



Luminescent solar concentrators to increase microalgal biomass productivity

By

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DECLARATION

I declare that this work is of my own research and contains no material previously published by another person. I certify this thesis contains work which has not been previously submitted for a degree at any tertiary institution.

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ABSTRACT

Light is the main limiting factor of any mass microalgal cultivation resulting in relatively low biomass productivity in raceway ponds. Microalgal cells in open ponds are normally photoinhibited on the surface and photolimited at the depth of the cultures where there is total darkness. Delivering light to the microalgal cells at the depth of cultures in large scale raceway ponds can increase biomass productivity. Luminescent solar concentrators (LSCs) can potentially be an economical light-diffusing system to be used in algal biotechnology. The main advantage of luminescent solar concentrators is that a solar tracking system is not needed. This results in less cost compared to other diffusing systems. Luminescent particles such as organic dyes or quantum dots (QDs) are the main constituents of LSCs. Luminescent particles absorb photons when light hits the surface of LSCs and the absorbed light is reflected internally and emitted from the edges at a longer wavelength. To the best of my knowledge, to date, there have been no attempts in using LSCs as a light guide for the growth of microalgae in any open system. Thus, the main aim of this study was to evaluate the effect of LSCs as a light guide to deliver light to the depth of microalgal cultures in raceway ponds to increase both biomass and high-value productivities.

To assess the viability and efficacy of the LSCs system in an algal raceway pond, it is first necessary to select the most suitable microalgae species for this purpose. Three species, *Arthrospira platensis* (MUR 129), *Scenedesmus* sp. (MUR 268) and *Chlorella* sp. (MUR 269). were chosen for a laboratory experiment to investigate the effect of red and blue LSCs on the productivity of cultures. *Arthrospira platensis* showed up to 9% higher productivity when red LSCs were used compared to control and blue LSCs. The biomass productivity of *Scenedesmus* sp. cultures under red LSCs was also 30%

and 4.5% higher compared to that in control and blue LSCs. The growth rate of *Chlorella* sp. cultures did not improve under red and blue LSCs. Furthermore, *Scenedesmus* sp. culture resulted in 30% higher cell density in cultures with red LSCs compared to that in control. Thus, *Arthrospira platensis* and *Scenedesmus* sp. were chosen as the most suitable species for further outdoor investigations using micro raceway ponds.

In the next stage, *Arthrospira platensis* and *Scenedesmus* sp., were grown using red and blue LSCs and compared with control cultures with no LSCs using micro raceway ponds (0.1 m²) with the final culture volume of 21.5 L. The LSCs were installed on the edge of raceway ponds to have 200 mm of a panel inside the raceway pond and 100 mm of the panel out of the pond facing the sun to collect visible and diffuse light from sunlight, downgrade and, transfer it to the depth of *A. platensis* cultures. The bottom part of LSCs inside the *A. platensis* culture was also laser-cut to have enough surface area to increase the irradiance. *Arthrospira platensis* cultures when grown with red LSCs, reached a significantly higher biomass yield ($1.77 \pm 0.014 \text{ g L}^{-1}$) compared to control ($1.53 \pm 0.002 \text{ g L}^{-1}$) and blue LSCs ($1.59 \pm 0.056 \text{ g L}^{-1}$). The biomass productivity of $57 \pm 3.2 \text{ mg L}^{-1} \text{ d}^{-1}$ ($12.2 \text{ g m}^{-2} \text{ d}^{-1}$) was obtained when *Arthrospira* cultures in raceway ponds were equipped with red LSCs. This was 24% and 26% higher than the biomass productivity of *Arthrospira* cultures when grown in raceway ponds with blue LSCs and control. There was no significant difference between the productivity of *Arthrospira* cultures with blue LSCs and control. Furthermore, the maximum phycocyanin productivity in *Arthrospira* cultures with red LSCs was $8.49 \pm 0.9 \text{ mg L}^{-1} \text{ d}^{-1}$, which was 14% and 44% higher than that in cultures with blue LSCs and control cultures. In addition, the phycocyanin content of *A. platensis* was 136 mg L^{-1} ($77 \text{ mg g biomass}^{-1}$) and 141 mg L^{-1} ($89 \text{ mg$

gbiomass⁻¹) under red and blue LSCs, respectively. The results of showed that red LSCs can significantly increase *Arthrospira*'s growth and productivity. Based on the outcome of this study, only red LSCs were applied to outdoor *Scenedesmus* sp. cultures in the next experiment.

When grown with red LSCs, *Scenedesmus* sp. cultures reached a higher cell density compared to the control. Furthermore, the maximum specific growth rate (μ) of *Scenedesmus* sp. cultures with red LSCs was 16% higher than control with no LSCs. The biomass productivity of $43.6 \pm 1.3 \text{ mg L}^{-1} \text{ d}^{-1}$ ($9.4 \text{ g m}^{-2} \text{ d}^{-1}$) was obtained for *Scenedesmus* sp. cultures equipped with red LSCs which was 18.5% higher than that for *Scenedesmus* sp. cultures when grown in raceway ponds with no LSCs. Further, the protein content of *Scenedesmus* sp. under red LSCs was $436 \text{ mg gbiomass}^{-1}$ (43.6%) which was 17.5% higher than that in control. The lipid content of *Scenedesmus* cultures under red LSCs ($133 \text{ mg gbiomass}^{-1}$) was also 10% higher compared to control with no LSCs. However, the carbohydrate content of *Scenedesmus* sp. cultures with red LSCs and control was not significantly different.

The results of all indoor and outdoor experiments showed that using red LSCs on *Arthrospira platensis* and *Scenedesmus* sp. cultures was promising. More light availability to microalgal cells into the depth of the cultures is the most likely reason for having higher productivity in cultures with red LSCs. From the energy perspective, the results showed that the total amount of photosynthetic active radiation (PAR) available for *A. platensis* and *Scenedesmus* sp. cells at the depth of each pond emitting from four red LSCs is $34 \mu\text{mol photons s}^{-1}$. In other words, using red LSCs in each outdoor raceway pond bring about $34 \mu\text{mol photons s}^{-1}$ more light to the depth of *A. platensis* and *Scenedesmus* sp. cultures. This means injecting $34 \mu\text{mol photons s}^{-1}$ deep into the *A. platensis* and *Scenedesmus* sp. cultures where it would otherwise

be in full darkness. This helps move the light from the photosaturated surface to the depth of the microalgal cultures. Moreover, based on the mixing rate, the thickness of the LSCs and surfaces of each red LSC, *A. platensis* and *Scenedesmus* sp. cells received brief bursts of light when they pass an edge and a surface of LSCs. For instance, considering PAR emitting from an edge of a red LSC ($110 \text{ Wm}^{-2}/506 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$), *A. platensis* and *Scenedesmus* sp. cells received around $506 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in 27 ms from each edge and $276 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in 218 ms when they pass each surface of a red LSC. In other words, it can be said that *A. platensis* and *Scenedesmus* sp. cells with red LSCs received brief bursts of light with different intensities for durations less than a second inside the cultures while there was total darkness for the cultures without LSCs.

Finally, the costs of biomass and phycocyanin production using luminescent solar concentrators as a light delivering system on an industrial scale raceway pond cultivation of *Arthrospira* was assessed. The results showed that using red luminescent solar concentrators would result in a biomass and phycocyanin production costs of AU\$ 3.16 and AU\$ 125 per kg, respectively, which are 14% and 35% lower than the corresponding costs in a conventional raceway pond with no LSCs. The biomass and phycocyanin production costs of *Arthrospira* cultivation in conventional raceway ponds (with no LSCs) were AU\$ 3.67 and AU\$ 187 per kg, respectively. These results showed that using LSCs for growing *Arthrospira* can significantly lower the cost of biomass and phycocyanin production if the same size production facility is used.

In conclusion, this study clearly showed that using LSCs in a raceway open ponds can be a promising method to increase the biomass productivity of a microalgal culture while reducing the production costs of biomass and the desired high-value product.

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ABBREVIATIONS

LSC	Luminescent solar concentrator
QD	Quantum dots
PBR	Photobioreactor
PAR	Photosynthetic active radiation
UV	Ultraviolet
IR	Infrared
PE	Photosynthetic efficiency
PI	Photosynthetic-Irradiance
LSE	Light saturation effect
PFD	Photosynthetic flux density
PPFD	Photosynthetic photon flux density
PSU	Photosynthetic unit
S/V	Surface to volume
LDOF	Light diffuser optical fibre
PMMA	Polymethyl methacrylate
GSC	Green solar collector
Capex	Capital expenses
Opex	Operational expenses
EISA	Energy Independence and Security Act
RFS	Renewable Fuel Standard
C-PC	C-Phycocyanin

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Chapter 1 Introduction

1 General Introduction

Light is considered as the main limit to any microalgae cultivation. Microalgae use the light energy in photosynthesis. Light is by far the main limit to the growth of any microalga. In any cultivation system, microalgae productivity depends on the amount of light that cells receive. For instance, in open raceway ponds, there is not enough light to microalgal cells at the depth of the culture and each cell spend most of the time in total darkness below 5 cm from the surface. Increasing the number of photons available to microalgal cells in ponds can increase biomass productivity. Light diffusing systems can be a potential way to provide more photons to microalgal cells in a raceway pond. Using a light delivering system can transfer photons to microalgal cells at the depth of a culture where there is total darkness and thus, increase the biomass productivity. Different light delivering systems for microalgae production to improve light availability to microalgal cells have been discussed in the following section and it has been published as a review paper in Renewable and Sustainable Energy Reviews Journal.

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Navid. R. Moheimani (15%)	Assisted in data analysis and critical revision of the manuscript.
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Luminescent solar concentrator panels for increasing the efficiency of mass microalgal production

1.1 Abstract

Raceway open ponds are preferred cultivation system for mass algal commodity production. For operational reasons, large-scale raceway ponds must be operated at a depth greater than 20 cm meaning that algal cultures are normally light limited as light cannot penetrate into the depth below 5 cm. For the efficient distribution of light into the culture, different light delivery systems such as temporal and spatial have been proposed. If the proper mixing created, the flashing light effect can be created and that would result in a significant increase in biomass productivity. However, to date, this method has not been achieved in outdoor raceway open ponds. On the other hand, spatial light dilution systems are found to be more effective and economical than temporal light dilution systems. Among spatial dilution systems, luminescent solar concentrator (LSC) panels have a potential to be commercialized for mass microalgae production. Luminescent solar concentrators combine spectrum shifting properties with spatial dilution to channel the light into the culture where it is needed. There is also the possibility of electricity production as well as higher algal biomass production when using LSC panels in open ponds or PBRs. Additionally, compared to other proposed methods, the lower capital cost can be expected when using LSCs in algal cultivation systems as there is no need to use a solar tracking system to track the sun. In this review article, the effects of photolimitation, photosaturation and, photoinhibition in concentrated microalgal cultures, as well as the impact of applying different light distribution systems on the biomass productivity and photosynthetic

efficiency as a result of having more uniform distribution of light into the culture, have been outlined.

1.2 Introduction

Since 1965, microalgae have been grown commercially in various fields such as high value products (e.g., β -carotene and astaxanthin), human and animal nutrition, pharmacy and cosmetics (Doucha & Lívanský, 2014; Masojídek et al., 2013; Raesossadati et al., 2014). Further, microalgae have the potential to be commercialized for commodity products such as biofuel and food (Becker, 2007), as well as a tool for carbon dioxide bioremediation (Raesossadati et al., 2015).

There are two main proposed microalgae cultivation systems, raceway open ponds and closed photobioreactors. To date, paddle wheel driven raceway ponds are found to be the most cost-effective cultivation systems, especially for large scale mass cultivation of commodity products (Costa & de Morais, 2013). Achieving higher yields per illuminated surface area and culture volume as well as shorter specific growth rates are primary goals in microalgal cultivation (Borowitzka & Moheimani, 2013). Large scale open ponds must be operated in depth of 20–30 cm, however, there is more availability of light into the depth of shallower ponds (Murphy et al., 2015). Solar energy plays a significant role in the growth and productivity of microalgae (Grobbelaar, 2007). In any cultivation system, culture productivity depends heavily on capturing light energy efficiently while the growth of microalgae is usually saturated at an irradiance of around $200 \mu\text{mol m}^{-2} \text{s}^{-1}$, which is about 1/10 of the maximum irradiance of a summer day (Vadiveloo et al., 2015). The main aim of any algal grower is to achieve maximum yield of targeted product at the shortest doubling time resulting in the highest productivity (Benemann, 2008). Considering that one would have to operate the culture at specific depth (Borowitzka, 1999) and biomass concentrations

are normally set at the highest achievable yield (Chisti, 2016), there is a very limited control on light availability to the cell in open ponds. Thus, using a light delivering system for algal cultivation systems with poor light availability to algal cells such as raceway open ponds is demanding.

There have been several systems for increasing light irradiance inside the microalgae cultures such as temporal light dilution (Abu-Ghosh et al., 2016), Fresnel lenses (Zijffers et al., 2008b), optical fibers (Xue et al., 2013) and, luminescent solar concentrators (Mohsenpour & Willoughby, 2013). These systems are discussed in detail in the following sections. The overarching goal of this review is to evaluate and compare various light distribution designs for photobioreactors and open ponds aiming to deliver incident light to microalgal cells more efficiently. The main target is to improve photosynthetic efficiency resulting in an increase of microalgal productivity. In addition, the effects of photolimitation, photosaturation and, photoinhibition in concentrated microalgal cultures are discussed.

1.3 Microalgae, Light and, Photosynthesis

Sun supplies an enormous amount of energy to the Earth with radiant power of 3.846×10^{26} W. The visible spectrum (390–750 nm), the infrared (IR) (0.7–300 μ m) and, ultraviolet (UV) radiation (10–390 nm) account for 52%, 42% and, 6% of solar energy (Ringsmuth et al., 2016). Photosynthetic active radiation (PAR), 400–700 nm, is the visible portion of light delivering around 3.9×10^6 EJ each year to the Earth (Crabtree & Lewis, 2007) which can be absorbed by photosynthetic pigments (Vadiveloo et al., 2016). The PAR contains 43% of the total solar energy (AM1.5) and mainly includes the visible spectrum (Ringsmuth et al., 2016). The Earth is covered by green plants and oceans containing photosynthetic organisms which transfer light energy into chemical energy via photosynthesis. However, the overall photosynthesis

conversion efficiency, the ability to convert light energy into biomass, is very low (1–2%) to make up the human demand for energy. It is to be noted that the maximum theoretical PE is 8–12% (Crabtree & Lewis, 2007).

In the process of photosynthesis, photosynthetic pigments are responsible for capturing light and using the absorbed energy to generate NADPH and ATP and convert CO₂ and water to carbohydrate (Razeghifard, 2013). Also, producing one mole of carbohydrate (CH₂O) and one mole O₂ requires 8 moles of light photons in the photosynthesis process (Walker, 2009). Thus, the maximum (theoretical) quantum yield can be the fixation of 0.125 mol CO₂ (or oxygen evolution) per mole photon absorbed (Tredici & Zlttelli, 1998). Considering that one mole of photons in the PAR region has the averaged energy content of 217 kJ, producing one mole of CH₂O requires the potential captured light energy of 1744 kJ. Knowing the fact that the energy contained in one mole of CH₂O is about 467 kJ and, 46 kJ mole⁻¹ PAR photons is the amount of energy lost as a result of PAR degradation to excitation energy at 700 nm (21% of absorbed PAR), the maximum theoretical photosynthetic solar energy conversion can be 12% (Tredici, 2010). Nevertheless, the maximum achieved photosynthetic efficiency of 3% has been reported for some microalgae species (Larkum, 2010). Such a low efficiency is due to loss of photons by reflection, respiration, photosaturation and, photoinhibition (Tredici, 2010).

Three major pigment groups present in microalgae are chlorophylls, carotenoids and phycobilins with chlorophyll *a* present in all species (Torzillo & Vonshak, 2013). These pigments are responsible for absorbing light in different parts of PAR. Chlorophylls absorb blue light (450–475 nm) and red light (630–680 nm) (Torzillo & Vonshak, 2013) and carotenoids (e.g., α - and β -carotenes, xanthophylls, lutein, and fucoxanthin) absorb light between 400 and 550 nm spectra (Moheimani & Parlevliet,

2013). On the other hand, phycobilins absorption is mainly between 500 and 650 nm (Gutierrez-Wing et al., 2014).

The quantum rate captured from the light source, which affects the rate of microalgal photosynthesis, is determined by light absorption properties of microalgae, as well as light quality and quantity (Walker 2009). The efficiency of photosynthesis is microalgal species specific. Photosynthetic biomass productivity is also a function of photosynthetic efficiency (Moheimani & Parlevliet, 2013). The photosynthetic rate is proportional to the captured photon rate and the efficiency of photosynthetic reactions to convert the absorbed light into the chemical energy. The photosynthesis can be photolimited, photosaturated or photoinhibited region (Tredici, 2010).

In well-mixed concentrated microalgal cultures, there is a complicated light field to which microalgae cells are exposed. In that light regime, light is declining exponentially from full sunlight at the surface to darkness at the depth according to the Lambert–Beer law (Brindley et al., 2016). In a concentrated microalgal culture, light can be categorized into four main zones (Figure 1-1) (Tredici, 2010):

- a) Photoinhibited region where the amount of light received at the surface is far greater than light saturation (I_s) resulting in photoinhibition;
- b) In the light saturated zone where the maximum photosynthetic rate (P_{max}) is achieved and irradiance is at I_s ;
- c) In the light limited zone where light is below I_s but above compensation light (I_c). In this condition, maximum light efficiency is achieved;
- d) In the dark zone where, net positive photosynthesis does not occur as irradiance is below I_c .

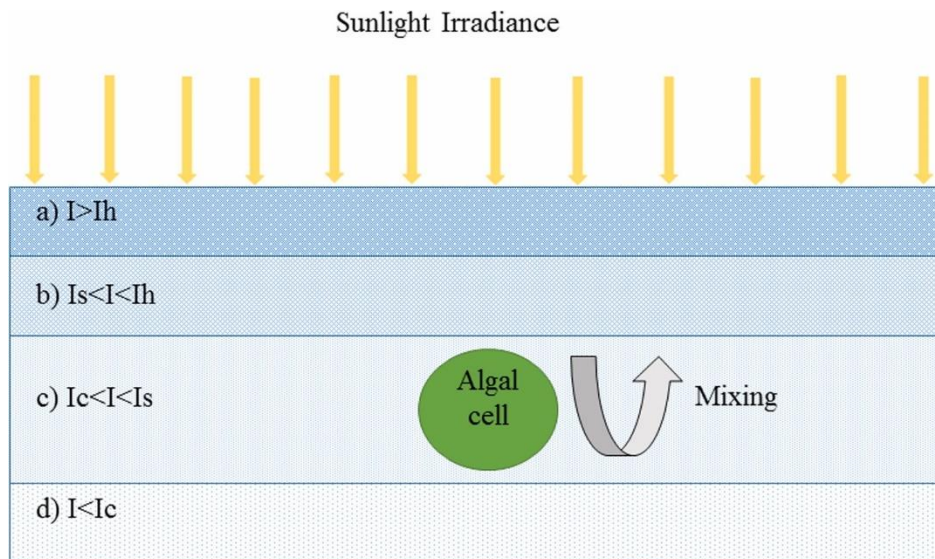


Figure 1-1. Light zones in high concentrated algal culture. a) The zone where photoinhibition occurs, b) The light saturated zone where the maximum photosynthetic rate (P_{max}) is achieved, c) The light limited zone where irradiance is lower than saturation point and c) The dark zone where photosynthesis does not occur (Tredici et. al., 2010). An algal cell shown in the figure above can be mixed across all zones.

