kHz</td>
<td>1/10.0</td>
<td>Matthijs et al., 1996</td>
</tr>
<tr>
<td><em>Phaeodactylum tricornutum</em></td>
<td>230</td>
<td>2800</td>
<td>1-1.55</td>
<td>1/1.0</td>
<td>Molina Grima et al., 2000</td>
</tr>
<tr>
<td><em>Chlorella kessleri</em></td>
<td>280</td>
<td>280</td>
<td>1-37 kHz</td>
<td>1/1 to 1/20</td>
<td>Park and Lee. 2000</td>
</tr>
<tr>
<td><em>Chlorella kessleri</em></td>
<td>50</td>
<td>78</td>
<td>10-50 kHz</td>
<td>1/1 to 1/10</td>
<td>Park and Lee. 2001</td>
</tr>
<tr>
<td><em>Phaeodactylum tricornutum</em></td>
<td>68</td>
<td>68</td>
<td>0.01-10</td>
<td>1/1.0</td>
<td>Walsh and Legendre. 1988</td>
</tr>
<tr>
<td><em>Chaetoceros calcitrans</em></td>
<td>10</td>
<td>188</td>
<td>0.1-10</td>
<td>1/5 to 1/20</td>
<td>Yoshimoto et al., 2005</td>
</tr>
</tbody>
</table>

LED technology can provide light at a specific wavelength with 50-95% of the total light emitted at that wavelength (Matthijs et al., 1996). Therefore, the use of LEDs for growing algae indoors has increased due to their much lower energy consumption compared to fluorescent and incandescent lamps. Matthijs et al. (1996) was able to grow *Chlorella pyrenoidosa* using red LEDs, with a peak emission at 659 nm, and an alternating light period of 5 µs and dark period of 45 µs (a light/dark ratio of 1/10) under flashing conditions of 20 kHz.

There is evidence of quantum absorbing memory in algal photosynthesis. In an early study conducted with *Chlorella pyrenoidosa*, a light flash for 1 ms at 61 µmol m² s⁻¹ supported photosynthesis for 20 ms (Philiph and Myers. 1954). More recently, studies have shown that the...
energy stored by *Chlorella vulgaris* in a flash of 500 ms and at a light intensity of 58-73 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) was sufficient to support photosynthesis (assimilating CO\(_2\) and releasing O\(_2\)) at maximum rate for 9.2 ms (Lee and Pirt, 1981; Pirt, 1986). Table 1.3 shows a summary of optimal light intensities for algal growth.

### Table 1.3. Optimal light intensity for growing algae.

<table>
<thead>
<tr>
<th>Algal specie</th>
<th>Min</th>
<th>Max</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chlorella pyrenoidosa</em></td>
<td>50</td>
<td>200</td>
<td>Ogbonna et al., 1999.</td>
</tr>
<tr>
<td><em>Chlorella pyrenoidosa</em> (outdoor)</td>
<td>200</td>
<td>450</td>
<td>Ogbonna et al., 1999</td>
</tr>
<tr>
<td><em>Euglena gracilis</em></td>
<td>50</td>
<td>450</td>
<td>Obbonna et al., 2001</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>10</td>
<td>250</td>
<td>Degen et al., 2001</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em> (Outdoor)</td>
<td>80</td>
<td>980</td>
<td>Degen et al., 2001</td>
</tr>
<tr>
<td><em>Chlamydomonas reinhardtii</em></td>
<td>60</td>
<td>200</td>
<td>Kim et al., 2006</td>
</tr>
<tr>
<td><em>Chlamydomonas reinhardtii</em></td>
<td>200</td>
<td>300</td>
<td>Kim et al., 2006</td>
</tr>
<tr>
<td><em>Phaeodactylum tricornutum</em></td>
<td>230</td>
<td>250</td>
<td>Fernández et al., 2003</td>
</tr>
<tr>
<td><em>Phaeodactylum tricornutum</em> (outdoor)</td>
<td>800</td>
<td>1100</td>
<td>Fernández et al., 2003</td>
</tr>
<tr>
<td><em>Porphyridium cruentum</em> (outdoor)</td>
<td>1600</td>
<td>2000</td>
<td>Rebolloso et al., 1999</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>3.8</td>
<td>12</td>
<td>Lababpour et al., 2004</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>3.8</td>
<td>12</td>
<td>Lababpour et al., 2005</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>2</td>
<td>12</td>
<td>Katsuda et al., 2006</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>21</td>
<td>49</td>
<td>Ranjar et al., 2008</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>23</td>
<td>60</td>
<td>Garcia-Malea et al., 2005</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>350</td>
<td>2500</td>
<td>Garcia-Malea et al., 2005</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em> (Inside the culture)</td>
<td>70</td>
<td>130</td>
<td>Garcia-Malea et al., 2006</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em> (outdoor)</td>
<td>50</td>
<td>2000</td>
<td>Garcia-Malea et al., 2006</td>
</tr>
<tr>
<td><em>Spirulina platensis</em></td>
<td>300</td>
<td>3000</td>
<td>Wang et al., 2007</td>
</tr>
<tr>
<td><em>Nannochloropsis sp</em></td>
<td>100</td>
<td></td>
<td>Zittelli et al., 1999</td>
</tr>
</tbody>
</table>

#### 1.1.5.2 PHOTOINHIBITION IN ALGAE

High light emission intensities can cause photoinhibition, thereby reducing algal growth rate. In a study conducted in the outdoors with *Porphyridium cruentum* it was observed that the photosynthetic activity showed variation during the day period. The lowest photosynthetic
activity was observed between 12:00 and 2:00 pm, when solar radiation is usually highest (Rebollos Fuentes et al., 1999).

Studies of the biomass production of the marine microalgae *Isochrysis galbana* under light intensities ranging from 820 to 3270 µmol m$^{-2}$ s$^{-1}$ showed a peak algal growth rate at 1630 µmol m$^{-2}$ s$^{-1}$. Above this light intensity, biomass production decreased due to photoinhibition (Molina-Grima et al., 1996). While biomass production of 19.2 mg L$^{-1}$ h$^{-1}$ at a light intensity of 3270 µmol m$^{-2}$ s$^{-1}$ was higher than that at 820 µmol m$^{-2}$ s$^{-1}$ (16.6 mg L$^{-1}$ h$^{-1}$), quantum yields decreased above light intensities of 820 µmol m$^{-2}$ s$^{-1}$ (Molina-Grima et al., 1997). See Table 1.4 for quantum yield absorption under different light intensities.

**Table 1.4.** Quantum yield in photon absorption by biomass under different photon flux.

<table>
<thead>
<tr>
<th>Light intensity (µmol m$^{-2}$ s$^{-1}$)</th>
<th>Quantum yield (g biomass/energy mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>820</td>
<td>0.603</td>
</tr>
<tr>
<td>1620</td>
<td>0.428</td>
</tr>
<tr>
<td>3270</td>
<td>0.087</td>
</tr>
</tbody>
</table>

*Source: Molina-Grima et al., 1997*
1.1.5.3 WEIGHT LOSS DURING NIGHT PERIOD

Growing algae in controlled environments has problems related to the day and night cycles as well as to variations of the light intensity during the day cycle. During night, photosynthetic cells switch their biochemistry from energy production autotrophic growth to energy consumption heterotrophic growth (Ogbonna et al., 1999). Consumption of this energy reserve during the night has been reported to result in a cell body weight loss of 17% in Chlorella pyrenoidosa (Ogbonna and Tanaka, 1996), and can represent up to 35% of the entire weight that is produced during the day photosynthetic period (Torzillo et al., 1991a; Torzillo et al., 1991b; Grobbelarar and Soeder, 1985). Experiments conducted with Spirulina platensis have shown that biomass losses during the night period ranged from 5 to 10% and which represented losses of 16-32% of the biomass daily production. These biomass losses are affected by growth temperature and light irradiance during the day growth (Torzillo et al., 1991).

1.1.6 ALGAE GROWTH UNDER ARTIFICIAL AND OUTDOOR CONDITIONS

1.1.6.1 GROWTH UNDER INDOOR ARTIFICIAL CONDITIONS

Open ponds and photobioreactor is the most implemented technology to growth algae (Olivieri et al., 2013). Algae can be grown under indoor artificial conditions using open ponds in greenhouse, tubular bubble column, continous stirred tanks, horizontal tubular and flat panel photobioreators. Studies with the algal Chlorella vulgaris growing in tubular bubble column and stirred tanks has produced standing biomass of 0.6-0.7 g L\(^{-1}\) (Sacasa-Castellanos, 2013). The algae Spirulina platensis grown indoors in a horizontal tubular photobioreactor with a nutrient media flow of 21 cm s\(^{-1}\) and illumination of 120 µmol m\(^{-2}\) s\(^{-1}\) produced a standing biomass of 10 g L\(^{-1}\) in 19 d. The same algal specie growing in an indoor open pond under similar conditions attained a standing biomass of only 1.5 g L\(^{-1}\) in 20 d (Converti et al., 2006).
1.1.6.2 GROWTH UNDER OUTDOOR CONDITIONS

Under outdoor conditions solar quantum yields during photosynthesis are less than 5%, and the maximum biomass production is 30-40 g m$^{-2}$ day$^{-1}$ or 2-2.7 g L$^{-1}$ day$^{-1}$ (Goldman, 1979). Experiments conducted during the summers 2003 and 2004 under outdoor conditions with the algal *Tetraselmis suecica* attained biomass production of 0.49-0.56 g L$^{-1}$ d$^{-1}$ (Chini Zittelli et al., 2006). Further experiments conducted with *Nannochloropsis sp* during the summer 1997 under outdoor conditions reported biomass production of 0.51-0.76 g L$^{-1}$ d$^{-1}$ (Chini Zittelli et al., 1999). Outdoor studies with the blue-green algae *Anabaena variabilis* (cyanobacteria) obtained biomass production during winter of 0.40 g L$^{-1}$ d$^{-1}$ (6 g m$^{-2}$ d$^{-1}$) and 1.13 g L$^{-1}$ d$^{-1}$ (17 g m$^{-2}$ d$^{-1}$) in summer, with photosynthetic activity occurring to 35 cm depth and temperatures controlled at 30-35°C (Fontes et al., 1987). Most recent outdoor experiments with *Tetraselmis suecica* and *Chlorella sp* have obtained productivity of 0.18 and 0.24 g L$^{-1}$ d$^{-1}$ respectively (Moheimani, 2013).

1.1.7 COMMERCIAL PRODUCTION OF ALGAE

There has been commercial production of microalgae since 1950 in Taiwan and Japan (Sivasubramanian et al., 2010; Muthukumaran et al., 2012). By 1997 there were approximately 110 commercial enterprises of microalgae in the Asia Pacific region and the most common algae produced were *Chlorella sp*, *Nannochloris sp*, *Dunaliella sp*, and *Spirulina sp*. (Lee, 1997). In 1996 the annual consumption of *Chlorella sp* in Japan was 2000 T of which 1057 T were produced locally and 943 T were imported (Lee, 1997).

The most common uses of *Chlorella sp* biomass are as food supplements and in the comestic and pharmaceutical industries (Sankar and Ramasubramanian, 2012); another significant use is oil production for biofuels (Lyon et al., 2013). The top four commercial species of algal biomass are 3000 T for *Spirulina sp*, 2000 T for *Chlorella sp*, 1200 T for *Dunaliella salina*, and 500 T for *Aphanizomenon sp* (Priyadarshani and Rath, 2012). Another important species is the marine alga *Nannochloropsis salina*, which has been considered for biodiesel production because its oil production can exceed 50% of the body mass (Silva et al., 2014).
Production of microalgal biodiesel has the potential to replace other sources of diesel fuel without using soils destined for agriculture (Rajvanshi and Sharma, 2012). The world’s algae production for biofuels can be divided in regions; 78% USA, 13% Europe and 9% in the rest of the world. The most implemented technology is the closed bioreactor at 52% followed by open ponds at 26%; only 22% of the production is grown under natural environments (Gendy and El-temtamy, 2013). Some commercial companies that produce algae for biofuel in the USA are Greenfuel Technologies Corporation, Solazyme, Livefuels, Solix Biofuels, and Inventure Chemical (Gao et al., 2012).

1.1.8 ALGAE USED DURING THE COURSE OF THIS RESEARCH

This research examined the growth of three marine algae and three fresh water algae. Detailed taxonomy of these algae is reported in Table 1.5.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Classification of the algae studied in this research</th>
<th>Marine algae *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domain Eukaryota</td>
<td>Eukaryota</td>
<td>Eukaryota</td>
</tr>
<tr>
<td>Kingdom Protista</td>
<td>Plantae</td>
<td>Plantae</td>
</tr>
<tr>
<td>Division Chlorophyta</td>
<td>Chlorophyta</td>
<td>Chlorophyta</td>
</tr>
<tr>
<td>Class Trebouxiophyceae</td>
<td>Chlorophyceae</td>
<td>Chlorophyceae</td>
</tr>
<tr>
<td>Order Chlorellales</td>
<td>Sphaeropleales</td>
<td>Chlamydomonadales</td>
</tr>
<tr>
<td>Family Chlorellaceae</td>
<td>Scenedesmaceae</td>
<td>Chlamydomonadaeae</td>
</tr>
<tr>
<td>Genus Chlorella</td>
<td>Scenedesmus</td>
<td>Chlamydomonas</td>
</tr>
</tbody>
</table>

Source: Arora et al., 2013; Assuncao et al., 2012; Huss et al., 1999; Krienitz et al., 1996.

The algae used during this research have different size and shape, Figure 1.1 shows the features of these algae.
1.2 FORMAT OF THE THESIS

The thesis is contained in 10 chapters: Chapter one is the introduction for the thesis and chapter two states the objectives and goal of the research. Chapter three contains the literature review including the evolution of the photosynthetic machinery anoxygenic, oxygenic, different types of photosynthetic organisms, as well as a detailed description of the architecture and function of PSII and PSI. Chapter four describes the growth of two marine and two fresh water algae under solar radiation, white light, blue LED light and red/blue LED light and the combination of the treatments to determine the optimal light treatment to support algae growth. Chapter five discusses further research in light treatments versus biomass production that combines 12 hours solar radiation against 24 hours red/blue LED and 12 hours solar plus 12 hours red/blue LED in six different algae species. Chapter six describes a study with *Chlorella vulgaris* under four different 24 hours LED light treatments; white, red, blue and red/blue we measured population density and biomass production. Chapter seven provides an artificial illumination experiment using light and dark periods in milliseconds: the flashing effect was used to growth one marine and one fresh water algae against the same algae under continuous red/blue LED. Chapter eight
consists in a group of experiments that measures different CO₂ concentration in the growth and biomass production of \textit{Chlorella vulgaris}, the goal of the experiment is to determine if CO₂ concentration in the air supply affects algae biomass production. Chapter nine describes the study of two marine and two fresh water algae under a flat panel reactor using natural light during day periods and artificial light during night periods, these study also measured the effect of CO₂ concentration and biomass production in an industrial scale. Chapter ten presents the overall conclusions of all six different studies in algae.
1.3 REFERENCES


CHAPTER 2 RESEARCH OBJECTIVES

2.1 GENERAL GOAL

The goal of this thesis research is to study the critical factors affecting the growth of marine and fresh water microalgae in a bench-top and pilot-scale. Factors studied were light source and intensity, CO₂ supply concentration and bioreactor design. Algal growth was accessed by measurements of growth rate, biomass production, cell size and cell weight. We also constructed and tested a large-scale flat panel photo-bioreactor that overcomes some of the biomass production limitations common to bubble column photo-bioreactors.

2.2 SPECIFIC RESEARCH OBJECTIVES

1. To measure growth of marine and fresh water algae under different light sources: solar, white, red, blue and the combination red-blue.
2. To evaluate the flashing light effects on the growth and biomass production of marine and fresh water algae.
3. To measure the effects of increasing CO₂ concentrations on algal growth kinetics and biomass production.
4. To design and test a flat panel photo-bioreactor for biomass synthesis using marine and fresh water algae.
CHAPTER 3  THE PHOTOSYNTHETIC MACHINERY: A DETAILED DESCRIPTION OF THE LIGHT REACTION; PSI, PSII, ATP SYNTHASE, STATE OF TRANSITION, PHOTO-DAMAGE, REGENERATION, AND MACHINERY ARCHITECTURE

3.1  ABSTRACT
The overall photosynthetic efficiency from solar radiation is in the range of 4-6%, this electromagnetic energy hits and energizes the antenna of photosynthetic organisms. The antenna pass the energy as vibrational energy from chlorophyll to chlorophyll until reaches the reaction center (RC), inside the reaction center the special pair of chlorophyll receive this energy and send it into an electron that passes from a ground state to an excite state. This electron leaves the reaction center using the mobiles carrier; via plastoquinone b, plastocyanin and cytochrome b6f in PSII. In PSI the route is via phylloquinone, ferredoxin, ferredoxin-NADP-reductase known as FNRI. In anoxygenic photosynthesis in the purple bacteria the route is from the reaction center PSII passing through Bacterio-Pheophytin-plastoquinone Qa to cytochrome bc1. The evolution of photosynthesis has changed from one that was anoxygenic and energized the stage PSII complex with a reverse electron transfer to produce NADPH, as in purple sulfur and non-sulfur bacteria, to only one energized state in green sulfur bacteria ending with oxygenic photosynthesis with two energize stages consisting in PSII and PSI, as found in cyanobacteria, algae and all plants including single cell and multicellular structures.

GLOSSARY

Anoxygenic photosynthesis: Photosynthesis that is performed in the absence of oxygen. The process requires light, a source of carbon (CO₂, acetate, lactate or pyruvate), an electron donor (H₂ or H₂S). In some cases water is produced as a byproduct. It is performed by bacteria and heliobacteria.
**Rhodopsin photosynthesis:** Photosynthesis performed under aerobic or anaerobic conditions; light absorption is driven by the protein called rhodopsin. It is performed by Archaea and Bacteria.

**Oxygenic photosynthesis:** Photosynthesis in the presence of oxygen. The process requires light, a source of carbon (CO₂), an electron donor (H₂O), and produces oxygen as byproduct. It is performed by cyanobacteria, algae and plants.

**Photosystem I (PSI):** Secondary machinery that energized the electron for the second time during oxygenic photosynthesis to produce NADPH.

**Photosystem II (PSII):** Primary machinery in oxygenic photosynthesis that splits the water molecule and energizes the electron from water to produce ATP.

**Light harvesting complex (LHCl, LHClI, or Antennae):** Structure that absorbs electromagnetic energy, converts this energy into vibrational energy and transfers the energy to the reaction center (RC).

**Reaction center (RC):** Protein structure where the electron is energized during photosynthesis for the production of ATP or NADPH.

**Water oxidation complex (WOC) and Oxygen evolution center (OEC):** structures where water is split and oxygen produce during oxygenic photosynthesis in PSII.

**D1 and D2 proteins:** Major proteins of PSII where the RC, pheophythin D1-D2, WOC, OEC, and other important structures are located including the plastoquinones Qa and Qb (mobile carriers that pass the energized electron to cytochrome b6f).

**PSaA and PSaB:** Major proteins of PSI where the RC, special pair of chlorophylls, Chlorophyll A0a, A0b and other structures are located.

**Light independent reaction (Calvin cycle):** In oxygenic photosynthesis this process fixes CO₂ and uses the energy from ATP and NADPH to synthetize G3P (Glyceraldehyde 3 phosphate), that is the precursor of glucose.

### 3.2 INTRODUCTION

The amount of solar radiation entering the earth’s atmosphere is ±1376 W m⁻² and at the earth’s surface on the equator is ± 1000 W m⁻² (Kruse et al., 2005). However, the total energy available for the photosynthetic activity of plants, cyanobacteria and algae is only 43% of this (430 W m⁻²), with 5% in the ultraviolet region and 52% in the infrared region. Part of the energy in the infrared region can be used in anoxygenic photosynthesis so that purple and green bacteria using
440 W m$^{-2}$ (Kruse et al., 2005). The efficiency of intercepted light by the photosynthetic pigments ranges from 6 to 11%, the overall efficiency considering 76% of interception is 4-8% (Wilson et al., 1991). Other estimates of the net photosynthetic efficiency are in the range of 4-6% (Zhu et al., 2010; Zhu et al., 2008). Mejia (2006) calculated an overall photosynthetic efficiency of 6.6% with an energy losses of 93.4%.

In photo-autotrophs, photosynthesis can operate in all earth environments where solar radiation is present, in oceans, lakes, land and soil, and in aerobic or anaerobic environments. The photosynthetic organisms are divided into oxygenic, anoxygenic and rhodopsin belonging the the archaea and bacteria domain (Staley, et al 2007). The most common photosynthetic organisms are aerobic which carried out oxygenic photosynthesis. In general photosynthesis captures light energy and converts this energy to oxidise water, reduce ADP to ATP and NADPH synthesis, as well as for O$_2$ production by splitting water. The entire system is based on the necessity for an inorganic carbon source, CO$_2$. The ATP and NADPH are used to synthetize G3P in the light independent reaction (Ferreira et al., 2004).

Oxygenic photosynthesis is performed by eukaryotic organisms such as plants, multicellular algae, single cell algae and bacteria like cyanobacteria. In oxygenic photosynthesis H$_2$O is used as an electron donor, CO$_2$ and the H$_2$ from the split of H$_2$O are used as electron acceptors in the formation of glyceraldehyde-3-phosphate (G3P) during the Calvin cycle. This process also produces O$_2$ during the splitting of H$_2$O in the light reactions, and therefore is called oxygenic photosynthesis. (Staley et al., 2007; Brock 1979).

Anoxygentic photosynthesis is carried out in a non-oxygen environment by a group of microorganisms called purple non-sulfur bacteria, green and purple sulfur bacteria, and by the heliobacteria. In anoxygenic photosynthesis H$_2$ or H$_2$S are the electron donors; they are oxidised and CO$_2$ is reduced during the Calvin cycle to form G3P, and H$_2$O is produced as a by-product instead of O$_2$. Anoxygentic photosynthesis by heliobacteria (bacteriochlorophyll g 788 nm) does not use CO$_2$ as carbon sources but uses monomers such as acetate, lactate and pyruvate. These bacteria grow in soils and do not grow photoautotrophically. There is also a group of organisms belonging to the archaea and bacteria domains that perform photosynthesis in aerobic and anaerobic environments. (Staley et al., 2007; Brock 1979).
Rhodopsin photosynthesis do not uses carotenoids neither chlorophyll nor bacterio-chlorophyll, to absorb light for photosynthesis. The photosynthetic machinery have Bacteriorhodopsin with absorption peak of 570 nm. These types of photosynthetic organisms do not have an electron transport chain to produce NADPH during the light reaction, but use bacteriorhodopsin molecules to create the proton gradient necessary to run the ATP synthase which makes ATP (Staley et al., 2007; Brock 1979).

3.3  EVOLUTION OF PHOTOSYNTHESIS: ANOXYGENIC AND OXYGENIC

The first photosynthetic organisms on earth appeared 3-3.5 billion years ago, and were anoxygenic organisms from the bacteria and archaea domains. These microorganisms included the purple and green sulfur and nonsulfur bacteria and those possessing rhodopsin photosynthesis, the organisms inhabited the anaerobic environment present on earth at that time. The first oxygenic organisms to evolve were from the cyanobacteria group, and appeared 2.6-2.8 billion years ago during the Proterozoic period (Staley et al., 2007). These cyanobacteria had the ability to survive in an anaerobic environment even though they produced O₂. All of the O₂ produced at that time oxidised Fe²⁺ to Fe₂O₃, a process that took place over 0.5 to 1 billion years. Oxygen began to accumulate and the ozone layer to appear in the atmosphere 2 billion years ago. There was an evolution among the purple sulfur bacteria and the green sulfur bacteria; the purple bacteria used a reverse electron transfer reaction to capture sufficient energy to synthetize NADPH; the green sulfur bacteria had a bacteriochlorophyll that allowed them to harvest energy to produce ATP and NADPH without using the reverse electron transfer reaction (Staley et al., 2007).

The photosynthetic machinery of the anoxygenic photosynthesis bacteria is much less complex than that of oxygenic photosynthesis. For example, purple bacteria has the same mechanisms to produce ATP as cyanobacteria with different photosynthetic pigments, however the purple sulfur and nonsulfur bacteria use the reverse electron transfer reaction to synthetize NADPH. The green sulfur bacteria and cyanobacteria do not need to use this mechanism because both have evolved to harvest sufficient energy to produce ATP and NADPH. Cyanobacteria use a two step electron
excitation, known as photosynthesis II (P 680) to produce the proton gradient for ATP synthesis, and photosynthesis I (P 700) to produce the remaining energy needed to synthetize NADPH; this represents an evolution from the purple bacteria (Staley et al., 2007).

The Eukarya domain appeared 600 million of years ago, and the photosynthetic organism that we know today (single cell algae, multicellular algae and plants) evolved from a symbiosis of eukaryotic primitive cells and cyanobacteria. It has been hypothesized that cyanobacteria enter to the cell and transformed into what we know today as chloroplast. Evidence from this symbiotic union comes from the 16S rRNA analysis which shows that chloroplast has their own DNA different from the eukaryotic cell’s nucleus. The chloroplast ribosome DNA is 70s which is a similar type of DNA found in cyanobacteria and all bacteria domain, besides chloroplast organelles have DNA with no nucleus envelope like prokaryotic cells. Another evidence is that a typical ribosome DNA from 16S rRNA in eukaryotic cells is 80S. In chloroplast the internal membranes containing the chlorophyll molecules, is called chlorosomes and they are found in algae and in plants cells with another name thylakoid disk. All this evidence show that chloroplast are an evolution of the cyanobacteria group and primitive eucaryotic cells. Chloroplast uses the same chlorophyll found in cyanobacteria; Chlorophyll a antenna and chlorophyll a reaction center to gather energy to excite an electron that goes in an electron transport chain to produce ATP and NADPH, this system is similar in cyanobacteria, algae and plants. The only difference is the size of the cyanobacteria cells which are much smaller than plants and algae cells. These evidence lead to the hypothesis that chloroplast one day were free living cyanobacteria and the symbiosis between eukaryotic organisms with cyanobacteria allow eukaryotic cells to have a photosynthetic mechanisms to produce energy from inorganic compounds. This explain why cyanobacteria and all the photosynthetic eukaryots perfomer the same photosynthetic mechanisms pathways (Staley et al 2007).

There is a special pair of chlorophylls in the reaction center in most of the photosynthetic organisms, the function of the reaction center is to charge an electron using the energy from the antenna, and to donate this excited-state electron to the pheophytin protein to drive the production of ATP and NADPH (Barber and Archer, 2001). The evolution of photosynthesis can be traced with the boost in the oxidation potential of the reaction center. The special pair of
chlorophyll in the reaction center of anoxygenic photosynthesis in green sulfur bacteria which is binding P840 has an oxidation potential of 0.4 V and in purple non sulfur bacteria the special pair of chlorophylls binding P870 has an oxidation potential of 0.45 V. The special pair is also present in oxygenic photosynthesis in the core of the reaction center of PSI binding the two P700 and has an oxidation potential of 0.49 V. In contrast the reaction center of PSII contains a tetramer of 4 chlorophylls known as P680, with an oxidation potential of 1.17V. Both PSI and PSII systems are present in oxygenic photosynthesis in plants, algae and cyanobacteria (Barber and Archer, 2001).

3.3.1 ANOXYGENIC PHOTOSYNTHESIS
Anoxygenic photosynthesis is carried out by a domain of bacteria groups which include, the purple nonsulfur bacteria, the purple sulfur bacteria, the green sulfur bacteria and the Heliobacteria. These bacteria use bacteriochlorophyll (a, b, c, d, e and g, depending of the bacteria group) as the main light harvesting molecules and uses complimentary pigments, such as carotenoids, to transfer the harvested energy to the bacteriochlorophyll. The bacteria have chlorosomes where the light gathering system is located, the counterpart in plants are the thylakoids located in the chloroplast where the light reaction and light independent reaction take place (Staley et al., 2007).

3.3.1.1 THE PURPLE NON-SULFUR BACTERIA
The purple nonsulfur bacteria are found in lakes, sediments and ponds. They use hydrogen as an electron donor (not water), and use the electrons from hydrogen to create a proton gradient similar to the cyanobacteria in oxygenic photosynthesis. This proton gradient is used to drive ATP synthase for production of ATP and these bacteria use a reverse electron transfer to produce NADPH. The light independent reaction reduces CO₂ to synthesize carbohydrates, however, these bacteria are capable of using several pathways for carbohydrate production, one being the Calvin cycle, however this pathway is not the main one as it is in oxygenic photosynthesis. During anoxygenic photosynthesis water is synthesised as by-product (Staley et al., 2007).
3.3.1.2 THE GREEN AND PURPLE SULFUR BACTERIA
The purple sulfur bacteria are similar to the purple nonsulfur bacteria: they use the same bacteriochlorophyll a and b. However, the purple sulfur bacteria use hydrogen sulfide as an electron donor and accumulate elemental sulfur in granules inside the cell, and is able to tolerate high levels of sulfur that are otherwise toxic for the purple nonsulfur bacteria. In contrast, the green sulfur bacteria possess a different type of bacteriochlorophyll c, d and e and another type of carotenoids as complementary pigments. Pigments such as chlorobactene and isorenieratene allow these bacteria to harvest more energy, and for this reason these type of bacteria do not require the reverse electron transfer to produce NADPH which is characteristic of purple bacteria. Another major difference is that the sulfur residues in the green sulfur bacteria are synthesized outside the cell and not inside the cell like in purple bacteria (Staley et al., 2007).

3.3.1.3 HELIOBACTERIA
The heliobacteria are gram positive bacteria found in soils and contains a bacteriochlorophyll g (788 nm) that has some similarities with the chlorophyll a present in plants. These bacteria do not have chlorosomes and the light gathering system is attached to the cytoplasmic membrane. These bacteria do not fix CO₂ but use simple organic compounds (acetate, lactate and pyruvate) thus perform photoheterotrophic growth, as carbon sources (Staley et al., 2007).

3.3.1.4 RHODOPSIN PHOTOSYNTHESIS
Rhodopsin is the type of photosynthesis present in the Archaea and bacteria domains growing under environmental conditions where there can be both aerobic and anaerobic conditions. Their photosynthetic machinery contains only bacteriorhodopsin (absorption peak 570 nm) with no accessory pigments (they are the only photosynthetic organisms which do not have complementary pigments). These organisms do not have an electron transport chain as do other photosynthetic organisms, but have a light-powered proton pump which produces the proton gradient necessary to drive ATP synthase (Staley et al., 2007).
3.3.2 OXYGENIC PHOTOSYNTHESIS

The oxygenic photosynthesis mechanism provides most of the chemical energy essential for living organisms. For eukaryotic organisms it includes plants, multicellular algae and microscopic algae, and for bacteria includes the cyanobacteria group. The architecture of photosynthesis in plants, algae and cyanobacteria is formed by two states of energy harvest: photosystem I and II. Photosystems I and II share similar architecture in which both have a light harvesting complex, known as antennas, containing chlorophyll and complementary pigments.

Each antenna has a reaction center (RC). In the antenna the photosynthetic pigments absorb electromagnetic wavelengths in the visible spectrum and transfer this vibrational energy to the RC. The vibrational energy charges an electron from a ground state to an excited state, and it then moves through an electron transfer chain, releasing its free energy to produce ATP and NADPH (Yan et al., 2003).

In oxygenic photosynthesis both eukaryotes and bacteria use chlorophyll as the main molecule to harvest light and convert it to chemical energy. Both domains also use accessory pigments to harvest light and to pass the energy to the chlorophyll a antenna also known as the chlorophyll a RC. The complementary molecules are carotenoids, carotenes, xanthophylls, phycobillins and their subdivisions (Campbell et al., 1999).

During oxygenic photosynthesis, H₂O is oxidised (split into H⁺ + H⁺ + O⁻₂, oxygen is released as O₂); the two electrons from water are energized twice, the first in the photosynthesis II complex to produce the proton gradient that generates ATP; the second is in the photosynthesis I complex to energize the ferredoxin NADP⁺ reductase (FNR) producing NADPH from NADP⁺ + P. These transformations are known as the light reaction and involve photosynthesis II (P680), photosynthesis I (P700), cytochrome b₆f, ferredoxin NADP reductase (FNR), ATP synthase and the mobile plastoquinones Qa and Qb, plastocyanin, and ferredoxin (Campbell et al., 1999).

The photosystem I with P700 chlorophyll, discovered in 1957, has a signal formation of 10 ns and flash decay time lasting 10-20 ms. PSII with P680 chlorophyll was discovered more recently.
as the flash decay lasted just 0.1-0.2 ms and was considered P700 noise in earlier studies. The absorption peaks for P700 are 438 and 700 nm, whereas they are 435 and 680 nm for P680. The oxidation potential of P680 is 1.17 V, which is greater than that necessary for the oxidation of water (1.0 V). P700 has an oxidation potential of 0.49 V, chlorophyll a 0.78 V and carotenoid 1.06 V (Barber and Archer, 2001).

There is an additional reaction occurring during photosynthesis that is independent of the light reaction and is known as the Calvin cycle. It uses 6 ATPs and 6 NADPHs to fix 3 CO₂ molecules and to produce 1 molecule of glyceraldehyde 3 phosphate (G3P). The Calvin cycle also requires an extra 3 ATPs to regenerate the ribulose bisphosphate (RuBP) that is the CO₂ acceptor. In total 9 ATPs and 6 NADPHs are used (Campbell et al., 1999).

The photosynthesis process has stabilized the fleeting charge separated state of energy into a controlled chemical redox state thereby transforming electromagnetic radiation into chemical energy. PSI in combination with PSII transfers the excited state electron from cytochrome C6 or plastocyanin to ferredoxin and to ferredoxin NADP reductase to store the energy for generating ATP by the PSII reaction whereas the energy required to synthesize NADPH from NADP is from PSI. The free energy stored as ATP and NADPH are used to produce G3P during the light independent reaction and this is the main product of photosynthesis (Di Donato et al., 2011; Lubner et al., 2011). The PSI is located in the stroma lamellae and PSII is located in the grana stacks. The light independent reaction takes place in the stroma of the chloroplast (Haldrup et al., 2001).

3.3.3 ARCHITECTURE OF THE PHOTOSYNTHETIC APPARATUS IN PLANTS AND ALGAE

The photosynthetic apparatus in eukaryotic cells such as plants and algae is located in a structure called the chloroplast. Inside the chloroplast is an aqueous fluid, called stroma, and an arrangement of disks of 300-600 nm diameter size. These disks are called thylakoids and they have an aqueous solution called lumen. The thylakoids are arranged in stacks of disks called grana, and the network between grana is called stroma lamellae or lamella (Kouril et al., 2012). See Figure 3.1 for details of chloroplast architecture.
The photosynthetic apparatus is located in the thylakoid membrane, and the cytochrome 6bf is located between the two membranes grana and stroma. PSI and PSII are located in the grana where 80% of the photosynthetic pigments are present and carry out processes in the electron transport chain. PSI and PSII are also present in the stroma lamellae where 20% of the total photosynthetic pigments and activity are organized in a cyclic electron transport chain. PSI has 57-60% of the total photosynthetic pigments, therefore has the ability to capture more of the light energy than does PSII (Albertsson, 2001). See Figure 3.2 for more detail of the thylakoid disk.
Figure 3.2. Thylakoid disk including PSI, PSII and ATP synthase.

A grana stack is composed of 4 thylakoid disks and a stroma lamellae that links grana stacks. Each thylakoid disk has a diameter close to 500 nm (appressed membrane and end membrane) and width of 60 nm. Approximately there are 500 PSII and 250 cytochrome b6f located in the appressed membrane, 210 PSI and 120 cytochrome b6f in the margin annulus, and in the stroma lamellae there are 190 PSI, 50 PSII and 80 cytochrome b6f. See Figure 3.3 for a normal state of transition of a thylakoid disk, however these ratios change depending of the pool sizes of ATP and NADPH. (Albertsson, 2001).

During the photosynthetic process 6 hydrogen ions (6H⁺) are added to the lumen: two from the oxidation of water, two from the activity of plastoquinones and two from cytochrome 6bf. The activities of plastoquinones and the cytochromes are driven by the free energy obtained from the two electrons charged during PS II. These two electrons are recharged in PSI to produce one NADPH, a process organized in a linear electron transport chain. However, 3-4 or more hydrogen ions are required to produce one ATP, and the light independent reaction requires 1.5 ATP for each NADPH produced. Therefore there is a cyclic electron transport chain between PSII and PSI that pumps the remaining hydrogen needed to produce the ATP for the light
independent reaction. The pool of ATP and NADPH or ATP:NADPH ratio induces changes in the stroma lamellae including the PSI and PSII ratio, and the efficiency of PSI and PSII required to harvest energy is also modified. These changes in amounts of PSI and PSII as well as the efficiency of each structure to capture energy are known as state of transition (Albertsson, 2001).

**Figure 3.3.** Organization of the thylakoid disk including the stroma lamellae.

### 3.3.4 GENERAL DESCRIPTION AND FUNCTION OF PHOTOSYSTEM II (PSII)

Photosynthesis II (PSII) is a membrane-bound protein complex (700 KDa) located on the thylakoid membrane, the protein complex is divided into 20 subunits which includes ~60 organic and inorganic molecules. PSII is formed by two main structures, the antenna and a reaction center (RC) (Service et al., 2011). PSII is formed from 27-28 proteins subunits, the antenna and
the reaction centre (RC) are attached with CP47 and CP43 proteins. The RC contains 35-36 molecules of chlorophyll and 11-12 β-carotenes. The system is organized in 6 arrays, each one with 5-6 chlorophylls and 1-2 β-carotenes (Alstrum-Acevedo et al., 2005).

The antenna is divided into a core antenna and a peripheral antenna; The core antenna containing xanthophylls and light harvesting complex II (LHClII), the peripheral antenna containing chlorophyll a and b, carotenoids, as well as 2-4 LHClII, subdivided into proteins; Lhcb1 (66%), Lhcb2 (24%), Lhcb3 (8%) and minor complexes Lhcb4, Lhcb5 and Lhcb6 (Kouril et al., 2012). The RC contains a core of two chlorophyll P680, chlorophyll D1 and chlorophyll D2, D1 and D2 proteins, pheophytin D1 and D2, plastoquinones Qa and Qb, water oxidation complex (WOC), which includes one redox active tyrosine (tyr161), the Mn4 cluster, oxygen evolve complex (OEC) and the remainder of the electron transport chain (Alstrum-Acevedo et al., 2005; Barber, 2002). The peripheral antenna functions in light absorbance, and in transporting the vibrational energy to the core antenna. It also has a photo-protective mechanism that dissipated excess energy as heat (Alstrum-Acevedo et al., 2005), the function of the reaction center (RC) is to oxidize water (H₂O), synthesis of oxygen (O₂) and electron excitation (energization) from the split of water (Kouril et al., 2012). There are two major routes for transferring the vibrational energy from the antenna to the RC. One route is from the light harvesting complex II (LHClII) attached protein CP26 to CP43 and to the RC. The other route is from the light harvesting complex II (LHClII) attached protein CP24 to CP29, then to CP47 and to the RC. Both routes from the antenna to the RC have to be through either CP43 or CP47 (Kouril et al., 2012). See Figure 3.4 for 3-D structure of the PSII antenna and RC.
In cyanobacteria PSII has 35 chlorophyll a, 2 pheophytin and 12 carotenoids. Some of the PSII carotenoids are xanthophyll, neoxanthin and lutein. There is a maintenance mechanism in PSII to repair degradation of the D1 protein in the core of the RC. D1 proteins have a half life of ~30 min and about every 60 min have to be replaced with a new copy. PSII has two copies of the D1 protein so when a D1 protein does not function the other copy absorbs the excited-state electron from the RC. Thus, the photosynthetic process does not stop during the repair cycle (Caffarri et al., 2009).

The photosynthetic process in PSII initiates when energy is absorbed by the 35-36 chlorophyll antennae and the 11-12 β-carotenes, and this excitation or vibrational energy is transferred to one of the two chlorophylls P680 located in the reaction center. The vibrational energy excites an electron inside of chlorophyll P680 and this excited state electron leaves the chlorophyll P680 or chlorophyll RC through one of the chlorophyll D1/D2 proteins which reduces one of the pheophytin D1/D2. The pheophytin passes the excited electron to the plastoquinone QA (mobile
carrier), and QA reduces QB therefore transferring the energy of the excited electron. Plastoquinone QB requires two excited electrons and two protons (2H⁺) from the stroma which are released from PSII to a lipid bilayer known as a lumen. The process in PSII ends when QB releases two protons into the lumen and transfers the two excited electron to the cytochrome b6f complex (Barber, 2008; Ferreira et al., 2004).

### 3.3.4.1 PSII REACTION CENTER PROCESSES: P680, D1-D2 PROTEINS, PHEOPHYTIN, OXYGEN EVOLUTION CENTER (OEC), WATER OXIDATION PROCESS (WOC) AND PLASTOQUINONES

Photosystem II is a plastoquinone-oxidoreductase reaction that occurs inside the RC. It is driven by light absorption in the antennae complex, which involves absorption of electromagnetic energy, charging of an electron to an excited state, releasing this excited electron to cytochrome 6bf using the mobile carrier plastoquinones a and b, and resulting in water oxidation and oxygen release. These processes can be separated into three reaction sequences:

1. Photon absorption by the antennae, transfer of this vibrational energy to chlorophyll P680 which excites an electron in the RC, and transfer of the excited electron to plastoquinone a and plastoquinone b.

2. Oxidation of water which takes place in the water oxidation complex (WOC) using tyrosine Yz, an intermediate redox carrier.

3. Reduction of plastoquinones which involves the transfer of an excited electron from the RC to the plastoquinones using the heterodimer proteins D1 and D2. The electron flow is from the D1 and D2 proteins, which have a special pair of chlorophyll PD1 and PD2, chlorophyll D1 and D2 and pheophytin D1 and D2, to plastoquinone a and plastoquinone b (Renger and Renger, 2008).

The photosynthetic electron transport chain in the PSII RC occurs in the sequence: (i) charging of the tetra dimer P680, (ii) excited electron flows to pheophytin (in a few picoseconds). (iii) The electron flows from pheophytin to plastoquinones Qa (200 pico seconds) and one by one, two
electrons flow to plastoquinone Qb (microseconds to milliseconds). And (iv) plastoquinone Qb is converted to plastoquinol and transports the two electrons to cytochrome b6f. Water oxidation and electron transport to P680 involves three steps: (i) water is oxidised by a Mn4 cluster which has five internal redox states, S0 to S4, (ii) the redox- active tyrosine (tyr161) oxidizes the Mn4 cluster. And (iii) the P680 oxidizes tyr161 (10 nanoseconds) extracting the excited electron, originally from water, and using it as an energy source to introduce the resonance energy captured by the antenna (Barber and Archer, 2001).

Figure 3.5. Proposed structure of PSII based on X-ray images.

The PSII does not have 2 special pair of chlorophyll common in PSI, and anoxygenic photosynthesis such as P840 and P870, therefore PSII cannot absorb light in the far red spectrum. The general structure of the PSII RC (Figure 3.5) contains four chlorophylls- P680 and 2 pheophytin chlorophylls, with D1 and D2 proteins sharing the P680 chlorophyll and each protein having one pheophytin. The D1 protein also has plastoquinone Qa, plastoquinone Qb, the oxygen evolution center (OEC) and the water oxidation complex (WOC) which includes one redox active tyrosine (tyr161) and the Mn4 cluster. In PSII the antenna is attached to the RC in
the following sequence: D1 and D2 proteins are attached to CP47 protein, CP47 protein is attached to cytochrome b559, cytochrome b559 is attached to PsbI, PsbTc, PsbW and then to the antenna. P680 is located in the middle of the PSII RC and functions as its primary electron donor. P680 in its oxidised state has a redox potential (1.12V) which more than sufficient to oxidise water (Barber, 2002). PSII contains an oxygen evolving site, the Mn3-CaO4 cluster, where the photooxidation of water occurs. The PSII contains 19 different proteins with 36 chlorophyll a in 3 subunits of 12 chlorophylls, 7 carotenoids, 1 reaction center (RC) with a pair of chlorophylls, 1 oxygen evolving center (OEC), 1 heme b and 1 heme c, 1 plastoquinone a and b, 2 pheophytins D1-CP43- Psbl and D2- CP47 -PsbX subunits. It has the following dimensions: depth 105 A, length 205 A and width 110 A (Ferreira et al., 2004).

3.3.4.2 OXYGEN EVOLUTION CENTER (OEC)

The evolution of anoxygenic to oxygenic photosynthesis has changed the electron donor from H₂S or H₂ to H₂O and it involves the production of O₂. The H₂O oxidation reaction takes place in the oxygen evolution complex (OEC) located inside PSII (Service et al., 2011). The PSII system extracts electrons from water in the OEC and transfers these electrons to the P680 in the core of the RC (Gilchrist et al., 1995). The OEC is located in the thylakoid membrane, nearby the PSII RC. The WOC has a CaMn₄ cluster (tetra nuclear clusters of manganese with calcium and chlorine) where O²⁻ + O²⁻ couples and forms molecular oxygen O₂ and it plays an active role in proton distribution after the oxidation (Liu et al., 2008).

The OEC is activated by an excited electron transfer from the RC, received as vibrational energy harvested by the chlorophyll antennae. The transfer of an excited electron from the RC to the plastoquinones QA produces in the P680 sufficient oxidised equivalents to trigger water oxidation in the OEC. This process uses the redox active tyrosine Yz as transport between the chlorophyll and the OEC (Liu et al., 2008). The WOC and OEC, (they have similar structures) are located in the D1 protein as a complex containing a tetra manganese cluster Mn₄ with chloride and calcium cofactors. It is in this structure that the oxidation of water takes place. The transfer of electrons from the OEC to the P680 uses two tyrosines, a redox-active tyrosine Yz (tyr 161) that transfers electrons from the manganese cluster to the P680 located in the D1
protein. The other is tyrosine $Y_D$ (tyr 160) which transfers electrons from the manganese cluster to another P680 located in the D2 protein. The RC of PSII has 4 P680- two associated with the D1 protein and two associated with the D2 protein. The location of the manganese cluster with respect to the two tyrosines is not symmetric. The distance from the Mn to the Tyr 161 Yz is 4.5 Å both located in the D1 protein, and the distance from the manganese cluster to the Tyr 160 YD is more than 4.5 Å (Gilchrist et al., 1995).

### 3.3.4.3 Architecture and Dynamics of the Water Oxidation Process (WOC)

The oxidation potential of P680 is 1.17 V which is greater than 1.0 V necessary for water oxidation; P700 has an oxidation potential of 0.49 V, The oxidation potential of chlorophyll a is 0.78 V, and the carotenoid is 1.06 V. In anoxygenic photosynthesis, P840 has an oxidation potential of 0.4 V, P870 0.45 V and bacteriochlorophyll 0.64 V (Barber and Archer, 2001). In photosystem II the water oxidation complex (WOC) architecture has 10 redox cofactors bound to the D1/D2 proteins and the catalytic site consists of the CaMnO$_4$ cluster and the redox active tyrosine Yz. The photosynthetic process is initiated in the antennae where electromagnetic energy is collected and transferred to the P680, which is the primary electron donor located in the RC and is made up of four chlorophylls. The P680 uses the energy transferred by the antenna to charge an electron and donates this electron to D1 protein using chlorophyll D1 and Pheophytin D1 as the electron transport chain. The P680 chlorophyll becomes considerable oxidized after the loss of the excited state electron, this oxidation potential is strong enough to extract a ground stage electron from the CaMnO$_4$ cluster in the core of the water oxidation complex (WOC). The CaMnO$_4$ cluster involves four steps and five oxidation stages S$_0$, S$_1$, S$_2$, S$_3$ and S$_4$ where S$_0$ is the most reduced state and S$_4$ the most oxidised. P680 oxidise the CaMnO$_4$ cluster and bring the oxidation from S$_0$ to S$_4$, when the WOC is at S$_4$ state, in this stage of oxidation the CaMnO$_4$ cluster has the potential to oxidise water extracting the electrons locate in the hydrogen-oxygen bonds, water is split and the complex returns to S$_0$ where the P680 start the oxidation process again oxidising the CaMnO$_4$ cluster. The electrons resulting from water oxidation flow to P680 located in the RC. In the RC, vibrational energy from the antenna
is used to excite an electron in the P680 and the electron transport chains starts over again in PSII. The WOC provides a pH of 5 and a voltage difference of 0.93V per electron, for a total of 3.72 eV required to oxidise one water molecule (Chen et al., 2011; McEvoy et al., 2005).

Each state of oxidation in the Mn4Ca cluster is a combination of Mn3+ and Mn4+; the S0 state spend 36% of the time and is the state after the oxidation of H2O and the release of O2; S1 state spend 62% of the time and has 2 Mn3+ plus 2 Mn4+; S2 state spend 2% of the time and has 1 Mn3+ plus 3 Mn4+; S3 and S4 states are negligible most of the time and the formation of these states triggers the oxidation of H2O and the release of O2 (Service et al., 2011).

3.3.4.4 MECHANISM OF ENERGY TRANSFER FROM PSII TO THE MOBILE CARRIERS

The PSII complex absorbs oxygen, oxidizes water and reduces plastoquinones. PSII has two branches located across a membrane that transfer the charged electron to plastoquinone, one branch being active at any specific time. The active branch contains the pheophytin D1 (PheoD1) protein which accepts two excited electrons and is the primary electron acceptor in the transfer chain to plastoquinones QA and QB. In the other hand the Pheophytin D2 (PheoD2) coupled to a central chlorine in the RC to assist with equilibrium of the excited RC state, thus Pheophytin D2 (PheoD2) is involved in the proper distribution of excitation energy throughout the chlorophyll in the excited RC. PheoD1 is involved in the transferring of the excited electrons from the RC to the Plastoquinones QA to QB that is the stable electron acceptor, and the PheoD2 is involved in stabilizing the charges in the RC in the excited state (Perrine et al; 2011).

The RC in PSII for purple bacteria and oxygenic photosynthesis in plants, algae and cyanobacteria contains 4 bacteriochlorophyll or chlorophylls and bacteriopheophytin or pheophytin plus 2 B-carotenes between chlorophylls. These molecules are arranged in two branches, with one branch active for charge separation and electron transfer at any one time. In the purple bacterium *Rhodobacter sphaeroides*, the light harvest 1 (LH 1) absorbs energy at a
peak wavelength of 875 nm, the energy collected charges an electron in the RC which is then transferred to bacteriochlorophyll on the active branch. There are two pathways in the subsequent electron flow: (i) an active branch using bacteriopheophytin, and (ii) a special pair of bacteriochlorophyll using the active branch. After an active branch is charged, it can not receive additional energy from the antenna. The two pathways have different rates of electron transfer and the one used depends of the intensity of light absorption and on the temperature of the environment. This allows the RC to cope with stressful situations and be able to adjust the rate of charged electron transfer to plastoquinone QA, a similar mechanism is present in the RC of plants (Romero et al., 2010).

3.3.4.5 ROLE OF PLASTOQUINONES IN PSII (MOBILE CARRIERS)

Plastoquinone A (QA) is the primary electron receptor of PSII, it is responsible for the transfer of the excited electron from the RC to plastoquinone B (QB) and then to cytochrome b6f. The reduced state of plastoquinone A (QA) increases as the concentration of xanthophyll (a complementary pigment) increases and the rates of epoxidation increase, and during this process the light harvesting process decreases. At the same time the composition of the polypeptides involved in the light harvesting process in the PSII RC are regulated by the redox state of plastoquinone A (QA). Light intensity and temperature produce similar effects in controlling and regulating xanthophyll production and the epoxidation state (Perrine and Sayre, 2011).

Two indicators have been identified responding to changes in light intensity and temperature during the photosynthetic growth and adaptation of the algae Chlorella vulgaris. One is the increase in the redox state of plastoquinones as light intensity increases, and the second indication is the change in pH of the lumen in the thylakoid disk. There are two pathways responsible for the transfer of excited electrons from the RC to plastoquinones QA, this being a strategy to regulate the rate of excited electron transfer depending on light and temperature conditions (Perrine and Sayre, 2011). An additional strategy for regulating photosynthetic rate is through the PSII/PSI ratio (Melis, 1998). The PSII: PSI optimizes the flow of electrons from the RC to cytochrome b6f and to plastocyanin, and then to the PSI complex (Wilson and Huner, 2000).
3.3.4.6 DAMAGE AND REGENERATION OF PSII

The regeneration of the RC of PSII is processed every 30 to 60 min under normal conditions caused by the oxidative damage from the splitting of water into $O_2^+ + O_2^-$ and the formation of $O_2$ before it is released (Liu et al., 2008; Barber and Archer, 2001). This process occurs inside the chloroplast of plants, algae and inside the PSII of cyanobacteria. The regeneration process is focused on the D1 protein as it is this protein that is in contact with the oxygen produced in the OEC. If the D1 protein becomes damaged, the P680 stops absorbing energy from the antenna due to lack of electrons supplied from the WOC. The P680 also has an oxidation protection mechanism, known as P680 tetramer carotenoid triplet quenching mechanism, in which B-carotene quenches the P680 to protect the chlorophyll from oxidation by $O_2$ (Barber and Archer, 2001).

The degradation process occurring during photo-damage develops first to the D1 protein, whose synthesis is encoded by the specific gene, psbA. The synthesis of the D1 protein can be accomplished only in the presence of light and there are additional proteins involved in the process. Degradation of the D1 protein is common during the process of photosynthesis, therefore the D1 protein is synthesized during a normal photosynthetic period (Mulo et al., 2012).

There are two types of maintenance processes of the RC machinery: the first process is called the novo assembly, that is the synthesis and replacement of functional sub-units damaged during photosynthesis, the second process is PSII repair whereby the D1 protein is repaired. Enzymatic degradation of the existing or damaged D1 protein occurs prior to the new copy of D1 replacing the degraded one (Mulo et al., 2012).