It is also noteworthy to mention that penetration of light varies with wavelength. For instance, green light penetrates into an algal culture 20-times more than blue and red light which are more important for photosynthesis than the green light (Figure 1-2) (Zittelli et al., 2013). Figure 1-2 shows three wavelength region (a) the blue region in which 440 nm is absorbed by chlorophylls and carotenoids; (b) the green region, which there is poor absorption by chlorophyll and carotenoids; and, (c) the red light region, which represents chlorophyll absorption at 678 nm (Richmond & Cheng-Wu, 2001). Obviously, penetration of green light is much deeper (20 times) than blue and red light. However, the green light is poorly absorbed by microalgae cells (Figure 1-2). Therefore, green light can play a significant role in concentrated algal cultures where there is not enough light available for cells and thereby, increasing the photic volume in the reactor (Richmond & Cheng-Wu, 2001).

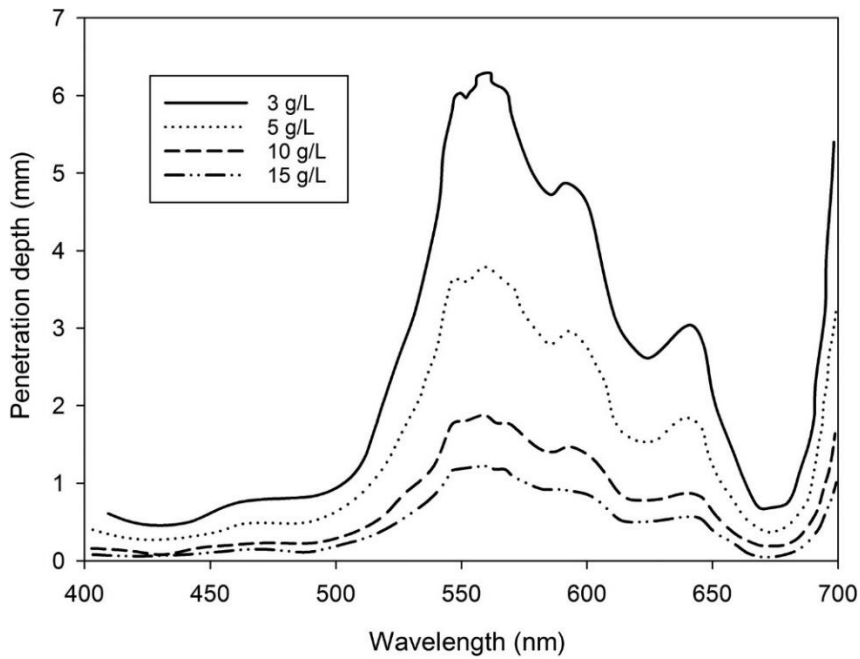


Figure 1-2. Penetration depth* spectra in *Nannochloropsis* sp. as a function of cell density in a 200 L flat plate glass photobioreactor, with a 10 cm light-path. *Light penetration depth was calculated from the attenuation coefficient of down-welling irradiance which is defined as the depth in which down-welling irradiance decreased tenfold. Reproduced from (Richmond & Cheng-Wu, 2001).

1.3.1 Photolimitation

Considering that light is strongly attenuated in concentrated microalgal cultures, its availability is not solely determined by incident radiation (I_0) on the reactor surface (Fernández Sevilla et al., 1998). Photolimitation stems from inadequate irradiance and, thus, microalgal cells will not receive enough irradiance resulting in low areal algal biomass productivity, especially in open ponds. Photolimitation can be reduced by increasing the input irradiance and decreasing the culture depth (Torzillo & Vonshak, 2013). For instance, Moheimani and Borowitzka (2007) showed that by reducing open pond depth from 21 to 13 cm in autumn, *Pleurochrysis carterae* productivity could be increased over fivefold from $0.012 \text{ g l}^{-1} \text{ d}^{-1}$ to $0.069 \text{ g l}^{-1} \text{ d}^{-1}$.

In the region where light is limited, photosynthesis is linearly proportional to irradiance and, the maximum photosynthesis rate could be achieved in this region (MacIntyre et al., 2002). The maximum efficiency of light conversion into biomass is determined in the initial part of the PI curve (α) (Figure 1-3). The maximum quantum yield of photosynthesis is also determined by the ratio between photosynthesis and irradiance in this region of the PI curve (Ralph & Gademann, 2005). If α is measured in a very concentrated culture (all light is absorbed), it can be considered as the measured absorbed light and thus, the maximum quantum yield of photosynthesis (Tredici, 2010).

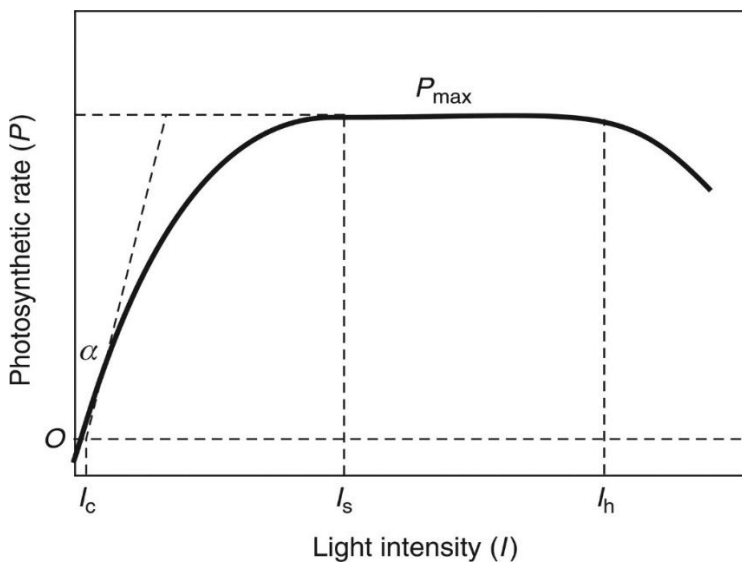


Figure 1-3. PI curve that is the response of light to photosynthesis. The maximum light utilization efficiency is shown as α which is the initial slope of the PI-curve. I_c , light compensation point; I_s , light saturation intensity; I_h , the light intensity at which photoinhibition occurs. (Copied from Richmond (2013) with permission).

1.3.2 Photosaturation

Photosaturation of microalgal cells occurs when light irradiance increases and microalgal cells cannot absorb the excess of photons which leads to no increase in

photosynthesis. At light-saturated region, the number of photons absorbed by chlorophyll is higher than the number of electrons transferred from water to CO₂ and, consequently, the photosynthetic rate is limited. Thus, the rate of light conversion efficiency into chemical energy declines at the end of the linear region ending up to the light saturated region of the PI-curve (Figure 1-3) (Tredici, 2010). There is a point (Ik), interception of α and Pmax, where irradiance is saturating and photosynthesis is light saturated indicating the photoacclimation status (Figure 1-3) (Masojídek et al., 2013).

The maximum photosynthetic efficiency is determined by photosaturation or light saturation effect (LSE) in outdoor concentrated microalgal cultures. The LSE can be represented by the ‘Bush equation’ (Goldman, 1979):

$$E_s = \frac{I_s}{I_0} \left[\left(\ln \frac{I_0}{I_s} \right) + 1 \right] \quad \text{Equation 1-1}$$

where E_s is the light utilization efficiency, I_s is the light saturated point and, I_0 is the incident irradiance. The ‘light utilization efficiency’ is based on the amount of light utilized by the microalgal cells and the total irradiance (Figure 1-4) (Goldman, 1979). High E_s can be potentially attained at low irradiances, but at high I_0/I_s ratios, E_s declines rapidly (Figure 1-4). Thus, the value I_0 is the main factor for determining the E_s in an outdoor algal culture. For example, at I_0/I_s of 20, E_s is approximately 0.2 and, thus, light utilization efficiency is about 20%. It can be simply concluded that a lower ratio of I_0/I_s is desirable to have higher E_s . I_s is crucially important to determine the productivity of outdoor algal cultures and it is highly advantageous to grow microalgal

species with high I_s values (Goldman, 1979). Nonetheless, the saturation irradiance of the most marine algae is below $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (~5% full sunlight) (Tredici, 2010).

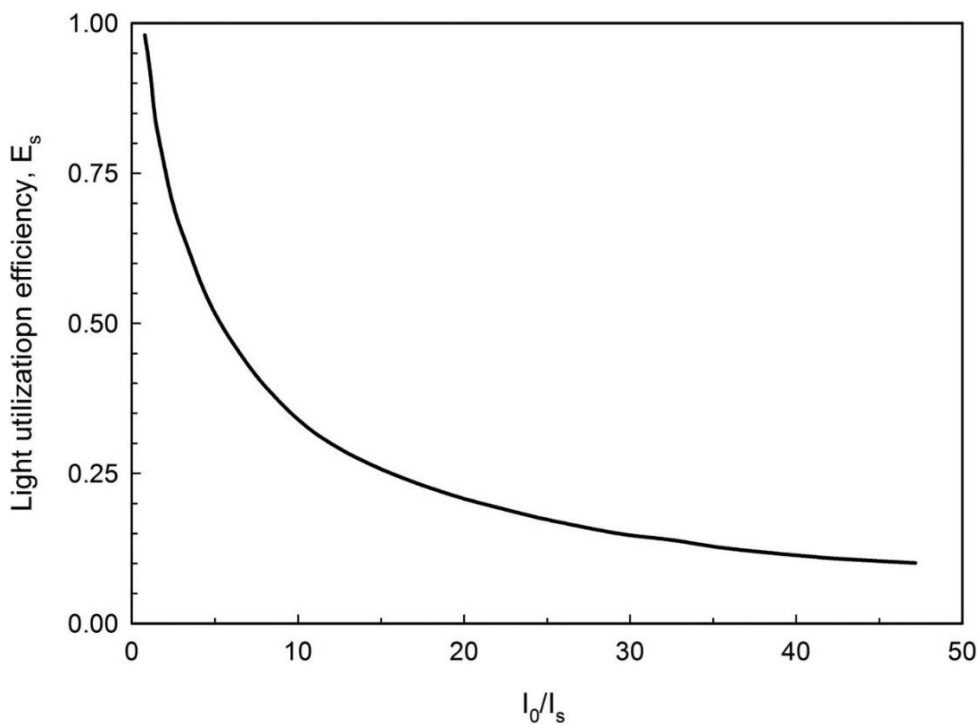


Figure 1-4. Light utilization efficiency (E_s) based on Bush equation (Equation 1-1) in a dense microalgae culture. Reproduced from (Goldman, 1979).

The light saturation effect would highly alleviate the photosynthetic efficiency of an outdoor mass culture of algae illuminated under full sunlight. Table 1-1. Minimum energy losses of total incident solar radiation in microalgae mass culture (Modified from (Tredici, 2010)). summarises the minimum energy losses of total sunlight irradiance in an outdoor microalgae culture from the beginning of receiving light by microalgae cells to carbohydrate formation. The actual photosynthetic efficiency of 7% of PAR has been reported at irradiance around half of the solar intensity (Zijffers et al., 2010); however, several microalgae species have shown the photosynthetic efficiencies of up to 24% of PAR (11% of total solar radiation) (Greenbaum, 1988).

Table 1-1. Minimum energy losses of total incident solar radiation in microalgae mass culture (Modified from (Tredici, 2010)).

Minimum energy losses	Energy remaining (%)
Total incident solar radiation	100
Radiation outside PAR (55%)	45
Degradation of absorbed PAR photons to excitation energy at 700nm (21%)	35.6
Conversion of excitation energy at 700nm to the chemical energy of glucose (65%)	12.4 (Maximum photosynthetic efficiency)
Reflection (10%)	11.2
Respiration (20%)	9
Photosaturation and photoinhibition (40%)	5.4

1.3.3 Photoinhibition

Photoinhibition (I_h) is defined as a decrease of photosynthesis at supra-saturating light intensity. It also results in declining maximum quantum yield of photosynthesis, light conversion efficiency and, the rate of photosynthesis mainly due to exposure of cells to high irradiance (Adir et al., 2003). Photosynthetic capacity is also reduced by photoinhibition due to damage caused by high irradiance (Parlevliet & Moheimani, 2014). In other words, photoinhibition occurs when the irradiance is higher than the light saturated irradiance and, then, photosynthesis is less than P_{max} (Moheimani & Borowitzka, 2006). Photoinhibition depends on both light intensity and duration of light exposure. In many microalgae species, irradiances in the range of 100–200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (approximately 10% of full sunlight) can cause photoinhibition (Tredici & Zltelli, 1998).

Photoinhibition is due to the inactivation of reaction centres and is one of the most important problems for achieving high photosynthetic efficiency (PE) in outdoor algal cultures (Tredici, 2010). Grobbelaar (2007) observed not only photoinhibition could reduce areal production rates by up to 30%, but also more than 60% of the reaction

centers could become inactive by photoinhibition in a low-density culture (Grobbelaar, 2007). Photoinhibition can be controlled by:

a) Increasing biomass concentration: Richmond (2000) showed that increasing biomass concentration in high density mass culture exposed to high light irradiance reduces photoinhibition which is due to increased self-shading.

b) Increasing the cycling between the light and dark zones by better mixing: Qiang and Richmond (1996) increased the rate of mixing of *Spirulina* culture in a 2.5L flat plate PBR from 0.6 vvm (L air per L culture per min) to 2.1 vvm and 4.2 vvm at a concentrated culture with biomass concentration of 5g l^{-1} . They found that biomass productivity increased from $55\text{ mg l}^{-1}\text{ h}^{-1}$ to $110\text{ mg l}^{-1}\text{ h}^{-1}$ at $500\ \mu\text{mol m}^{-2}\text{ s}^{-1}$. Moreover, for the highest photosynthetic flux density (PFD) used, i.e., $1800\ \mu\text{mol m}^{-2}\text{ s}^{-1}$, biomass productivity of cell mass obtained at this energy flux indicated a sensitive response to the rate of mixing; an increase in mixing rate from the minimal 0.6 to 4.2 vvm increased biomass productivity from $90\text{ mg l}^{-1}\text{ h}^{-1}$ to $400\text{ mg l}^{-1}\text{ h}^{-1}$ (Qiang & Richmond, 1996);

c) The use of intermittent light pulses: this method contains using a system to provide intermittent light irradiance. However, this approach can be useful for microalgae cultures with low cell densities where there is no mutual shading effect (Lunka & Bayless, 2013). This method is most likely not going to be useful for mass algal cultures where achieving high productivity is the main objective as mutual shading increases, and consequently, there is less availability of light to algal cells (Zou & Richmond, 2000).

d) The use of a continuous light source and moving the cells in and out in the illuminated region at a high frequency. By having high frequency, the illuminated cells

will be replaced by dark cells and more cells, specifically in a concentrated culture, are exposed to flashes of light per unit time (Richmond, 2013);

e) The use of microalgae species with a shorter antenna (de Mooij et al., 2014; Doucha & Lívanský, 2009; Doucha et al., 2005). The photon absorption in a microalgae photosynthetic system with less light harvesting chlorophyll is fewer at a high light intensity, and thus, photon waste is also fewer (Beardall & Raven, 2013; Benemann, 2004) and;

f) The use of filters to remove unnecessary light wavelengths and pick specific useful wavelength for microalgae, thus reducing the total light irradiance (Gutierrez-Wing et al., 2014). Vadiveloo et al. (2015) investigated the effect of spectrally limited light on the growth and photosynthesis rate *Nannochloropsis sp.* using filters on top of the microalgae cultures. They found the highest specific growth rate of 0.30 d^{-1} under pink light and the highest biomass productivity of $1.93 \text{ mgL}^{-1}\text{d}^{-1}$ ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)⁻¹ under blue light for *Nannochloropsis sp.* (Vadiveloo et al., 2015). The advantage of this system on microalgae culture was to select the particular wavelength to increase the biomass productivity as well as the potential ability to use the remainder wavelength for electricity production.

1.3.4 Photoacclimation

Photoacclimation is a physiological response of phototrophic microalgae to changes in light intensity which happens in relatively short periods of time (Vonshak & Torzillo, 2007; Zou & Richmond, 2000). In mass microalgal cultures, acclimation of microalgal cells to high light depends on biomass yield, depth of the culture and, mixing rate (Torzillo et al., 2012). The main problem in concentrated cultures is that cells do not receive enough light most of the time during the growth period and

consequently, a very large antenna will be assembled due to low light acclimation. This is due to either producing photosynthetic unit (PSU) size in a larger size or higher number within the cell (Masojídek et al., 2013). This results in a significant attenuation of light into the depth of the culture in which there is a very complex irradiance regime due to different culture depth, cell concentration and, mixing rate (Richmond et al., 2003). During photoacclimation, the quantum efficiency increases when irradiance decreases, but I_k and P_{max} decline (Figure 1-5). This leads to a lower capacity to use high irradiances efficiently. The microalgal cells adapted to low light due to self-shading-effect, absorb photons in large excess when they are in the irradiated layers, and then, there is a three possible consequences: a) they cannot use the excess of light efficiently and waste it as they are photosaturated; b) they may be photoinhibited ; and c) they do not allow light to penetrate to the cells at the depth due to the shading effect (Tredici, 2010). This is the reason that productivity increases minimally while irradiance increased significantly even for algal cultures operated at optimum conditions. Interestingly, high-light adapted microalgae cells can re-adapt to low light condition quickly (Zittelli et al., 2013).