Thermo-degradation of the D1 protein in the RC of PSII occurs when cells are subjected to temperatures higher than their biological normal. During the normal photosynthetic process, D1 protein regeneration follows the following steps: (i) disassembly, enzymatic degradation and removal of the damaged D1 protein, and (ii) synthesis and assembly of the new D1 protein at the same location as the damaged D1 protein. Under normal conditions rates of degradation and
regeneration are equal but this steady-state becomes uncoupled when there is a permanent change in the normal temperature. An increase from the normal temperature increases the electron transfer flux in plastoquinones Qa-Qb, and this increases the reactive oxygen species (ROS) inside the photosynthetic machinery. A high concentration of ROS induces D1 protein oxidation and degradation, thereby decreasing D1 protein concentrations, and this overall reduces photosynthetic activity. An increase in temperature enhances photo-respiration which causes additional damage to the photosynthetic machinery, especially to D1 regeneration (Dinamarca et al., 2011).

An excess of electromagnetic radiation produces high amounts of ROS, the three principal species being super oxide radical O$_2^-$, hydrogen peroxide H$_2$O$_2$ and hydroxyl radical OH', and this inhibits the synthesis of the novo protein necessary to repair the PSII machinery. Specifically ROS reduces the rate of D1 protein synthesis thereby slowing the replenishing of the D1 protein. ROS does not damage the photosynthetic machinery, however it decreases the regeneration and repair rates of the machinery. ROS targets the PSbA genes -there are 3 PSbA genes- that encode for the novo D1 protein synthesis. H$_2$O$_2$ targets the initiation and elongation in translation of PSbA mRNAs, thus affecting the binding process of PSbA mRNAs to ribosomes. ROS not only affects the synthesis of the D1 protein but also inhibits synthesis of the majority of the proteins in the photosynthetic machinery in PSII. The most important ROS is formed in the PSI when there is excess light. The first ROS formed is the super oxide radical O$_2^-$, part which forms H$_2$O$_2$ and some forms the OH': Hydrogen peroxide is the most abundant ROS and though it is the least reactive it causes the most damage to the D1 protein regeneration process (Nishiyama et al., 2001).

### 3.3.4.7 ROLE OF PSB27 IN THE REGENERATION OF PSII

Psb27 is one of the 20-23 proteins present in the PSII complex located in plants, algae and cyanobacteria. The PS II performs the harvesting of light, the splitting of water, the excitation of electrons from the energy obtained from light, and the reduction of plastoquinones. Psbp27 has a molecular weight of 11000 daltons and is located in the thylakoid lumen. There is evidence that plants able to synthetize Psbp27 recover faster from photo-inhibition damage than do those
plants that cannot synthetize Psbp27. The PSII containing Psbp27 do not have Mn$_4$Ca in the RC water splitting region, but contain a D1 protein (D2 protein is absent). When there is a photo-damage to the proteins of PSII in cyanobacteria, the bacteria synthetize Psbp27 to conduct photosynthesis during the period of repair to damaged proteins. When the repair is completed Mn$_4$Ca and D2 protein is synthetized in the RC splitting water site and Psbp27 is inactivated. The Psbp27 protein is used during the formation of the Mn$_4$Ca complex and D2 protein in the PSII RC and in the repairing process of the PSII complex caused by photo-damage. The presence of Psbp27 is an evolutionary advantage for photosynthetic cells to be able to survive adverse environmental conditions with excess of light and low temperature (Mabbitt et al., 2009).

3.3.5 GENERAL ARCHITECTURE AND FUNCTION OF PSI

The architecture of PSI consists of membrane proteins made up of 11-13 polypeptides and subunits of these proteins, the most important of which are PSaA and PSaB. They bind the core of the antenna with electron transfer cofactors of the RC, including the primary donor P700 pigments and the iron sulfur clusters FX. The antennae consist of three major pools containing 90-100 chlorophyll molecules and 22 carotenoids, and in plants are contained in a structure called a light harvesting complex I (LHCI) (not found it in cyanobacteria). The LHCl is a peripheral structure surrounding the complex containing the antennae and has 100 chlorophylls, including 77 chlorophyll a and 23 chlorophyll b and 20 xanthophyll molecules. The antenna including the light harvesting complex initiate the electron transfer process by first forming a stable charge-separated state. The excited state electrons are then transferred via ferrodoxin to the ferrodoxin NADP reductase (FNR) which synthetize NADPH (Di Donato et al., 2011; Yan et al., 2003; Chitnis, 2001; Palson et al., 1998).

There are two models to explain the excited electron energy transfer dynamics in PSI: the first is the trap-limited model and the second is transfer via a trap kinetic scheme. The stromal protein, known as PSaC, binds the terminal iron clusters FA and FB, the latter having a relatively long half life ~50 ms and a midpoint potential of -580 mV which is used to reduce protons to H$_2$ (Lubner et al., 2011).
PSI has been described as a light-driven engine located in the thylakoid membrane of prokaryotic cells like cyanobacteria or in the chloroplast of algae and plants. PSI is a plastocyanin-P700-ferrodoxin redox system which functions to energize an electron and transfer it to ferredoxin reductase (FNR) for the synthesis of NADPH. The PSI complex has 11-12 proteins in cyanobacteria and 11-14 proteins in algae and plants, plus organic and inorganic cofactors.

The P700 RC is made up of a pair of chlorophylls a, which converts the vibrational energy by exciting an electron. This excited electron leaves the P700 RC and flows to chlorophyll a (also called A0) through to phylloquinones (A1) to the FX, then to the Fa and Fb cofactors. Fb is the terminal electron donor and it reduces ferredoxin, a mobile carrier that provides the excited electron for NADPH synthesis (Chitnis, 2001).

3.3.5.1 PSI REACTION CENTER CHLOROPHYLL P700

Chlorophyll P700 in the core of the PSI RC is made up of a pair of chlorophylls Pα and Pβ and located adjacent to an accessory pair of chlorophylls, referred to as a special pair, for a total of 4 chlorophylls. The RC has two functional branches A and B, each protected by a protein, and referred to as branch PSaA and branch PSaB, respectively. Inside PSaA is chlorophyll Pα and attached to chlorophyll Pα is a special pair a1A. Special pair a1A is attached to chlorophyll A0. Chlorophyll A0 is attached to phylloquinone A1 which in turn is attached to an iron sulfur cluster ferredoxin FX. In total, the PSI complex is made up of 28 chlorophyll Pα and 72 chlorophyll Pβ, 98 reside in the antenna and 2 are in the RC. The energy flow follows the sequence: energy captured by the antenna is transmitted as vibrational energy to the RC, and this energy excites an electron located in each chlorophyll P700. Chlorophyll P700 Pα or Pβ donates an excited-state electron to the special pair 1A or 1B, and the special pair transfers the electron to chlorophyll A0 or B0, then its flows to phylloquinone A1 or B1. Phylloquinone A or B transfers the electron to the iron sulfur cluster FX, and then on to ferredoxin reductase FNR where the energy from the electron is used to synthesize NADPH (Saito and Ishikita, 2011). Figure 3.6 shows the structure of the photosystem I.
Each of the 90-100 chlorophylls a of the antennae of PSI are separated by a distance ranging from 7 to 16Å, and the distance is increased to 19Å from the central chlorophylls. The antennae also have complementary pigments, 20 β-carotenes. The core of the antenna has 4 chlorophylls, two chlorophyll cC and cC’ located at distance of 14Å from the central chlorophylls eC3 and eC3’. The central chlorophyll transfers the vibrational energy to P700 located in the RC. The RC has 6 chlorophylls, 2 phylloquinones A1 and A2 and three iron sulfur clusters. The core of the RC contains a dimer of chlorophyll, known as P700, 2 special pair of chlorophyll A and B, and two chlorophyll a’s. The six chlorophylls are known as the A0 primary electron donor. The
excited electron flows from A0 to phylloquinones A1 and then on to the iron sulphur cluster (4Fe-4S) in a sequence FX, FA and FB. They are located near to the stroma where ferredoxin reductase (FNR) receives the excited electron and generates NADPH. From the time of energy absorption by the antenna to the transfer of this energy to the RC takes 20-30 Ps (10-12 S) for plants, cyanobacteria and algae. The time required to transfer vibrational energy between chlorophyll in the antenna is 200 fs (10-15 S) (Palson et al., 1998). See Figure 6 for PSI organization.

The ~98-100 chlorophylls of the antenna in PSI have an absorption peaks ranging from 660 to 700 nm, 8 of which have an absorption peak ~703 nm. The antennae are located 30-50 Å from the RC. The RC has two active branches, PSaA and PSaB, which pass the excited state electrons to ferredoxin FX, contrary to PSII where only one branch pass excited stated electron the D1 protein branch. The antenna excitation decay varies depending on the organism species, and in the case of cyanobacteria lasts for 19-24 ps (Pico second 1*10^-12 seconds). Under normal levels of radiatation the time from light absorption in the antenna and transmission of the energy to P700 takes 20-25 ps (open antenna 23.5 ps and closed antenna 24 ps), however under conditions of high irradiance the time can be reduced to 4 ps. Excited electron transfer from P700 to chlorophyll a A0 takes 1-3 ps and from A0 to phylloquinone A1 takes 20-50 ps (Savikhin et al., 2000).

### 3.3.5.2 REDOX POTENTIAL OF PSI

The redox potential of PSI has increased during biological evolution as evident by the redox potential being different between P700 and P700^+ among photosynthetic organisms. There is an increasing trend in redox potential from simple prokaryotic organisms to more complex eukaryotic organisms and multi cellular organisms. Prokaryotic cyanobacteria, the first oxygenic organisms, have a P700 to P700^+ redox potential between +398 and +454 mv. The PS1 redox potential in red algae, a eukaryote, varies from +431 to +433 mv and in green algae is +469 mv. Plants a multicellular organims have a redox potential of +470 mv which is the highest for the PSI of any photosynthetic organism. All organisms with chloroplasts have a higher redox potential in PSI than that of eukaryotic organisms without chloroplasts. In general, the redox
potential in the RC of PSI P700 to P700$^+$ is $+398 \text{ mv}$ to $+470 \text{ mv}$ for the heterodimer; 2 P700, the two special pair of chlorophylls, in the chlorophyll a A0 part of the RC has a redox of $+800 \text{ mv}$ (Nakamura et al., 2011).

### 3.3.6 MECHANISMS TO MINIMIZE PHOTO-DAMAGE IN PSI AND PSII

Intense light radiation can damage the pigments contained in the antennae of PSI and PSII. The photosynthetic apparatus has mechanisms to dissipate excess energy as heat or to store it and use it later for photosynthesis when less illumination energy is available. In plants the P700$^+$ cation radical acts as to cool the antenna by absorbing the longer wavelength radiation. In plants, 70-90% of P700 can be in the P700$^+$ state for managing the excess energy. In cyanobacteria the core antenna contains chlorophylls, Chl 735 nm, Chl 711 nm, and Chl 714 nm, which absorb the excess energy. The excess energy is transferred from chlorophyll to chlorophyll and then to the P700 chlorophylls by resonance mechanisms (Shubin et al., 2008).

In a typical photosynthetic reaction, the rate of the process is controlled by the rate of electron flow through the electron transport chain. The rate at which cytochrome b6f oxidises plastoquinones regulates the flow of electrons that are transferred from PSII to PSI. If an antenna absorbs more energy than the available electrons can carry from PSI to ferredoxin, the quenching or heat dissipation mechanism is activated in the antenna or in the reaction center to avoid damage to the machinery (Shubin et al., 2008).

### 3.3.7 STATE TRANSITION PSII AND PSI SYNTHESIS RATIO AND PHOTOSYNTHETIC ACTIVITY

The products of the light reaction in photosystem I and II are NADPH and ATP. NADPH is produced in the stroma of the chloroplast and its energy is derived from the flow of electrons from PSI. ATP is synthetized by a proton gradient from the lumen to the stroma through the ATP synthase and the energy is derive from PSII. PSI and PSII have different architectures and light absorption characteristics. Whereas both photosystems capture light in the blue and red
regions, PSI absorbs more in the far red region with the result that it needs to transmit more energy to the electron to produce NADPH. The light harvesting efficiency and quantity of PSI and PSII complexes are constantly regulated to balance both the electron flow between the two systems and the ratio of ATP and NADPH synthesis. Known as the state of transition, it is a mechanism for balancing the PSI and PSII ratio occurring due to changes in the light intensity and wavelength and optimizes the photosynthesis process. (Minigawa, 2011).

The state of transition process works with the phosphorylation of the LHCII in PSII or the movement of LHCII from the PSII to PSI, and not additional synthesis of LHCII in PSII. This process is a key component for the optimum performance of PSI and PSII. Depending on the light spectra, the photosynthetic cell increases the efficiency of either of the two systems to balance the electron flow for the production of ATP and NADPH. There are two states of transition. During state 1 there is more fluorescence and more energy in the red region that is absorbed by PSII, therefore there is more activity in PSII than PSI and the plastoquinone pool spends longer in the reduced form. This activates the LHCII protein kinase by phosphorylation moving LHCII from PSII to PSI and increasing the PSI light absorption efficiency. During state 2 there is less fluorescence yield and more absorption in the far red region therefore the plastoquinone pool is oxidised for longer periods the LHCII protein kinase is not as activated and the LHCII synthesis remains in PSII and there is more activity in PSI than PSII. Thus this mechanism is driven by the redox condition of the plastoquinone pool activating a protein that moves LHCII from PSII to PSI (Haldrup et al., 2001).
3.3.8 BACTERIA STATE OF TRANSITION

The antennae of purple non-sulfur bacteria *Rhodobacter sphaeroides* contain a light harvesting complex 1 and 2 (LHC1, LHC2); the LHC1 has 24-30 bacteriochlorophylls that form an arc in the RC. LHC1 also has a pair of plastoquinones Qa and a polypeptide Pufx. The function of Pufx is to ensure the oxidation of plastoquinone Qa by stabilizing the oxidation state of plastoquinone Qb. The oxidation of plastoquinone Qa is critical as plastoquinone Qb obtains the excited-state electrons derived from the core of the RC through the bacterio-pheophytin-plastoquinone Qa and transports the two electrons to cytochrome bc1. Without Pufx plastoquinone Qb would be stabilized by LHC1 and this would prevent electron flow from plastoquinone Qa to plastoquinone Qb and ultimately stop the photosynthesis process (Stahl et al., 2011).
3.3.9 CONCLUSIONS

In oxygenic and anoxygenic photosynthetic ecept heibacteria the organisms use a source of hydrogen (H₂, H₂S or H₂O) during the light reaction to transform electromagnetic energy into chemical energy and store this energy as ATP and NADPH. The photosynthetic machinery collects solar energy in its antennas and passes this energy to reaction centers where a ground state electron passes to an excited state and leaves the RC. The energy from the excited-state electron drives the entire photosynthetic process in the light reaction. During the light independent reaction the ATP and NADPH formed in the light reaction are used to produce G3P. Contrary to respiration, the end product of the photosynthetic process is the formation of G3P, and not the consumption of these carbohydrates.
3.4 REFERENCES


CHAPTER 4  GROWTH OF TWO MARINE ALGAE AND TWO FRESH WATER ALGAE UNDER SOLAR AND LED ILLUMINATION

4.1 ABSTRACT

Growth experiments with two marine algae, *Tetraselmis sp* and *Nannochloris sp*, and two fresh water algae, *Chlorella vulgaris* and *Scenedesmus sp*, were conducted to study the effects of five light treatments on growth rate during a 20-24 d incubation. The light treatments were: daily solar radiation, daily solar plus 12 h white light, 24 h blue LED light, 24 h red-blue LED light, and daily solar plus 12 h red-blue LED light. Exponential growth was observed during the first 18 d of incubation and was followed by a stationary phase. Specific growth rates were highest in treatments which included daily solar illumination, reaching a final population density of $10^7$ cells mL$^{-1}$ after 18 d and standing biomass of 1.200 mg L$^{-1}$. ANOVA and T test at $\alpha = 0.05$ in cell density, cumulative growth and biomass production indicated a significant difference between treatments that included daily solar radiation and those treatments that included 24 h LED illumination. There was no significant difference in growth rate response to light treatment between algal species.

*Keywords*: growth rate; algal biomass; population density; cell weight

4.2 INTRODUCTION

Cultivation of algae has gained importance because of the increased in demand for fossil fuels, food and the increase of greenhouse gases resulted in global warming (Hill et al., 2006). Algae have great potential as renewable source fuels, feedstock for fish and animals, food productions, food supplements, antioxidants, and other important biochemicals (Pittman et al., 2011; Spolaore et al., 2006).
Algae could become an alternative for reduction of carbon dioxide emissions in power generation plants, cement plants, and for atmospheric carbon dioxide sequestration (Fan et al., 2007; Yun and Park, 1997). Algae growth rate is controlled by various factors including medium nutrient content, temperature, pH, disturbance, illumination source and intensity. Algal species have evolved growing under solar radiation, which provides energy in the ultraviolet (5%), visible (43%) and infrared (52%) electromagnetic spectrum (Kruse et al., 2005). The visible light spectrum is divided into 6 main colors: violet 380-450 nm, blue 450-495 nm, green 495-570 nm, yellow 570-590 nm, orange 590-620 nm and red 620-750 nm. Not all wavelengths are equally absorbed by the algae’s main photosynthetic pigments. Chlorophyll a and chlorophyll b preferentially absorb blue and red light, while the other wavelengths are absorbed in lower magnitude by the complementary pigment carotenoids (Matthijs et al., 1995).

Algae have been grown under both photoautotrophic and heterotrophic conditions in outdoor ponds and in open and closed photo-reactors exposed to varying light wavelengths and intensity. Photo-reactors have combined exposure to solar radiation during the day with exposure to white light during the night (Ogbonna et al., 2001). Lately algae have been grown under LED illumination which provides specific wavelengths that match the algae absorption peaks (Yeh and Chung, 2009). Many experiments have studied algal growth exposed to specific wavelengths of red, blue, yellow, green, and white light (Wang et al., 2007). Marine algae such as Tetraselmis suecica, and Nannochloropsis sp have been study for the production of feed stock in aquaculture, polyunsaturated fatty acids (PUFA) for food supplements (Abiusi et al., 2013), fatty acid concentration and composition for the production of biodiesel (Bondioli et al., 2013; Park et al., 2013). Different light spectra has been tested with T. suecica for cell biochemical composition, cell size and biomass production. Cell size has been reduced, eicosapentaenoic acid and cell motility increased while exposing the algae under red light for 9 d (Abiusi et al., 2013).

Studies in fresh water alga Scenedesmus sp and Scenedesmus obliquus have been focused on maximization of biomass production, increased in lipid concentration and changes in lipid composition for renewable fuel production (Nayak et al., 2013; El-Sheekh., 2013).
Methodologies in biomass sonication to increase carbohydrates availability for hydrogen and ethanol fermentation have been studied using the alga *Scenedesmus sp* (Jeon et al., 2013). The studies have concluded that *Scenedesmus sp* has great potential for the production of renewable fuels such as biodiesel, ethanol and hydrogen. A two stage growth methodology one for biomass production and the second one with nitrogen deprivation for lipid accumulation has been tested using the alga *Chlorella protothecoides* to increase its potential for biodiesel production (Li et al., 2013). A novel methodology to process biomass from *Chlorella vulgaris* has been tested for antioxidant extraction (lutein) for food supplements and for fatty acid synthesis necessary for biodiesel production (Prommuak et al., 2013). This study examined the growth rates, cell density, and biomass production of marine algae *Tetraselmis sp*, *Nannochloris sp* and fresh water algae *Scenedesmus sp*, *Chlorella vulgaris* during a 20-24 d incubation under different illumination treatments including solar radiation, white light, LED blue light and a combination of red and blue LEDs. The objective of the research was to evaluate the effect of light on growth for different algae species and to compare biomass production among marine and fresh water algae growing under similar conditions.

### 4.3 MATERIALS AND METHODS

#### 4.3.1 MICROORGANISM

The algae cultures used in this study were obtained from the algal culture collection located at the University of Texas, Austin, TX. They included *Chlorella vulgaris* (26), *Nannochloris sp* (1268), *Scenedesmus sp* (1589), *Tetraselmis sp* (2767).

Cultures were established in 1 L batch cultures for 15 d and then transferred to 4-L flasks. The population density was measured using direct microscopic counting techniques. The population density at the start of the experiment was measured to ensure accurate results of growth rate, because the study was carried on with four different algae with different cell size, cell weight and cell population. The reactors were set to 4 L at the beginning of the experiment.
4.3.2 LIGHT SOURCE

4.3.2.1 EXPERIMENT 1 (DAILY SOLAR RADIATION)

For this experiment four 4 L reactors were set outside in an area exposed to only direct solar radiation during the day and no artificial lighting during night period. The solar radiation fluctuated from 8 µmol m\(^{-2}\)s\(^{-1}\) at 6:00 pm to 2985 µmol m\(^{-2}\)s\(^{-1}\) at 12:00 pm, the daily average solar radiation measured during the course of the study was 1271 µmol m\(^{-2}\)s\(^{-1}\). For all the experiments light was measured using a Quantum meter (Model MQ, Apogee Instruments Inc, Logan UT, USA).

4.3.2.2 EXPERIMENT 2 (DAILY SOLAR PLUS 12 H WHITE LIGHT)

For this experiment daily average solar radiation 1271 µmol m\(^{-2}\)s\(^{-1}\) was supplemented after 6:30 pm until 6:00 am with white light provided by two 45 watt fluorescent cool white lamps individual output 1850 µmol m\(^{-2}\)s\(^{-1}\) located 10 cm from the reactors. In addition, one 65 watt cool white lamp 2680 µmol m\(^{-2}\)s\(^{-1}\) output was located 2 m above the 4 L reactors.

4.3.2.3 EXPERIMENT 3 (24 H BLUE LEDs)

This experiment was conducted in a room kept darkened. Blue illumination (425-435 nm) was supplied with a 4.8 watt reflector containing 48 blue LEDs placed 15 cm from each reactor. The illumination intensity measured at the reactor surface was 2850 µmol m\(^{-2}\)s\(^{-1}\). This experiment was conducted using 4 L reactors.

4.3.2.4 EXPERIMENT 4 (24 H RED + BLUE LEDs)

For this light treatment LED strips (LED 3m bands, ALAS, Shanghai, China) providing radiation in the red 26 LEDs 0.08 W bulbs (620-625 nm) and blue 26 LEDs 0.1 W bulbs (425-430 nm) wavelengths were used. The LEDs were contained in a clear plexiglass cylinder that was placed...
in the center of each 4 L reactor, in total 52 bulbs with a power consumption of 4.7 W were used per reactor. Light intensity, measured using a Quantum meter, was 2143 µmol m\(^{-2}\) s\(^{-1}\) from the blue LEDs and 1345 µmol m\(^{-2}\) s\(^{-1}\) for the red LEDs. The illumination system was supplied with 12 V and 8 amp power (Model SA 201-3485, ASTEC, California, USA).

4.3.2.5 EXPERIMENT 5 (DAILY SOLAR PLUS 12H RED AND BLUE LEDS)

Four 4 L reactors containing the four algae were placed in an outside area to receive direct solar radiation averaging 1271 µmol m\(^{-2}\)s\(^{-1}\) during the day which was supplemented with 12 h red and blue LEDs during the night. LEDs were placed inside the reactor, the same type of LED illumination was used as described in experiment 4.

4.3.3 LIGHT DISPERSION

The LEDs used in this study produced light emission intensity of 1700 to 2800 µmol m\(^{-2}\) s\(^{-1}\), which is far greater than that required for optimal algal growth, which it is 50-250 µmol m\(^{-2}\) s\(^{-1}\), there is a high probability of photo-inhibition, and even photo-damage, that will reduce the photosynthetic activity of the cell (Kim et al., 2006; Degen et al., 2001; Ogbonna et al., 1999; Merchuk et al., 1998; Fernandez-Sevilla et al., 1998; Molina –Grima et al., 1996; Garcia-Sanchez et al., 1996; Qiang and Richmond, 1994; Aiba, 1982; Bannister, 1979; Eppley and Coatsworth, 1996). Notwithstanding that the light emission from the LEDs is intense, light dispersion occurs over short distances, and placement of the light source away from the reactor reduces its intensity to a more favourable range for algal growth. The distance from the LEDs to the algal suspension was 7 mm for the studies with red and blue light, and 10 cm for blue light alone and 15 cm for the white light treatment receiving solar radiation. These distances were sufficient to reduce the light intensity at the reactor surface to < 300 µmol m\(^{-2}\) s\(^{-1}\) (Ogbonna et al., 1999).
4.3.4 MIXING, QUANTIFYING CARBON SOURCE UNDER AUTOTROPHIC GROWTH AND TEMPERATURE MEASUREMENTS

Five turbines (Model AC-9904, Resun, China) were used to inject bubbles with carbon dioxide and to provide gentle mixing of the algal medium, 1125 mL air min⁻¹ L⁻¹ of algal suspension was supplied with a CO₂ concentration 0.0350% (350 ppm ± 50) measured by a CO₂-Temperature monitor (Model 7001, Telaire-General Electric, California, USA).

Temperatures for outside experiments was 24°C ± 3 for treatments 1, 2, 5 and 22°C ± 0.9 for treatment 3, 4 as measured in laboratory and outside conditions using a meter (Model 7001, Telaire-General Electric, California, USA) and for liquid algal media as measured by an Infrared thermometer (Model Fluke 62, Fluke Corporation, Everett, Washington, USA). Carbon dioxide was measured every 30 min and temperature every h, outside treatments reached higher temperature due to direct solar radiation into the reactors.

4.3.5 GROWTH MEDIA PREPARATION

The growth media for algal culture was prepared using a commercial synthetic fertilizer (Solucat 25-5-5, Atlantica Agricola, Villena, Spain), which contains total nitrogen - 25% (as ammonium-14.2% plus nitrates-10.8%), P₂O₅ - 5%, K₂O - 5%, Fe - 0.020%, Mn - 0.010%, Zn - 0.002%, B - 0.010%, and Cu - 0.002%. The medium for both the fresh water and marine algae contained 0.8 g fertilizer/L, initial electrical conductivity of the medium was 0.95 ± 0.01 mS measured by EC monitor (Model HI 991301, Hanna, Michigan, USA). 35 g/L of sodium chloride salt was added to the medium culture for the two marine algal species to simulate marine conditions at 3.5% salinity. Volume culture were corrected by adding sterilized fresh media every time a sample was taking, initial volume and final volume of the experiments were the same due to volume correction.
4.3.6  CELL DENSITY ANALYSIS

The alga cell density was measured daily throughout the time of the experiments by direct microscopy using the Neubauer chamber. The methodology used is describe in 8 steps: 1) Shake each algae reactor to make sure homogenous algal distribution before taking a 20 mL sample with syringe, 2) for very concentrate sample dilute the sample with distillate water with a 1 to 10 ratio, 3) fill the Neubauer chamber with algal media, 4) depending of the cell size use the microscopy 10X or 40X lens, 5) count the cells in the chambers A and B, for the small cells (smaller than 6µm) count those cells in the chamber C, 6) for cells in chamber A and B use this equation \( C = N \times 10^4 \times \text{dil} \), where \( C = \text{cells mL}^{-1} \), \( N = \text{cells average in 1mm}^2 (1\mu\text{L}) \) and dil = dilution factor, 7) For small cells use the results in chamber C and this equation; \( C = (N/4\times10^{-6})\times\text{dil} \), where \( C = \text{cells mL}^{-1} \), \( N = \text{cell average in the 5 C chambers} \) and dil = dilution factor, 8) Repeat steps 1 to 7 three times in order to have three repetitions.