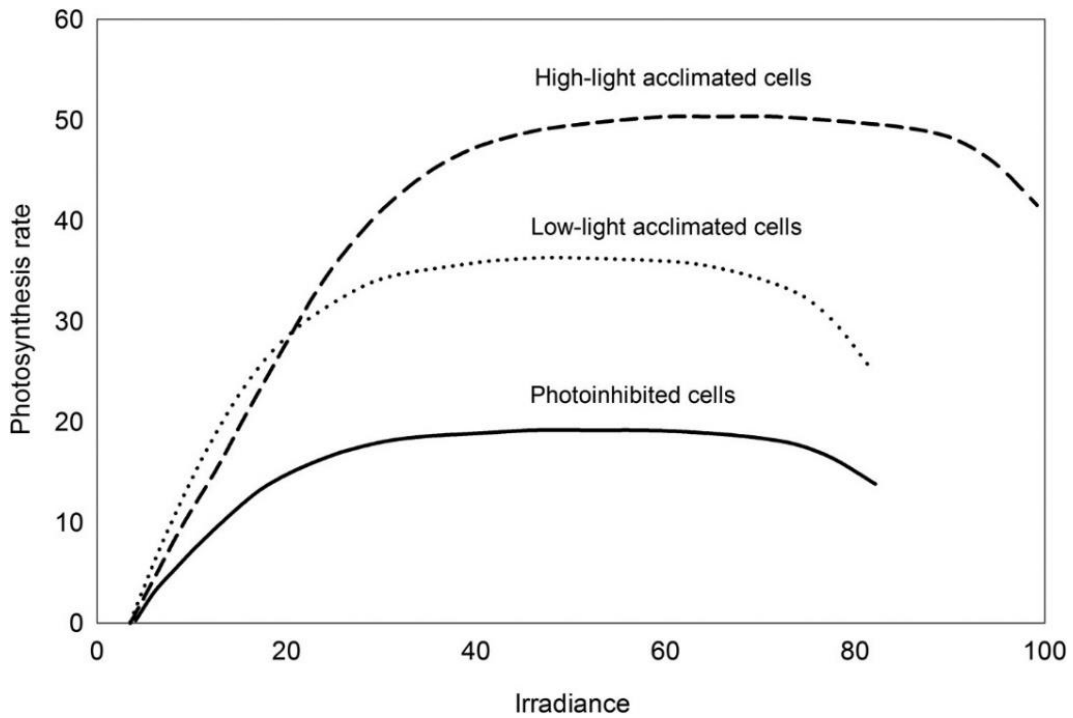


Figure 1-5. The effect of photoinhibition and photoacclimation to low light in dense algal cultures on the light-response curve of photosynthesis. Reproduced from (Tredici, 2010).

Torzillo et al. (2012) carried out an outdoor experiment on the mass culture of *Phaeodactylum tricornutum* grown at a closed tubular photobioreactor at two biomass concentrations (0.3 and 0.6 g l⁻¹) to study the photoacclimation of *P. tricornutum*. The highest stress occurred for cultures grown at 0.3 g l⁻¹. As a result of that, photosynthesis parameters and chlorophyll fluorescence were changed dramatically, and areal productivity also decreased significantly while more concentrated cultures (0.6 g l⁻¹) did not show considerable changes in the photosynthetic parameters. They concluded that high-irradiance stress affected the diadinoxanthin cycle negatively and increased non-photochemical quenching, which lowered biomass productivity in the less concentrated culture (Torzillo et al., 2012).

1.4 Light and microalgae growth

1.4.1 Microalgal irradiance-growth model

In recent years, several microalgal irradiance-growth and productivity models have been developed (Béchet et al., 2013; Darvehei et al., 2018). The light availability of microalgal cells inside a culture depth determines the productivity. The PAR irradiance inside a microalgae culture at a depth of z (m) from the culture surface can be estimated

$$\text{by } I = I_0 \exp(-\varepsilon X z) \quad \text{Equation 1-2}$$

where: I_0 (W m^{-2}) is PAR irradiance, ε ($\text{m}^2\text{gdw}^{-1}$) is the extinction coefficient, X (gm^{-3}) is the biomass concentration (Doucha & Lívanský, 2014).

The average light irradiance inside a microalgae culture with a depth of h can be

$$\text{summarized in } I_{mean} = \frac{1}{h} \int_0^h I dz = \frac{I_0 - I_h}{\varepsilon_{mean} X h} \quad \text{Equation 1-3}$$

where $I_h = I_0 \exp(-\varepsilon_{mean} X h)$ is the amount of light that is not absorbed in the culture depth, and ε_{mean} is the mean extinction coefficient (Doucha & Lívanský, 2009). Doucha and Lívanský (2014) used Equation 1-3 to measure the relationship between I_h/I_0 inside *Chlorella* sp. culture at different cell concentrations. The following correlation was also found by Doucha and Lívanský (2009) for *Chlorella* sp. culture: $\varepsilon_{mean} = \varepsilon_0 (1 - a_1 h/2) (1 - a_2 X)$, with values of empirical coefficients: ε_0 ($\text{m}^2 \text{g dw}^{-1}$) = 0.175; $a_1 = 46.165$; $a_2 = 9.664 \cdot 10^{-6}$. They showed that increasing cell concentration of *Chlorella* sp. leads to decreasing the mean light intensity inside the culture depth (Figure 1-6) (Doucha & Lívanský, 2014). It was also shown that *Chlorella* sp. cells absorbed almost all of light incident in the top 6 mm of pond depth when grown at 5 g L^{-1} yield of the culture (Doucha & Lívanský, 2014).

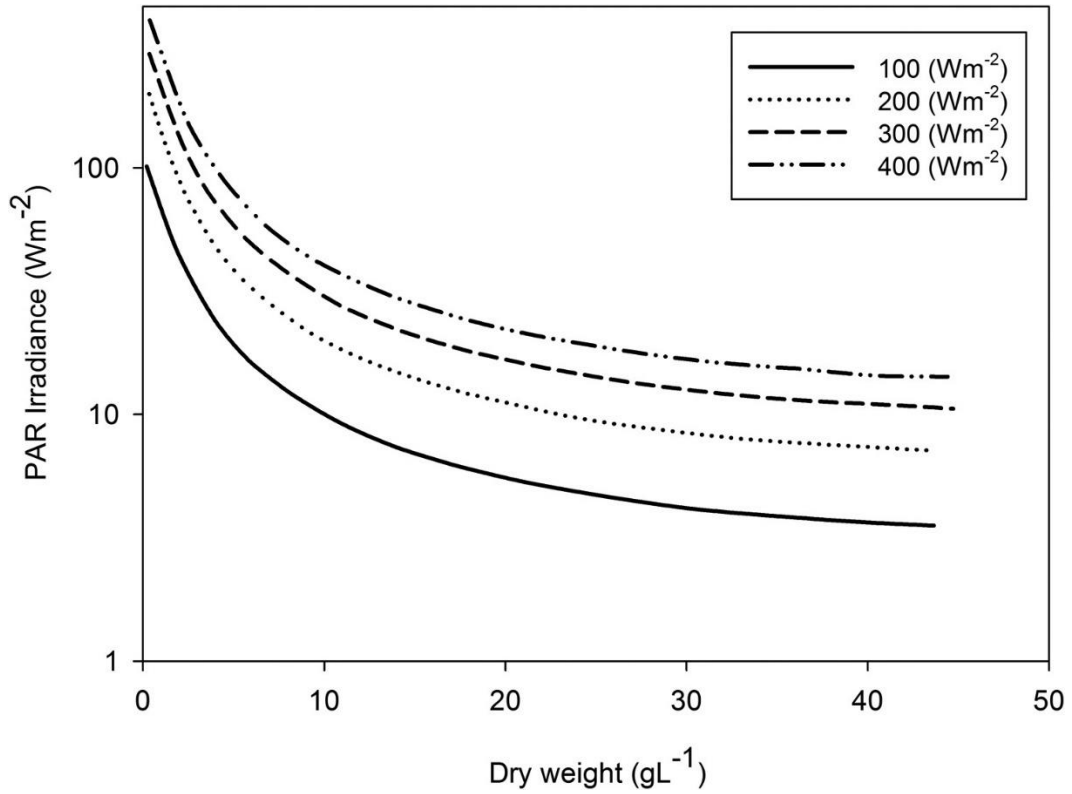


Figure 1-6. Dependence of the mean light intensity inside of an 8 mm thick culture layer on *Chlorella* sp. dry weight. Reproduced from (Doucha & Lívanský, 2014).

The biomass production efficiency of microalgae regrading using light energy can be expressed according to Equation 1-4

$$Y_{dw,E} = \frac{P_{dw}}{PFD_d} \times \frac{V}{A} \quad \text{Equation 1-4}$$

where $Y_{dw,E}$ (g (mol photon)⁻¹) is the biomass yield per light energy, P_{dw} (g m⁻³ d⁻¹) is microalgal volumetric productivity of, PFD_d (mol photon m⁻² d⁻¹) is the total photon flux density, and V/A (m³ m⁻²) is the volume to surface ratio of the microalgae culture.

The photosynthetic efficiency of a microalgae culture (%) can also be calculated using

$$PE = Y_{dw,E} \times \frac{C_B}{E} \times 100\% \quad \text{Equation 1-5}$$

where PE (%) is the photosynthetic efficiency, C_B (kJ g^{-1}) is the microalgal calorific content, and E ($\text{kJ (mol photon)}^{-1}$) is the energy input from the conversion of irradiance (Gutierrez-Wing et al., 2014).

Equation 1-4 and 1-5 show the dependency of photosynthetic efficiency and biomass productivity on light conversion efficiency. They also indicate that higher light conversion efficiency leads to higher biomass productivity and yield. Furthermore, the relationship of light irradiance and microalgal specific growth rate can be described by the Steele's kinetics model shown in the following equation (Benson et al., 2007; Engqvist & Sjöberg, 1980).

$$\mu = \mu_{max} \frac{I_a}{I_{opt}} e^{1 - \frac{I_a}{I_{opt}}} \quad \text{Equation 1-6}$$

that μ (d^{-1}) is the specific growth rate, I_a ($\mu\text{mol m}^{-2} \text{s}^{-1}$) is the mean irradiance, I_{opt} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) is the optimum irradiance which results in achieving μ_{max} . In this model, the specific growth rate declines when irradiance is increased to a value higher than the optimum irradiance (Figure 1-7). The model is appropriate for microalgal cultures with medium density (Gutierrez-Wing et al., 2014). The optimum irradiance is dependent on species and strain cultivated. For example, *Selenastrum minutum* have the optimum irradiance of $365 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Bouterfas et al., 2006), *Selenastrum capricornutum* at $391 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Benson & Rusch, 2006), *Spirulina platensis* at $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Qiang & Richmond, 1996), and *Chlorella sp.* at $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Kumar et al., 2010).

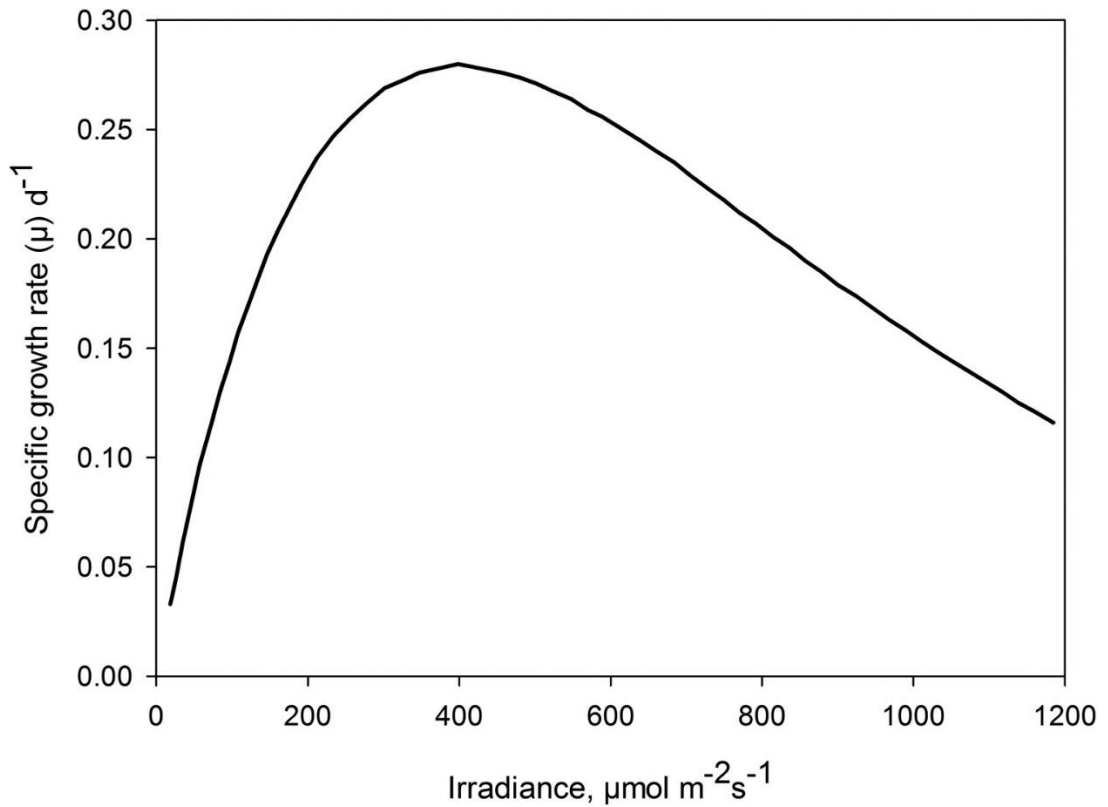


Figure 1-7. Curves fitted to experimental specific growth rate versus irradiance for a *Chlorella vulgaris/Leptolyngbya* sp. co-culture under Steele kinetics. Reproduced from Gutierrez-Wing et. al., (2014).

Many models have been developed for light scattering in a high density microalgal culture, but the most common model for the light attenuation in depth of a concentrated culture is mainly based on the Lambert-Beer law (Benson et al., 2007). Light availability to cells reduces in the first couple of centimetres in a concentrated algal culture. In PBRs, there is more homogenous light availability to microalgal cells but photoinhibition is the side effect. The average irradiance in the reactor can be obtained by the following equation:

$$I_a = \frac{1}{d} \int_0^d I(z) dz = \frac{I_0(1 - e^{-k_0 d})}{k_0 d} \quad \text{Equation 1-7}$$

where: I_a is the average light irradiance received by microalgal cells, d is the reactor depth, z is the aiming depth at which irradiance is calculated, I_0 is the irradiance at the

culture surface, and k_0 is the attenuation coefficient for overall coefficient (from water and biomass):

$$k_0 = k_w + k_b X \quad \text{Equation 1-8}$$

where: k_w and k_b are the attenuation coefficient for water and biomass respectively, and X is the biomass concentration (gm^{-3}).

Air, water and the density of culture attenuate the amount of irradiance received by microalgae cells. Microalgae cells can be either photo-limited or photo-inhibited in a culture with no mixing. On the other hand, when there is an appropriate mixing system in culture, microalgae cells are exposed to a cycle of high and low light irradiance and therefore receive similar average irradiance within the cultivation system.

The more homogeneous light distribution can be found in a cultivation system with a shorter light path. However, they are more prone to photoinhibition. On the other hand, the light irradiance regime is more complicated in different parts of the depth but, it is less prone to photoinhibition (Gutierrez-Wing et al., 2014).

1.4.2 Light and microalgae cultivation systems

Highest areal productivity is the objective of mass microalgal cultivation. Several obstacles and limitations (e.g., mixing, cooling, environmental conditions, etc.) prevent the industrial exploitation of microalgae for mass production of commodity products such as feed, food, and biofuel (Zittelli et al., 2013). Algae must be grown in a container/cultivation centre. Open ponds and closed photobioreactors are two types of cultivation systems, both having advantages and disadvantages. In here, the relative pros and cons of each system when it comes to light and biomass productivity have been addressed. Readers can refer to (Borowitzka, 1999; Moheimani et al., 2015; Zittelli et al., 2013) for more detailed reviews on algal cultivation systems.

1.4.2.1 Closed photobioreactors

There are numerous design of closed PBRs including stirred tank (Ye et al., 2016), vertical tubular (Ashokkumar et al., 2015), bubble column (Khoo et al., 2016), airlift (Jeffryes et al., 2016), horizontal tubular (Valiorgue et al., 2014) and, flat panel (Sun et al., 2016). Reducing the costs of biomass production is the main goal of any PBR (Borowitzka, 1999). To achieve that, favouring a sufficient amount of light to the PBR is critical (Gupta et al., 2015). There are some benchmarks by which a good PBR can be described; a) using light irradiance efficiently; b) having a uniform illumination and reducing mutual shading and c) providing a fast mass transfer of fertilizers, CO₂ and, O₂ (Moheimani et al., 2015). Hence, understanding the effects of environmental parameters such as light on the biomass production within the PBR is required to design an efficient PBR (Chiang et al., 2016; Gupta et al., 2015).

The amount of light irradiance in a PBR decreases with increasing culture density. One of the typical solutions for that is to use high light intensity at the PBR surface which leads to photoinhibition. Besides, there is a sharp attenuation of light inside the culture along the light path causing photolimitation. Having a reactor with a high surface to volume (S/V) ratio, therefore, is beneficial to distribute the light more uniformly in the reactor (Jain et al., 2015). As a result, there is a more uniform distribution of light into the reactor, more productivity, and more photosynthetic efficiency (Brindley et al., 2011; Richmond et al., 2003). Jain et al. (2015) designed a PBR with integrated waveguides to deliver light evenly across the reactor. The highest volumetric and areal production rate of 22 mg l⁻¹ d⁻¹ and 2.55 g m⁻² d⁻¹ were attained, respectively at the intensity of 86 μmol m⁻² s⁻¹ (Jain et al., 2015). This productivity was two to four times higher than what previously obtained in conventional flat-plate PBR with the light path of 3 cm (Jung et al., 2014).

Although different closed PBRs have been widely used for microalgae growth and have several advantages such as better control on growth conditions, less contamination to the culture, more light availability for microalgal cells and better mixing rates, there are some significant drawbacks that make PBRs economically and environmentally unfeasible for low cost by-product (Gupta et al., 2015). The operational cost of PBRs (Tredici, 2010) and maintenance issues such as cleaning and sterilization (Borowitzka, 1999), as well as scaling up difficulties (Moheimani et al., 2015) are restricting the commercialization of PBRs. Most importantly, the amount of energy that is required for suitable mixing and thus, efficient mass transfer in PBRs such as air-bubbled is more than 100 W m^{-3} (approximately $2000 \text{ MJ ha}^{-1} \text{ day}^{-1}$) which equals to 50% of the biomass energy content (Moheimani et al., 2015).