4.3.7  GROWTH RATE METHODOLOGY

The growth of the algae during the incubations was calculated using the specific growth rate equation \( K = \ln (N_2/N_1)/(t_2-t_1) \), where \( K \) is the specific growth rate constant (h\(^{-1}\)), \( N_1 \) is the algal population at time 1 (cells mL\(^{-1}\)), \( N_2 \) is the algal population at time 2 (cells mL\(^{-1}\)), \( T_1 \) is the time at the start of exponential growth (h), \( T_2 \) is the time after a period of exponential growth (h). Divisions per day \( D = (K'/\ln 2) \), Generation Time (Doubling time) \( GT = 1/D \). Accumulative or cumulative growth \( CG = (\text{Total K}/\ln 2) \) (Andersen, 2005; Levasseur et al 1993; Dykhuizen, 1983).

4.3.8  BIOMASS AND CELL WEIGHT METHODOLOGY

The dry weight methodology was used for biomass quantification, this methodology is divided in 7 steps: 1) Dry a 15 mL glass tubing until reach constant weight, 2) fill out the tubing with 10 mL of the alga media from the experiment, 3) centrifuge the tubing at 4000 rpm for 15 min, 4) pour off the water in the vial, 5) dry the tubing (105 °C) with the algae in an oven until reaches constant weight, 6) weigh the tubing with the algae and subtract the weight of the tubing without
the algae, and 7) the resulting value is the alga biomass in a volume of 10 mL. This procedure was repeated in duplicate each time alga biomass was measured. Cell weight was calculated for each alga by dividing biomass (g L\(^{-1}\)) by cell population (cells L\(^{-1}\)).

### 4.3.9 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The study was set up using 20 bubble-column reactors (12 cm internal diameter and 40 cm in length), each containing 4 L of growth media and exposed to five light treatments. Four different algal species were used for each light treatment. Triplicate samples for cell density were collected daily, measurements of final standing biomass were taken at the end of the experiment in triplicate. Cell weight was calculated from the measurements of cell density and standing biomass. ANOVA was performed using Biostatistics 1.0 at \(\alpha \leq 0.05\) for cumulative growth rate, final population density of five different light treatments and four algae species, and for biomass production of four algae growing under daily solar + 12h red-blue LEDs and 24h red-blue LEDs. T test was used to compare differences among the specific light treatments.

### 4.4 RESULTS AND DISCUSSION

Figures 4.1 to 4.5 show the growth curves for the four different algal species growing under five different light treatments. Most curves showed a sigmoid growth pattern and completed their exponential growth stage within the 15 d incubation period and started the stationary stages after approximately 16 d. Exceptions were those algae growing under daily solar radiation or growing under 24 h LED red and blue. The highest population densities were achieved with those treatments that combined solar radiation with exposure to a artificial light treatment. Growth pattern of the two marine algae and two fresh water algae are similar for any specific light treatment.
4.4.1 ALGAL GROWTH UNDER DAILY SOLAR RADIATION

Figure 4.1. Algal growth under daily solar radiation.

4.4.2 ALGAL GROWTH UNDER DAILY SOLAR RADIATION + 12 H WHITE LIGHT

Figure 4.2. Algal growth under daily solar radiation + 12 h white light.
4.4.3 ALGAL GROWTH UNDER 24 H BLUE LEDS IN 4 L REACTORS

Figure 4.3. Algal growth under to 24 h blue LEDs in 4-L reactors.

4.4.4 ALGAL GROWTH UNDER 24 H BLUE + RED LEDS
4.4.5 ALGAL GROWTH UNDER DAILY SOLAR RADIATION + 12 H BLUE + RED LEDs

![Graphs of algal growth under different light treatments.]

Figure 4.5. Algal growth under daily solar radiation + 12 h blue + red LEDs.

4.4.6 FINAL POPULATION DENSITY FOR FOUR ALGAL SPECIES GROWING UNDER 5 LIGHT TREATMENT

The population densities developed by the marine algae during the course of this experiment was $6 \times 10^6$ to $9 \times 10^7$ and are in agreement with those previously reported with other algae species. Studies with the marine alga *Isochrysis galbana* reached a population density of $3 \times 10^7$ cells mL$^{-1}$ after 12 d of incubation (Fidalgo et al., 1998). In experiments with the marine alga *Chaetoceros calcitrans* in different types of closed photo-bioreactors, a population density of $9 \times 10^7$ cells mL$^{-1}$ was measured (Krichnavaruk et al., 2007). Experiments with *Nannochloropsis sp* reported population densities of $1 \times 10^8$ Cells mL$^{-1}$ (Fabregas et al., 2004) and with *Tetraselmis suecica* densities of $1.3 \times 10^6$ and $7.8 \times 10^6$ cells mL$^{-1}$ (Fabregas et al., 1985; Fabregas et al., 1984).
commercial production the marine alga *Dunaliella salina* has attained a population density in the order of $10^9$ cells mL$^{-1}$ (Borowitzka, 1999).

Studies of fresh water algae have reported population densities in the same magnitude to those presented in this study. $1.6\times10^6$ to $3\times10^7$. Experiments with the red fresh water alga *Porphyridium purpureum* has reached population density of $3\times10^7$ cells mL$^{-1}$ in 16 d of incubation (Baquerisse et al., 1999). Studies with the fresh water alga *Chlorella ellipsoidea* has reached population density of $1.3\times10^8$ cells mL$^{-1}$ (Cho et al., 2007). Experiments with the fresh water algae *Haematococcus pluvialis* have reported a population density of $3.9\times10^6$ cells mL$^{-1}$ (Garcia-Malea et al., 2006).

**Table 4.1.** Population densities (cells mL$^{-1}$) after 18 days of incubation for four different algae under five different light treatments.

<table>
<thead>
<tr>
<th>Light treatment/Algae</th>
<th>Daily solar</th>
<th>Daily solar and 12h white light</th>
<th>24h Blue LEDs</th>
<th>Daily solar and 12h LEDs (Red and Blue)</th>
<th>24h LEDs (Red and Blue)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tetraselmis sp</em></td>
<td>2.65E+07</td>
<td>5.36E+07</td>
<td>8.96E+06</td>
<td>6.55E+07</td>
<td>2.49E+07</td>
</tr>
<tr>
<td><em>Nannochloris sp</em></td>
<td>4.74E+07</td>
<td>7.14E+07</td>
<td>9.17E+06</td>
<td>5.69E+07</td>
<td>2.13E+07</td>
</tr>
<tr>
<td><em>Scenedesmus sp</em></td>
<td>8.39E+06</td>
<td>2.02E+07</td>
<td>5.46E+06</td>
<td>1.39E+07</td>
<td>8.65E+06</td>
</tr>
<tr>
<td><em>Chlorella sp</em></td>
<td>1.06E+07</td>
<td>1.85E+07</td>
<td>1.60E+06</td>
<td>3.17E+07</td>
<td>7.80E+06</td>
</tr>
<tr>
<td>Mean</td>
<td>2.32E+07</td>
<td>4.09E+07</td>
<td>6.30E+06</td>
<td>4.20E+07</td>
<td>1.56E+07</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.80E+07</td>
<td>2.59E+07</td>
<td>3.56E+06</td>
<td>2.36E+07</td>
<td>0.69E+06</td>
</tr>
<tr>
<td>Coefficient of Variation</td>
<td>0.775</td>
<td>0.634</td>
<td>0.566</td>
<td>0.563</td>
<td>0.556</td>
</tr>
</tbody>
</table>

Independent of the algal species, light treatments that included daily solar radiation reached population densities in the magnitudes of $10^7$ cells mL$^{-1}$, see Table 4.1. The five light treatments were tested for ANOVA and no significant differences were found; $F = 2.9826$ and $p = 0.0537$. However the ANOVA test that include 4 treatments; all 24 h experiments including daily solar plus artificial illumination and 24 h artificial illumination resulted in statistical differences with $F = 3.9358$ and $p = 0.0362$. Individual treatments were tested with the T tests analysis of variance; Statistical differences were found in 24 h blue LEDs versus daily solar + 12 h cool white light with $T = 2.643$ and $p = 0.038$. A significant difference was found in 24 h blue LEDs versus daily
solar + 12 h red and blue LED with $T = 2.993$ and $p = 0.024$. The $T$ test 24 h blue LED against daily solar radiation showed no significant difference with $T = 1.842$ and $p = 0.115$. Further, the $T$ test showed that there is no statistical difference in daily solar radiation versus any treatments that included daily solar illumination.

The effects of the different light sources on algal growth can also be examined with reference to their specific growth rates as calculated during the exponential growth phase. Although the growth rate and the cumulative growth rate is an important measurement, factors such as the initial population, natural cell size of alga, turbulence of the growth media can affect the specific growth rate. Another measure of the effectiveness of the treatments is standing biomass of the final population density. For the four algae, *Tetraselmis sp*, *Nannochloris sp*, *Scenedesmus sp* and *C. vulgaris* the best performance in terms of population densities were achieved when grown under a combination of solar radiation (12 h) + artificial light for an additional 12 h. Under these conditions, all four algae reached final population densities in the order of $10^7$ cells mL$^{-1}$ after 18 d growth. From the results of this study solar radiation plays a key role on the growth of high population density in algae, mainly because this illumination contains all photosynthetic spectra 390-700 nm plus near infrared and provides the alga with sufficient energy reaching 3000 µmol m$^{-2}$s$^{-1}$ with a daily average of 1271 µmol m$^{-2}$s$^{-1}$. However solar radiation is only available half of the time, therefore when complemented resulted in a higher population density, as seen in the results of this study. The two light treatments tested as complements of solar radiation increased population density, however there was no conclusive difference which light complement performed best.

### 4.4.7 SPECIFIC GROWTH RATE FOR VARIOUS LIGHT TREATMENTS

The marine algae, *Tetraselmis sp* and *Nannochloris sp*, obtained higher final populations under the light treatments that included daily solar radiation, reaching a population density in the order of $10^7$ cells mL$^{-1}$, and were similar in the white light and red + blue LED treatments. *Tetraselmis sp* developed the highest cumulative growth rate of 7.6 in 18 d under treatments consisting of daily solar radiation and 12 h white light. Though the initial population for this treatment was the
lowest of all the five treatments, *Nannochloris sp* had a similar result under the same light treatment, the cumulative growth rate was 7.4 in 18 d and the initial population for this treatment was not the lowest. *Tetraselmis sp* attained the highest population density under exposure to 12 h solar radiation plus 12 h red (625 nm) + blue (430 nm) LEDs. The final population density of this treatment was 6.55 *10^7 cells mL^{-1}. Under exposure to daily solar radiation plus 12 h white light, *Nannochloris sp* reached a final population density of 7.1*10^7 cells mL^{-1}, which was the highest of the four algae. See Table 4.2 for more details

**Table 4.2.** Growth rate (d\(^{-1}\)) under different light treatments after 18 d of incubation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Daily Solar</th>
<th>Daily solar and 12h white light</th>
<th>24h Blue LEDs</th>
<th>Daily solar and 12h LEDs (Red and Blue)</th>
<th>24h LEDs (Red and Blue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial population cells mL(^{-1})</td>
<td>9.00E+05</td>
<td>2.75E+05</td>
<td>7.86E+05</td>
<td>6.60E+06</td>
<td>3.60E+06</td>
</tr>
<tr>
<td>Final population cells mL(^{-1})</td>
<td>2.65E+07</td>
<td>5.36E+07</td>
<td>8.96E+06</td>
<td>6.55E+07</td>
<td>2.49E+07</td>
</tr>
<tr>
<td>Mean</td>
<td>0.220</td>
<td>0.371</td>
<td>0.170</td>
<td>0.146</td>
<td>0.133</td>
</tr>
<tr>
<td>Max</td>
<td>0.656</td>
<td>1.306</td>
<td>0.693</td>
<td>0.540</td>
<td>0.578</td>
</tr>
<tr>
<td>Min</td>
<td>0.050</td>
<td>0.004</td>
<td>0.000</td>
<td>0.001</td>
<td>0.020</td>
</tr>
<tr>
<td>Total in 18 days</td>
<td>3.364</td>
<td>5.272</td>
<td>2.886</td>
<td>2.295</td>
<td>1.932</td>
</tr>
<tr>
<td>Cumulative growth</td>
<td>4.854</td>
<td>7.605</td>
<td>4.163</td>
<td>3.311</td>
<td>2.787</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Daily Solar</th>
<th>Daily solar and 12h white light</th>
<th>24h Blue LEDs</th>
<th>Daily solar and 12h LEDs (Red and Blue)</th>
<th>24h LEDs (Red and Blue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial population cells mL(^{-1})</td>
<td>2.88E+05</td>
<td>4.25E+05</td>
<td>3.25E+06</td>
<td>6.65E+06</td>
<td>4.75E+06</td>
</tr>
<tr>
<td>Final population cells mL(^{-1})</td>
<td>4.74E+07</td>
<td>7.14E+07</td>
<td>9.17E+06</td>
<td>5.69E+07</td>
<td>2.13E+07</td>
</tr>
<tr>
<td>Mean</td>
<td>0.345</td>
<td>0.363</td>
<td>0.062</td>
<td>0.133</td>
<td>0.089</td>
</tr>
<tr>
<td>Max</td>
<td>1.739</td>
<td>0.809</td>
<td>0.175</td>
<td>0.352</td>
<td>0.290</td>
</tr>
<tr>
<td>Min</td>
<td>0.048</td>
<td>0.004</td>
<td>-0.009</td>
<td>0.016</td>
<td>0.000</td>
</tr>
<tr>
<td>Total in 18 days</td>
<td>5.083</td>
<td>5.124</td>
<td>1.037</td>
<td>2.147</td>
<td>1.498</td>
</tr>
<tr>
<td>Cumulative growth</td>
<td>7.333</td>
<td>7.392</td>
<td>1.496</td>
<td>3.097</td>
<td>2.161</td>
</tr>
</tbody>
</table>
The two fresh water algae, *Scenedesmus sp* and *C. vulgaris*, followed similar growth patterns. For *Scenedesmus sp*, the treatments that included solar radiation plus artificial lighting reached population densities of $10^7$ cells mL$^{-1}$. Other treatments reached population densities of $10^6$ cells mL$^{-1}$. The highest growth rates were recorded for algae exposed to daily solar radiation + 12 h white light, reaching a final population density of $2.2\times10^7$ cells mL$^{-1}$ and a cumulative growth rate of 5.8 in 18 d. However, the initial population of this treatment was the lowest of all five treatments. For *C. vulgaris* the best treatments was daily solar radiation + 12 h red and blue LEDs. The final population of this treatment was $3.17\times10^7$ cells mL$^{-1}$ and the cumulative growth rate 2.8 in 18 d. The highest cumulative growth rate, 5.1, was obtained under daily solar radiation + 12 h white light, however this treatment, as well as the treatment with *Scenedesmus sp*, had the lowest initial population density of all treatments.
The ANOVA test of the cumulative growth rate for five light treatments and four algal species resulted in significant differences with $F = 7.0543$ and $p = 0.0021$. The individual T tests for specific treatments showed significant differences with 99% confidence in 4 of 10 possible tests: The light treatment daily solar + 12 h white light against 24 h blue LEDs resulted in significant differences $T = 4.669$ with $p = 0.003$. Another significant difference was for daily solar + 12 h white light against 24 h red and blue $T = 6.707$ with $p = 0.000$. Daily solar + 12 h white light against daily solar + 12 h red and blue LEDs showed a significant differences $T = 5.656$ with $p = 0.001$. Significant difference were found for the light treatment daily solar + 12 h red and blue LEDs against 24 h red and blue LEDs with $T = 3.647$ and $p = 0.011$. Overall the light treatments affected significantly the growth and cell population of alga, the best treatment in cell density and growth rate for two marine and two fresh water algae was the combination of daily solar + 12 h white light.

4.4.8 BIOMASS PRODUCTION

The standing biomass of alga growing under 24 h red-blue LEDs ranged from 910 mg L$^{-1}$ to 1160 mg L$^{-1}$, whereas alga growing under daily solar + 12 h red-blue LEDs developed biomass in the range of 1090 mg L$^{-1}$ to 1250 mg L$^{-1}$. See Figure 4.6 for details.
Figure 4.6. Standing biomass of four alga species growing under two light sources.

Algal species growing under daily solar + 12 h red-blue LED illumination developed a higher standing biomass than those alga species growing under 24 h red-blue LEDs alone. There was a statistically significant difference in standing biomass $F = 7.0049$, with $P = 0.0382$ (more than 96% of confidences) between these two light treatments in the four alga species.

4.4.9 CELL WEIGHT

Algal cells weight ranged from 18 to 130 pico grams (pico gram $1 \times 10^{-12}$ g), and differed between algal species. The results show that algal cells are heavier when grown under 24 h LEDs illumination than under daily solar + 12 h LEDs. The algae *Tetraselmis sp* and *Nannochloris sp* had double the weight growing under the light source of 24 h LEDs; for *C. vulgaris* the difference in weight was 3 times higher when the alga were grown under 24 h LED illumination in comparison to daily solar + 12 h LEDs. See Table 4.3 for details.
The results of this study show that alga cell weight is altered by light treatments. A study conducted with the alga *Tetraselmis suecica* under different LEDs showed that cell weight was different when alga were grown under blue + red illumination (Abiusi et al., 2013). An explanation for greater alga cell weight under 24 h LEDs and higher cell population and biomass under daily solar + 12 h LED may be because of availability of space. At high cell populations, culture space for growth in volume becomes limiting, therefore cell weight is smaller. Space efficiency is better used by packing more cells per volume resulting in less free space and therefore, more biomass. Another explanation may be to avoid mechanical damage as in a highly populated system there is more possibility for cell collisions than in a less populated culture. In addition smaller cell size would sustain less mechanical damage during collisions than would bigger cells. From the observation of this study, the standing biomass and cell density is high when alga cell weight of is low.

### 4.5 CONCLUSIONS

The nature of the light source used for illumination in algal reactors has a significant effect on algal growth rate, cell density, biomass production and cell weight. The results of this study confirm that light treatments affect these parameters in both marine and fresh water algae species.
We hypothesized that growth rate would be higher in those treatments exposed to 24 h illumination compared to daily solar radiation. However, this study showed that a critical component of the light treatment is exposure to solar radiation. Light treatments that included solar radiation performed better than those that didn’t. The light treatments of daily solar radiation performed better than 24 h blue LED and 24 h red + blue LED, presumably because solar radiation provides all the light wavelengths required for algae growth.

Independent of algal species, the light treatments that produced the highest cell density, growth rate and standing biomass are those that combined daily solar radiation with 12 h artificial illumination during the night period. In these treatments degradation of algal biomass during the night period due to algae respiration (Ogbonna et al., 1999; Ogbonna and Tanaka, 1996) are minimized because photosynthetic processes are kept active during night time.

Alga cell weight is higher and standing biomass is lower in those algae growing under 24 h LEDs illumination. As a result of the low cell population there is more space for alga cells to grow, and this free space also reduced the probabilities of cell collisions of large heavy cells and causing less mechanical damage, however space efficiency is low because there is a lot empty space that can be filled with algae.

Further studies which combine 12 h LED illumination with daily solar radiation should be conducted to establish a clear dependence of solar radiation as a key factor to ensure more efficient algal growth. This study showed that daily solar radiation performed better than 24 h exposure to artificial light.
4.6 REFERENCES


CHAPTER 5  BIOMASS PRODUCTION OF SIX ALGA SPECIES GROWING UNDER LED AND SOLAR RADIATION

5.1 ABSTRACT

Three marine and three fresh water alga species were grown under two light treatments: a 24 h continuous exposure to red, blue and red-blue LED illumination and a daily exposure to solar radiation supplemented with nightly 12 h LED illumination. The algae were grown for 49 d during which biomass measurements were taken every 7 d. The marine algae *Nannochloris sp* produced an average biomass of 81-94 mg L⁻¹ d⁻¹; *Tetraselmis sp* produced 78-82 mg L⁻¹ d⁻¹ and *Dunaliella salina* produced 44-59 mg L⁻¹ d⁻¹. The fresh water alga *Chlamydomonas sp* produced 73-86 mg L⁻¹ d⁻¹; *Chlorella vulgaris* produced 60-86 mg L⁻¹ d⁻¹ and *Scenedesmus sp* produced 61-81 mg L⁻¹ d⁻¹. We found statistical differences in biomass production between algae species and among light treatments (p = 0.0000), we also found statistical differences between marine algae treatments (p = 0.0000) and between fresh water algae treatment (p = 0.0014).

5.2 INTRODUCTION

Systems for alga production have advanced during the past three decades from opens ponds to closed tubular systems, to thin layer, airlift flat panel photo-bioreactors. Algal production in closed reactors is 20 to 90% higher than in open systems. Thin layer, airlift flat panel photo-bioreactors are more efficient in biomass production than open pond systems and tubular photo-bioreactors because they provide better light utilization by reducing exposure periods of shadow in the reactor. Also these reactors usually differ in media turbulence, velocity, oxygen saturation, light source and intensity and duration of light exposure, including the flashing light effect (Goldman, 1979).
Light supply is an important factor controlling algal growth as it influences cell density and biomass production. Open outdoor reactors have evolved in the past 30 y and are capable of supporting biomass production rates of 15-25 g m$^{-2}$ d$^{-1}$ or 0.6-1 g L$^{-1}$ d$^{-1}$, with production peaks of 30-40 g m$^{-2}$ d$^{-1}$ or 1.2-1.6 g L$^{-1}$ d$^{-1}$ (Goldman, 1979). Reports of commercial production of *Skeletonema sp* in open systems have recorded biomass production rates ranging from 1.8 to 22.3 g m$^{-2}$ d$^{-1}$ or 0.072-0.89 g L$^{-1}$ d$^{-1}$, with peaks of 30 g m$^{-2}$ d$^{-1}$ or 1.2 g L$^{-1}$ d$^{-1}$ in June-August (Pauw et al., 1983).

Development of closed-tubular bioreactor systems has evolved in Italy during the past 17 y, doubling biomass production rates. Carlozzi (2008) reported a production increase from 25 g m$^{-2}$ d$^{-1}$ or 1.0 g L$^{-1}$ d$^{-1}$ in 1986 to 47.7 g m$^{-2}$ d$^{-1}$ or 1.9 g L$^{-1}$ d$^{-1}$ in 2003 for the algae *Arthrospira platensis* and production rates of 0.49 g L$^{-1}$ d$^{-1}$ for *Tetraselmis suecica*. Comparable biomass production rates for the algae *Phaeodactylum tricornutum*, 1.2 g L$^{-1}$ d$^{-1}$, were obtained using a horizontal tubular air-lift photobioreactor exposed to solar radiation (Acien Fernandez et al., 2001). Table 1 summarizes biomass production rates for different algal species.
Other systems for algal production include flat panel photo-reactors varying in reactor inclination and depth, and airlift reactors. Production of *Spirulina platensis* was 0.3 g L⁻¹ d⁻¹ with a 10.4 cm reactor depth, increasing to 4.3 g L⁻¹ d⁻¹ when the depth of the reactor decreased to 1.3 cm (Hu et al., 1996). A vertical photo-bioreactor of 3 cm depth and containing baffles to provide a flashing light exposure resulted in *Chlorella vulgaris* biomass production of 1.95 g L⁻¹ d⁻¹, with production peaks of 2.6 g L⁻¹ d⁻¹ (Degen et al., 2001). Biomass production of *Chlorella sp* in an outdoor, thin layer photo-bioreactor reached 30 g m⁻² d⁻¹ or 1.2 g L⁻¹ d⁻¹, the annual biomass production estimated to be 80-100 tons ha⁻¹ y⁻¹ (Doucha and Livansky, 2009).
The objective of this study was to evaluate biomass production of three marine and three fresh water algal species grown under two light treatments: a 24 h continuous exposure to LED illumination and a daily exposure to solar radiation supplemented with nightly 12 h LED illumination.

5.3 MATERIALS AND METHODS

5.3.1 MICROORGANISM

Five of the six algal cultures used in this study were obtained from the algal culture collection located at the University of Texas, Austin, TX. They included *Chlorella vulgaris* (26), *Nannochloris* sp (1268), *Scenedesmus* sp (1589), *Tetraselmis* sp (2767), and *Dunaliella salina* (1644). A species of fresh water alga, *Chlamydomonas* sp, was isolated from a local liquid manure tank (Fredonia, Colombia), and identified from photographic records.

Cultures were established in 1 L batch cultures for 15 d and then transferred to 4-L reactors. The population density was established using direct microscopic counting techniques. The population density at the start of the experiment was set at 4.5-5.0x10^5 cells mL^{-1} by adjusting the volume of medium in the reactors.

5.3.2 BIOREACTOR SYSTEM

Glass containers of 4 L capacity were filled with 3.6 L of nutrient media. Six replicate containers were kept in a darkened room and provided continuous illumination from a LED source; 6 replicate containers were placed outdoors and received daily solar radiation and 12 h LED illumination during the night.

5.3.3 LIGHT SOURCE

The LED lighting systems, contained in a sealed, transparent plexiglass container to protect against water damage, were placed in the center of each bioreactor. The light emission intensity
of each LED was 2143 μmol m\(^{-2}\) s\(^{-1}\) for blue LEDs and 1345 μmol m\(^{-2}\) s\(^{-1}\) for red LEDs. The lighting system in each reactor contained 26 red LEDs and 26 blue LEDs for a total light power of 4.94 W (2.34 W for red and 2.6 W for blue) or a power/bioreactor volume ratio of 1.37 W L\(^{-1}\). Light emission intensity was measured in μmol m\(^{-2}\) s\(^{-1}\) using a quantum meter (Model MQ, Apogee Instruments Inc., Logan, UT, USA).

5.3.4 LIGHT DISPERSION

LEDs of 0.09-0.1W can produce emission from 1345 to 2143 μmol m\(^{-2}\) S\(^{-1}\), this radiation is more than the optimum radiation for photosynthesis activity; 50-250 μmol m\(^{-2}\) S\(^{-1}\) therefore the radiation from the LED could cause photo-inhibition and photo-damage which leads to the reduction of the photosynthetic activity (Kim et al., 2006; Degen et al., 2001; Ogbonna et al., 1999; Merchuk et al., 1998; Fernandez-Sevilla et al., 1998; Molina –Grima et al., 1996, Garcia-Sanchez et al., 1996; Qiang and Richmond, 1994; Aiba, 1982; Bannister, 1979; Eppley and Coatsworth, 1966). The dispersion of light in algae media develops in short distances, in this experimental setup the distance from the LED bulb to the algal in suspension is 7 mm. Enough distance to reduce the light intensively from 2143 μmol m\(^{-2}\) s\(^{-1}\) to 300 μmol m\(^{-2}\) s\(^{-1}\) or even lower, therefore avoiding photo inhibition process, however the distance is enough to support optimum algal phototropic growth under artificial conditions (Ogbonna et al., 1999).