1.4.2.2 Open ponds

Open ponds offer a straightforward and profitable approach. Large shallow ponds, circular ponds, tanks, and raceway ponds are the most commonly used open pond systems (Borowitzka & Moheimani, 2013; Gupta et al., 2015). Raceway ponds are efficient and inexpensive and have been used in the production of algae commercially (Borowitzka, 1999). Open raceway ponds have been the most common reactors for commercial microalgal production in the last 60 years (Craggs et al., 2011). A raceway pond has a closed-loop shape with 25-30cm depth and the surface to volume ratio of up to 10 m^{-1} . This is one of the main disadvantages of open ponds compared with the surface to volume ratio of closed photobioreactors (up to 50 m^{-1} for flat plate PBRs) (Jacobi & Posten, 2013). The S/V ratio can be increased by decreasing the depth which will improve light penetration but having a large scale raceway pond with the depth of less than 25cm is not feasible (Borowitzka & Moheimani, 2013; Chiaramonti et al.,

2013). Although easy construction and operation are the main advantages of open ponds compared to closed PBRs, the major constraint is poor light utilization by the cells (Borowitzka, 1996; Chisti, 2007). Additionally, lower biomass productivity and light dilution to the cells stem from insufficient mixing (Cuello et al., 2015).

The light absorption by microalgal cells is affected by various factors such as the cell position, density of the culture and, pigmentation of the cells (Moheimani & Parlevliet, 2013; Richmond et al., 2003). The irradiance (I_L), at depth (L) of the culture, can be estimated by Equation 1-9 (Chisti, 2016):

$$I_L = I_0 e^{-K_a C_x L} \quad \text{Equation 1-9}$$

where K_a ($\mu\text{E m}^{-2} \text{s}^{-1}$) is the light absorption coefficient which is alga-dependent (can be calculated based on the light-depth profile of an alga at specific cell concentration) and C_x is the biomass concentration. The equation shows that there is a rapid decline in irradiance with increasing depth and biomass concentration as expected (Chisti, 2016). However, to define the precise culture performance of an open pond, the relationship between light received by algal cells and photosynthesis of the culture needs to be understood. For example, light can only penetrate in 5cm of an algae culture with the density of 0.45 g/L leaving most of the cultures in complete darkness (Ono & Cuello, 2004).

Various systems have been introduced to overcome the undesirable effects of poor utilization of light or excess of light irradiance in outdoor algal cultures by using of light distribution systems to increase biomass productivity and photosynthetic efficiency (Doucha & Lívanský, 2014) which are discussed in the following sections in details.

1.5 Light distribution systems

1.5.1 Temporal light dilution (Flashing light effect)

Temporal dilution is based on turbulent mixing which results in light/dark frequency and dilution of photosynthetic photon flux density (PPFD) over time. In this phenomenon, microalgal cells are exposed to high light intensity in a short period followed by a longer period in the dark, therefore, decreasing the average intensity below the saturation point. (Laws et al., 1983). For the first time, Kok (1953) applied rapid mixing method for algae cultures (Kok, 1953). He observed that when algal cells are provided by high intensity millisecond flashes followed by a long dark period, the energy conversion efficiency is significantly high (Kok, 1953). This is because only one photon is captured by a photosynthetic unit in a flash of high intensity up to I_{solar} . Thus, the time-averaged light intensity is below I_{sat} (Dye, 2010). It has been widely argued and investigated that flashing light can effectively increase algal biomass production by a factor of three (Abu-Ghosh et al., 2015a; Abu-Ghosh et al., 2015b; Combe et al., 2015; Grobbelaar, 1991; Grobbelaar, 1994; Iluz et al., 2012; Stuart & Hincapie, 2015; Yang et al., 2014; Zhang et al., 2015). Optimal flashing light conditions can result in enhancing algal productivity parameters. Moreover, the advantage of using a flashing light system is to have a shorter cooling period over continuous light which will reduce electrical energy consumption and costs (Abu-Ghosh et al., 2016).

The flashing light is characterized by three main parameters which are the intensity and frequency of light and the light/dark cycle (Nedbal et al., 1996). Consequently, the cycles of mixing can be significantly different and change by order of magnitudes between a millisecond to longer times. Laws et al. (1983) designed arrays of foils in

48 m² algal culture flume with 4150 L working volume to create systematic mixing. Flowing of water over and under the foils created a pressure differential and thus vortices. Vortices with rotation rates of 0.5-1.0 Hz were produced in a flume having a flow rate of 30 cm/s resulted in an increase in the solar energy conversion efficiencies in the culture of *P. tricornutum* by 2.2-2.4 fold and averaged 3.7% over a three-month period (Table 1-2) (Laws et al., 1983). Besides, Zhang et al. (2015) designed a novel raceway pond with a working volume of 412 L equipped with flow deflectors and wing baffles to enhance the effect of flashing light and reduce the dead zone. They found that the pressure loss lowered by 14.58%, fluid velocity increased by 26.89% and dead zone decreased by 60.42%. Moreover, the average L/D cycle also shortened from 14.05 s to 4.42 s, and significant swirling flow was produced. They proved that *Chlorella* sp. had 30.11% more biomass productivity when cultured in a raceway open pond with wing baffles compared to the control pond in outdoor cultivation (Table 1-2) (Zhang et al., 2015). Lunka and Bayless (2013) also used flashing light on *Scenedesmus dimorphus* culture in a thin flat-plate bioreactor. A constant photon flux of 75 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and three flashing light intensities of 375, 275, and 175 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ were used. They found that the lowest energy consumption (9.6 % less power) and the highest biomass productivity (2.86 times higher productivity) were achieved when the photon flux of 375 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ was used (Table 1-2) (Lunka & Bayless, 2013).

Table 1-2. Summary of different temporal and spatial light dilution systems used for microalgae cultivation systems.

Light Dilution system		Reactor	Volume (L)	Species	Produced Frequency	solar energy conversion efficiency enhancement	Biomass enhancement	Photosynthetic efficiency enhancement	Ref
Temporal	Pressure differential	Algal culture flume	4150L (48m ²)	<i>P. tricornutum</i>	0.5-1.0Hz	2.2-2.4 fold (3.7%)	-	-	(Laws et al., 1983)
	Flow deflectors and wing baffles	Open pond	412L	<i>Chlorella sp</i>	Shortened L/D cycle period from 14.05 to 4.2s	-	30.11% higher productivity	-	(Zhang et al., 2015)
	flashing light	Flat plate	-	<i>Scenedesmus dimorphus</i>	10Hz	9.6%	2.86 times higher productivity	-	(Lunka & Bayless, 2013)
	Cones	Open pond	2000L	<i>Chlorella</i>	-	-	27g/l to 38g/l	-	(Mayer et al., 1964)

Fresnel lenses	Spatially diluted PBR	18L	<i>Neochloris oleoabundans</i>	-	-	2.5 times higher productivity	-	(Dye et al., 2011)
optical fiber	Bubble column	2.5L	<i>Synechococcus</i> sp.	-	-	4.2 times higher productivity	-	(Takano et al., 1992)
	Airlift	130L	<i>Spirulina platensis</i>	10Hz	-	43% higher productivity	-	(Xue et al., 2013)
PMM* tubes	Airlift	130L	<i>Scenedesmus dimorphus</i>	10Hz	-	38% higher productivity	-	(Xue et al., 2013)
	Flat plate	3.3	<i>Chlorella vulgaris</i>	-	2-6.5 times higher average light intensity	23.42%	12.52%	(Sun et al., 2016)
fluorescent dyes	Flat plate	270ml	<i>Chlorella sp</i>	-	-	10% higher productivity	Higher Chl <i>a</i> content from 27*10 ⁶ cellml ⁻¹ to 48*10 ⁶ cellml ⁻¹	(Delavari Amrei et al., 2014)

Luminescent solar concentrator panels	Flask	250ml	<i>Chlorella vulgaris</i>	-	-	Higher growth rate ($\mu = 0.29$ compared to $\mu = 0.23$)	Lower doubling time (td=2.44 d compared to td =2.98 d)	(Detweiler et al., 2015)
	Open pond	50L	<i>D. salina</i>	-	-	-	Higher Chl <i>a</i> content	(Detweiler et al., 2015)
	Luminescent acrylic PBR	450ml	<i>Chlorella vulgaris</i>	-	-	Higher biomass concentration and biomass productivity (max=1.49g/l and 0.135g/l/d)	-	(Mohsenpour et al., 2012)
	Luminescent acrylic PBR	450ml	<i>Gloeothece membranacea</i>	-	-	Higher biomass concentration and biomass productivity (max=2.27/l and 0.132g/l/d)	-	(Mohsenpour et al., 2012)
	Open pond	-	-	-	-	230% increase (2445*10 ⁴ cells/ml compared to 1000*10 ⁴ cells/ml)	-	(Falber, 2013)

* Polymethyl metacrylate

Overall, a flashing light system is effective in a microalga open pond cultivation system as long as the mixing velocity is optimized in the culture. That means that the microalgae culture should be harvested and diluted over the time to keep the cell density at an optimum concentration (Abu-Ghosh et al., 2016). However, conventional mixing systems in outdoor open ponds do not effectively enhance the conversion efficiency of light by flashing light effect. To achieve an optimum L/D cycle with the timescale of the flashing light, a sophisticated mixing system is required for an algal cultivation system which is technically not feasible and may induce high operational costs (Tredici, 2010).

1.5.2 Spatial light dilution

Spatial light dilution is a method to decrease photon flux density lower than 10% of full sunlight by using light distribution systems (Gordon, 2002; Tredici & Zlttelli, 1998). One potential advantage of spatial dilution compared to the flashing light system is that the conventional mixing can be used. It seems that temporal light dilution requires simpler optical system and fewer capital costs than spatial dilution but the operational costs may be considerably higher due to having a turbulent mixing facility to induce high frequency light/dark cycle (Dye, 2010). Obtaining the irradiance below the saturation intensity by applying spatial dilution systems requires optical concentrators and diffusers such as optical fibres (Xue et al., 2013), trough systems (Fernández-García et al., 2010), parabolic dishes (Chiang et al., 2016), green solar collector (Zijffers et al., 2008a) and, luminescent solar concentrator panels (Mohsenpour et al., 2012).

Mayer et al. (1964) cultivated a 2000 L mass culture of *Chlorella* in an open pond with 1 m depth. They could increase the biomass productivity of the culture from 27 g

d^{-1} to 38 g d^{-1} by using translucent Perspex cones as a light diffusing system into the open pond culture (Table 1-2) (Mayer et al., 1964). The similar study was carried out by Badby (2010) to investigate the effect of diffusers to increase light irradiance into the pond and enhance microalgal productivity. The diffusers increased the amount of light supplied to a concentrated culture up to 20% but did not increase areal productivity. The possible reasons were likely due to carbon limitation and oxygen saturation within the algal culture (Badby, 2010). Furthermore, Dye et al. (2011) designed a diluted photobioreactor (sdPBR) cultivation system with 18 L to concentrate and distribute light over the larger area. They used Fresnel lenses as the solar concentrators, and the planar waveguides to transfer the light into the photobioreactor which resulted in a 2.5 times higher productivity (Table 1-2) compared to conventional systems.

1.5.2.1 Optical fibres

Using fibre optics is another method to carry light to the PBR (Chen et al., 2006). The use of fibre optics systems for microalgal photobioreactors can potentially address two important criteria in the design of a lighting system for algal photobioreactors: (a) electrical energy efficiency; and (b) lighting distribution efficiency (Ono & Cuello, 2004). Takano et al. (1992) investigated the construction of 661 light diffuser optical fibre (LDOF) bundles in the middle of a bubble column PBR with 2.5L working volume for *Synechococcus* sp. culture. They found that increasing light intensity from $2.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ using LDOF will increase biomass yield by 4.2 fold to the total yield of 0.97 g/L (Table 1-2) (Takano et al., 1992). Xue et al. (2013) also designed an airlift PBR with 130L working volume by using optical fibres which were fixed vertically inside the reactor. They showed an increase of 43% and 38% in

productivity for *Spirulina platensis* and *Scenedesmus dimorphus*, respectively, as a result of having an even distribution of light/dark frequencies being over 10 Hz (Table 1-2) (Xue et al., 2013). Although optical fibres can be made in different designs and they are separate from the reactor resulting significantly higher productivity (Chen et al., 2008; Ono & Cuello, 2004), delivering light into mass cultivation of algae through optical fibres can be very inefficient (Xue et al., 2011). It has also been argued that fibre prices are exceedingly high around tens of (US) dollars per linear meter suggesting the use of fibre optics as the economic bottleneck in such systems (Gordon, 2002). Besides, other issues such as high installation and maintenance fees and high capital costs make the use of optical fibres unachievable in a large scale cultivation system (Xue et al., 2011).

Sun et al. (2016) designed a 3.3 L flat-plate PBR equipped with polymethyl methacrylate (PMMA) tubes inside the reactor as light guides for *Chlorella vulgaris* cultivation. The average light intensity and biomass production were increased by 2-6.5 times and 23.42%, respectively (Table 1-2). The photosynthetic efficiency of *Chlorella vulgaris* was also increased to 12.52% (Sun et al., 2016). The other spatial light distribution method is the potential use of green solar collector (GSC) modeled and designed by Zijffers et al. (2008) to collect the sunlight and deliver it into the photobioreactor via flat rectangular PMMA. The design is based on the capture of sunlight by Fresnel lenses on top of the GSC that can rotate to follow the sun and is directed to the photobioreactor through light guides. Their design showed a better efficiency compared to previous attempts to capture sunlight through optical fibres. The GSC system has several advantages compared to optical fibres including no loss of light in transport into the system and lowers costs and construction consideration for large scale systems due to using ease of construction and maintenance and the use

of cheap material (PMMA). However, setting up the tracking sun system and positioning the lenses are the major drawback of the system which makes this system economically unfeasible. Furthermore, incident angles of sunlight vary greatly during a day and, therefore, a uniform distribution of light on the surface of the distributor is not achievable (Zijffers et al., 2008b).

1.5.2.2 Luminescent Solar Concentrator

Luminescent solar concentrators (LSCs) for concentrating and converting sunlight into electricity through photovoltaic cells have been first reported by Weber and Lambe (1976). The advantage of LSCs is that there is no need for an expensive solar tracking system as LSCs can absorb direct and diffuse light (Debijs & Verbunt, 2012). LSCs consist of luminescent particles such as organic dyes (Cheng & Baojun, 2015), quantum dots (QDs) (Bomm et al., 2011), or semi-conducting polymers dispersed uniformly inside it (Slooff et al., 2007) (Figure 1-8). The sunlight is absorbed by the surface of a luminescent panel through luminescent dyes. The absorbed light undergoes total internal reflection towards the edges and is emitted at a longer wavelength (Cheng & Baojun, 2015; Corrado et al., 2013).

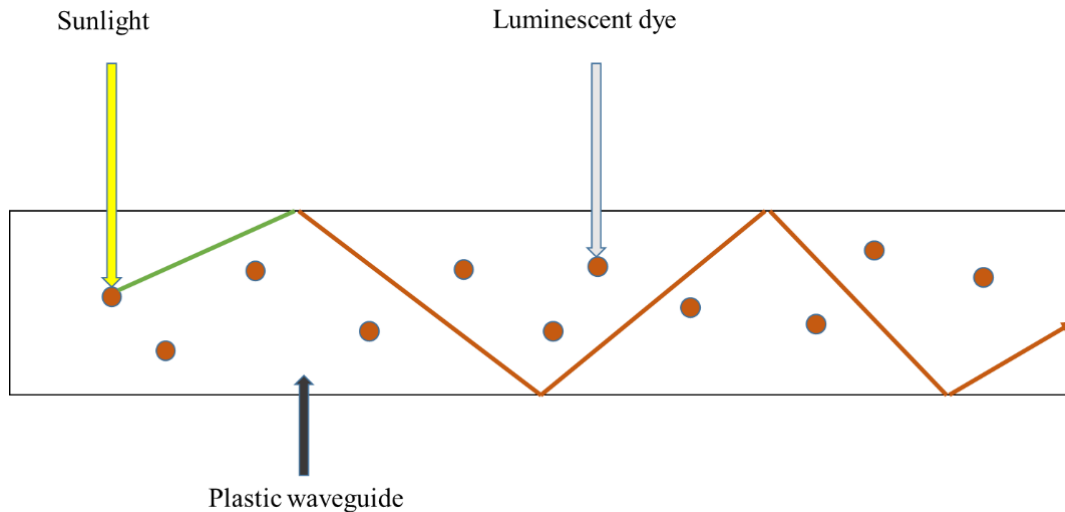


Figure 1-8. The luminescent solar concentrator. Incident light (yellow arrow) is absorbed by luminescent dyes (red circles) inside the waveguide and re-emitted at a longer wavelength to the edge(s) by total reflection (Debije & Verbunt, 2012).

Using LSCs for microalgae cultivation systems have been reported in the literature (Delavari Amrei et al., 2015; Mohsenpour & Willoughby, 2013; Sforza et al., 2015; Wondraczek et al., 2013). Delavari Amrei et al. (2014) investigated the effect of fluorescent material coated on a 270 ml flask to enhance the growth rate *Chlorella* sp. The two absorption and emission peaks of the coated layer were at 370-380 nm and 435-465 nm, respectively. They showed that the biomass productivity of *Chlorella* sp. increased 10% by using coated reactors with shifter layers compared to control. It was also found that number of cells increased from 27×10^6 cell ml⁻¹ to 48×10^6 cell ml⁻¹ due to removing UV-A radiation (Delavari Amrei et al., 2014).

A similar study was carried out by Detweiler et al. (2015) cultivating four strains of microalgae as *Chlorella vulgaris*, *D. salina*, *Chlamydomonas reinhardtii*, *Botryococcus sudeticus* and a cyanobacteria (*Spirulina platensis*) in a 250 ml flask with 100 ml working volume under greenhouse building covered by LSCs panel. They used red LSC panels that had an absorption peak at 400 nm and emission spectra at

600–700 nm range. The results showed that growth rate increased and doubling time decreased significantly for *C. vulgaris* under the red LSC panel ($\mu=0.29 \text{ d}^{-1}$; $t_d=2.44 \text{ d}$) compared to the control reactor ($\mu=0.23 \text{ d}^{-1}$; $t_d=2.98 \text{ d}$) (Table 1-2) (Detweiler et al., 2015).

Mohsenpour and Willoughby (2013) also cultivated *Chlorella vulgaris* and *Gloeotheca membranacea* in bubble column PBRs coated with luminescent filters in blue, green, yellow, orange and red with working volume of 450 ml at different initial culture densities (Table 1-2). The results indicated that the biomass productivity increased in red luminescent PBRs by 1.14 and 1.62 times in *C. vulgaris* ($0.135 \text{ g l}^{-1} \text{ d}^{-1}$) and *G. membranacea* ($0.184 \text{ g l}^{-1} \text{ d}^{-1}$) cultures, respectively. The chlorophyll production increased in *C. vulgaris* by green light; however, light conditions did not affect chlorophyll production in *G. membranacea* cultures. The highest chlorophyll content of 1.98% of biomass was produced by *C. vulgaris* under green light compared to 1.14% for control which shows the effect of green light on pigmentation (Mohsenpour & Willoughby, 2013).

A large-scale open pond study using LSCs was reported by Falber (2013) who invented a bioreactor comprised of luminescent solar concentrator panels with triangular shaped bags. The algae were grown inside the LSCs panel while the inverted triangular spaces between panels were filled with water to be used as a light path. Additionally, the water was considered as a thermostat. In the summer, the heat is taken away from the system by replacing water and in the winter, the water can keep the temperature of the algae at the level required for algae. By using this system in an open pond with LSCs panel, he produced 230% more algae biomass compared to the control system without luminescent panels (approximately $2445 \times 10^4 \text{ cells ml}^{-1} \text{ d}^{-1}$ compared to $1000 \times 10^4 \text{ cells ml}^{-1} \text{ d}^{-1}$) (Table 1-2) (Falber, 2013). This system showed

a significant increase in biomass concentration; however, it requires a huge amount of water.

On the other hand, Miglio and Palmery (2015) used a flat plate PBR with 750 ml volume made of a red luminescent solar concentrator and resulted in no significant difference in specific growth rate and photosynthetic efficiency of *Nanochloropsis* culture (Miglio & Palmery, 2015).

Overall, spatial light dilution systems seem to be a better and cheaper option than temporal light dilution due to better efficiency in microalgal growth. Among all spatial light dilution systems, LSC panels appear to be a suitable method to be used in microalgal culture systems to have a better efficiency. The advantages of LSC panels are easy to construct, cost-effectiveness, no need for a sun tracking system, feasibility to be used in outdoor open pond systems and, the ability to produce electricity. However, any diffusers design will need to be easily scalable to a commercial scale. Fouling and durability issues of diffusers will also need to be tested at the scale. Due to the wide range of other factors and limitations constantly interacting with an outdoor algal culture, it is likely that much more research is needed to determine the light diffusers true value to different commercial cultivation species.

1.6 Techno-economical and Policy Analysis

1.6.1 Techno-economical analysis

Microalgal large-scale cultivation started with *Chlorella* in Japan and Taiwan in the 1960s and continued with *Spirulina* (in 1960s) and *Dunaliella* (in 1970s). Nowadays, these large-scale ponds are spread all around the world (Ravikumar, 2014) with the largest plant based in Hutt lagoon in Western Australia (700 ha un-mixed pond) (Borowitzka & Moheimani, 2013). There are two major algal cultivation systems, open

ponds and closed photobioreactors (PBRs). Cultivation of microalgae in closed PBRs results in high biomass productivity (Zittelli et al., 2013) and low contamination risks but very high CAPEXs and OPEXs. Open ponds such as paddle wheel driven raceway ponds are less expensive, but have a lower biomass productivity (maximum average annual = $20 \text{ g m}^{-2} \text{ d}^{-1}$) (Borowitzka & Moheimani, 2013). Raceway ponds are the preferred commercial microalgal cultivation system for production of *Arthrospira*, *Chlorella*, *Haematococcus*, and *Dunaliella* (Benemann, 2013). The estimated cost of algal biomass achieved in large scale raceway ponds and PBRs for different species are summarized in Table 1-3 (Borowitzka, 2013). The main advantages of using raceway open ponds for microalgal mass cultivation are a) no need for a cooling system, b) lower hydrodynamic stress and, c) lower capital and operational costs (Moheimani et al, 2015).

Table 1-3. cost estimation of algal biomass grown in raceway ponds from different studies (All costs are adjusted to 2018 US inflation rate) (Reproduced from (Borowitzka, 2013).

Algae species	Culture system	Culture area/volume	Productivity (g m ⁻² day ⁻¹)	Estimated Cost (\$US kg ⁻¹)	References
<i>Scenedesmus</i>	Raceway	4 ha	20	7.56	(Becker & Venkataraman, 1980) ^a
<i>Chlorella</i> (Photoautotrophic)	Raceway	10	25–30	12.42	(Kawaguchi, 1980) ^b
<i>Chlorella</i> (Mixotrophic)	Raceway	10 ha	25–30	12.64	(Kawaguchi, 1980) ^c
<i>Spirulina</i>	Raceway	2 ha	12	12.57	(Jassby, 1988)
<i>Porphyridium</i>	Tubular PBR	10 ha	16	10.21	(Tapie & Bernard, 1988)
<i>Spirulina</i>	Raceway	5 ha	3.2	20.20	(Jassby, 1988)
<i>Dunaliella salina</i>	Raceway	2 ha	4	12.75	(Mohn & Contreras, 1990)
<i>Chlorella</i>	Thin-layer Cascade	1 ha	18	23.71	Data from Pilot-scale facility at Dongara, Western Australia ^d
Microalgae	Tank Culture	20,000 L	–	79.57	(Fulks & Main, 1991)
Microalgae	Biocoil	2400 L	0.06 g/L d ⁻¹	27.50	Unpublished Data ^e
<i>Spirulina</i>	Raceway	1.5 ha	15	13.35	(M. Tanticharoen et al., 1993)
<i>Nannochloropsis</i>	Raceway	0.2 ha	16 (summer), 8 (winter)	54.99	(Zmora & Richmond, 2004) ^g

^a Based on experience of Indo-German project in Mysore, India.

^b Freeze-dried.

^c Spray-dried.

^d Includes harvesting and spray-drying costs – no depreciation of capital costs.

^e Does not include harvesting and drying costs – no depreciation of capital costs.

^f Grown on sago starch factory wastewater.

^g Only biomass production cost. Harvesting costs etc. not included.

Economic is the main challenge of cultivating microalgae in large scale raceway ponds for biofuels production. To have economically feasible biofuel from microalgae, there needs to be a sharp reduction in production costs (Carriquiry et al., 2011). One potential way to the overall cost of biomass by an order of magnitude is to increase biomass productivity as it would significantly affect the economics of a large scale

microalgal production (Benemann, 2013). Capital and operational costs of microalgal growth in raceway ponds with 30 and 60 g m⁻² d⁻¹ productivities are summarized in Table 1-4 (Carriquiry et al., 2011) and the estimated cost of microalgal oil has been calculated between \$51 and \$90 per barrel (Benemann & Oswald, 1996) for two different yields and CO₂ supply methods (Table 1-4). It is to be noted that, so far the highest achieved microalgal annual average biomass productivity has been reported to be only 20 g m⁻² d⁻¹ (Borowitzka & Moheimani, 2013). Although the productivities reported in

Table 1-4 could theoretically be possible, such a high yield has to be obtained in practice consistently (Borowitzka & Moheimani, 2013; Carriquiry et al., 2011).

Table 1-4. Capital and operating costs for a microalgae open pond system with two different biomass productivity. (All costs are adjusted to 2018 US inflation).

	30 g m ⁻² /d Remotely supplied CO ₂	109 tonnes/ha/yr On-site flue gas	60 g m ⁻² /d Remotely supplied CO ₂	218 tonnes/ha/yr On-site flue gas
Capital costs (\$)	113,446	106,561	159,727	143,816
\$/tonne-yr biomass	1040	979	734	658
Operating costs(\$) ^a	23,210	16,631	25,504	23,362
Capital charge (15%)	16,982	16,064	23,944	21,573
Total annual costs (\$)	40,192	32,695	49,448	44,935
\$/tonne biomass	369	300	226	206
\$/barrel of algal oil	105	86	64	60
\$/L of algal oil	0.67	0.54	0.40	0.37

^aLabor and overhead would amount to about \$4590 and \$6119 for the low and high productivity cases respectively. *Source:* Reproduced from (Carriquiry et al., 2011)

Carriquiry et al. (2011), also has estimated the impacts of biomass productivity on production cost of biofuel from microalgae (Figure 1-9). The importance of high microalgal productivity on reducing production costs as well as improving oil yields is also summarised in Figure 1-9. Such a theoretical value would certainly result in producing economically sustainable algal biofuel at less than USD 0.7 (Figure 1-9).

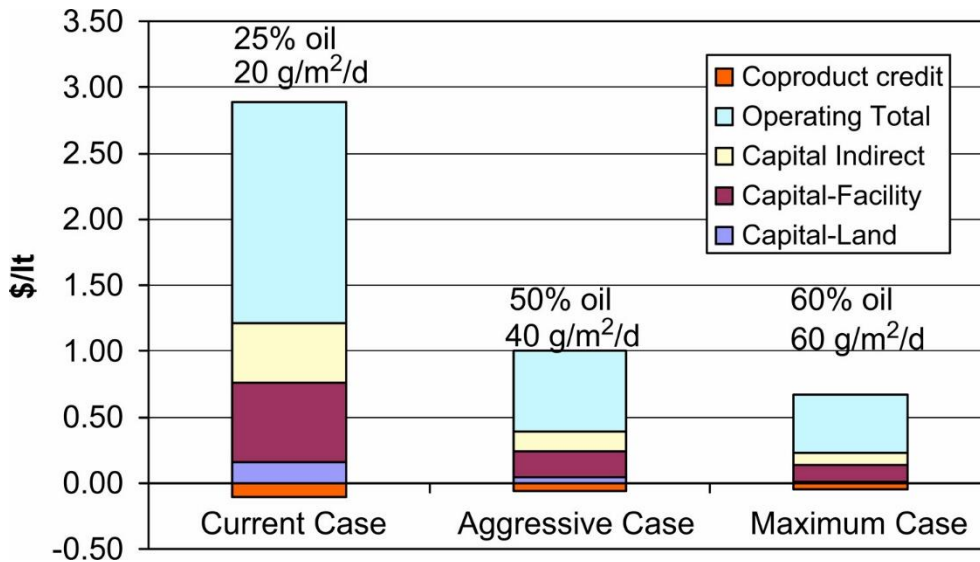


Figure 1-9. Effect of productivity on costs of oil production. (Copied from (Carriquiry et al., 2011) with permission).

The maximum biomass productivities reported in Table 1-4 are based on the photosynthetic conversion efficiency of 10% of solar energy (Benemann & Oswald, 1996) while the achievable photosynthetic efficiency in microalgae is 2–3% in practice (Tredici, 2010). As discussed previously, one solution to increase microalgal productivity is to use an appropriate light delivering system. Such a method can significantly increase the availability of light to algal cells hence increase photosynthetic efficiency. In other words, a better light delivery system into the microalgae cells can increase algal biomass productivity. It is to be noted that such a method would certainly increase the capital expenses of the process but if the productivity is increased significantly, such a method would result in reducing the overall production cost and for the same amount of product a smaller number of ponds would be required. Furthermore, there is also a chance of reducing energy cost by co-producing electricity using light delivering systems such as luminescent solar concentrator panels (Vadiveloo et al., 2015). The potential advantage of using

luminescent solar concentrator panels for microalgae production is the production of electricity using photovoltaic cells as well as delivering the light into the microalgae culture and thus, reducing the cost of energy and biomass production.

1.6.2 Policy constraints

There is no doubt that worldwide the policies of using energy is encouraging utilization of renewable energy (Carrquiry et al., 2011). The US Energy Independence and Security Act (EISA) of 2007 specify a production of advanced biofuel at 79.5 billion liters by 2022 as a part of second Renewable Fuel Standard (RFS2) (Carrquiry et al., 2011). The main challenge of producing microalgal biofuel is economics. When using conventional growth systems such as raceway ponds, cost of microalgal biofuel production is too high compared to fossil fuel (Carrquiry et al., 2011). Increasing biomass productivity in large scale cultivation systems is a promising way to lower the biofuel production. The application of using luminescent solar concentrator panels in microalgae cultivations is in early stage specifically in outdoor cultures. There is a very limited study on using LSCs in outdoor microalgae cultures which makes the economic assessment of this method very difficult. Another obstacle for using LSCs for algae raceway ponds is the design of the panels. Design of the luminescent panels can have a significant effect on biomass productivity of outdoor ponds which affects the capital costs accordingly. Furthermore, there should be an exclusive study of using luminescent panels on specific algae species in an outdoor pond to be able to find the suitability and true potential of the panels for the outdoor algal cultures. Therefore, we need more investigations on using luminescent solar concentrator systems in algal ponds in terms of application and economics.

1.6.3 Future perspective

As highlighted earlier, light is the main limits to the growth and productivity of algae. There is no doubt that distributing light more evenly and increasing light availability to algal cells will enhance the biomass productivity and photosynthetic efficiency in outdoor raceway ponds. Among spatial light dilution systems, LSCs seem to be one of the most economical and effective systems to be applied in raceway open ponds. LSCs can be solving the poor light availability issue of algal cells in raceway open ponds. However, it should be noted that the technology of using LSCs for algal cultivation is still at very early stages and needs further investigation for finding the potential of this technology in commercial scale microalgal cultivation.

1.7 Conclusion

It has been argued that microalgae culture is yet unable to supply basic human needs that stem from the incapability of utilizing solar energy efficiently (Donham et al., 2011; Grobbelaar, 2012). Photolimitation, photosaturation and, photoinhibition are crucial factors which may happen during a growth of concentrated microalgae cultures specifically those being cultivated outdoor under sunlight. By using filtering and light dilution systems, the photoinhibition and photolimitation can be reduced. This leads to a higher productivity culture. There are mainly two dilution systems, temporal and spatial, for distributing light into the microalgae culture. Among spatial dilution systems, LSCs seems to have a good potential to be used in commercial microalgae cultivation systems. They potentially combine spectrum shifting properties with spatial dilution to channel the light into the culture where it is needed. However, only a limited number of studies have been done on LSC for microalgae cultivation, and further studies need to be carried out to find out the true potential of LSC panels.

1.8 References

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1.9 Aim of the study

To the best of my knowledge, to date there has been no information on the use of luminescent solar concentrators as a method to deliver specific light to the depth of an algal pond. To test the effectiveness of LSCs as light guide, laboratory and outdoor experiments are needed. Red and blue portion of the light are the most appropriate regions of PAR for photosynthesis. Therefore, commercially available red and blue LSCs were selected for this study.

The overarching aim of this study was to evaluate the effect of these LSCs to increase the number of photons available to microalgal cells at the depth of an algal culture in a raceway pond. If successful, this would result in improving biomass productivity of selected species.

The objectives of the current PhD study are as follows:

- Screening microalgal species to find the feasibility of using LSCs on those and finding the most suitable species under red and blue LSCs.
- Testing the selected species under outdoor conditions in raceway ponds using red and blue LSCs.
- Estimating the biomass production cost analysis of the most suitable microalga using LSCs.

Thus, in this study, the feasibility experiment was carried out using red and blue LSCs for growth of *Arthrospira platensis*, *Scenedesmus* sp. and *Chlorella* sp. (Chapter 2). Then, in Chapters 3 and 4, *Arthrospira platensis* and *Scenedesmus* sp., were cultivated under outdoor conditions in micro raceway ponds using LSCs. Further, to obtain the economic feasibility of using LSCs in a large-scale raceway pond, a production cost analysis for biomass and phycocyanin of *Arthrospira* was carried out in systems with and without LSCs. Finally, the overall outcome of the thesis was discussed in Chapter 6 with the future directions regarding to the current study.

2 Red and blue luminescent solar concentrators for screening growth of *Arthrospira*, *Scenedesmus* and *Chlorella*

2.1 Abstract

Considering light as the main limiting factor in any mass microalgal production open system, increasing light availability to microalgal cells can improve biomass productivity. Applying a light diffusing system is a method to deliver the light to microalgae cultures. Using luminescent solar concentrators (LSCs) as a light diffusing system can be an economical method to increase the available light to microalgae cells. In this chapter, the efficacy and viability of using red and blue LSCs on three microalgae species, *Arthrospira platensis* (MUR 129), *Scenedesmus* sp. (MUR 268) and *Chlorella* sp. (MUR 269), has been assessed. The species were cultured in a small photobioreactors with the working volume of 1 L equipped with red and blue LSCs. The results showed that *Arthrospira platensis* had 5% to 9% higher productivity when red panels used compared to control and blue LSCs. The biomass productivity of *Scenedesmus* sp. cultures was also 30% and 4.5% higher under red LSCs ($92 \text{ mg L}^{-1} \text{ d}^{-1}$) compared to that in control ($70 \text{ mg L}^{-1} \text{ d}^{-1}$) and blue LSCs ($88 \text{ mg L}^{-1} \text{ d}^{-1}$) while the growth rate of *Chlorella* sp. cultures did not improve under red and blue LSCs. Furthermore, cell count analysis of *Scenedesmus* sp. resulted in 30% higher maximum cell density in cultures with red LSCs compared to that in control. The results of this study clearly showed that the growth of *Arthrospira platensis* and *Scenedesmus* sp. can be improved under red and blue LSCs and therefore, they were selected for further outdoor studies.

2.2 Introduction

Light, temperature and nutrient are the main limits to any mass microalgal cultivation (Borowitzka & Vonshak, 2017). If a microalgal cultivation is in a temperate region

and the culture has been provided with enough nutrients, light will be the main growth limiting factor (Vonshak et al., 2014). Consequently, light would be the main cause of relatively low biomass productivity in raceway open ponds where microalgal cells spend most of their time in a region with no available light (Tredici, 2010). Photolimitation and photoinhibition are also the major drawbacks for microalgal cells growing in raceway open ponds (Borowitzka & Moheimani, 2013). Using light diffusers is a potential method to solve the light limitations of microalgal cultures in open raceway ponds. Different light diffusing systems have been proposed such as optical fibres (Xue et al., 2013), trough systems (Fernández-García et al., 2010), parabolic dishes (Chiang et al., 2016), green solar collector (Zijffers et al., 2008b) and, luminescent solar concentrators (LSCs) (Raeisossadati et al., 2019). There is no need of a solar tracking system when using LSCs which is an advantage compared to other systems and results in less cost (Raeisossadati et al., 2019). Luminescent particles such as organic dyes or quantum dots (QDs) are the main constituents of a LSC (Debije & Verbunt, 2012). Luminescent particles absorb photons when light hits the surface of a LSC and the absorbed light is reflected internally and emitted from the edges at a longer wavelength (Corrado et al., 2013). In this study, the effect of red and blue LSCs on the growth and productivity of three microalgae species, *Arthrospira platensis* (MUR 129), *Scenedesmus* sp. (MUR 268) and *Chlorella* sp. (MUR 269) to find the most suitable microalgae species for further investigations. These species were chosen based on their applications in algal biotechnology. *Arthrospira* is a blue-green cyanobacterium being commercially produced in different parts of the world which is of supreme importance in producing phycocyanin as high value product and can be used as protein source for human food (Belay 1997). *Scenedesmus* and *Chlorella* are green microalgae and extensively being used for wastewater treatment. They have a

huge potential to treat different wastewaters and the produced biomass can be used as animal feed (Raeisossadati et al, 2019).