5.3.5 EXPERIMENT 1: 24 H RED + BLUE LEDS

The illumination was provided by LEDs (Alas, China) and consisted of red emitting diodes at 620-625 nm and blue at 425-430 nm. The illumination system was driven by a power supply of 12 V and 8 amp (Model SA 201-3485, ASTEC, California, USA).

5.3.6 EXPERIMENT 2: DAILY SOLAR + 12 H LEDS

Six reactors were exposed to daily solar radiation and provided red (620-625nm) and blue (425-430nm) LED illumination for 12 h during the night.
5.3.7 MIXING, QUANTIFYING CARBON SOURCE UNDER AUTOTROPHIC GROWTH AND TEMPERATURE MEASUREMENTS

Two Resun turbines (Model AC-9904, Resun, China) were used to supply CO₂ to the reactors and for gentle mixing of the algal culture, one located inside and the other outside. Each turbine provide a volume of 12 L min⁻¹ in 21.6 L of algal suspension (air/media volume ratio was 556 mL of air L⁻¹ min⁻¹ growing media). The CO₂ concentration supplied in the air to the two experiments ranged from 0.0323% to 0.0428%, measured by CO₂ meter (Model 7001, Telaire-General Electric, California, USA).

The temperature (inside and outside) fluctuated from 23°C ± 2 as measured by an Infrared thermometer (Model Fluke 62, Fluke Corporation, Everett, Washington, USA).

5.3.8 CULTURE MEDIUM

Commercial fertilizer (Solucat 25-5-5, Atlantica Agricola, Villena, Spain) containing nitrogen 25% as ammonia 14.2% plus nitrates 10.8%. P₂O₅ 5%, K₂O 5%, Fe 0.020%, Mn 0.010%, Zn 0.002%, B 0.010%, Cu 0.002%. We used 0.8 g of fertilizer per litre of alga suspension for both fresh water and marine alga, at the beginning of the experiment the electrical conductivity was 0.95 ms ± 0.01 ms, measured by EC sensor (Model HI 991301, Hanna, Michigan, USA). The three marine algae were amended with 3.5% of NaCl after the application of the solucat fertilizer.

5.3.9 BIOMASS DETERMINATION

For biomass quantification we used the dry weight methodology which is divided in 7 steps:

1. Dry a 15 mL glass tubing until reach constant weight.

2. Fill out the tubing with 10mL of the algae media from the experiment.
3. Centrifuge the tubing at 4000 rpm for 15 min.

4. Separate the water residues in the tubing.

5. Dry the tubing (105 °C) with the algae in an oven until reaches constant weight.

6. Weigh the tubing with the algae and subtract the weight of the tubing without the algae.

7. The resulting value is the alga biomass in a volume of 10mL. We repeated this procedure in duplicate each time alga biomass was measured.

5.3.10 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experiment measured standing biomass for six algae species; three marine *Tetraselmis sp*, *Dunnaliella salina* and *Nannochloris sp* and three fresh water *Chlorella vulgaris*, *Scenedesmus sp* and *Chlamydomonas sp*. The algae were growing under two different light conditions; Experiment 1; Algae growing under 24 h red and blue LEDs and experiment 2; algae growing under daily solar radiation and 12 h red and blue LEDs. The algae were grown for 49 d, every 7 d the biomass was measured before and after harvesting half of the reactor volume (1.8 L per reactor). The growing media was 0.8 g L\(^{-1}\) of Solucat fertilizer for both experiments. The light power ratio was 1.37 W L\(^{-1}\) and the air ratio was 556 mL L\(^{-1}\) for both experiments. The biomass results were analyzed using statistical methods such as standard deviation, coefficient of variance, and one way ANOVA and T test using Biostatistics 1.0.

5.4 RESULTS

5.4.1 BIOMASS PRODUCTION FOR FRESH WATER ALGAE GROWING UNDER TWO DIFFERENT LIGHT TREATMENTS

The biomass production for three fresh water algae during 49 days of incubation period ranged from 320 to 1070 mg L\(^{-1}\) week\(^{-1}\) and the average was from 423 to 627 mg L\(^{-1}\) week\(^{-1}\). The standing biomass ranged from 900 to 1180 mg L\(^{-1}\), thus the biomass recovery after harvest was 7 d, showing a doubling rate of 6-7 days, and the coefficient of variance of the biomass production
fluctuated from 9.6% to 32%. For all algal cultures, the light treatment consisting of daily solar radiation and red + blue LEDs during the night resulted in more biomass production than continuous illumination with red + blue LEDs. The cumulative biomass production during 49 d is shown in Table 5.2. was for *Chlorella sp* 24 h LED 2960 mg L\(^{-1}\) in solar-LED 4230 mg L\(^{-1}\), for *Scenedesmus sp* in 24 LED 3030 mg L\(^{-1}\), in solar-LED 3990 mg L\(^{-1}\), for *Chlamydomonas sp* in 24h LED 3590 mg L\(^{-1}\), in solar-LED 4390 mg L\(^{-1}\). See Table 5.2 for details.

The experiment conducted on the 6 algae developed similar standing biomass; biomass production ranged from 190 to 776 mg L\(^{-1}\) week\(^{-1}\), the average was from 317 to 659 mg L\(^{-1}\) and the maximum standing biomass 1310 mg L\(^{-1}\). The alga *Nannochloris sp* developed the highest biomass production 4614 mg L\(^{-1}\) under the light treatment solar-LED and 3980 mg L\(^{-1}\) under LED. *Tetraselmis sp* developed more biomass under LED 4016 mg L\(^{-1}\) and 3840 mg L\(^{-1}\) under solar-LED. The lowest biomass production recorded of all 6 algae was *Dunaliella salina* 2220 mg L\(^{-1}\) under LED and 2870 mg L\(^{-1}\) under solar-LED. The growth of the marine algae is more stable than the growth of the fresh water algae, therefore the coefficient of variance 7.6% to 22% is lower than the coefficient of variance of the fresh water algae.

The algae *Chlamydomonas sp* developed the highest biomass production for the fresh water algae, whereas *Nannochloris sp* produced more biomass than the other two marine algae and the three fresh water algae including *Chlamydomonas sp*. The marine algae *Nannochloris sp* produced highest biomass of all six algae grown in the present study. See Table 5.3 for more information.
## Table 5.2. Biomass production for three fresh water algae under two light sources.

<table>
<thead>
<tr>
<th>Incubation days</th>
<th>Chlorella vulgaris</th>
<th>Scenedesmus sp</th>
<th>Chlamydomonas sp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LEDs 24h Daily</td>
<td>LEDs 24h Daily</td>
<td>LEDs 24h Daily</td>
</tr>
<tr>
<td></td>
<td>Biomass mg L⁻¹</td>
<td>Biomass mg L⁻¹</td>
<td>Biomass mg L⁻¹</td>
</tr>
<tr>
<td>0-7 (1-7)</td>
<td>480</td>
<td>490</td>
<td>430</td>
</tr>
<tr>
<td>7–14</td>
<td>380</td>
<td>690</td>
<td>420</td>
</tr>
<tr>
<td>15-21</td>
<td>500</td>
<td>560</td>
<td>480</td>
</tr>
<tr>
<td>21–28</td>
<td>370</td>
<td>710</td>
<td>500</td>
</tr>
<tr>
<td>28–35</td>
<td>470</td>
<td>590</td>
<td>480</td>
</tr>
<tr>
<td>35–42</td>
<td>470</td>
<td>590</td>
<td>480</td>
</tr>
<tr>
<td>42–49</td>
<td>340</td>
<td>590</td>
<td>320</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2960</strong></td>
<td><strong>4230</strong></td>
<td><strong>3030</strong></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>422.9</strong></td>
<td><strong>604.3</strong></td>
<td><strong>570.0</strong></td>
</tr>
<tr>
<td><strong>Max</strong></td>
<td><strong>500.0</strong></td>
<td><strong>490.0</strong></td>
<td><strong>510.0</strong></td>
</tr>
<tr>
<td><strong>Min</strong></td>
<td><strong>340.0</strong></td>
<td><strong>710.0</strong></td>
<td><strong>320.0</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td><strong>61.8</strong></td>
<td><strong>520</strong></td>
<td><strong>510.0</strong></td>
</tr>
<tr>
<td><strong>Coefficient of Variation</strong></td>
<td><strong>0.146</strong></td>
<td><strong>75.2</strong></td>
<td><strong>380.0</strong></td>
</tr>
<tr>
<td>Max standing biomass mg L⁻¹</td>
<td><strong>1010</strong></td>
<td><strong>0.125</strong></td>
<td><strong>470.0</strong></td>
</tr>
</tbody>
</table>

## Table 5.3. Biomass production for three marine algae under two light sources.

<table>
<thead>
<tr>
<th>Incubation days</th>
<th>Tetraselmis sp</th>
<th>Nannochloris sp</th>
<th>Dunaliella salina</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LEDs 24h Daily</td>
<td>LEDs 24h Daily</td>
<td>LEDs 24h Daily</td>
</tr>
<tr>
<td></td>
<td>Biomass mg L⁻¹</td>
<td>Biomass mg L⁻¹</td>
<td>Biomass mg L⁻¹</td>
</tr>
<tr>
<td>0-7 (1-7)</td>
<td>560</td>
<td>520</td>
<td>590</td>
</tr>
<tr>
<td>7–14</td>
<td>350</td>
<td>550</td>
<td>570</td>
</tr>
<tr>
<td>15-21</td>
<td>776</td>
<td>540</td>
<td>560</td>
</tr>
<tr>
<td>21–28</td>
<td>590</td>
<td>490</td>
<td>480</td>
</tr>
<tr>
<td>28–35</td>
<td>600</td>
<td>540</td>
<td>620</td>
</tr>
<tr>
<td>35–42</td>
<td>550</td>
<td>580</td>
<td>610</td>
</tr>
<tr>
<td>42–49</td>
<td>590</td>
<td>620</td>
<td>550</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>4016</strong></td>
<td><strong>3840</strong></td>
<td><strong>3980</strong></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>573.7</strong></td>
<td><strong>548.6</strong></td>
<td><strong>568.6</strong></td>
</tr>
<tr>
<td><strong>Max</strong></td>
<td><strong>776.0</strong></td>
<td><strong>620.0</strong></td>
<td><strong>770.0</strong></td>
</tr>
<tr>
<td><strong>Min</strong></td>
<td><strong>350.0</strong></td>
<td><strong>490.0</strong></td>
<td><strong>480.0</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td><strong>124.5</strong></td>
<td><strong>41.8</strong></td>
<td><strong>46.7</strong></td>
</tr>
<tr>
<td><strong>Coefficient of Variation</strong></td>
<td><strong>0.217</strong></td>
<td><strong>0.076</strong></td>
<td><strong>0.082</strong></td>
</tr>
<tr>
<td>Max standing biomass mg L⁻¹</td>
<td><strong>1230</strong></td>
<td><strong>0.076</strong></td>
<td><strong>0.110</strong></td>
</tr>
</tbody>
</table>
5.4.2 MEAN AND TOTAL BIOMASS PRODUCTION

This study shows higher biomass production in five of the six algae species grown under daily solar radiation + 12 h LED illumination. Three of the algae, a marine and two fresh water, produced a weekly mean of more than 600 mg L\(^{-1}\) of biomass and a cumulative biomass production reached 4 g L\(^{-1}\) during the 49 d of incubation (Figure 5.1 and 5.2).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure51.png}
\caption{Mean weekly algal biomass production under two light treatments during 49 d of incubation.}
\end{figure}

The highest weekly mean biomass production in 49 days was 659 mg L\(^{-1}\) obtained by the algal *Nannochloris sp* under the light treatment; daily solar plus 12 h LED during night periods. The ANOVA test for the entire experiment six algae species growing under two light treatment with 7 repetitions of seven days resulted in F = 9.3594 with p = 0.0000, this test prove that there is
differences among algae treatment and light treatment. Figure 5.2 shows total biomass production during the 49 days experiment.

**Figure 5.2.** Total algal biomass production under two light treatments.

ANOVA was performed to compare biomass production of algal species growing under the two light treatments. The results for the three fresh water algae $F = 5.0021$ with $p = 0.0014$ and the ANOVA for the three marine algae $F = 20.6136$ with $p = 0.0000$. These ANOVA tests show that there is a statistically significant difference among light treatments and algae species with $p < 0.01$. We also performed an ANOVA for each alga to evaluate the different light treatments. Table 5.4 shows statistical differences in light treatment for the algae *Chlorella sp*, *Scenedesmus sp* with $p < 0.01$ and for *Nannochloris sp* and *Dunaliella salina* with $p < 0.02$. 

![Total Algal Biomass (mg L$^{-1}$) During 49 d of Incubation](chart.png)
Table 5.4. ANOVA test for total biomass production.

<table>
<thead>
<tr>
<th>Algae ANOVA light treatments (24 h LEDs against daily solar plus 12 h LEDs in six algae)</th>
<th>Chlorella vulgaris</th>
<th>Scenedesmus sp</th>
<th>Chlamydomonas sp</th>
<th>Tetraselmis sp</th>
<th>Nannochloris sp</th>
<th>Dunaliella salina</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>24.2907</td>
<td>F = 19.3885</td>
<td>F = 1.9406</td>
<td>F = 0.2567</td>
<td>F = 7.6842</td>
<td>F = 8.3553</td>
</tr>
<tr>
<td>P</td>
<td>0.0003</td>
<td>p = 0.0009</td>
<td>p = 0.1889</td>
<td>p = 0.6216</td>
<td>p = 0.0169</td>
<td>p = 0.0136</td>
</tr>
</tbody>
</table>

Note: Statistical differences in bold p < 0.05

We performed 60 T tests to identify statistical differences between each individual algae and for each light treatment. The T test for the light treatment 24 h LED showed differences between algae resulting in statistical difference with p < 0.05 in 11 of the 15 test. The only cases where there were no differences were for *Chlorella sp* against *Scenedesmus sp*, *Chlamydomonas sp* against *Scenedesmus sp*, *Chlamydomonas sp* against *Nannochloris sp* and *Tetraselmis sp* against *Nannochloris sp*. For the treatment of daily solar radiation plus 12 h LED and comparing algae against algae, we found statistical differences in 7 of the 15 tests. For the T test in algae with 24 h LEDs against algae with daily solar plus 12 h LED, 11 out of the 15 tests showed statistical differences with p < 0.05. For the test for algae with the light treatment daily solar plus 12 h LED illumination against algae provided 24 h LED showed 7 out of the 15 with statistical difference. In total 36 of the 60 individual tests comparing algal against algal resulted in statistical difference with p < 0.05.

5.4.3 BIOMASS VARIANCE

The weekly mean production of marine algal biomass showed a lower coefficient of variance than that of the fresh water algae, though the variance was lower in the fresh water algae growing under continuous LED illumination. The marine algae developed lower average biomass variance under daily solar radiation plus 12 h LED. *Chlamydomonas sp* and *Dunaliella salina* have the highest coefficient of variance of the algae species studied (Figure 5.3).
5.5. DISCUSSION AND CONCLUSIONS

The light treatment combining daily solar radiation plus LED during the night produced greater standing biomass and greater daily biomass production among five of the six algae species during the 49 d experiment. There were statistical differences in biomass production for the two light treatments among four of the six algae. The combination of solar radiation plus LED illumination during the night periods produced more biomass than continuous LED illumination.

There were also differences in biomass production among algae species; *Nannochloris* *sp* under solar-LED produced 4614 mg L$^{-1}$ with an average daily production of 94 mg L$^{-1}$ d$^{-1}$; under

**Figure 5.3.** Biomass variance during the experiment period.
continuous LED illumination biomass production was 3980 mg L\(^{-1}\) and daily average of 81 mg L\(^{-1}\)d\(^{-1}\). The second largest production was 4390 mg L\(^{-1}\) by *Chlamydomonas sp* under combined solar-LED illumination and 3590 mg L\(^{-1}\) under continuous LED. *Chlorella vulgaris* produced 4230 mg L\(^{-1}\) under solar-LED and 2960 mgL\(^{-1}\) under continuous LED. *Scenedesmus sp* developed 3990 mgL\(^{-1}\) under solar-LED and 3030 mgL\(^{-1}\) under continuous LED. The marine alga *Tetraselmis sp* adapted well to the artificial illumination producing 4016 mg L\(^{-1}\) under continuous LED exposure and 3840 mg L\(^{-1}\) under solar-LED. The lowest biomass production was registered in *Dunaliella salina*, 2870 mg L\(^{-1}\) under solar-LED illumination and 2200 mg L\(^{-1}\) under continuous LED. Statistical analysis shows significant differences with P >0.001 in biomass production among fresh water algae, marine algae, and the 6 algae species together.

The mean daily biomass production by *Nannochloris sp* under the present study 81-94 mg L\(^{-1}\) d\(^{-1}\) is higher than the 20 mg L\(^{-1}\) d\(^{-1}\) reported by Lee et al., (2011) but is lower than that reported by Bondioli et al. (2012) of 396 mg L\(^{-1}\) d\(^{-1}\) , 1011 mg L\(^{-1}\) d\(^{-1}\) by Park et al. (2012); 116 mg L\(^{-1}\) d\(^{-1}\) by Su et al. (2011) and 520 mg L\(^{-1}\) d\(^{-1}\) by Huerlimann et al. (2010). The daily biomass production by *Chlamydomonas sp* 73-86 mg L\(^{-1}\) d\(^{-1}\) is much lower than the 820-2000 mg L\(^{-1}\) d\(^{-1}\) reported by Kong et al. (2009). The mean biomass production of *Chlorella vulgaris* at 60-86 mg L\(^{-1}\) d\(^{-1}\) is similar to 70 mg L\(^{-1}\) d\(^{-1}\) reported by Zhao et al. (2013), but higher than 50 mg L\(^{-1}\) d\(^{-1}\) reported by Kong et al. (2011), also higher than 13 mg L\(^{-1}\) d\(^{-1}\) reported by Liang et al. (2009) and lower than 100-1440 mg L\(^{-1}\) d\(^{-1}\) reported by Ramos and Roux (1986). The alga specie *Scenedesmus sp* produced a mean biomass of 61-81 mg L\(^{-1}\) d\(^{-1}\) which is higher than 1.3-1.5 and 26 mg L\(^{-1}\) d\(^{-1}\) reported by Goswami (2011) and Andrade et al. (2009), but lower than 870 mg L\(^{-1}\) d\(^{-1}\) reported by Sanches et al., 2008). The marine alga *Tetraselmis sp* developed a mean daily biomass of 78-82 mg L\(^{-1}\) d\(^{-1}\) which was higher than 64 mg L\(^{-1}\) d\(^{-1}\) reported by Lee et al. (2011) and much lower than that reported by Bondioli et al. (2012) at 304 mg L\(^{-1}\) d\(^{-1}\), 1350 mg L\(^{-1}\) d\(^{-1}\) by Garcia et al. (2012), 214 mg L\(^{-1}\) d\(^{-1}\) by Go et al. (2012) and 1800 mg L\(^{-1}\) d\(^{-1}\) by Huerlimann et al. (2010). The alga *Dunaliella salina* grew in a slower rate than the other algal species in this study. Its mean biomass production was 44-59 mg L\(^{-1}\) d\(^{-1}\) which was lower than results previously reported at 80-1000 mg L\(^{-1}\) d\(^{-1}\) by Garcia-Gonzales et al. (2005) and Cortinas et al. (1984) (Table 5.1).
The marine algae *Tetraselmis sp* was better adapted for growth under artificial illumination producing greater biomass under continuous LED exposure than under the daily solar-LED combination. Biomass production by *Tetraselmis sp* under continuous LED illumination was the highest among the 6 algal species, though it was among the lowest under solar-LED illumination.

The coefficient of variance ranged from 7 to 32%; the fresh water algae *Chlamydomonas sp* developed the highest coefficient of variance of the 6 algae species under solar-LED the CV was 32% and 14.2 % under continuous LED. The marine algae *Tetraselmis sp* had a CV 21.7% under continuous LED and 7.6% under solar-LED.

From this research we can conclude with statistical support that growing algae with illumination that combines both solar radiation and artificial LED lighting during the night results in greater biomass production than continuous artificial illumination alone. This is because algae has evolved with solar illumination.
5.6 REFERENCES


9. Dev-Goswami, R. C. 2011. *Scenedesmus dimorphus* and *Scenedesmus quadricauda* two potent indigenous microalgae strains for biomass production and CO\textsubscript{2} mitigation – a study on their growth behavior and lipid productivity under different concentration of urea as nitrogen source. J. Algal Biomass Utln. 2-4: 42-49.


CHAPTER 6 EFFECT OF USING DIFFERENT LEDs LIGHTS ON THE GROWTH AND BIOMASS PRODUCTION OF CHLORELLA VULGARIS

6.1 ABSTRACT

Use of artificial light for indoor algae production is expensive so it is critical that the source of the light energy be efficient for cost-effectiveness. The effects of continuous exposure to four different LED illumination systems on the growth of *Chlorella vulgaris* were studied during a 15 d incubation. The light sources evaluated supplied white (380-760 nm), blue (430 nm), red (625 nm) and a combination red+blue wavelengths. The growth parameters measured included cell density and biomass production. Exposure to white and to red+blue lights resulted in the highest growth rates, reaching a population density of 1.8*10^7 cells mL^-1, with a standing biomass of 1 g L^-1 and a maximum doubling rate of 1.1 d^-1. Red light exposure resulted in the lowest cell density of 1.04*10^7 cells mL^-1 with a standing biomass of 0.57 g L^-1 and maximum doubling rate of 0.72 d^-1. This study showed that light sources combining wavelengths absorbed by the photosynthetic pigments of *C. vulgaris* have potential to maximize growth.

*Key words*: Artificial light; illumination system; algal production; light wavelength absorption

6.2 INTRODUCTION

Alga biomass has become an important source of high-valued products for a wide variety of uses (Hejazi and Wijffels, 2004; Sanches et al., 2002; Lorenz and Cysewski, 2000). Their products include carotenoids and β-carotene photosynthetic pigments, astaxanthins, canthaxanthin, lutein, phycocyanin, which can be used as natural colorants and antioxidants (Garcia-Gonzalez et al., 2005; Del campo et al., 2004; Lorenz and Cysewski, 2000; Vandamme, 1994), proteins for
human and animal feed stocks and enzymatic reactions, and therapeutics for a source of renewable pharmaceuticals (Borowitzka, 1995; Schwartz et al., 1995). Some of the products are antibacterial, antiviral, antitumor, and antifungal (Cannell, 1993). Algae also produce polyunsaturated fatty acids rich in Omega 3, 6 and 9 and eicosapentanoic acid (Viswanath et al., 2010; Belarbi et al., 2000) that can be used for nutritional application and biofuel production.

The alga *Chlorella sp* have been used in the pharmaceutical industry as a source of carbohydrate extract for the immune system in the production of anti-flu (Pulz and Gross, 2004). Studies have reported the use of *Chlorella sp* extracts for their antibiotic properties against gram positive and gram negative bacteria, and for the growth inhibition of four cancer cell lines (Ordog et al., 2004; Metting and Pyne, 1986). *Chlorella sp* have also been used in preparations of health food supplements and as a source of amino acids (Benemann et al., 1987). Global production of this species at 2000 tons year\(^{-1}\) is second largest for single algal cell culture (Pulz and Gross, 2004).

Light energy has a central role for controlling algal growth under a phototropic regime and, when these organisms are grown under artificial conditions, light becomes the most expensive single input. Therefore, the quality of the light, the wavelength of the photon, as well as photon intensity and duration (referred to as the flash effect or light/dark cycles), are important for induction of phototropic growth and regulation of growth rate (Wang et al., 2007; Katsuda et al., 2006; Jeon et al., 2005, 2006). Light emitting diodes (LEDs) can be used to supply photons at the wavelengths that the algae absorb and at an appropriate intensity to support phototropic growth (Yeh and Chung, 2009). Thus, LED technology is efficient at converting electrical energy into light energy and this is an advantage for algae production (Michel and Eisentraegar, 2004). This study examined the growth and biomass production of *Chlorella vulgaris* under 4 LED light sources: red (625 nm), blue (430 nm), red+blue, and white (380-760 nm) over a 15-d incubation.
6.3 MATERIALS AND METHODS

6.3.1 MICROORGANISM

*Chlorella vulgaris* (UTEX 26) was obtained from the algal culture collection located at the University of Texas, Austin, TX. The culture was established in 1 L batch cultures for 15 d and then transferred to 4-L reactors. The population density was determined using direct microscopic counting techniques. The population density at the start of the experiment was set at $4.5 \times 5 \times 10^5$ cells mL$^{-1}$ by adjusting the volume of medium in the reactors.

6.3.2 LIGHT SOURCE

Four light sources providing blue, red, white and red+blue illumination were evaluated. The light sources were 0.1 W LEDs obtained from ALAS™ with the following emission parameters: red, 620-625 nm wavelength at 1345 µmol m$^{-2}$ s$^{-1}$ intensity; blue, 425-430 nm wavelength at 2143 µmol m$^{-2}$ s$^{-1}$; white, 380-760 nm wavelength at 1680 µmol m$^{-2}$ s$^{-1}$ intensity. Each light source contained 52 LEDs fixed into a clear plexiglass tube (internal diameter, 4 cm) and placed in the centre of the 4-L culture flask (internal diameter 12 cm). The illumination system was powered by a transformer (Model SA 201-3485, ASTEC, California, USA) supplying 12 V and 8 amp. To ensure that external illumination would not affect the experiment, the laboratory was kept dark throughout the incubation period.

6.3.3 LIGHT EMISSION INTENSITY

Light intensity was measured using a quantum meter (Model MQ, Apogee Instruments Inc., Logan, UT, USA), and with a Lux Meter (Model MS6610, VIA Instruments, Shanghai, China). Light emission from a LED is intense, a 0.1 W bulb produces from 1700 to 2300 µmol m$^{-2}$ s$^{-1}$ and this amount of energy greatly exceeds the maximum photosynthetic light absorption capacity of algae, which is 50-250 µmol m$^{-2}$ s$^{-1}$. This could cause photo-inhibition and damage to the photosynthetic system which would reduce the photosynthetic activity of the cell (Kim et al.,
Since the intensity of light decreases as an inverse square of the distance from the source, the LEDs were positioned at a distance from the algal suspension to provide an emission intensity level acceptable for supporting photosynthetic growth (Table 6.1). In our study the distance from the LED source to the algal suspension was 5 mm, a sufficient distance to reduce the light intensity to less than 300 µmol m\(^{-2}\) s\(^{-1}\), which is where the photo inhibition process begins, yet optimal for phototropic growth (Ogbonna et al., 1999). Zhou and Richmond (1999) reported optimal growth of *Nannochloropsis sp* in a vertical reactor located 10 cm from the light source.