2.3 Materials and Methods

2.3.1 Microorganism and culture medium

Three species were chosen for this study as *Arthrospira platensis* (MUR129), *Scenedesmus* sp. (MUR 268) and *Chlorella* sp., (MUR 269) with having high commercial applications. Microalgae were cultured in small photobioreactors with dimensions of 14.5×10×10 cm with the working volume of 1 L (Figure 2-1). The cultures were mixed by aerating the cultures. The Zarrouk medium (Zarrouk, 1966) was used for *Arthrospira platensis* cultures and Bold Basal medium (Stein et al., 1973) was used for *Scenedesmus* sp. and *Chlorella* sp. The LED light source was used for all cultures.

2.3.2 Cultivation design

Red and blue luminescent solar concentrators (LSCs) were installed in reactors. LSCs were purchased from Evonic company (<https://www.plexiglas-shop.com/Home/>) as PLEXIGLAS fluorescent red and blue sheets. Each reactor was equipped with two LSCs with a size of 200×100×3 mm. The LSCs were installed on the edge of reactors to have 110 mm of the panel inside a reactor and 90 mm of the panel out of a reactor facing the LED source, with the intensity of 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, to collect light, shift and, transfer it to the depth of cultures (Figure 2-1). The four treatments were red, blue, red & blue LSCs and, control reactors with no panels.



Figure 2-1. Structural view of photobioreactors equipped with red and blue luminescent solar concentrators with the culture volume of 1 L for each reactor. In this study, four reactors were used as with a) red & blue LSCs, b) two red LSCs, c) two blue LSCs and d) control with no LSCs for each species of *Arthrospira platensis*, *Scenedesmus* sp. and *Chlorella* sp. In a batch mode. The cultures were mixed by aeration.

2.3.3 Growth measurement

The cultivations of all species were carried out in a batch culture for about 20 days from 25/03/2017 to 17/04/2017. Dry weight of each culture was determined every second day using GF/C filters. The filters were first pre-combusted at 100 °C in an oven and then the microalgae cultures were added to the filters in a filter unit. Then after, the filters containing microalgae biomass were removed from the filter unit and dried in the oven at 100 °C. Finally, dried filters containing microalgae were weighted for dry weight determination (Moheimani et al., 2013).

2.4 Results and discussion

2.4.1 Light source and luminescent solar concentrator specifications

The spectrometric analysis was done by StellarNet Inc spectrometer, (USA). The spectrometric analyses of red and blue LSCs under LED light are summarised in Table 2-1 and Figure 2-2. Red LSCs (27% transmission with conversion efficiency of 11.6%) had a peak at 650 nm which is desirable for microalgal growth, while the blue LSCs (85% transmission) had a wide peak in the visible area meaning that most of light passes through blue LSCs (Fig 2). In other word, Fig 2 shows that both red and blue LSCs reduced the total amount of light transmitted through them. The red LSCs converted a lot of the higher energy photons to red photons. However, most of the light passed through the blue LSCs rather than being shifted to the desired blue wavelength.

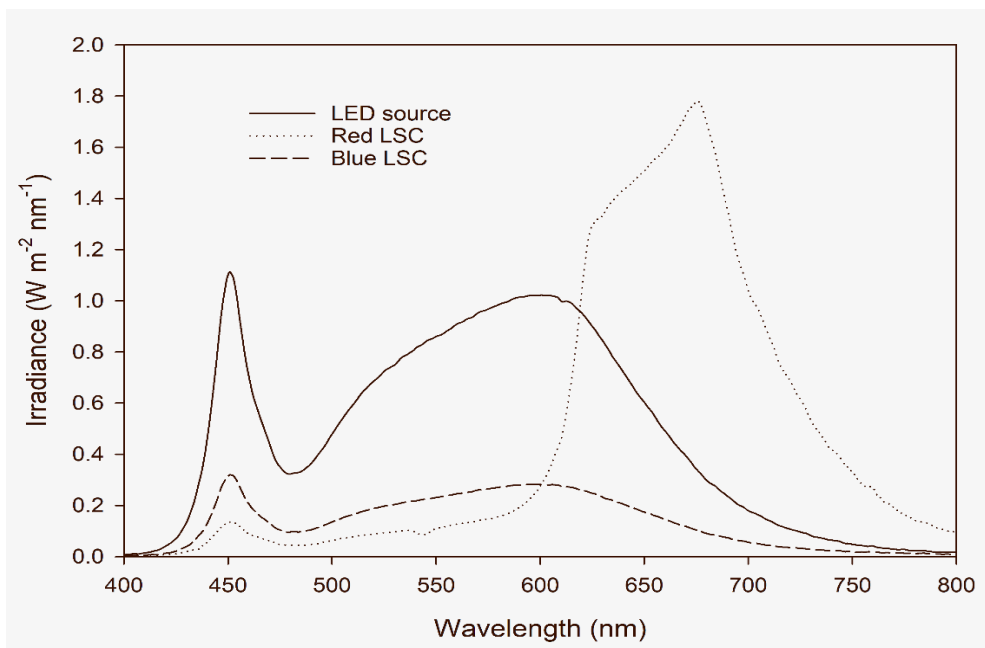


Figure 2-2. Spectral emission of LED source, red LSCs and blue LSCs in the range of 400-800 nm.

Table 2-1. Light source (LED) and red and blue LSCs specifications.

Parameter	LED (Wm ⁻²)	Red LSC (Wm ⁻²)		Blue LSC (Wm ⁻²)	
		Edge	Surface	Edge	Surface
PAR	41.39	28.72	6.27	15.10	14.62
400-500nm	10.56	1.5	0.41	3.90	3.30
500-600nm	18.78	3.1	0.51	6.87	6.40
600-700nm	12.05	23.99	5.35	4.33	4.31
700-800nm	1.106	6.58	0.83	0.43	0.49

2.4.2 Growth rate and productivity

Three species, *Arthrospira platensis* (MUR 129), *Scenedesmus* sp. (MUR 268) and *Chlorella* sp. (MUR 269) were chosen for the indoor experiment to investigate the effect of red and blue LSCs on the productivity. The aim of this study was to assess the suitability of the chosen species and select the most suitable microalgae species under LSCs for further studies in an algal raceway pond outdoors. The growth of *A. platensis* and *Scenedesmus* sp. showed around 5% and 18% higher maximum biomass yield under red LSCs during the cultivation period respectively compared to control with no LSCs while *Chlorella* sp. had a similar growth rate in all treatments (Figure 2-3).

A. platensis also showed 5% to 9% higher productivity when red panels used compared to control and blue LSCs. The biomass productivity of *Scenedesmus* sp. cultures was also 30% and 4.5% higher under red LSCs (92 mg L⁻¹ d⁻¹) compared to that in control (70 mg L⁻¹ d⁻¹) and blue LSCs (88 mg L⁻¹ d⁻¹) while the growth rate of *Chlorella* sp. cultures did not improve under red and blue LSCs. Furthermore, cell count analysis of *Scenedesmus* sp. resulted in 30% higher cells in cultures with red

LSCs compared to that in control. The maximum specific growth rate of *Arthrospira* and *Scenedesmus* were also higher under red LSCs and blue LSCs respectively compared to other treatments (Table 2-2).

The reason for having better growth under red LSCs might be due more photons to algal cells. As it is shown in Figure 2-2, red LSCs had a peak at 680 nm while blue LSCs and LED source had a peak 450 nm. That means that red LSCs absorbed the visible light from LED and emitted photons in the wavelength of 680 nm while blue LSCs emitted more photons in the wavelength of 450 nm to microalgal cells. The results of this study showed that biomass productivities and yields of *Arthrospira platensis* and *Scenedesmus* sp. were improved by LSCs and thus, *Arthrospira platensis* and *Scenedesmus* sp. were chosen as the most suitable species for further outdoor investigations.

Table 2-2. Maximum specific growth rate of microalgae cultures under different LSCs conditions.

Specific growth rate (d ⁻¹)	<i>Arthrospira</i>	<i>Scenedesmus</i>	<i>Chlorella</i>
Red & blue LSCs	0.08	0.26	0.21
Red LSCs	0.1	0.28	0.22
Blue LSCs	0.084	0.32	0.25
Control	0.082	0.28	0.24

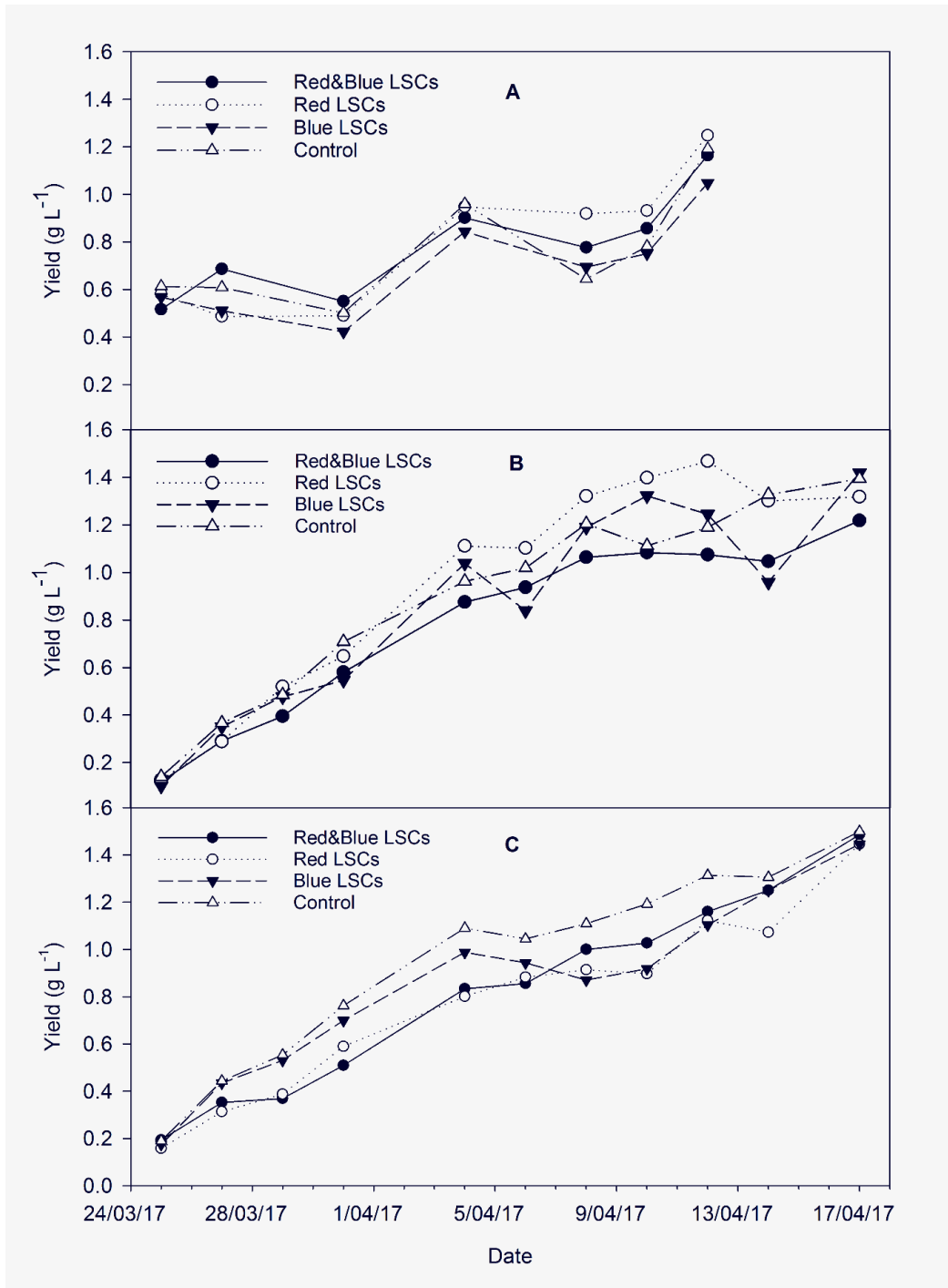


Figure 2-3. Growth curves of A) *Arthrospira platensis*, B) *Scenedesmus* sp., C) *Chlorella* sp. in photobioreactors equipped with red and blue luminescent solar concentrators with and control with no LSC with the culture volume of 1 L for each reactor. The cultures were run in a batch mode.

2.5 Conclusion

The main aim of this scoping study was to investigate to see if the use of LSCs for microalgae cultures works. This study was used to find out an indication of effectiveness of using LSCs for microalgae cultures and then to be tested in outdoor real-life conditions to find the true potential of LSCs on microalgal growth. In this scoping study, the growth of *Arthrospira platensis* and *Scenedesmus* sp. were improved under red and blue LSCs. The results showed that *Arthrospira platensis* and *Scenedesmus* sp. had a better yield and productivity under red and blue LSCs compared to that in control. Thus, *Arthrospira platensis* and *Scenedesmus* sp. were selected for further investigations using red and blue LSCs under outdoor conditions to find the true potential of the species for a large-scale cultivation.

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Link to the next chapter

The results of feasibility experiment showed that red and blue LSCs could increase biomass productivity of *Arthrospira* and *Scenedesmus* cultures. Thus, in the next experiment, the effect of red and blue LSCs on the growth of *Arthrospira platensis* in raceway ponds outdoors has been investigated. The aim of the next study was to maximize the biomass and phycocyanin productivity of *Arthrospira* cultures grown outdoors in raceway pond by using red and blue LSCs with a unique design.

Chapter 3

The effect of red and blue luminescent solar concentrators on biomass and phycocyanin productivity of *Arthrospira platensis*

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Red and blue luminescent solar concentrators for increasing *Arthrospira platensis* biomass and phycocyanin productivity in outdoor raceway ponds

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Author contribution

Contributor	Statement of Contribution
Mohammadjavad Raeisossadati (70%)	Designed and conducted all experiments. Analysed and processed the data and wrote the manuscript.
Navid. R. Moheimani (15%)	Proposed the concept, assisted in the data analysis and revision of the manuscript.
David Parlevliet (15%)	Proposed the concept, assisted in the data analysis and revision of the manuscript.

Supervisors confirmation

I hereby confirm and certify the authorship of this manuscript and the contribution of the first author.

Name	Signature	Date
Dr Navid Moheimani	NR Moheimani	11/09/2019
Dr David Parlevliet		12/09/2019

3 Red and blue luminescent solar concentrators for increasing *Arthrospira platensis* biomass and phycocyanin productivity in outdoor raceway ponds

3.1 Abstract

Achieving high biomass productivity is critical for establishing a successful large-scale algal facility. Microalgae cultures in raceway ponds are normally light limited. To achieve high biomass productivity, there is a need to develop a system to deliver light into the depth of microalgal cultures in raceway ponds. We investigated red and blue luminescent solar concentrators (LSCs) in outdoor raceway ponds to downgrade the sunlight, re-emit and, deliver it into the depth of *Arthrospira platensis* culture operated at 21 cm depth. When red LSCs were used, the biomass productivity ($12.2 \text{ g m}^{-2} \text{ d}^{-1}$) and phycocyanin productivity ($8.5 \text{ mgL}^{-1}\text{d}^{-1}$) of *A. platensis* increased 26% and 44%, respectively. However, using blue LSCs resulted in no significant increase in *A. platensis* biomass productivity. Therefore, for generating same phycocyanin productivity using red LSCs, 44% less cultivation area would be required. This can lead to a significant reduction in the cost of phycocyanin production.

3.2 Introduction

When enough nutrient is available, light and temperature are the most critical limits to the growth of microalgae. Microalgae grown in temperate regions have an optimum temperature for growth and thus, light is the primary limiting factor in those regions (Vonshak et al., 2014). When grown in outdoor open ponds, microalgal cultures face two different light/dark schemes. The first one which happens quickly is caused by turbulent mixing in a pond resulting in the light/dark cycle (Laws et al., 1983). This cycle occurs in a timescale of second and algal cells are exposed to full sunlight at the top surface of the culture to total darkness at the depth of the culture below 5 cm (Moheimani & Borowitzka, 2007). The second scheme happens when solar irradiance

changes from sunrise to sunset during a day. The acclimatization of outdoor microalgae cells to light depends on these two light regimes. There is also a self-shading effect that determines the availability of light to each cell in dense microalgal cultures (Vonshak et al., 2014).

Raceway ponds are the most recommended cultivation system for mass microalgal production of some species such as *Arthrospira* and *Chlorella* (Borowitzka & Moheimani, 2013). The main aim of any algal farmer is to achieve the highest biomass production in the shortest possible time resulting in the highest productivity (Borowitzka & Moheimani, 2013). Considering the operational depth of 20 cm or more for large scale raceway ponds and light can only penetrate the top 3-5 cm of the pond (Raeisossadati et al., 2019), there is very small amount of light available to microalgal cells. Therefore, photolimitation is one of the main obstacles for microalgae grown in raceway ponds resulting in rather low biomass productivity (Tredici, 2010). Hence, light delivering systems such as diffusers have been proposed as a potential method to increase the availability of light to microalgal cells. There are two light diffusing systems, temporal and spatial, to increase light availability to microalgal cells (Raeisossadati et al., 2019). Temporal light diffusion is based on inducing high light irradiance in an instant by turbulent mixing resulting in higher light/dark frequency called flashing light effect (Laws et al., 1983). The flashing light effect can be an effective system for algal growth as long as the optimum mixing velocity is provided. However, the conventional mixing using paddle wheels cannot provide efficient turbulent mixing velocity required for flashing light effect (Tredici, 2010). Therefore, there is a need to develop more advanced mixing system which is most likely not technically and economically feasible (Tredici, 2010). Spatial light diffusion is based on providing more efficient light by reducing or increasing light irradiance by using

light distribution systems (Zijffers et al., 2008). Spatial light dilution systems can be used with conventional mixing system leading to less overall capital cost (Dye et al., 2011). Different light distribution systems have been proposed such as optical fibres (Xue et al., 2013), trough systems (Fernández-García et al., 2010), parabolic dishes (Chiang et al., 2016), green solar collector (Zijffers et al., 2008) and, luminescent solar concentrators (LSCs) (Raeisossadati et al., 2019).

The main advantage of luminescent solar concentrators is that a solar tracking system is not needed resulting in less cost compared to other systems (Raeisossadati et al., 2019). Luminescent particles such as organic dyes or quantum dots (QDs) are the main constituents of a LSC (Debijs & Verbunt, 2012). Luminescent particles absorb photons when light hits the surface of a LSC and the absorbed light is reflected internally and emitted from the edges at a longer wavelength (Corrado et al., 2013). There have been some small scale studies on using LSCs in closed algal photobioreactors (Delavari Amrei et al., 2015; Mohsenpour & Willoughby, 2013; Sforza et al., 2015) all of which have used LSCs as a light shifter. However, to date, to the best of author's knowledge, no studies have been carried out on using LSCs as a light guide for growth of *Arthrospira platensis* in an outdoor raceway pond.