**Table 6.1.** Effect of distance from the LED light source on light power.

<table>
<thead>
<tr>
<th>Distance from the light source (mm)</th>
<th>Light intensity (µmol m(^{-2}) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>blue</td>
</tr>
<tr>
<td>0</td>
<td>2143</td>
</tr>
<tr>
<td>1</td>
<td>568</td>
</tr>
<tr>
<td>5</td>
<td>206</td>
</tr>
<tr>
<td>10</td>
<td>95</td>
</tr>
<tr>
<td>15</td>
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<td>40</td>
</tr>
<tr>
<td>25</td>
<td>32</td>
</tr>
<tr>
<td>30</td>
<td>26</td>
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<td>35</td>
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<td>40</td>
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<td>45</td>
<td>14</td>
</tr>
<tr>
<td>50</td>
<td>11</td>
</tr>
</tbody>
</table>
6.3.4 REACTOR MANAGEMENT

Two air turbines (Model ACO-9720, Hailea, Guangdong, China) each with an output of 30 L min\(^{-1}\), were used to mix the algal suspension. Carbon dioxide at a concentration of 3.7% \(\pm 0.18\%\) was supplied at a rate of 864 mL min\(^{-1}\) L\(^{-1}\) of algal suspension from a compressed air tank equipped with a mass flow regulator (Model 191 AR-60, Gentec Corporation, Shanghai, China). The concentration of dissolved carbon dioxide in the algal suspension was measured at 5 s intervals with a Telaire CO\(_2\) monitor (Model 7001). Room temperature 22.4°C \(\pm 0.5\), was measured with a CO\(_2\) temperature monitor (Model 7001, Telaire-General Electric, California, USA); the algal suspension temperature was measured with a infrared thermometer (Model Fluke 62, Fluke Corporation, Everett, Washington, USA).

6.3.5 CULTURE MEDIUM

The culture medium was prepared using a commercial synthetic fertilizer as the nutrient source, Solucat (25-5-5 + trace elements), by dissolving 0.8 g in 1 L distilled water. At the start of each experiment, the culture medium contained 114 mg L\(^{-1}\) ammonium-N, 86.4 mg L\(^{-1}\) nitrate-N, 40 mg L\(^{-1}\) phosphate, 40 mg L\(^{-1}\) potash, 160 \(\mu\)g L\(^{-1}\) iron, 80 \(\mu\)g L\(^{-1}\) manganese, 80 \(\mu\)g L\(^{-1}\) boron, 16 \(\mu\)g L\(^{-1}\) zinc, and 16 \(\mu\)g L\(^{-1}\) copper; the electrical conductivity was 0.95 \(\pm 0.01\) mS, measured by EC meter (Model HI 991301, Hanna, Michigan, USA).

6.3.6 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experiment was set out in 24-4 L glass containers (internal diameter 12 cm), each containing 3 L of culture medium. Each light source was replicated 6 times and the illumination was on for the entire time of the experiment. Algal cultures were kept at room temperature (~22°C) and incubated for 15 d. A one-way ANOVA and T test were conducted on the data using the software Biostatistics 1.0 with \(\alpha \leq 0.01\). Figure 6.1, shows the physical set up of the experiment.
6.3.7 ALGAL POPULATION, LIGHT ABSORPTION PEAKS AND BIOMASS ANALYSIS

The population density was measured daily throughout the incubation by direct microscopy and a cell-counting plate using the Neubauer chamber. The methodology is described below;

- Shake each algae container to make sure homogenous algal distribution before taking a 20 mL sample with syringe
- For very concentrated sample, dilute the sample with distillate water a ratio of 1:10
- Fill the Neubauer chamber with algal media
- Depending of the cell size, use a microscopic magnification of 10X or 40X
- Count the cells in the chambers A and B, for the small cells (smaller than 6µm) count those cells in the chamber C.
- For cells in chamber A and B use this equation \( C = N \times 104 \times \text{dil} \), where \( C = \text{cells mL}^{-1} \), \( N = \text{cells average in 1mm}^2 \) (1µL) and dil = dilution factor.
• For small cells use the results in chamber C and this equation; $C = \frac{N}{4 \times 10^6} \cdot \text{dil}$, where $C = \text{cells mL}^{-1}$, $N = \text{cell average in the 5 C chambers}$ and $\text{dil} = \text{dilution factor}$.

Light absorption of each algal culture was measured over wavelengths ranging from 400 to 700 nm using the Shimadzu UV 1600 spectrophotometer. The colorimetric determination methodology was used to calculate the standing biomass production at the end of the incubation. Light absorption was measured using a spectrophotometer (Shimadzu UV 1600). A calibration plot relating algal biomass with 7 biomass measurements from 0.25 g L$^{-1}$ to 3.49 g L$^{-1}$ against light absorption measured at 680 nm resulted in linear equation $Y = 0.7015X + 0.0362$ with a $R^2 = 99.87\%$. The resulting equation was used to determine the standing biomass of the 24 replicates.
6.4. RESULTS AND DISCUSSION

6.4.1 THE EFFECT LIGHT IN THE ALGAL ABSORPTION SPECTRA

The light absorbance pattern of an algal culture depends on the nature and quantity of the specific photosynthetic pigments it produces to capture energy. Figure 6.2 shows the absorption spectra of *C. vulgaris* grown under blue, red, red+blue and white light after 15 d incubation.

![Absorption Spectra](image)

**Figure. 6.2.** Algal absorption spectra after 15-d incubation exposed to blue, red, red+blue and white LED lights.

The absorption spectrum of cultures exposed to white light can be used as a reference to evaluate the effects of the other light sources on the formation of photosynthetic pigments. As shown in Fig. 6.2, the alga contains photosynthetic pigments able to absorb light over the entire spectral range, thus the main curvature is caused by the light absorption peaks of the main photosynthetic pigments; chlorophyll a and b. Growth under white light showed the highest absorbance 1.55 ± 0.019 with 1.52% coefficient of variance (CV), followed by that under red+blue 1.45 ± 0.010
with 0.83% CV; growth under blue light showed an absorbance of $1.15 \pm 0.016$ with 1.59% CV and that under red light $0.85 \pm 0.007$ with 1.01% CV.

These data suggest that *C. vulgaris* growing under white light synthesized greater quantities of photosynthetic pigments than when grown under light sources of restricted wavelengths.

The absorption spectrum of *C. vulgaris* does not appear to be altered by exposure to light of different wavelengths. Since *C. vulgaris* is a fresh water species inhabiting shallow water bodies, it may have evolved the ability to absorb solar visible radiation over a broad waveleght range. Previous studies of the in vivo absorption spectra for *Chlorella* species, including *Chlorella vulgaris*, have reported similar results. Minor differences in the blue and red absorption peaks were observed in that the red absorption peak was higher than the blue peak (Kamiya, 1988; Shioi and Sasa, 1984; Ley and Mauzerall, 1982; Shibata et al., 1954). The results of this study suggest that *C. vulgaris* does not have a mechanism for altering it’s photosynthetic pigments to be able to respond to radiation different from those wavelengths shown in Fig. 6.2.

**6.4.2. POPULATION GROWTH OF *C. VULGARIS* EXPOSED TO DIFFERENT LIGHT SOURCES**

Population growth under the four light sources shows a classical sigmoid curve (Fig. 6.3), with slight differences in the adaptation time and period of exponential growth. The initial population density was $4.5 - 5*10^5$ cells mL$^{-1}$. Under white light, the growth rate during the exponential growth phase was 0.67 d$^{-1}$ and reached a maximum cell density of $1.80*10^7$ cells mL$^{-1}$. Under blue light the growth rate was 0.64 d$^{-1}$ with a maximum population density of $1.71*10^7$ cells mL$^{-1}$. Growth under red light was the slowest with a growth rate at 0.43 d$^{-1}$ and a population density of $1.04*10^7$ cells mL$^{-1}$. Exposure to red + blue light resulted in a growth rate of 0.56 d$^{-1}$ and a maximum population density of $1.75*10^7$ cells mL$^{-1}$, which was not significantly different from that under white light.
Figure 6.3. Growth curves of the four light treatments with 6 replicates of each treatment.

The maximum population density obtained in this study is similar to that reported in previous studies. Yi et al. (2000) obtained a cell density of $10^6$ cells mL$^{-1}$ and Mandalam and Palsson (1995) reported cell densities ranging from $10^6$ to $10^7$ cells mL$^{-1}$. Typical cell densities for *Chlorella sp* under open ponds, bubble column reactors and static mixer reactors are in the range of $3*10^4$ to $1.38*10^9$ cells mL$^{-1}$ (Cho et al., 2007; Borowitzka, 1999). Studies conducted under heterotrophic growing conditions have obtained cell densities of $3.8*10^8$ cells mL$^{-1}$ (Killam and Myers, 1956). Lee and Palsson (1996) reported cell densities ranging from $1*10^7$ to $5*10^7$ cells mL$^{-1}$ in a growth study exposed to red (680 nm) light.
6.4.3.3 STANDING BIOMASS PRODUCTION UNDER DIFFERENT LIGHT SOURCES

6.4.3.3.1 STANDING BIOMASS

The effects of light wavelength on biomass production are shown in Table 6.2. The highest biomass production was 1044 mg L\(^{-1}\) in 15 d growing under white light followed by red + blue at 1003 mg L\(^{-1}\), blue at 827 mg L\(^{-1}\) and least under red with a biomass of 571 mg L\(^{-1}\). This biomass is higher than 560 mg L\(^{-1}\) in red (660 nm) after 8 days incubation (Zhao et al., 2013), the Zhao research also report biomass production in white 500 mg L\(^{-1}\), and in blue (460 nm) 370 mg L\(^{-1}\), those values are lower than the biomass obtained in this study. Several studies of \textit{C. vulgaris} have shown that the typical standing biomass under both phototropic and heterotrophic growth after 12-15 days of incubation is between 250 and 1700 mg L\(^{-1}\) (Bhola et al., 2010; Liang et al., 2009); Chinnasamy et al., (2009) has reported a biomass of 210 mg L\(^{-1}\) growing in wastewater. A most recent study has reached 2730 mg L\(^{-1}\) growing \textit{C. vulgaris} under blue LED at 200 µmol m\(^{-2}\) s\(^{-1}\) and photoperiod of 12h light and 12h dark for 312 h (Atta et al., 2013).

**Table 6.2.** Standing biomass of \textit{C. vulgaris} growing under white, blue, red and red + blue light.

<table>
<thead>
<tr>
<th>Repetition</th>
<th>Blue</th>
<th>Red</th>
<th>Red-Blue</th>
<th>White</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>813</td>
<td>568</td>
<td>987</td>
<td>1026</td>
</tr>
<tr>
<td>2</td>
<td>851</td>
<td>565</td>
<td>1007</td>
<td>1058</td>
</tr>
<tr>
<td>3</td>
<td>821</td>
<td>576</td>
<td>1006</td>
<td>1034</td>
</tr>
<tr>
<td>4</td>
<td>830</td>
<td>578</td>
<td>1005</td>
<td>1038</td>
</tr>
<tr>
<td>5</td>
<td>827</td>
<td>565</td>
<td>1001</td>
<td>1053</td>
</tr>
<tr>
<td>6</td>
<td>823</td>
<td>574</td>
<td>1014</td>
<td>1061</td>
</tr>
</tbody>
</table>

| Mean       | 828  | 571 | 1003     | 1045  |
| Standard Deviation | 13  | 6   | 9       | 14   |
| Coefficient of Variation | 0.016 | 0.010 | 0.009 | 0.014 |

White light supported a greater standing biomass than the other light treatments because this light source provided radiation over the entire range of photosynthetic pigments. Interestingly the combination of red + blue light produced almost as much standing biomass as the white light. Since this is the only treatment that combined more than one photosynthetic wavelength, \textit{C.}
*vulgaris* adapted better than the single wavelength treatment of either red or blue alone. Growth of *C. vulgaris* under blue light was better than that under red because the absorption spectra has one of the important peaks located in 430 nm and the other at 680 nm. From the growth and biomass production we can propose that the algal do not appear to be able to change its photosynthetic pigment composition to adapt to this specific wavelength.

In contrast to our study, Wang et al. (2007) reported that highest biomass production in *Spirulina platensis* was achieved under red light (620-645 nm) followed by growth under white light (380-760 nm), followed by green (515-540 nm), yellow (587-595 nm) and the least growth in blue (460-475 nm). In our study *C. vulgaris* produced more biomass when expose to multiple wavelengths such as the white light and the combination of red-blue, and a single wavelength light not performing as well. The blue light (430 nm) is the best single wavelength light treatment because it matches the wavelength absorption peak of the photosynthetic pigment of this specie. Other studies in single wavelength light have been conducted to increase biomass and astaxanthin concentration testing red and blue LEDs separately. The highest biomass production was obtained using the blue LED 380-470 nm (Lababpour et al., 2005; Lababpour et al., 2004). A study which compared blue 370-430 nm, red >580 nm and blue +red at an intensity of 20-40 µmol m\(^{-2}\) s\(^{-1}\) for astaxanthin formation in the alga *Haematococcus pluvialis*, showed that blue light and blue + red performed better than red light alone (Kobayashi et al., 1992).

This study analysed four LED light treatments with six repetitions per treatment. One way ANOVA test was performed using the software Biostatistics 1.0. The ANOVA test resulted in \(F = 2305\) with \(p = 0.0000\) showing statistical difference among the four treatments. Thus, the exponential coefficient of variation was very low 1.27%. In addition, to complement the Analysis of Variance (ANOVA test), we choose the student’s T test from the unequal variance T-test, Mann-Whitney. We tested each treatment individually to one another using a Student’s t-test at \(P \leq 0.01\), the reference value for the Student’s at \(p = 0.01\) is \(T \geq 4.6041\). The results of these analyses show that the biggest value is 100, made in red light against red-blue light, and the
lowest value is 6.05 from the test made in red-blue light against white light. From the results of the T test there is statistical difference at 99% confidence that each individual treatment differed from each other.

6.4.3.4 EFFECT OF LIGHT SOURCE ON ALGAL CELL WEIGHT

Individual alga cell weight was calculated from the population cell density by dividing by the standing biomass (Table 6.4). It ranged from ~49 to 64 pg (1 pg = 1*10^{-12} g) in cultures grown under the four light sources. The heaviest cells were achieved under the white light at 63 pg cell^{-1}, followed by those grown under red + blue light at 61 pg cell^{-1}, blue at 50 pg cell^{-1} and red at 55 pg cell^{-1}. A study conducted by Krzywicka and Wagner (1975) reported cell weights ranging from 7.0*10^{-11} to 8.2*10^{-11} g cell^{-1}. In a similar study, also using an inorganic fertilizer medium, Griffiths (1963) reported cell weights ranging from 3.5*10^{-11} to 9.7*10^{-11} g cell^{-1}. Chimiklis and Karlander (1973) recorded cell weights ranging 0.9*10^{-11} from 2.5*10^{-11} g cell^{-1} in a growth study of Chlorella sorokiniana.

Table 6.4. Cell weight of C. vulgaris grown under 4 different light sources.

<table>
<thead>
<tr>
<th>Repetition</th>
<th>Red (Grams Cell(^{-1}))</th>
<th>Blue (Grams Cell(^{-1}))</th>
<th>Red + Blue (Grams Cell(^{-1}))</th>
<th>White (Grams Cell(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.33E-11</td>
<td>4.90E-11</td>
<td>5.97E-11</td>
<td>6.22E-11</td>
</tr>
<tr>
<td>2</td>
<td>5.43E-11</td>
<td>5.11E-11</td>
<td>6.22E-11</td>
<td>6.43E-11</td>
</tr>
<tr>
<td>3</td>
<td>5.62E-11</td>
<td>4.93E-11</td>
<td>6.13E-11</td>
<td>6.27E-11</td>
</tr>
<tr>
<td>5</td>
<td>5.54E-11</td>
<td>4.98E-11</td>
<td>6.03E-11</td>
<td>6.25E-11</td>
</tr>
<tr>
<td>6</td>
<td>5.54E-11</td>
<td>4.89E-11</td>
<td>6.02E-11</td>
<td>6.39E-11</td>
</tr>
</tbody>
</table>

Mean: 5.51E-11, Standard Deviation: 1.11E-12, Coefficient of Variation: 0.0201
The ANOVA test for cell weight was $F = 227$ with $p = 0.0000$ resulting in statistical difference, the coefficient of variance was 1.69%. These tests suggests that the nature of the light source had a significant influence on cell weight of *Chlorella vulgaris*.

### 6.5 CONCLUSIONS

Biomass production of the fresh water algal specie *C. vulgaris* was highest under white light, presumably because this light source provided a larger wavelength photosynthetic spectra than a single color light such as blue and red. The results of this study also show that *C vulgaris* cannot change its photosynthetic machinery significantly to absorb light from other wavelengths different from that of its evolutionary wavelength absorption spectra.

Biomass production under the four light treatments was significantly different from one another ($F > 99\%$ of probability). Though the growth curves showed a similar pattern, the cell density and growth rate was different for each individual light treatment, confirming the result of the biomass production. Further, the different light sources significantly altered the cell weight of *C. vulgaris*.

Further studies of algal growth under red light (680 nm) are recommended to confirm that light supplied at a single wavelength can develop more algal biomass than from multiple wavelength light sources.
6.6 REFERENCES


CHAPTER 7  BIOMASS PRODUCTION OF MARINE AND FRESH WATER ALGA GROWING UNDER FLASHING AND CONTINUOUS LED LIGHT

7.1  ABSTRACT

A marine alga *Nannochloris sp* and a fresh water alga *Scenedesmus sp* were grown under flashing light conditions at 10Hz with a light dark ratio of 1:10. The illumination was prounded by LED red 625 nm and blue 430 nm, the same algae were also grown under continuous light using the same LED technology. The marine alga *Nannochloris sp* under flashing light reached a cell density of $2.43 \times 10^7$ cell mL$^{-1}$, developed a standing biomass of 1041 mg L$^{-1}$ and the average cell weight was $4.98 \times 10^{-11}$ g cell$^{-1}$. Under continuous light the cell density was $5.69 \times 10^7$ cells mL$^{-1}$, the standing biomass 892 mg L$^{-1}$ and the cell weight was $2.26 \times 10^{-11}$ g cell$^{-1}$. In contrast the fresh water alga *Scenedesmus sp* under flashing conditions developed a cell density of $1.65 \times 10^7$ cells mL$^{-1}$, standing biomass of 1130 mg L$^{-1}$ and an average cell weight of $8.50 \times 10^{-11}$ g cell$^{-1}$. Under continuous light the cell density was $1.42 \times 10^7$ cells mL$^{-1}$, the standing biomass 1018mg L$^{-1}$ and the average cell weight was $5.05 \times 10^{-11}$ g cell$^{-1}$. Both algae produced more final standing biomass and more cell weight under flashing conditions, there is significant difference in daily biomass production $F = 81.7668$ with $P = 0.0000$ between flashing and continuous light in *Nannochloris sp*, however there is no significant difference in *Scenedesmus sp*.

7.2  INTRODUCTION

Continuous light may cause photo damage and photo inhibition which decreases photosynthetic activity therefore flashing light is an alternative to reduce this damage (Fathurrahman et al., 2013). The flashing effect increases algal biomass production, maximize energy efficiency (Kong et al., 2013), and has been tested for different algal species. A study made in *Chlorella sp* show an increase in cell density when the population was exposed to flashing light.
(Fathurrahman et al., 2013). Two independent studies using high frequency flashes of 0.5 to 1.0 Hz and 0.01 to 10 Hz with solar radiation and simulating solar radiation in *Phaeodactylum tricornutum* produced an average of 3.7% more energy into biomass conversion over a 3 month period and higher photosynthetic efficiency (Walsh and Legendre, 1988; Laws et al., 1983). Similar studies simulating solar radiation with *Chlorella sp* have been made producing biomass from 240 mg L\(^{-1}\) to 2720 mg L\(^{-1}\) (Pirt et al., 1980). More studies with the algal *Chlorella kessleri* using flashing light effect of 10 KHz, 20 KHz and 50 KHz with light: darks of 1:9, 2:8, 3:7, 4:6 and 5:5 were conducted to determine the effect of flashing effect in cell volume (Park and Lee, 2001). The flashing effect was tested to analysed cell density; low and high frequency flashes from 1Hz to 100Hz, and from 1KHz to 100 KHz with light period from 40 to 80% were tested using the algal *Isochrysis galbana*, flashes of 10 KHz developed the highest cell density (Yago et al., 2012).

The flashing effect can change algae pigment composition; flashes of 10 µS of light periods with 33-100 miliseconds of dark periods changed the chlorophyll concentration from 1 to 5% in the algal *Chlorella pyrenoidosa* (Myers and Graham, 1971). Flashes of 1 to 100 Hz were tested using the algal *Chlamydomonas reinhardtii*; flashes of 1 to 10Hz decreased the biomass production in 10%, and cycles of 100Hz increased the biomass production in 35% compared to continuous light (Vejraska et al., 2012). Thus flashes of 5 Hz to 37 KHz against continuous light with the algal *Chlorella kessleri* were tested, higher cell density and oxygen production was observed under flashes of more than 1KHz and 20% increase in cell density was achieved at 37 KHz (Park and Lee, 2000).

Medium frequency flashes have been studied too with the algae *Chlamydomonas reinhardtii* and *Chlorella sorokiniana*, flashes of 13 to 87 s with light/dark fraction from 0.32-0.88 increases chlorophyll content but decreased the biomass production of the algae. The results of the study show that a dark period of less than 4.4 s between flashes does not affect biomass production (Janssen et al., 1999). The algae *Chlorella sp* and *Scenedesmus sp* have been studied using medium rate flashes of 1.2-260 s (Grobbelaar, 1991). Light periods of 0.52 s at 20 Wm\(^{-2}\) can
support *Chlorella sp* at maximum photosynthetic activity for 9.2 s in the dark (Molina-Grima et al., 1996; Pirt, 1986; Lee and Pirt, 1981), previous studies show that light periods of 1 ms at 24 \text{Wm}^{-2} support photosynthetic activity in *Chlorella sp* for 20 ms (Philiph and Myers, 1954).

Lower frequency flashes has been tested, 27, 60 and 110 s cycle, with the algal *Porphyridium sp* using air lift and bubble column reactor concluded that a dark period of 6 s does not affect alga performance (Merchuk et al., 1998). In conclusion flashing light may increase cell density, cell volume, pigment composition, oxygen production, photosynthetic activity and biomass production. The present investigation evaluated flashes of 10 Hz with light dark ratio of 1:10 using red and blue LEDs to measure cell density and biomass production against treatments with continuous light using the marine algal *Nannochloris sp* and the fresh water algal *Scenedesmus sp*.

### 7.3 MATERIALS AND METHODS

#### 7.3.1 MICROORGANISM

The two alga species one marine and one fresh water used in this study are from the culture collection of algae at the University of Texas at Austin UTEX; *Nannochloris sp* (1268) and *Scenedesmus sp* (1589).

#### 7.3.2 PHOTO REACTOR

The reactor had the following measurements: 30 cm diameter, and 110 cm length, it was filled with the nutrient media to 80 cm of height with a active volume of 56 L. For the continuous light experiment, we used a glass container of 4 L capacity filled with 3.6 L of nutrient media.
7.3.3 LIGHT SOURCE AND FLASHING DEVICE

For the flashing light experiment a special computer device with a PLC was constructed to allow control of 100 flashes per second, with different light dark ratios. This experiment was adjusted to 10 flashes s\(^{-1}\) or 10 Hz with a light dark ratio of 1:10. In each flash cycle the lights were on for 10 millisec and off for 90 millisec for a total 100 millisec on and 900 millisec off s\(^{-1}\). The light source used was LED of 0.09-0.1 W (ALAS, China), red 620-625 nm at 0.09 w per bulb and blue 425-430 nm 0.1 W per bulb. It was used 400 red LEDs and 400 blue LEDs with a total power consumption of 36 W in red and 40 W in blue with a combine total power of 76 W of light in 56 L of algae suspension, and the power ratio was 1.35 W L\(^{-1}\). The LEDs were placed in four square towers submerged in the alga suspension located at 8 cm from each other, a transparent plexi glass protected the LEDs from water damage. The light emission per LED are 2143 µmol m\(^{-2}\) s\(^{-1}\) for blue and 1345 µmol m\(^{-2}\) s\(^{-1}\) for red. Light intensity was measure using a quantum meter (Model MQ output in µmol m\(^{-2}\) s\(^{-1}\), Apogee Instruments Inc., Logan, UT, USA). For the continuous light experiments we used the same type and brand of LEDs, 26 red and 26 blue with a light power of 2.34 W for red and 2.6 W for blue for a total of 4.94 W in 3.6 L reactor. The power ratio was 1.37 W L\(^{-1}\). The LEDs were placed in the center of each container with in a transparent plexi glass protection against water damage.

7.3.4 LIGHT DISPERSION

The LED technology is very efficient at producing light emissions in the photosynthetic spectra from 1345 to 2143 µmol m\(^{-2}\) s\(^{-1}\) from a bulb of 0.09-0.1 W. This amount of light energy is more than the algae species can process 50-250 µmol m\(^{-2}\) s\(^{-1}\), producing photo-inhibition and photo-damage which ends in the reduction of the photosynthetic activity (Kim et al., 2006; Degen et al., 2001; Ogbonna et al., 1999; Merchuk et al., 1998; Fernandez-Sevilla et al., 1998; Molina –Grima et al., 1996, Garcia-Sanchez et al., 1996; Qiang and Richmond, 1994; Aiba, 1982; Bannister, 1979; Eppley and Coatsworth, 1966). The dispersion of light occurs in a short distance, the distance from the bulb to the algal in suspension is 7 mm, sufficient distance to reduce the light intensively from 2143 µmol m\(^{-2}\) s\(^{-1}\) to a power lower than 300 µmol m\(^{-2}\) s\(^{-1}\) which is where the
photo inhibition process begins. Thus the resulting light intensity is optimal for alga phototropic growth under artificial conditions (Ogbonna et al., 1999).

7.3.5 MIXING, QUANTIFYING CARBON SOURCE UNDER AUTOTROPHIC GROWTH AND TEMPERATURE MEASUREMENTS

For carbon supply and gentle mixing we used in total three turbines (Model AC-9904, Resun, China); For the flashing experiments we used one AC-9904 for each reactor, the air volume we introduced was 9 L min⁻¹ in 56 L, the air ratio was 161 mL of air L⁻¹ of algae suspension min⁻¹. For the continuous light experiments we used one AC-9904 with volume of 9 L min⁻¹, however we only used two of the four outputs for the algae in this experiments, in total we used 4.5 L min⁻¹ in 7.2 L of algae suspension, the air ratio was 625 mL of air L⁻¹ min⁻¹ in the growing media. The CO₂ concentration in the two experiments ranged from 0.0323% to 0.0428% and growing media temperature was 23°C ± 2 measured by mini Infrared thermometer (Model Fluke 62, Fluke Corporation, Everett, Washington, USA).