The annual *Arthrospira* (*Spirulina*) estimated production is around 8000 MT (Vonshak et al., 2014) which costs about US\$10-\$20 kg⁻¹ in average (Borowitzka, 2013b). The C-phycocyanin market value is also US\$ 500 to 100,000 kg⁻¹ depending on the purity of the product (Borowitzka, 2013a). One potential way to reduce the cost of produced phycocyanin is by increasing productivity of *Arthrospira* cultures. Increasing light availability to *Arthrospira* cells at the depth of a raceway open pond by using an efficient light delivering system can be a possible method to achieve such a goal.

In this study, the use of red and blue luminescent solar concentrators with a novel design to enhance the biomass and phycocyanin productivity of *Arthrospira platensis* was investigated. The overarching aim was increasing the number of photons in blue or red spectrum available to the *Arthrospira platensis* cells at the depth of cultures operating in outdoor raceway ponds. Apart from phycocyanin, biochemical compositions of *Arthrospira platensis* grown using red and blue LSCs compared to the control were also studied. Furthermore, the response of *Arthrospira platensis* maximum quantum efficiency of PSII under blue and red LSCs was also investigated. To the best of author's knowledge, this is the first time that the use of LSCs as a light guide was evaluated for growth of any microalga in outdoor paddle wheel driven raceway ponds.

3.3 Materials and methods

3.3.1 Microorganism and culture medium

Arthrospira platensis (MUR129) used in this study was sourced from Murdoch University, Algal Culture Collection Centre. This species was cultured in outdoor 0.1 m² paddle wheel driven raceway ponds each of which had dimensions of 13 × 26 × 80 cm (W×H×L) and final culture volume of 21.5 L (Figure 3-1). The culture was mixed by a four-blade paddle wheel and operated at a depth of 21 cm resulted in mixing rate of 11 cm s⁻¹. The Zarrouk culture medium was used for all studies (Zarrouk, 1966).

3.3.2 Cultivation design

Red and blue luminescent solar concentrators (LSCs) were installed in raceway ponds in this study (Figure 3-1). LSCs were purchased from Evonic company (<https://www.plexiglas-shop.com/Home/>) as PLEXIGLAS fluorescent red and blue

sheets with a size of $300 \times 200 \times 3.00$ mm. The LSCs were installed on the edge of raceway ponds in order to have 200 mm of the panel inside the raceway pond and 100 mm of the panel out of the pond facing the sun to collect visible and diffuse light from sunlight, downgrade and, transfer it to the depth of *A. platensis* culture (Figure 3-1). The bottom part of LSCs inside the *A. platensis* culture was also laser-cut to have sufficient surface area in order to increase the irradiance (see section 3.3.3). The three treatments were red and blue LSCs and, control ponds with no panels. Each treatment was conducted in triplicates (Figure 3-1C).

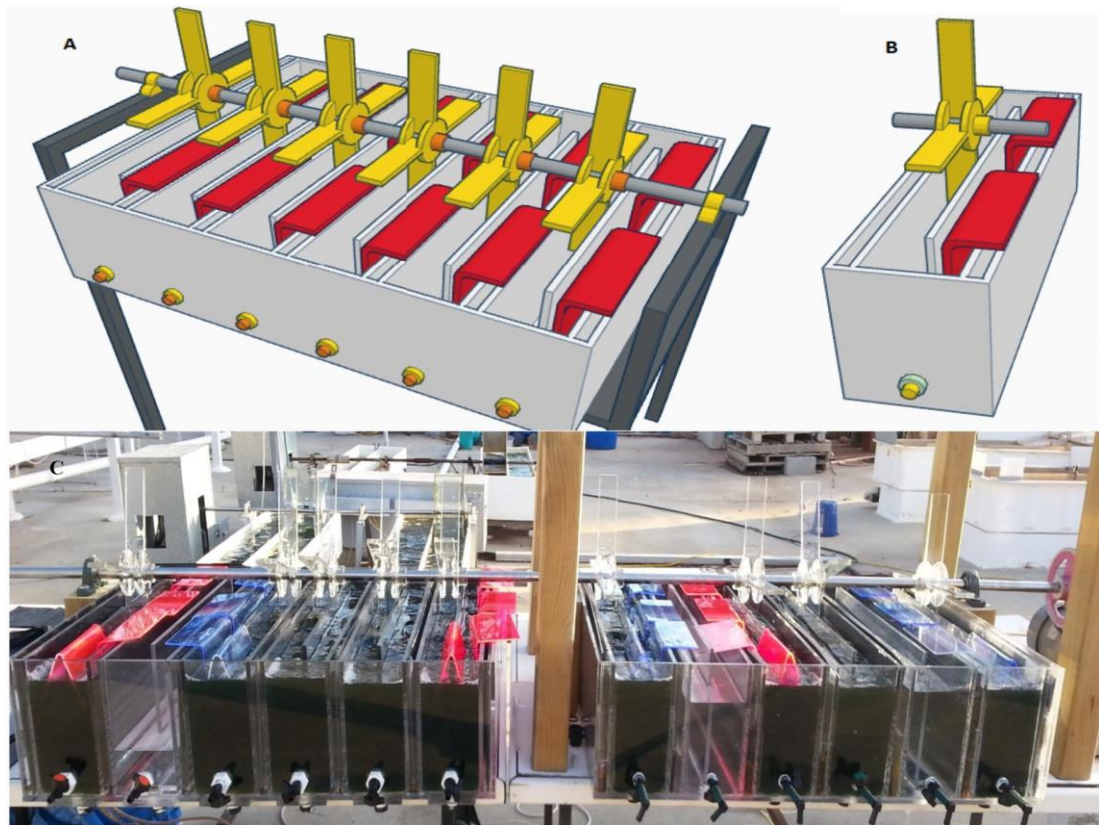


Figure 3-1. A) Schematic, B) A singular and C) structural view of raceway ponds equipped with luminescent solar concentrators with the culture volume of 21.5 L for each pond. In this study, nine raceway ponds were used for three treatments (red and blue LSCs and, control with no panel) in three replicates in semi-continuous mode starting with a batch culture followed by four harvests. All raceway ponds were covered from all sides during the cultivation period to simulate the condition of a large-scale raceway pond receiving sunlight only from the top part.

3.3.3 Luminescent solar concentrator specifications

The spectrometric analyses of red and blue LSCs under sunlight are summarised in Table 3-1 and Figure 3-2A. Red LSCs (27% transmission) had a peak at 650 nm which is desirable for microalgal growth, while the blue LSCs (85% transmission) had a wide peak in the visible area meaning that most of sunlight passes through blue LSCs (Figure 3-2A) and is not downgraded in the desired blue wavelength. In other words, Figure 3-2A shows that both red and blue LSCs reduced the total amount of light transmitted through them. The red LSCs converted a lot of the higher energy photons to red photons but is largely transparent to IR. However, over 85% of the sunlight passed through the blue LSCs rather than being shifted to the desired blue wavelength. That is because the commercial blue LSCs are lighter than the red LSCs in terms of dye concentration and did not have as much organic dyes concentration as the red LSCs.

As there is a different spectral emission for red and blue LSCs, they emit a different number of photons. Considering 17 cm of each LSC inside the algal culture (Figure 3-2B), the corresponding surface area of all edges (S) inside the algal culture is the length of all edges (1900 mm) (10 long edges and 5 small edges, Figure 3-2B) multiplied by the thickness of a LSC edge (3 mm) ($S = 1900 \text{ mm} \times 3 \text{ mm} = 5700 \text{ mm}^2 = 5700 \times 10^{-6} \text{ m}^2$) Equation 3-1):

$$S = 1900 \text{ mm} \times 3 \text{ mm} = 5700 \text{ mm}^2 = 5700 \times 10^{-6} \text{ m}^2 \quad \text{Equation 3-1}$$

Then, the total photosynthetic active radiation (PAR) emitting from all edges (Figure 3-2B) of a red LSC ($T_{\text{PAR, Red}}$) equals PAR emitting from edges of a red LSC (Table 3-1) multiplied by the surface area of all edges inside the algal culture ($(T_{\text{PAR, Red}}) = 110 \text{ Wm}^{-2} \times 5700 \times 10^{-6} \text{ m}^2 = 0.627 \text{ W}$ Equation 3-2)

$$(T_{\text{PAR, Red}}) = 110 \text{ Wm}^{-2} \times 5700 \times 10^{-6} \text{ m}^2 = 0.627 \text{ W} \quad \text{Equation 3-2}$$

Therefore, having four red LSCs in each pond results in 2.51 W ($0.627 \text{ W} \times 4$) or 11.53 $\mu\text{mole photons s}^{-1}$ (2.51×4.6 (conversion factor for W m^{-2} to $\mu\text{mole photons m}^{-2} \text{ s}^{-1}$)). Doing the same PAR calculations for surfaces (Figure 3-2B) of each red LSC (Table 3-1) inside the algal culture would result in 4.90 W / 22.52 $\mu\text{mole photons s}^{-1}$. Thus, the total amount of PAR emitting from four red LSCs in each pond leads to having approximately 34 $\mu\text{mole photons s}^{-1}$ available to algal cells.

Based on the data in Table 1 for blue LSCs and the same calculations mentioned above, the total PAR emitting from edges and surfaces of four blue LSCs inside each raceway pond is 4.5 $\mu\text{mole photons s}^{-1}$ which is less than the total emitted PAR from red LSCs.

The reason is due to a lower concentration of pigments in blue LSCs.

Table 3-1. Solar radiation and red and blue LSCs specifications. The spectrometric analysis was done at midday under the open sun (The assumption is that the solar irradiance variation was negligible during the period of the study).

Parameter	Solar irradiance (Wm^{-2})	*Red LSC (Wm^{-2})		Blue LSC (Wm^{-2})	
		Edge	Surface	Edge	Surface
Total	803	188	92	52	68
PAR	443	110	60	34	48
400–500 nm	128	–	–	7	10
500–600 nm	159	–	–	14	20
600–700 nm	155	104	45	13	18
700–800 nm	134	30	21	12	14

* The light irradiance emitted from a red LSC was negligible in the range of 400–600 nm.

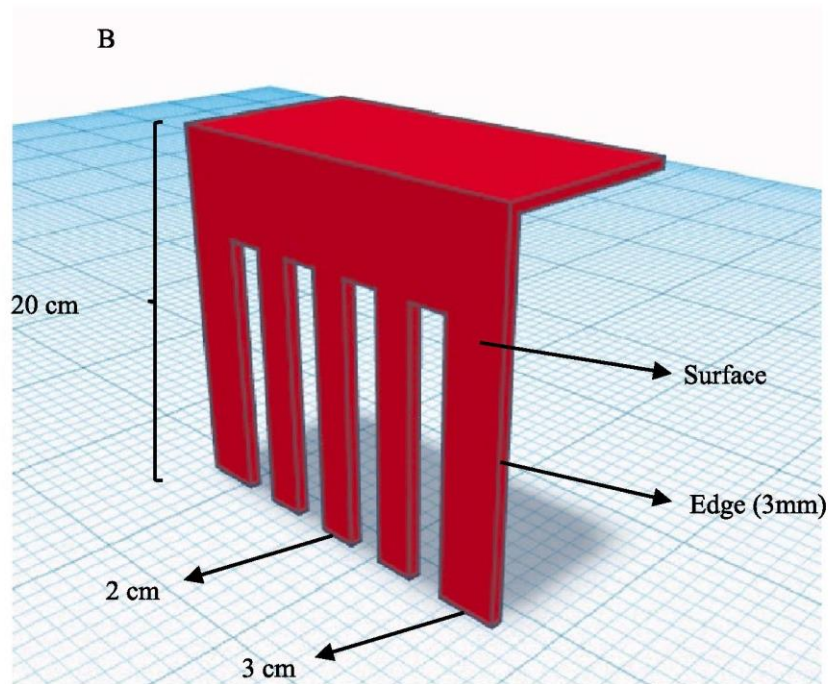
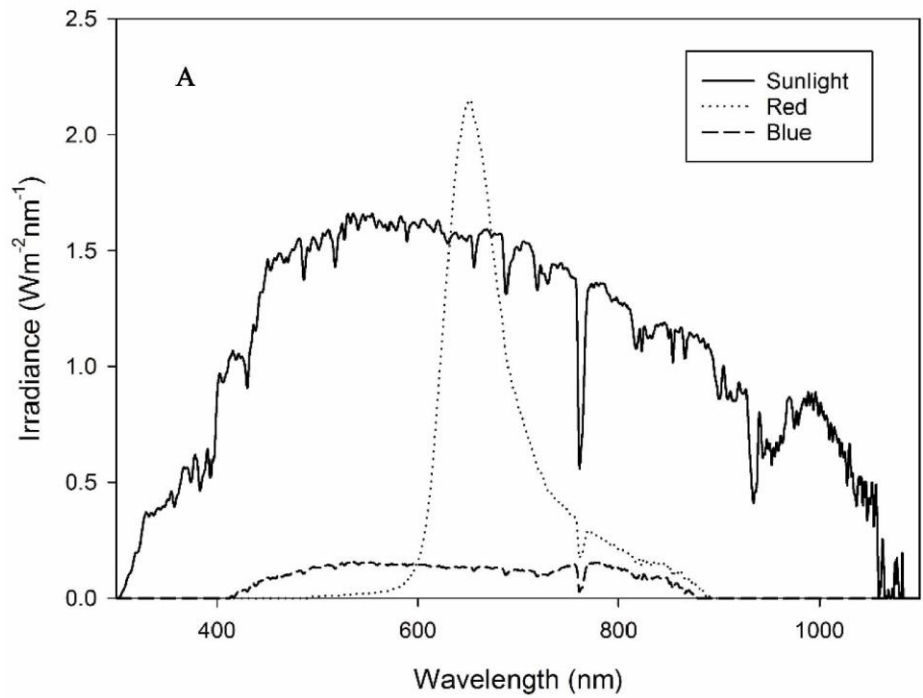


Figure 3-2. A) Solar spectrum and the spectrum of red and blue luminescent solar concentrators (photosynthetic active radiation (PAR) region is in the range of 400–700 nm). The spectra were obtained under open sun at midday, B) schematic design of a red LSC inside *Arthrospira* cultures. The blue LSCs were designed the same as red LSCs. 17 cm of each LSC was inside the algal culture.

3.3.4 Growth measurement

The outdoor cultivation of *A. platensis* was carried out for 75 days between 15/12/2017 and 03/03/2018. The cultures started as batch mode followed by semi-continuous mode with four harvests and the culture period of 15 days. The harvest and dilution frequencies were determined based on the growth rate at the late logarithmic growth phase. In each harvest, the required culture was replaced by fresh Zarrouk medium. The harvested biomass was used to measure biochemical composition as well as biomass and phycocyanin productivities. Dry weight was determined using methods previously described by Moheimani et al. (2013). The cultures temperatures were measured using a Tinytag TG-4100 (Gemini Data Loggers, UK) temperature loggers.

3.3.5 Chlorophyll fluorescence measurement

The different activities of photosynthetic apparatus were estimated by using variable chlorophyll fluorescence. The two common parameters are F_o' and F_m' representing the minimum and maximum fluorescent yield when the sample was light-adapted. The maximum variable fluorescence yield in actinic light ($F_v' = F_m' - F_o'$) was also measured to show maximum photochemical efficiency and the stress on photosynthetic apparatus (Cosgrove & Borowitzka, 2010). The effective quantum efficiency of PSII photochemistry (F_v'/F_m') of *A. platensis* samples (3ml) was measured by AquaPen AP 100 (Photon Systems Instrument, Czech Republic).

3.3.6 Protein, lipid, Chlorophyll *a* and phycocyanin determination

Protein and lipid content of biomass were determined using the methods previously described by Moheimani et al. (2013). The chlorophyll extraction method of Jeffrey and Humphrey was used for chlorophyll *a* extraction (Moheimani et al., 2013). The

concentration of phycocyanin was also determined using MacColl and Guard-Friar (1987) method.

3.3.7 Statistical analysis

The statistical analyses were carried out using Sigmaplot 14, One Way ANOVA to determine the significant difference between treatments ($P < 0.05$).

3.4 Results and discussion

To the best of author's knowledge, this is the first study of its kind assessing the suitability of LSCs to increase the light availability in the depth of algal culture. It is to be noted that in this study not only is sunlight delivered to the depth of algal cultures, but also the un-used part of sunlight such as UV is converted to photosynthetically active radiations (i.e. blue or red). Obviously, the overarching aim was to increase biomass productivity. *Arthrospira* was chosen to grow as this alga is usually mass-produced using raceway ponds. Further to biomass production, the other aim was to investigate the effect of shifted and delivered sunlight by LSCs on the high-value pigment (phycocyanin) production of this microalga.

3.4.1 Growth and productivity

The maximum irradiance of 1600 W m^{-2} was observed in day 20 which was approximately 81% higher than the lowest irradiance in day 25 with the intensity of about 300 W m^{-2} (Fig 3A). The daily variation in air and pond temperatures were from 37°C to 11°C , and 32°C to 10°C , respectively (Figure 3-3B&C). The highest daily evaporation was 0.65 mm in day 28 and the maximum average rainfall was 5 mm in day 1 (Figure 3-3D&E).

The main aim of the study was maximising biomass productivity, phycocyanin productivity and yield of *A. platensis* as a result of providing more irradiance to the cells by using red and blue LSCs. *Arthrospira platensis* cultures when grown with red LSCs, reached a significantly higher biomass yield (1.77 ± 0.014 g L⁻¹) compared to control (1.53 ± 0.002 g L⁻¹) and blue LSCs (1.59 ± 0.056 g L⁻¹) (One-way ANOVA $P<0.05$) (Figure 3-3F, Table 3-2). No significant differences were found between the biomass yield of *Arthrospira* when grown in control and raceway ponds with blue LSCs (One-way ANOVA $P>0.05$). The biomass yield is an important growth measurement tool for assessing algal growth. Higher biomass yield means less energy required for dewatering (Pahl et al., 2013). The biomass yield obtained for *Arthrospira* in this study under red LSCs is in accordance with studies grown *Arthrospira* in outdoor paddle wheel driven raceway ponds (Andrade & Costa, 2008; Magro et al., 2018). Andrade and Costa (2008) obtained the biomass yield of 1.73 g L⁻¹ for *Arthrospira* cultured in an outdoor raceway pond with working volume of 6 L. Magro et al. (2018) also showed *Spirulina platensis* biomass yield of 1.24 g L⁻¹ in a raceway pond with a working volume of 10 L and mixing rate of 10 cm s⁻¹.