7.4.5 CULTURE MEDIUM

Commercial fertilizer (Solucat 25-5-5, Atlantica Agricola, Villena, Spain) was dissolved at 0.8 g in 1 L of distilled water. The fertilizer has the following composition; nitrogen 25% in ammonia 14,2% plus nitrates 10.8%. P₂O₅ 5%, K₂O 5%, Fe 0.020%, Mn 0.010%, Zn 0.002%, B 0.010%, Cu 0.002%. Electrical conductivity was monitoring at 0.95 ms +- 0.01 mS using a EC sensor (Model HI 991301, Hanna, Michigan, USA).

7.4.6 BIOMASS DETERMINATION

For the flashing light experiments, we used the biomass colorimetric determination methodology to calculate the life standing biomass of the treatments. We used the spectrophotometer (Shimadzu UV-1600, Shimadzu Scientific Instruments Inc, Columbia, USA) to prepare a calibration curve, and measured the absorbance at 680 nm. A linear regression was made
resulting in the following equation for *Nannochloris sp* \( Y = 0.8586x + 0.1464 \) with a \( R^2 = 0.9854 \) and for *Scenedesmus sp* \( Y = 0.81x + 0.0461 \) with a \( R^2 = 0.9813 \).

For the continuous light experiments we made a regression between biomass and cell density. To measure the biomass we used the dry weight methodology that consist; dry a 15 mL glass tubing until reach constant weight, fill the tubing with 10 mL of the algae media from the experiment, centrifuge the tubing at 4000 rpm for 15 min, separate the water residues in the tubing, dry the tubing with the algae in an oven at 105 °C until reaches constant weight, weight the tubing with the algae substrate and weight the tubing without the algae, the resulting value is the algae biomass in 10 mL. We repeated this procedure in duplicate each time we measured algae biomass. For the cell density we used a microscope with a Neubauer chamber and we followed the procedure for cell counting. A regression between cell density and biomass resulted in the following equation for *Nannochloris sp* \( Y = 9 \times 10^{-14} X^2 + 2 \times 10^{-05} X - 3.0797 \) with \( R^2 = 0.9997 \) and for *Scenedesmus sp* \( Y = 8 \times 10^{-05} X - 117.94 \) with \( R^2 = 1 \). The equations were used to determine standing biomass from cell density.

### 7.4.7 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experiment measured standing biomass for two algae; one marine *Nannochloris sp* and one fresh water *Scenedesmus sp* growing under daily solar radiation and 12 h artificial light; red and blue LEDs flashing at 10 HZ, and growing under daily solar radiation and 12 h continuous lighting red and blue LEDs. The algae were grown for 13 d in both experiments in a medium of Solucat fertilizer 0.8 g L\(^{-1}\), for the flashing experiment the light power ratio was at 1.35 W L\(^{-1}\), the air intake 161 mL L\(^{-1}\) and for the continuous light experiment the light power ratio was 1.37 W L\(^{-1}\) and the air intake 625 mL L\(^{-1}\). The results were analyzed using statistical methods such standard deviation, coefficient of variance, and one way ANOVA and T test using Biostatistics 1.0.
7.5 RESULTS AND DISCUSSION

7.5.1 STANDING BIOMASS UNDER DIFFERENT LIGHT

The marine algal *Nannochloris sp* reached a standing biomass of 1041 mg L⁻¹ under flashing treatment, this overall biomass production is higher than the standing biomass reached under continuous light treatment 892 mg L⁻¹. The fresh water algal *Scenedesmus sp* reached a standing biomass of 1130 mg L⁻¹ under flashing light, the same algal under continuous light reached a lower standing biomass 1018 mg L⁻¹. In both algae species the flashing light treatments developed more biomass production during the 13 d of the study than the continuous light. See figure 7.1 for details of standing biomass during production during the experiment.

**Figure 7.1.** Standing biomass production under two light treatments for a marine algal *Nannochloris sp* and a fresh water alga *Scenedesmus sp*. 
7.5.2 DAILY BIOMASS PRODUCTION

The daily biomass production have a high index of fluctuation because the treatments include 13 daily solar radiations, and depending of the weather conditions the reactor receives more or less light during the day photoperiod, and as a result in more or less photosynthesis ratio and biomass production. The coefficient of variation for the daily biomass production under flashing light was 0.5 for *Nannochloris sp* and 0.8 for *Scenedesmus sp*, in continuous light the coefficient of variation was 0.7 for *Nannochloris sp* and 1.0 for *Scenedesmus sp*.

There are statistical differences between flashing and continuous light treatment in the alga *Nannochloris sp* resulting in ANOVA with $F = 32.8889$ and $p = 0.0002$, and T test with $T = 8.967$ and $p = 0.000$. However no statistical differences between treatments in *Scenedesmus sp* and between algae species were found. Flashing and continuous light treatments induced different biomass production for *Nannochloris sp* but not for *Scenedesmus sp*.

7.5.3 CHANGES IN POPULATION DENSITY

The marine alga *Nannochloris sp* developed the highest population density of this study under continuous light $5.69 \times 10^7$ cells mL$^{-1}$, under flashing light the cell density was $2.43 \times 10^7$ cell mL$^{-1}$. These results are contrary to these of the fresh water alga *Scenedesmus sp* which developed more cell density under flashing light $1.65 \times 10^7$ cells mL$^{-1}$ than under continuous light $1.42 \times 10^7$ cells mL$^{-1}$. See Figure 7.2 for details.
Figure 7.2. Population density of two algae under two light treatments.

The growth rate of *Nannochloris sp* under flashing light treatments was 2.08 in 13 d, and the average daily growth rate 0.160 and under continuous light the growth rate was 2.03 in 13 d, and the average daily growth rate 0.196. There is statistical difference with $F = 6.2567$ and $P = 0.0314$ during the first 6 days of incubation in the growth rate of *Nannochloris sp* under flashing light and continuous light. In the other hand *Scenedesmus sp* growth rate under flashing light was 1.48 and the daily average 0.148, and under continuous light the growth rate was 2.04 and the daily average 0.194. No statistical differences was found in the growth rate of *Scenedesmus sp* under flash light and continuous light. The growth rate of both algae fluctuate from 1.48 to 2.08 during the 13 d of incubation. In all two treatments *Nannochloris sp* grew faster and developed more cell density than *Scenedesmus sp* because the cell size of *Nannochloris sp* is 2.5-3.2 µm in diameter, smaller than the cell size of *Scenedesmus sp* 7 µm in width and 16 µm in lenght. See Figure 7.3 for analysis of cell size.
The cell morphology of *Nannochloris sp* is round 2.5-3.2 µm in diameter, and the nucleus is 1.5 µm in diameter. The *Scenedesmus sp* cell morphology is capsule, the algae typically are grouped in 2-4 cells, the cell size is 16 µm in length, 7 µm in width and the nucleus is 4 µm in diameter. The cell has a recognisable inner nucleus of 2 µm in diameter, chloroplast structure and flagella can be recognised as well in some cells, the nucleus of *Scenedesmus sp* is bigger than *Nannochloris sp* cells.

### 7.5.4 CELL WEIGHT

During the 13 d experiments the cell weight was calculated using the cell density measurements and the cell biomass, a total of 8 samples were taken for cell density and 8 samples for cell biomass in each treatment. Table 7.1 shows the cell weight for the two algae species under two light treatments.
### Table 7.1. Cell weight of two algae species under two light treatments.

| Repetition | Nannochloris sp | | Scenedesmus sp | |
|------------|-----------------|--------|-----------------|
|            | Flashing        | Continuous | Flashing | Continuous |
| 1          | 5.33E-11        | 2.84E-11 | 1.15E-10     | 1.62E-11  |
| 2          | 6.10E-11        | 2.62E-11 | 1.09E-10     | 3.36E-11  |
| 3          | 5.49E-11        | 2.50E-11 | 7.41E-11     | 3.93E-11  |
| 4          | 4.56E-11        | 2.37E-11 | 8.50E-11     | 4.81E-11  |
| 5          | 4.43E-11        | 2.12E-11 | 8.12E-11     | 6.05E-11  |
| 6          | 4.39E-11        | 1.95E-11 | 7.90E-11     | 6.35E-11  |
| 7          | 5.42E-11        | 1.91E-11 | 8.62E-11     | 7.13E-11  |
| 8          | 4.13E-11        | 1.76E-11 | 5.08E-11     | 7.17E-11  |
| Mean       | 4.98E-11        | 2.26E-11 | 8.50E-11     | 5.05E-11  |
| Standar Deviation | 6.94E-12 | 3.82E-12 | 2.00E-11 | 1.98E-11 |
| Coefficient of Variance | 0.14 | 0.17 | 0.24 | 0.39 |

The average cell weight of *Scenedesmus sp* under the flashing light is 8.50*10^{-11} g and for continuous light 5.05*10^{-11} g, almost double the weight of *Nannochloris sp* cell which under flashing treatment reached 4.98*10^{-11} g and for continuous light 2.26*10^{-11} g. There is a statistical difference in cell weight in *Nannochloris sp* between flashing and continuous light with $F = 94.2495$, $T = 9.708$ and $p = 0.000$. Similar results in cell weight were obtained with *Scenedesmus sp* with $F = 11.9953$, $T = 3.463$ and $p = 0.004$. The cell weight is higher under the flashing conditions, not due to the light treatment, but could be as a result of the turbulences. The flashing light reactor is a bigger reactor 56 L with an air ratio of 161 mL of air L^{-1}, on the other hand the continuous light reactor is 3.6 L with an air ratio of 625 mL of air L^{-1}, this air ratio produce more turbulence in the smaller reactor which affect directly the size of the cell. Turbulences system develop smaller cell because of the increasing probability of mechanical damage as the cell grow, therefore the differences of cell size in not produced by the light treatment but the difference of turbulences of the two systems.
7.6 DISCUSSION AND CONCLUSIONS

The standing biomass obtained in this study under flashing and continuous light after 13 days of incubation for both algae *Nannochloris sp* and *Scenedesmus sp* were in the range of 892-1130 mg L\(^{-1}\), these biomass production is similar to those obtained by Bhola et al., 2010 and Liang et al., 2009 which confirm that 12-15 days of incubation of *Chlorella vulgaris* produces 250 to 1700 mg L\(^{-1}\) of biomass. However there is a big potential in biomass production using flashing light effect, a study made with *C. vulgaris* in a Taylor vortex reactor has reached a standing biomass of 10000 mg L\(^{-1}\) in just 4 days of incubation (Kong et al., 2013). In relation with the size of the algae and the population density; *Nannochloris sp* produces more cell density 2.43*10\(^7\) to 5.69*10\(^7\) cell mL\(^{-1}\) with a smaller cell size 2.5-3.2 µm in diameter and *Scenedesmus sp* produce less cell density 1.42*10\(^7\) to 1.65*10\(^7\) cells mL\(^{-1}\) with a bigger cell size 7 µm width and 16 µm length.

The growth rate of the two algae is different because they produce similar standing biomass (14% differences) with different cell size (3x-7x); therefore the algal that has a smaller cell (*Nannochloris sp*) produce more cell density in order to produce similar biomass. Thus *Nannochloris sp* cell weight range from 2.26*10\(^{-11}\) to 4.98*10\(^{-11}\) g Cell\(^{-1}\) smaller weight than *Scenedesmus sp* cell weight 5.05*10\(^{-11}\) to 8.50*10\(^{-11}\) g Cell\(^{-1}\).

The ANOVA test confirm that growing *Nannochloris sp* under flashing light produce more biomass and growth rate during the first 6 days of incubation than continuous light. The daily biomass production and growth rate in the alga *Scenedesmus sp* for two different treatments (continuous light and flashing light) resulted in no statistical difference. However we cannot conclude that flashing light does not have any effect in more or less biomass production because *Scenedesmus sp* produced more standing biomass under flashing light than under continuous light treatment.
This study found statistical difference in growth rate and daily biomass production for the marine algae *Nannochloris sp*, however did not find any significant difference in daily biomass and growth rate for the fresh water alga *Scenedesmus sp*. The two algae are morphologically different; *Nannochloris sp* is smaller, produce more cell density and has a higher growth rate. However the standing biomass of *Scenedesmus sp* under flashing light is 8.5% higher than the standing biomass of *Nannochloris sp*, and for the continuous light is 14% higher. Both algae increased their cell weight as the turbulence of the media decrease.
7.8 REFERENCES


CHAPTER 8  
EFFECT OF CARBON DIOXIDE CONCENTRATION ON THE GROWTH RESPONSE OF CHLORELLA VULGARIS UNDER FOUR DIFFERENT LED ILLUMINATION

Sebastián Mejía Rendón       Advisor: R. Paul Voroney

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8.1 ABSTRACT

This experiment examined the growth response of Chlorella vulgaris exposed to CO₂ concentrations increasing from ambient to 8-9% and under white, blue, red and red+blue lights after 15 days incubation. Biomass production increased with increasing CO₂ concentrations under all light sources. The highest biomass production, 1.59 g L⁻¹, was obtained when the algae were supplied with 8-9% CO₂ and exposed to white light. Biomass production under blue, red and red+blue light was 1.53 g L⁻¹, 0.45 g L⁻¹ and 1.27 g L⁻¹, respectively. The research suggests that C. vulgaris is not able to adapt production of its photosynthetic pigments to absorb light sources different that it is normally has evolved to.

Keywords: Chlorella vulgaris, Photobioreactor, Biomass production, CO₂ concentration, Artificial light.

8.2 INTRODUCTION

Phototrophic algal growth requires light, mineral nutrients, water and an inorganic source of carbon (CO₂). While there are algae, such as Chlorella sp, that can grow relatively rapidly under the ambient air concentration of CO₂ (0.037%) (Usui and Ikenouchi, 1997; Hirata et al., 1996), maximizing biomass production for the purpose of CO₂ capture requires that higher concentrations of CO₂ be provided. Algae have been grown in closed atmospheres at high CO₂
concentrations (10-20% and higher) with the objective of CO₂ fixation (Cheng et al., 2006; Brown, 1996; Zeiler et al., 1995). The alga *C. vulgaris* is a good candidate for biomass production under high CO₂ concentrations because it is able to fix up to 74% of the original CO₂ with only 2 seconds of CO₂ residence time (Keffer and Kleinheinz, 2002).

Recently designs of closed photo-bioreactors for the purpose of enhancing light-use efficiency, CO₂ fixation, as well as biomass production, have received more attention because algae are efficient photosynthetic organisms (Kajiwara et al., 1997) and have the potential to reduce atmospheric CO₂ levels (Yoshihara et al., 1996), or reduce emissions from a gas or coal power plant. Thus *C. vulgaris* produce compounds of economical value such as antioxidant (β-carotenes), natural colorants, oils such as omegas 3, 6 and 9, and proteins and carbohydrates. This study evaluated standing biomass production of the alga *C. vulgaris* growing under normal and elevated CO₂ concentrations and exposed to 4 different light sources.

### 8.3 MATERIALS AND METHODS

#### 8.3.1 MICROORGANISM

The algal specie, *Chlorella vulgaris* (UTEX 26), was obtained from the culture collection of algae at the University of Texas at Austin (UTEX, 205 W, 24th St., Austin, Texas, TX 78712, USA). The culture was established in 1 L batch for 15 d and then transferred to 4 L reactors. The population density was established using direct microscopic counting techniques. The population density at the start of the experiment was set at 4.5-5*10⁵ cells mL⁻¹ by adjusting the volume of medium in the reactors.

#### 8.3.2 LIGHT SOURCE

Sources providing white, blue, red, and red-blue light were evaluated. They were 0.1 W LEDs obtained from ALAS™ (China) with the following emission parameters: red, 620-625 nm
wavelength at 1345 µmol m\(^{-2}\) s\(^{-1}\) intensity; blue, 425-430 nm wavelength at 2143 µmol m\(^{-2}\) s\(^{-1}\); white, 380-760 nm wavelength at 1838 µmol m\(^{-2}\) s\(^{-1}\) intensity.

Each lighting system contained 52 LEDs fixed into a clear square plexiglass tube (dimension 4*4 cm and 33 cm in length) and placed in the centre of the 4-L culture flask (internal diameter 12 cm). The illumination system was powered by a transformer (Model SA 201-3485, ASTEC, California, USA) supplying 12 V and 8 Amp. To ensure that external illumination would not affect the experiment, the laboratory was kept dark (0 µmol m\(^{-2}\) s\(^{-1}\)) throughout the incubation period.

Light intensity was monitored during the incubation using 2 different light meters: photosynthetic light was measured using a quantum meter (Model MQ output in µmol m\(^{-2}\) s\(^{-1}\), Apogee Instruments Inc., Logan, UT, USA) and visible light was measured using a lux meter (Model MS6610 output in Lux, VIA Instruments, Shanghai, China)

### 8.3.3 LIGHT DISPERSION

The LEDs light emissions are very powerful; a bulb of 0.1 W produces light emissions in the photosynthetic spectra from 1300 to 2140 µmol m\(^{-2}\) s\(^{-1}\) depending in the wavelength. This amount of energy surpasses the maximum photosynthetic light absorption from algae species that is 50-250 µmol m\(^{-2}\) s\(^{-1}\) causing photo-inhibition and photo-damage (Kim et al., 2006; Degen et al., 2001; Fernandez-Sevilla et al., 1998; Merchuk et al., 1998) Nevertheless the dispersion of this light happens in a short distance, thus the algal in the present study receives lower light emissions than the maximum value for photosynthetic growth. The distance from the bulb to the algal in suspension is 7 mm enough distance to reduce the light intensively lower than 300 µmol m\(^{-2}\) s\(^{-1}\) that is where the photo inhibition process begins, thus the resulting light intensity is perfect for algal phototropic growth under artificial conditions (Ogbonna et al., 1999). See Table 8.1 for more details about light dispersion versus distance in water measured during the course of this experiment with the Apogee quantum meter.
Table 8.1. LED light dispersion and light reducing intensity through distance.

<table>
<thead>
<tr>
<th>Distance from source (mm)</th>
<th>Light dispersion from the source</th>
<th>Light emission in (μmol m⁻² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Blue</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>2143</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>568</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>206</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>95</td>
</tr>
</tbody>
</table>

8.3.4 PHOTOBIOREACTOR AND CO₂ SUPPLY

The photobioreactor is a bubble-column glass container of 36 cm length, 12 cm diameter, and volume 4 L. Carbon dioxide at concentrations of 0.035% (350 ppm ± 50), 1.1%, 3.7% and 8.5% ± 0.18% was supplied at a rate of 864 mL min⁻¹ L⁻¹ of algal suspension from a compressed air tank equipped with a mass flow regulator (Model 191 AR-60, Gentec Corporation, Shanghai, China). Two turbines, (Model ACO-9720, Hailea, Hailea Industrial Zone, Guangdong, China) each with an output of 30 L min⁻¹, were used to mix the algal suspension. The concentration of dissolved CO₂ in the inlet gases was measured at 5 s intervals with a CO₂ monitor (Model 7001, Telaire-General Electric, California, USA). Room temperature was measured with a temperature monitor (Model 7001, Telaire-General Electric, California, USA). Periodically during the experiment the algal suspension temperature was measured with an infrared thermometer (Model Fluke 62, Fluke Corporation, Everett, Washington, USA), Both room temperature and algae suspension temperature were about 22°C ± 0.9.

8.3.5 CULTURE MEDIUM

The culture medium was prepared using a commercial synthetic fertilizer (Solucat 25-5-5, Atlantica Agricola, Villena, Spain) by dissolving 0.8 g in 1 L distilled water. At the start of each
experiment the culture medium contained 114 mg L\(^{-1}\) ammonium-N, 86.4 mg L\(^{-1}\) nitrate-N, 40 mg L\(^{-1}\) phosphate, 40 mg L\(^{-1}\) potash, 160 μg L\(^{-1}\) iron, 80 μg L\(^{-1}\) manganese, 80 μg L\(^{-1}\) boron, 16 μg L\(^{-1}\) zinc, and 16 μg L\(^{-1}\) copper; the electrical conductivity was 0.95 ± 0.01 mS measured using an EC sensor (Model HI 991301, Hanna, Michigan, USA).

8.3.6 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

Each experiment was set out in 24 units of 4 L photobioreactors, exposed to four different light treatments (white, blue, red and red-blue) and 6 replicates, and incubated for 15 d. Each photobioreactor contained 3 L of culture medium. The experiment was repeated for the four different CO\(_2\) concentrations.

8.3.7 BIOMASS ANALYSIS

Colorimetric determination methodology was used to calculate the standing biomass production at the end of the incubation. Light absorption of each sample was measured at 680 nm with a spectrophotometer (Shimadzu UV-1600, Shimadzu Scientific Instruments Inc, Columbia, USA). For the calibration of this methodology seven samples with different biomass concentrations of C. vulgaris, ranging from 0.25 to 3.49 g L\(^{-1}\) were used to establish a relationship between standing biomass and absorbance. The resulted equation Y = 0.7015x + 0.0362 with R\(^2\) of 0.9987 was used to determine the biomass from 96 replicates in this study.

8.3.8. STATISTICAL ANALYSIS

The results were analyzed using ANOVA test with the software Biostatistics 1.0, and Stats Pad version 1.3 at α≤ 0.01. An ANOVA was performed for different CO\(_2\) concentrations as well as for different light treatments.
8.4 RESULTS AND DISCUSSION

8.4.1 STANDING BIOMASS PRODUCTION UNDER INCREASING CO₂ CONCENTRATIONS

Standing biomass of *C. vulgaris* increased with increasing concentrations of CO₂ under the four different light sources (Figure 8.1). The highest biomass production, 1.59 g L⁻¹, was found when the algal culture were supplied with 8.5% CO₂ and exposed to white light. Biomass production under blue, red and red+blue light was 1.53 g L⁻¹, 0.45 g L⁻¹ and 1.27 g L⁻¹, respectively. An experiment growing *Chlorella sp* under increasing concentrations of CO₂ found that the standing biomass increased from 0.5 to 5.7 g L⁻¹ reaching the highest standing biomass when the CO₂ reached 10% (Sung et al., 1998). A similar study growing *Chlorella sp* at different CO₂ concentrations resulted in standing biomass of 2 g L⁻¹ at 10% CO₂ (Maeda et al., 1995). Other study with *Chlorella* showed a standing biomass of 3 g L⁻¹ when the algal was grown at 10% CO₂, also good growth was reported with *Chlorella sp* at CO₂ concentrations from 10 to 50% (Sung et al., 1999), reaching 2 g L⁻¹ when the CO₂ concentration range from 5 to 40% (Sakai et al., 1995). Standing biomass of 2 g L⁻¹ also was obtained growing *Chlorella sp* at 5% CO₂ (Ryu, et al., 2009), concentration of 2% and 10% CO₂ has resulted in biomass synthesis of 1.67 to 1.5 g L⁻¹ after 6 days of incubation (Chiu et al., 2011). Several studies conducted with *C. vulgaris* have reported a typical standing biomass between 0.25 g L⁻¹ and 1.7 g L⁻¹ under phototropic growth after 12-15 days of incubation (Bhola et al., 2010; Liang et al., 2009), growing *Chlorella sp* under digested manure had produced standing biomass of 1.7 g L⁻¹ after 21 days of incubation (Pitman et al., 2011; Wang et al., 2010). The lowest levels of standing biomass 0.21 g L⁻¹ were obtained growing *C. vulgaris* in waste water (Chinnasamy et al., 2009), however using artificial waste water *C. vulgaris* had developed standing biomass of 1.6 g L⁻¹ after 11 days of incubation (Feng and Zhang, 2011).
Figure 8.1. Changes in standing biomass under different concentration of CO₂ and different wavelength illumination.

8.4.2 STATISTICAL ANALYSIS OF THE STANDING BIOMASS

The present statistical analysis is a two way ANOVA comparing two variables; the four CO₂ concentration treatments and the four light treatments. The ANOVA test results in a F in the samples = 13319, columns = 38194 and interaction = 2480 with p = 0.0000, these results show significant differences in the two variables CO₂ and light. In addition, a one way ANOVA was used to compare individual treatments of CO₂ and light to one another. For example; the algae growing in four CO₂ concentration treatments under white light to analysis possible differences in the standing biomass as the CO₂ concentration changes. The second analysis is comparing the same CO₂ concentration under different light treatment with the objective of determining if the wavelength of the light affects standing biomass. Table 8.2 compares the effect of CO₂ concentration in the standing biomass in four different type of illumination.
Table 8.2. Analysis of variance of the standing biomass for four different CO$_2$ concentration under constant light sources.

<table>
<thead>
<tr>
<th>Color</th>
<th>Red</th>
<th>Blue</th>
<th>Red-Blue</th>
<th>White</th>
<th>F table</th>
</tr>
</thead>
<tbody>
<tr>
<td>Different CO$_2$</td>
<td>3966</td>
<td>12466</td>
<td>13256</td>
<td>11174</td>
<td>4.94</td>
</tr>
</tbody>
</table>

The ANOVA shows a statistical difference at $p = 0.01$ in the standing biomass of the algae *C. vulgaris* growing under increasing CO$_2$ concentrations (0.035%, 1.1%, 3.7% and 8.5% CO$_2$) and 4 different light wavelengths. The F value from the experiment are between 1117 and 13256 and the F of the table at $p = 0.01$ is 4.94. The statistical analysis confirms that the differences in standing biomass production with increasing CO$_2$ concentrations and under the four different light sources are significant. For the next ANOVA test (Table 8.3) we leave the CO$_2$ concentration constant to see changes in the standing biomass if the light wavelength changes and the light power remains constant.

Table 8.3. Analyses of the standing biomass if the light regime changes and the CO$_2$ concentration remains constant.

<table>
<thead>
<tr>
<th>CO$_2$ Concentration</th>
<th>0.0350%</th>
<th>1.10%</th>
<th>3.70%</th>
<th>8.5%</th>
<th>F table</th>
</tr>
</thead>
<tbody>
<tr>
<td>Different color</td>
<td>1579</td>
<td>3983</td>
<td>2308</td>
<td>7405</td>
<td>4.94</td>
</tr>
</tbody>
</table>

The ANOVA for light source shows statistical differences at $p = 0.01$. The tests for the four CO$_2$ concentrations gave an F ranging from 740 to 3982 and the F of tables is 4.94. There is statistical difference in *C. vulgaris* standing biomass when is grown under different concentration of CO$_2$ and exposed to light sources of different wavelength.
8.4.3 LIGHT SOURCE EFFECTS ON BIOMASS PRODUCTION

Exposure of the algae to white LED light and supplied with 8.5% CO₂ concentration resulted in the highest standing biomass of this study 1.6 g L⁻¹, even higher than 0.16 g L⁻¹ obtained in a study growing Scenedesmus dimorphus at 10% CO₂ (Lunka and Bayless, 2013). Different results were obtained growing Nannochloropsis sp under LED illumination, the results of this study show that blue 470 nm developed the highest growth rate follow by white, green 550 nm and red 680 nm (Das, et al., 2011). Contrary to the rest of the lights treatments, biomass production under red light was highest 0.57 g L⁻¹ when the cultures were exposed to 3.7% CO₂ and not at the highest CO₂ concentration 8.5% where biomass was 0.44 g L⁻¹. The red 625 nm always developed less biomass than the rest of the lights treatments, these results confirm the study made with Isochrysis galbana which demonstrated that a shorter wavelength such as blue 460 nm is more photosynthetic efficient than a longer wavelength as red 670 nm (Jeon, et al., 2013). However a study made with C. vulgaris growing in synthetic waste water and under different LED illumination, conclude that red 660 nm developed higher biomass 0.28 g L⁻¹ than white 0.25 g L⁻¹, yellow 590 nm 0.21 g L⁻¹, purple 410 nm 0.16 g L⁻¹ blue 460 nm 0.15 g L⁻¹ and green 550 nm 0.1 g L⁻¹ (Yan, et al., 2013). Red 660-680 nm match better the C. vulgaris absorption peak than red 625 nm, however it was expected C. vulgaris adaptation to this light spectra by modifying its chlorophylls or by producing more complementary pigments such as carotenoids in the absorption peak of 620-630 nm, little evidence of light spectra adaptation is suggested from the results of this study.

The highest biomass production in cultures exposed to blue light 1.5 g L⁻¹ were obtained when the algal grew at 8.5% of CO₂. However biomass production under blue light was higher than the other light sources tested in this study for CO₂ concentrations of 1.1% and lower when the standing biomass is less than 0.6 g L⁻¹. Since the photosynthetic part of algae are the chloroplast, light reaching the rest of the algae body is not been used for photosynthesis, therefore increases in standing biomass increase light shadowing. The shadowing effect is light blocked and not used for photosynthetic process by algae located near to the light source resulting in less light
reaching those algae farther away from the light source. This effect causes loss of photons, therefore the amount of energy lost is greater at lower wavelength when the light photon has more energy (Das et al., 2011). Since blue 425 nm light has more energy per photon \((6.68 \times 10^{-19} \text{ J})\) than do the other light sources for example red 625 nm \((3.18 \times 10^{-19} \text{ J})\), losses of light energy caused by light shadowing would be greatest under blue light exposure. From the data in Table 8.4, the limit where the biomass production responds less to blue light switches between 0.6 to 0.8 g L\(^{-1}\). At this algal biomass concentration, the light source combination of red-blue performed better than blue light alone because the red light losses for shadowing effect are less than blue, and red light complements the blue light resulting in a higher standing biomass. White light travels further and would have reached more distant algae, therefore would perform better when the standing biomass is greater than 0.8 g L\(^{-1}\).

**Table 8.4.** Mean standing biomass production under increasing CO\(_2\) concentrations and exposed to four different light sources.

<table>
<thead>
<tr>
<th>CO(_2)/Light source</th>
<th>0.035%</th>
<th>1.10%</th>
<th>3.70%</th>
<th>8.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>0.148 ± 0.009</td>
<td>0.254 ± 0.008</td>
<td>0.571 ± 0.006</td>
<td>0.446 ± 0.005</td>
</tr>
<tr>
<td>Blue</td>
<td>0.429 ± 0.007</td>
<td>0.610 ± 0.006</td>
<td>0.828 ± 0.013</td>
<td>1.531 ± 0.014</td>
</tr>
<tr>
<td>Red-blue</td>
<td>0.309 ± 0.003</td>
<td>0.483 ± 0.003</td>
<td>1.003 ± 0.009</td>
<td>1.271 ± 0.016</td>
</tr>
<tr>
<td>White</td>
<td>0.296 ± 0.006</td>
<td>0.548 ± 0.006</td>
<td>1.045 ± 0.014</td>
<td>1.592 ± 0.021</td>
</tr>
</tbody>
</table>

**Note:** ± Standard deviation.

### 8.5. CONCLUSIONS

Production of *C. vulgaris* biomass increased when supplied with increasing CO\(_2\) concentrations up to 8.5% under the four light sources. Growth of the algae was better under blue light when algae were supplied with lower CO\(_2\) concentrations and the standing biomass was low. The results of this study show that *C. vulgaris* does not adapt production of their photosynthetic pigments to absorb light from a wavelength spectrum different from one that they would normally be exposed to.
Further studies with more CO\textsubscript{2} concentration are recommended to establish the limit where CO\textsubscript{2} is no longer a factor for increasing biomass production. It is further recommended to compare \textit{C. vulgaris} growth under red light at 660 nm, 670 nm with that under red at 625 nm.

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8.6 REFERENCES


CHAPTER 9  BIOMASS PRODUCTION OF TWO MARINE AND TWO FRESH WATER ALGAE GROWING IN AN INDUSTRIAL-SCALE THIN FLAT PANEL PHOTO-BIOREACTOR UNDER FOUR CARBON DIOXIDE CONCENTRATIONS

9.1  ABSTRACT

Two marine and two fresh water algae were grown in 750 L flat panel photo-bioreactors under four CO₂ concentrations, 395 ppm, 1500 ppm, 4000 ppm and 10,000 ppm, to evaluate CO₂ concentration effects on growth rate, population density and biomass production. The fresh water alga, *Chlorella vulgaris* reached an average standing biomass of 2582 mg L⁻¹ when the CO₂ concentration was 10,000 ppm, and 760 mg L⁻¹ growing at 380-410 ppm of CO₂. The algae *Scenedesmus sp* increased its biomass from 424 to 2428 mg L⁻¹ with CO₂ concentrations increasing from 395 to 10,000 ppm. The marine alga *Nannochloris sp* increased its biomass from 594 to 1796 mg L⁻¹ as the CO₂ concentration raised from 395 to 10,000 ppm, and *Tetraselmis sp* changed its average standing biomass from 710 to 1115 mg L⁻¹ when CO₂ changed from 380-410 to 10,000 ppm. The trend of increasing in biomass with increasing CO₂ concentrations was tested with ANOVA repeated measurements resulting in statistical difference in three of the four algae.

*Keywords:* Biomass production, population density, Carbon dioxide uptake, artificial lighting.

9.2  INTRODUCTION

Flat panel photo-bioreactors (FPB) represent the latest evolution in technology for industrial-scale, engineered systems for production of algal biomass. Biomass production under these systems greatly exceeds those achieved in open or closed ponds, and in closed bubble systems or closed tubular photo-bioreactors. The main advantage of FPB technology is the thickness of the system; a thin layer (5-70 mm) of growing medium which increases light exposure to the algae allowing it’s photosynthetic machinery to operate more efficiently. Another advantage of the
technology is the higher efficiency of oxygen release during photosynthesis, thereby reducing oxidative damage to the photosynthetic machinery (Sierra et al., 2008). Increases in biomass production from 0.34 to 4.4 g L\(^{-1}\) d\(^{-1}\) have been reported for FPB thickness decreases from 104 mm to 13 mm (Hu et al., 1996).), standing biomass of 16 g L\(^{-1}\) was attained in a FPB with a thickness of 13 mm, biomass decreased below 3.8 g L\(^{-1}\) when the thickness increased to 52 mm growing the marine algal *Nannochloropsis sp* (Zou and Richmond, 1999).

Open ponds have a useful media thickness about of 15-30 cm as light of appropriate intensity is not able to reach the algae population below these depths. Productivity of open pond systems is typically 0.06-0.1 g L\(^{-1}\) d\(^{-1}\) with a standing biomass of 1 g L\(^{-1}\) (Pulz, 2001; Becker, 1993). Open pond systems are also subject to contamination (Pulz, 2001). In bubble systems and closed tubular photo-bioreactors the algal medium limits the light intensity reaching the growing algae, however there have been major improvements in biomass production compared to those from open ponds because in closed systems the risk of contamination is reduced and biomass production is increased. Further, the functioning cost of closed photo-bioreactors is 43% compared to 57% of open ponds and the financial returns of photo-bioreactors are 3% more (Richardson et al., 2012).

A FPB of 6-7 mm thickness can achieve a biomass production of 5.6 g L\(^{-1}\) d\(^{-1}\) and standing biomass of 40 g L\(^{-1}\) (Doucha and Livansky, 2009). A thin layer reactor 10 mm thick achieved a standing biomass of 10 g L\(^{-1}\) and biomass production of 1.7 g L\(^{-1}\) (Livasky and Doucha, 1997). With a FPB of 200 mm thickness, a specific growth rate of 0.52 d\(^{-1}\) has been reported with the alga *Haematococcus pluvialis* (Issarapayup et al., 2009). The efficiency of a FPB of 6 mm thickness reached 49% CO\(_2\) absorption with a net efficiency of 4.4 kg of CO\(_2\) to produce 1 kg of dry biomass. This represents a net absorption efficiency of 38.7% of the supplied CO\(_2\); 10.3% is absorbed and lost from medium and 51% is not absorbed at all (Doucha et al., 2005).
Though FPB have a higher efficiency in biomass production and carbon uptake efficiency per medium volume than open or closed pond and tubular photo-bioreactors, they have a lower medium volume per square meter bioreactor area. Further, the cost of this technology is much higher on an area and volume basis than other technologies. This study examined algal growth in a FPB which addressed the volume/area problem by positioning the bioreactor vertically.

### 9.3 MATERIALS AND METHODS
#### 9.3.1 MICROORGANISM
The four algal species grown this study are from the culture collection kept at the University of Texas, Austin, TX; *Chlorella vulgaris* (26), *Nannochloris sp* (1268), *Scenedesmus sp* (1589) and *Tetraselmis sp* (2767).

#### 9.3.2 FLAT PANEL REACTOR
The FPB designed and constructed for this study contained 8 units, each unit consisting of 15 flat panels constructed from polypropylene and connected to a 500 L tank. Each flat panel was located 35-40 cm between one another, which simulated a 30-m tube, was comprised of 15 channels, each 2 m in length, 2.4 m in height and 30-50 mm in width. The entire unit contained 750 L of algal medium. A submersible pump (500 watt) set in the tank was capable of circulating medium at 7,000 -7,500 L h⁻¹. Each flat panel bioreactor held 30-33 L of medium with a flow rate of 7-8 L⁻¹ min⁻¹ reactor⁻¹ (the flow of medium in the reactor was 10-12 cm sec⁻¹). The distribution of the algal media was 250-260L resting in the 500L tank and 450-495L moving in the 15 flat panel photo bioreactors.

A pump was used raise the medium to the top of the reactor and it flowed down through the under gravity. The exposure time of the algal medium was 4 min per flow through the reactor and in total there were 8-10 complete medium cycles per hour, considering the time that the medium was in the tank before being pumped up to the top of the reactor. Figure 9.1 shows the FPB set up.
Figure 9.1. Reactor unit from experimental set up.

9.3.3 ILLUMINATION AND TEMPERATURE

During daytime the system was exposed to solar radiation, the polyethylene-covered greenhouse allowing 90% of the total solar radiation to reach the bioreactors. A portion of the solar UV radiation was filtered by the greenhouse cover and by the polypropylene panels, the solar radiation reaching the reactors through each day ranged from 71 to 1500 µmol m$^{-2}$ s$^{-1}$, outside the greenhouse the maximum radiation registered was 2550 µmol m$^{-2}$ s$^{-1}$, measured by a quantum meter (Model MQ, Apogee Instruments Inc, Logan, UT, USA). During the night time artificial illumination was provided to each reactor by a fluorescent cold white lamp (180 watts). In addition there were two blue (425 nm) 52 watt reflectors made up of LEDs and supplying 2,800 µmol m$^{-2}$ s$^{-1}$, measured 5 cm from the reflector. In total 1544 watts were supplied to 6,000 L of medium (750 L * 8 reactors = 6000 L) for an average input of 0.257 W L$^{-1}$, See Figure 9.2 for night illumination. Temperature in the media was 23°C ± 2 during the entire time of the experiments. Temperature sensors were used to measure temperature outside of the greenhouse, temperature inside of the greenhouse and temperature of the reactors media at all time.
9.3.4 CARBON DIOXIDE SUPPLY

Carbon dioxide was supplied to the FPB using a air turbine (Model GF-180, Resun, China) which provided 0.9 m³ min⁻¹ at 11.7 kPa. The turbine combined air from the atmosphere with pure carbon dioxide from a supply tank, and introduced the air-CO₂ mixture into the eight 750 L reactors. The air-CO₂ mixture was measured continuously with a CO₂ sensor (Model 7001, Telaire-General Electric, California, USA). Four CO₂ concentrations were supplied: 395 ppm (atmospheric air), 1,500 ppm, 4,000 ppm and 10,000 ppm CO₂.

9.3.5 CULTURE MEDIUM

The culture medium was prepared using a commercial synthetic fertilizer, Solucat (25-5-5 + trace elements), by dissolving 0.8 g in 1 L distilled water. At the start of each experiment the culture medium contained 114 mg L⁻¹ ammonium-N, 86.4 mg L⁻¹ nitrate-N, 40 mg L⁻¹ phosphate, 40 mg L⁻¹ potash, 160 µg L⁻¹ iron, 80 µg L⁻¹ manganese, 80 µg L⁻¹ boron, 16 µg L⁻¹ zinc, and 16 µg L⁻¹ copper; the electrical conductivity was 0.95 ± 0.01 mS, measured by EC.
sensor (Model HI 991301, Hanna, Michigan, USA). This EC level was maintained throughout the experiment. For the two marine algal species, NaCl was added to the medium to increase the salt concentration to 3.5%, simulating marine conditions.

9.3.6 BIOMASS DETERMINATION

A colorimetric methodology was used to estimate production of algal biomass. A calibration curve was prepared by measurement of the absorbance at 680 nm (Shimadzu UV-1600, Shimadzu Scientific Instruments Inc, Columbia, USA), for 7 known algal biomass ranging from 0.17-3.49 g L\(^{-1}\) in suspension medium. Linear regression analysis of the data for each alga gave the following equations: for *Nannochloris sp*, \(Y = 0.8586x + 0.1464\) with \(R^2 = 0.9854\), for *Tetraselmis sp*, \(Y = 1.054x + 0.2538\) with \(R^2 = 0.9985\), for *Scenedesmus sp*, \(Y = 0.81x + 0.0461\) with \(R^2 = 0.9813\), and for *Chlorella vulgaris*, \(Y = 0.7015x + 0.0362\) with \(R^2 = 0.9887\).

We used the following methodology to measure the biomass: (1) shake the 15 flat panel in each unit to suspend algae growing in the walls of the flat panel, (2) wait 3-5 minutes to ensure that the system is homogenous, (3) take a sample from the nutrient supply tanks with a 20 mL syringe, (4) introduce the sample into a spectrophotometer vial, (5) take the absorbance reading at 680 nm, (6) repeat steps 4 to 5 and compare results to ensure that they are similar and take the average of the two results, (7) in the case of different in the results repeat the six steps before.

9.3.7 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experiment measured population density and standing biomass of four algae species supplied with four different CO\(_2\) concentrations over an 18 d incubation. The algae were assigned to the reactors at random. Each algal treatment was replicated twice, with the 8 reactor units receiving the same concentration of CO\(_2\) concentration during the incubation. The results were analyzed using a one and two way ANOVA and repeated measures ANOVA using the software Biostatistics 1.0 and Stats Pad version 1.3.
9.4 RESULTS

9.4.1 CHANGES IN ALGAL CELL DENSITY UNDER AIR SUPPLY (380-410 PPM OF CO₂)

The four algae species showed a typical sigmoid growth curve, confirming that the industrial flat panel system was sufficiently well-designed to support algae growth under these conditions (Figure 9.3). The marine algae *Tetraselmis sp* developed the highest population density $3.58 \times 10^7$ cells mL$^{-1}$; the marine algal *Nannochloris sp* produced a population density of $2.44 \times 10^7$ cells mL$^{-1}$. The two fresh water species produced less cell density, though their cell sizes are larger. The cell density of *Scenedesmus sp* reached $1.04 \times 10^7$ cells mL$^{-1}$ whereas *C. vulgaris* developed the lowest population density $1.31 \times 10^7$ cells mL$^{-1}$, though it produced a higher biomass.

![Population density of algae species](image)

*Figure 9.3.* Population density of four alga species growing in a flat panel industrial scale reactor.
Growth rate of the fresh water algae *C. vulgaris* was 1.47 in 18 d\(^{-1}\) and for *Scenedesmus sp* was 1.45 in 18 d\(^{-1}\); on the marine algae growth rate for *Tetraselmis sp* was 1.36 in 18 d\(^{-1}\) and for *Nannochloris sp* was 1.43 in 18 d\(^{-1}\).

### 9.4.2 BIOMASS PRODUCTION WITH INCREASING CO\(_2\) TREATMENTS

Figure 9.4 shows the substantial increase in algal biomass production after an 18-d incubation when exposed to increasing CO\(_2\) concentrations. This trend is most evident in the two fresh water species: *C. vulgaris* increased its biomass 3.4-fold, from 760 mg L\(^{-1}\) to 2582 mg L\(^{-1}\), whereas *Scenedesmus sp* increased its biomass from 424 mg L\(^{-1}\) to 2428 mg L\(^{-1}\), a 5.7 fold increase. The marine algae *Nannochloris sp* increased its biomass from 691 mg L\(^{-1}\) to 1796 mg L\(^{-1}\) and *Scenedesmus sp* increased its biomass from 710 mg L\(^{-1}\) to 1115 mg L\(^{-1}\).

**Figure 9.4.** Biomass production of two fresh water algae *C. vulgaris, Scenedesmus sp* and two marine algae *Tetraselmis sp, Nannochloris sp* exposed to increasing CO\(_2\) concentrations in an FPB.
9.4.3 BIOMASS COMPARISON OF ALGAE GROWING AT DIFFERENT CO$_2$ CONCENTRATIONS

The fresh water algal $C. vulgaris$ in general produced more biomass than the other algal species. At a CO$_2$ concentration of 380 - 410 ppm, $C. vulgaris$ produced a standing biomass of 761 mg L$^{-1}$ with a peak of 1009 mg L$^{-1}$, which is similar to $Tetraselmis sp$ at 766 mg L$^{-1}$ with a peak of 867 mg L$^{-1}$. When the CO$_2$ concentration raised to 1000-2000 ppm $C. vulgaris$ biomass was 1284 mg L$^{-1}$ with a peak of 1946 mg L$^{-1}$ and the highest standing biomass was from $Nannochloris sp$ 1330 mg L$^{-1}$ with a peak of 2038 mg L$^{-1}$. $C. vulgaris$ produced the highest standing biomass 1741 mg L$^{-1}$ when the CO$_2$ concentration was 4000 ppm with a peak of 2067 mg L$^{-1}$. When the CO$_2$ concentration raised to 10,000 ppm the standing biomass of $C. vulgaris$ was 2316 mg L$^{-1}$ with a peak of 3057 mg L$^{-1}$ and the highest average standing biomass was 2357 mg L$^{-1}$ produced by $Scenedesmus sp$ with a peak of 2665 mg L$^{-1}$. Consistently $C. vulgaris$ produced the highest or second highest standing biomass at all CO$_2$ concentration treatments.

A two way ANOVA test was made to compare four different algae and four different CO$_2$ concentration, resulting in statistical differences with F in the samples = 19.4936, columns = 63.5129, and interaction = 6.38448 and p = 0.000. In addition the two variables; algae species and CO$_2$ concentration, were analysed separately. An ANOVA repeated measurements showed that there were statistical differences in biomass production with increasing CO$_2$ concentrations in $C. vulgaris$ F= 24.5948 with p =0.0130, in $Scenedesmus sp$ F = 123.1045 with p = 0.0012, in $Nannochloris sp$ F = 23.1414 with p = 0.0141, though it was not significantly different in $Tetraselmis sp$ F = 3.4339 with p = 0.1690. Therefore, increases in CO$_2$ concentration increased algal biomass production significantly when the changes are from 400 ppm to 10,000 ppm of CO$_2$ in three of the four species of algae tested under the present research.

A one way ANOVA was performed to compare the biomass of the four algae species at specific CO$_2$ concentration; for the four algae growing at 380-400 ppm the results were F = 5.0169 with p = 0.0058, at 1000-2000 ppm F = 5.0732 with p = 0.0055, at 4000 ppm of F = 22.7575 with p = 0.0000 and for 10,000 ppm we got F = 43.2615 with p = 0.0000. As a result of these tests it was
found statistical differences in the biomass production from algae to algae growing at the same \( \text{CO}_2 \) concentration, therefore each alga produced difference quantity of biomass growing at the same time in equal flat panel reactors at equal \( \text{CO}_2 \) concentration.

9.5 DISCUSSION AND CONCLUSION

The biomass production reached by the four algae species in an 18-d incubation growing in an FPB with thickness of 30-50 mm were from 424 mg L\(^{-1}\) to 2428 mg L\(^{-1}\). This biomass is similar to those obtained in previous studies. A study conducted with \( C. \) vulgaris reached a standing biomass of 1700 mg L\(^{-1}\) after 12-15 days of incubation (Liang et al., 2009). A study with \( C. \) vulgaris growing in an FPB of 15 mm and 30 mm thickness generated a standing biomass of 1950 mgL\(^{-1}\) and 1800 mgL\(^{-1}\) respectively (Degen et al., 2001). A most recent study resulted in more standing biomass 900 mg L\(^{-1}\) a lower reactor thickness 50-100 mm after 30 days incubation, and a maximum standing biomass of 4640 mg L\(^{-1}\) after 100 days incubation (Yoo et al., 2013). Another study growing \( C. \) vulgaris in a FPB of 3 mm reached 12450 mg L\(^{-1}\) in just 11 days of incubation (Choi et al., 2013). In the present study, \( C. \) vulgaris produced more biomass than the other three algae species growing under to similar \( \text{CO}_2 \) concentrations.

Although the FPB is a reliable system to grow algae for high biomass production in industrial scale, more studies are needed to developed its maximum potential in biomass production per volume and to increase the volume-area ratio.

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9.6 REFERENCES


CHAPTER 10: GENERAL CONCLUSIONS

During the course of this research, we evaluated biomass production and population density of marine and fresh water algae growing under different parameters such as light quality, CO₂ concentration and different types of photo-bioreactors. For light treatments, 6 algae species, three marine and three fresh water algae, were grown under solar radiation, blue-red LEDs and fluorescent illumination. The alga Chlorella vulgaris was grown under four different LED lights, blue, red, white and blue-red, and one marine and one fresh water algae were grown under flashing LED lights. For CO₂ concentration we grew the algae Chlorella vulgaris under four different CO₂ concentration and four different light treatments. We grew two marine and two fresh water algae under a polypropylene flat panel reactor and measured the biomass production of the algae for 5 months under four different CO₂ concentrations.

All light treatments that included solar radiation showed a faster growth rate and higher biomass production than those experiments that did not include solar radiation and relied only on artificial illumination. The treatments that produced the highest biomass were obtained when we combined solar radiation during the day with artificial illumination during the night period. In general, these treatments also performed better than those treatments with just 24 h artificial illumination. The experiments which compared different wavelengths resulted in more biomass production when the algae Chlorella vulgaris grew under white LED, the cell weight was higher under white light illumination, and the one way ANOVA test confirmed these results. The second best treatment was the combination of red-blue LEDs, which produced the second highest biomass production and the second highest cell weight. The flashing light experiment for a marine alga Nannochloris sp and fresh water alga Scenedesmus sp showed more standing biomass and higher cell weights in both algae under flashing lights compared against continuous light. There was a statistical difference in daily biomass production and growth rate under flashing light against continuous light in the marine alga Nannochloris sp, however there was not
conclusive evidence from this study for the fresh water alga *Scenedesmus sp* because the ANOVA test resulted in no significant difference in daily biomass production and growth rate.

The experiments conducted with the alga *Chlorella vulgaris* grown under different concentrations of CO\(_2\) resulted in more standing biomass with increasing CO\(_2\) concentrations. We also tested different light treatments and confirmed that *Chlorella vulgaris* growth was better under white LED and Blue LED. The ANOVA test confirms the fact of more algal standing biomass as the concentration of CO\(_2\) in the inlet air rise from 0.035% to 9%.

We tested for five months a new flat panel photo-bioreactor with two marine and two fresh water algae. These industrial scale experiments confirm what we previously study in *Chlorella vulgaris*, more biomass production as the CO\(_2\) concentration in the inlet air raise. The two marine algae tested *Nannochloris sp*, *Tetraselmis sp* and the two fresh water algae *Chlorella vulgaris*, *Scenedesmus sp* increased their biomass production when the CO\(_2\) in the inlet air increased from 380 ppm, 1000-2000 ppm, 4000 ppm and 10,000 ppm, thus the ANOVA test confirm this trend. The flat panel photo-bioreactor produced more biomass using *Chlorella vulgaris* at 1% of CO\(_2\) than some of the previous experiments using the bubble column photo-bioreactor at 8-9% of CO\(_2\) concentration, thus the standing biomass obtained growing the two marine and the two fresh water algae in the flat panel photo-bioreactor were the highest during the course of this study.

During the course of our experiments we worked with red (625 nm) illumination and this light treatment showed the worst performance in all experiments. The algae has an absorption peak at around 660 nm and the mean chlorophyll reaction centers are 670 and 680 nm. For this reason we suggest more studies comparing blue and white lights against red 660-690 nm, and all three treatments together. These experiments would provide conclusions on the effects of different light wavelengths on algal biomass production for selection of the best light source or combination of lights. We also suggest that more experiments should be conducted with a flashing light source because this technology decreases energy consumption up to 90% and increases growth rate and biomass production. The research with CO\(_2\) concentrations should be
expanded to evaluate increasing the concentration to 15, 20, 25, 30%, and higher concentrations of CO₂ to determine the optimal concentration for algal growth and for highest CO₂ removal rate.

The experiments with the flat panel reactor showed the need for further research to increase biomass production and robustness of the technology for industrial scale. However biomass production in the flat panel reactor is sufficient for commercial production of algae. Algal biomass can be used as a component of feedstocks for fish, cattle and pigs. The oils contained in the algae, such as omegas 3, 6 and 9, are valuable products for human food supplement. The fast growing alga *C. vulgaris* is rich in high value molecules such as lutein and canthaxanthin. These two powerful antioxidants are currently marketed as human supplements, making these algal species attractive for use as a crop.

In general the six algal species tested in this study contain oils that can be transformed into biodiesel and the carbohydrates can be fermented for commercial ethanol production. The ability of algae to rapidly fix high quantities of CO₂, plus the importance of the constituents of the algae such as proteins, high value molecules for food supplements, fats and carbohydrates for biofuels, make algae an alternative for a new generation of crops. They can be established on land that is unsuitable for agriculture, such as deserts, and grown using contaminated water or sea water.

This research has obtained interesting results, some conclusive and some not, although there is potential to further increase biomass production efficiencies. The biomass production obtained is enough to sustain commercial algal production. Critical factors such as illumination intensity and composition, CO₂ concentration in the growing media and reactor design have an impact on biomass production. The flat panel reactor at 8% CO₂ air intake, and the combination of solar radiation and multiple LEDs lights (red, blue and white) with the flashing effect during the night period maximizes biomass production. The combination of these critical factors is the most efficient way to grow algae from the findings of this research.