The most important algal growth measurement tool is biomass productivity (Borowitzka & Moheimani, 2013). The biomass productivity of 57 ± 3.2 mg L⁻¹ d⁻¹ (12.2 g m⁻² d⁻¹) was obtained when *Arthrospira* cultures in raceway ponds were equipped with red LSCs. This was 24% and 26% higher than *Arthrospira* cultures when grown in raceway ponds with blue LSCs and control (One-way ANOVA $P<0.05$) (Table 2). However, there was no significant difference between productivity of *Arthrospira* cultures with blue LSCs and control (One-way ANOVA $P>0.05$) (Table 3-2). More light availability to algal cells into the depth of the cultures is the most

likely reason for having higher productivity in cultures with red LSCs. The spectrum of red light (600-700nm) has the longest wavelength and the lowest energy level meaning that the photons cannot penetrate into the depth of dense cultures (Mohsenpour & Willoughby, 2013). The red absorption maximum of Chl *a* is observed at 678 nm for *A. platensis*, while the absorption peak for phycocyanin is at 622 nm (Vonshak, 2014). The preliminary results also indicated that no light penetrates past 5 cm depth of *A. platensis* cultures when grown at a yield of 1.5 g L⁻¹ (data are not shown). This clearly means that 16 cm of the control cultures was in complete darkness at all time. As discussed in 3.3.3, blue LSCs were lighter than the red LSCs in terms of dye concentrations and most of the sunlight passes through the blue LSCs rather than being shifted to the desired blue wavelength. This is the most possible reason that there was no significant difference between the productivity of *A. platensis* cultures with blue LSCs and control.

There are two ways of expressing the amount of light entering the system through LSCs; 1) From an energy perspective which gives an overall picture of the total amount of light entering the system, 2) The second way indicates how often the algal cells will pass an edge of LSCs receiving the peak intensity of light by taking mixing rate into account. From the energy perspective, the total amount of PAR available for *A. platensis* cells at the depth of each pond emitting from four red LSCs is 34 μmol photons s⁻¹ (see 3.3.3 for detailed calculations). In other words, using red LSCs in each pond bring about 34 μmol photons s⁻¹ more light to the depth of *A. platensis* cultures. This means injecting 34 μmol photons s⁻¹ deep into the *A. platensis* culture where it would otherwise be in full darkness. This helps move the light from the photosaturated surface to the depth of the culture.

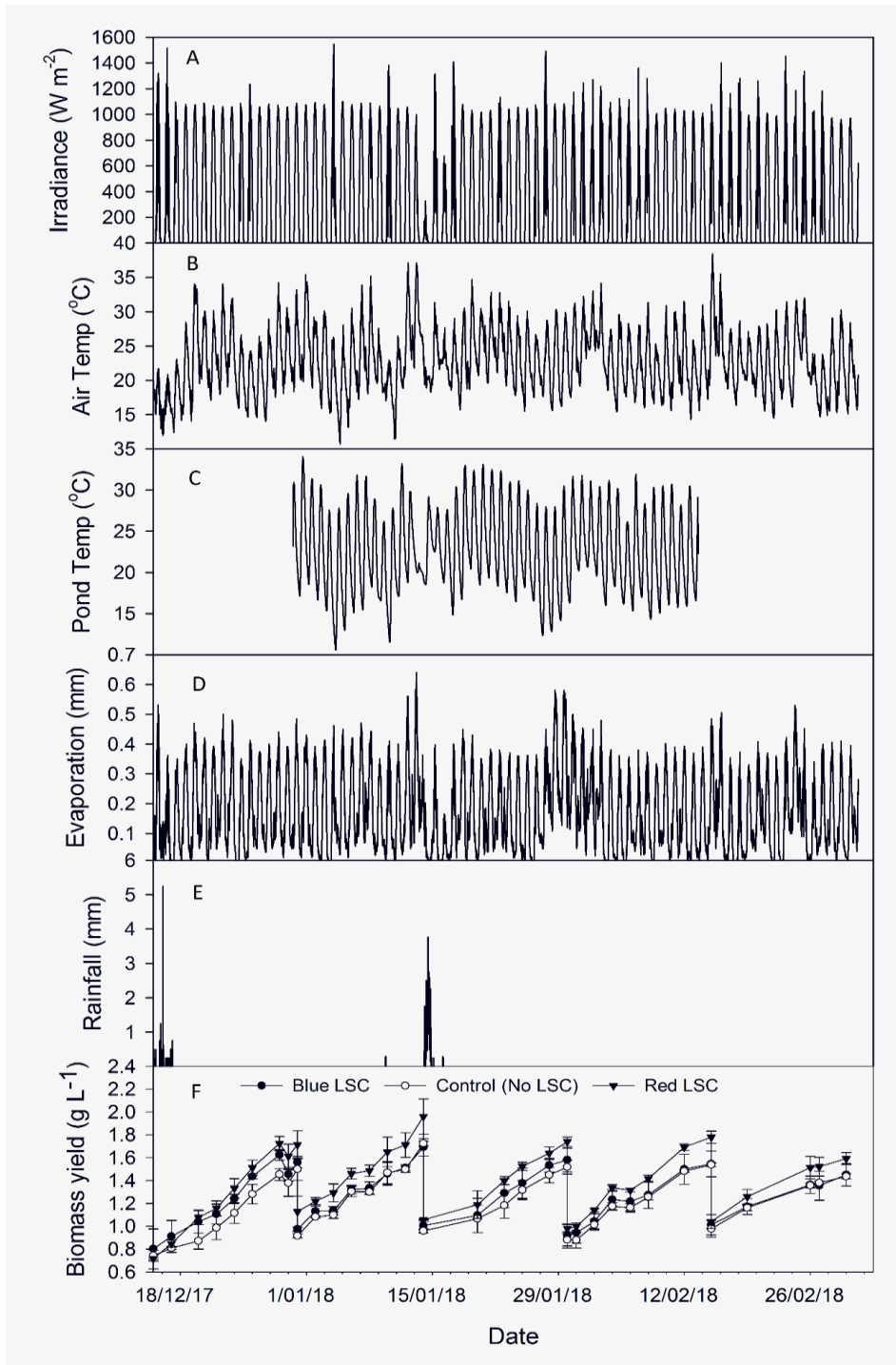


Figure 3-3. Growth of *A. platensis* culture during 75 days. A) Solar irradiance, B) air temperature, C) *ponds temperature D) evaporation, E) rainfall, F) growth rate. The cultures were run in raceway ponds outdoors with three treatments as red and blue LSCs and the control culture with no panel. The experiment was done from 15th of Dec 2017 to 3rd of March 2018 in a semi-continuous mode starting with a batch culture followed by four harvests. *Culture temperatures of all ponds were not significantly different, and the average values are shown here.

Moreover, based on the mixing rate (11 cm s^{-1}) and the thickness of the LSCs (3 mm), *A. platensis* cells pass an edge of a LSC in 27 ms. Considering PAR emitting from an edge of a red LSC ($110 \text{ W m}^{-2} / 506 \mu\text{mol m}^{-2} \text{ s}^{-1}$, Table 3-1), *A. platensis* cells receive around $506 \mu\text{mol m}^{-2} \text{ s}^{-1}$ in 27 ms from each edge (it should be noted that there are 10 edges in each LSC). A surface of each red LSC also emits $60 \text{ W m}^{-2} / 276 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and considering 2.4 cm as the average width of the surface (the width of one piece of a forked LSC, Figure 3-2B) and mixing rate of 11 cm s^{-1} , *A. platensis* cells received $276 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in 218 ms when they pass each surface of a red LSC. In other words, it can be said that *A. platensis* cells with red LSCs received brief bursts of light with different intensities for durations less than a second inside the cultures while there was total darkness for the cultures without LSCs (control cultures).

This should be noted that the outdoor raceway ponds in this study had a more uniform and turbulent mixing pattern (more vertical mixing) compared to large scale raceway ponds and thus, more *Arthrospira* cells could be exposed to sunlight. But even with such a turbulent mixing, *Arthrospira* cultures with red LSCs had a significantly higher biomass and phycocyanin productivity compared to control with no LSCs. It clearly demonstrated that there is a high possibility of higher biomass productivity when using red LSCs in a larger raceway pond where the uniform mixing is almost impossible. In large raceway ponds, there is only vertical and turbulent mixing in the vicinity of paddle wheels followed by a long laminar flow along the channel which leaves the most of microalgal cells in darkness. The region of laminar flow increases with longer channels where there is little vertical mixing. That means cells at a lower depth receive very little light while cells on the surface are photoinhibited (Borowitzka & Vonshak, 2017).

The P_{\max} and I_k levels of *Arthrospira* are highly dependent on high or low light intensities that also affects the photoadaptation of the cells (Vonshak, 1997). Vonshak (1997) reported the light saturation of three *Arthrospira* strains from 115-165 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Having approximately further 34 $\mu\text{mol photons s}^{-1}$ at the depth of *A. platensis* cultures with red LSCs means that algal cells received more PAR light as well as having pulses from red LSCs edges inside the culture which led to 27% higher biomass productivity in cultures with red LSCs.

The areal productivity obtained in this study is similar to productivity achieved by Earthrise Farms for mass culture of *Arthrospira*. The average biomass productivity of 8.2 $\text{g m}^{-2} \text{d}^{-1}$ (19 $\text{kg m}^{-2} \text{yr}^{-1}$) has been reported for the mass culture of *Arthrospira* by Earthrise Farms in California as annual average areal production in 1991 (Belay, 1997) which is comparable with the productivity of 12.2 $\text{g m}^{-2} \text{d}^{-1}$ achieved in this study under red LSCs Table 3-3. Richmond and Grobbelaar (1986) also grew *Spirulina* outdoors in a glass-fibre oval container with a surface area of 1.78 m^2 and resulted in the biomass productivity of 10 $\text{g m}^{-2} \text{d}^{-1}$. Further, Magro et al. (2018) reported biomass productivity of 11.75 $\text{g m}^{-2} \text{d}^{-1}$ for *Spirulina platensis* in 0.2 m^2 raceway pond with a mixing rate of 10 cm s^{-1} . Different large scale cultivations of *Spirulina* studies have also reported the biomass productivity of 10-20 $\text{g m}^{-2} \text{d}^{-1}$ for mass production of *Spirulina* (Ayala et al., 1988; Jiménez et al., 2003; Olgún et al., 2003; Pushparaj et al., 1997). However, using CO_2 for algal cultivation could potentially increase biomass production (Raeesossadati et al., 2014; Raeesossadati et al., 2015).

There are also some studies on growing cyanobacteria in closed photobioreactors using filters to shift the light into different wavelengths for algal cells. Mohsenpour and Willoughby (2013) used photobioreactors made of red LSCs to grow *Gloeotheca membranacea* and found a higher growth rate for *G.membranacea* under red

LSCs (Mohsenpour & Willoughby, 2013). Wang et al. (2007) also used different LED sources with different wavelengths to grow *Arthrospira* and found that red LEDs had the maximum specific growth rate of 0.4 day^{-1} under $3000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and the best economic efficiency of energy to biomass. They concluded that providing *Arthrospira* cells with red LEDs can increase the economic efficiency of energy to biomass from 70 to 110 (g L^{-1}) $\text{\$}^{-1}$ (Wang et al., 2007). Further, Markou (2014) reported the highest biomass productivity of $30.7 \text{ mg L}^{-1} \text{ d}^{-1}$ *Arthrospira platensis* when grown under red LED light which was two times higher than that compared to control with white LEDs. Similar studies on the effects of shifting light on green microalgae and diatoms can also be found in the literature (Burak et al., 2019; Fu et al., 2017). It has to be noted that the aforementioned studies were all used passive light diffusing systems. However, an active light delivering system has been used in the current study. Passive light systems do not increase the number of photons but allow a proportion of light to go through algal cultures by shifting it using filters or light sources with different wavelengths. On the other hand, the active light diffusing system could deliver a proportion of sunlight by delivering it to the depth of algal cultures using light delivering systems.

Table 3-2. Biomass yield, biomass and phycocyanin productivity, phycocyanin content, Chl *a* and biochemical composition of *Arthrospira* cultures with red and blue LSCs and control. Data are value \pm SD (One-way ANOVA $P < 0.05$, $n = 3$). Different letters show a significant difference in each row.

<i>Parameter</i>	<i>Red LSCs</i>	<i>Blue LSCs</i>	<i>Control</i>
* <i>Biomass yield (g L⁻¹)</i>	1.77 \pm 0.01 ^a	1.59 \pm 0.06 ^b	1.53 \pm 0.002 ^b
<i>Volumetric productivity (mg L⁻¹ d⁻¹)</i>	57 \pm 3.2 ^a	46 \pm 5 ^b	45 \pm 1 ^b
<i>Areal productivity (g m⁻² d⁻¹)</i>	12.2 ^a	9.89 ^b	9.67 ^b
<i>Max phycocyanin productivity (mg L⁻¹ d⁻¹)</i>	8.49 \pm 0.9 ^a	7.42 \pm 0.8 ^a	5.90 \pm 0.09 ^b
<i>Max C-Phycocyanin (mg L⁻¹)</i>	136 \pm 12 ^a	141 \pm 3.4 ^a	114 \pm 5.3 ^b
<i>Chl a (mg gbiomass⁻¹)</i>	11.7 \pm 0.3 ^a	11.4 \pm 0.6 ^a	10.8 \pm 0.2 ^a
** <i>Lipid (mg gbiomass⁻¹)</i>	107 \pm 1.8 ^a	120 \pm 5.3 ^a	101 \pm 11.5 ^a
** <i>Protein (mg gbiomass⁻¹)</i>	450 \pm 3.4 ^a	475 \pm 22 ^a	479 \pm 11.5 ^a

* This is the average of the maximum yields at the end of each growth period (batch and semi-continuous) before.

**The data are the average of values in batch and semi-continuous runs.

Table 3-3. Biomass productivities of *Spirulina* grown outdoors in open raceway ponds.

Culture volume (L)	Culture period (months)	Areal productivity (g m ⁻² d ⁻¹)	Volumetric productivity (g L ⁻¹ d ⁻¹)	Species	Location	References
750	+3	15-27	0.06-0.18	<i>Spirulina platensis</i>	Israel	(Richmond et al., 1990)
-	12 ^a	8.2	-	<i>Spirulina platensis</i>	USA (California)	(Belay, 1997)
13200-19800	12	14.5 (5.8–24.2)	0.03–0.12	<i>Spirulina platensis</i>	Antofagasta, Chile	(Ayala et al., 1988)
282	+3	14.47	0.183	<i>Spirulina platensis</i>	Italy	(Pushparaj et al., 1997)
135000	+3	2–17	0.006–0.07	<i>Spirulina</i> sp.	Spain	(Jiménez et al., 2003)
-	+3	9–13	-	<i>Spirulina</i> sp.	Mexico	(Olguín et al., 2003)
21.5	+2	12.2	0.057	<i>Arthrospira platensis</i>	Perth, Western Australia	Current study

a This figure is the annual average productivity at Earthrise farms with the growth period of 8 months.

3.4.2 Phycocyanin productivity

The highest commercial potential of *Arthrospira* is from phycocyanin as a food pigment (Borowitzka, 2013a). The phycocyanin molecules can store energy by absorbing visible light that could not be utilized by chlorophyll molecules. This energy will then be used by chlorophyll *a* in photosynthetic reaction centre (Vonshak, 1997). However, *Arthrospira* cells can also store this pigment in cytoplasmic granules for storing nitrogen (Vonshak, 1997). One of the reasons for mass *A. platensis* cultivation is to produce phycocyanin (Borowitzka, 2013a). Therefore, obtaining high phycocyanin productivity is of great importance in mass cultivation of *Arthrospira*. In this study, the maximum phycocyanin productivity in *Arthrospira* cultures with red LSCs was $8.49 \pm 0.9 \text{ mg L}^{-1} \text{ d}^{-1}$, which was 14% and 44% higher than that in cultures

with blue LSCs and control cultures (One-way ANOVA $P < 0.050$) (Table 3-2). Based on the results achieved in this study, the quality and quantity of light had a pivotal role in phycocyanin production by using different LSCs. The red LSCs had a large photon emission in phycocyanin absorption peak of 622nm (Figure 3-2A). Furthermore, the higher phycocyanin productivity for cultures with red LSCs could be due to having more light irradiance in the red region at the depth of *A. platensis* cultures (see sections 3.3.3 and 3.4.1).

The increase in phycocyanin productivity as a result of using red LSCs is significantly (>40%) higher than the value Mohsenpour and Willoughby (2013) obtained for *G. membranacea*. They reported the phycobilins production increase by only 2.3% under red LSCs as a light shifter for growing cyanobacteria *G. membranacea* in a photobioreactor. Walter et al. (2011) also used chromatic light for growing *Spirulina platensis* and resulted that using red light leads to higher purity of phycocyanin production.

In addition, the phycocyanin content of *A. platensis* was 136 mg L^{-1} ($77 \text{ mg g biomass}^{-1}$) and 141 mg L^{-1} ($89 \text{ mg g biomass}^{-1}$) under red and blue LSCs, respectively which is comparable to the results in the literature. Prates et al. (2018) resulted in a phycocyanin content of $58.56 \text{ mg g biomass}^{-1}$ under red LEDs. Lima et al. (2018) showed 121.01 mg g^{-1} phycocyanin in dry biomass of *A. platensis* cultured under illumination of red LEDs. In another study, Wicaksono et al. (2019) investigated the effect of red and blue light on phycocyanin production of *Spirulina platensis* and resulted in the highest production of phycocyanin under red light (5.1 mg g^{-1} biomass).

The importance of the phycocyanin results in this study is that applying red LSCs to a large-scale *A. platensis* culture to mass produce phycocyanin can bring about a significant increase in phycocyanin productivity while decreasing the plant size

required for the cultivation down to 44%. Capital expenses are the major costs for any commercial algae production (Tanticharoen et al., 1993). A 44% increase in phycocyanin productivity using red LSCs can significantly reduce the capital and operational costs of phycocyanin production. However, a detailed techno-economic analysis is required for assessing the economics of this technology for mass production of phycocyanin (A detailed discussion is in section 3.4.6).

3.4.3 Maximum quantum yield of photosystem II (F_v/F_m')

The effect of LSCs on the effective quantum efficiency of PSII photochemistry in outdoor *A. platensis* cultures was studied by measuring the diurnal changes in the F_v/F_m' of cultures in three different days of the batch cultivation period. The *A. platensis* tested showed the highest F_v/F_m' during the night period (before sunrise and after sunset at night) (Figure 3-4B). *A. platensis* cultures were stressed when sunlight irradiance increased during the day. *A. platensis* F_v/F_m' reduced by almost 40% at midday where the highest photoinhibition can occur (sunlight irradiance = 1000 W m⁻², Figure 3-4A). The F_v/F_m' recovered from midday to late night as solar irradiance was reduced. The results showed that there was no significant difference between *A. platensis* F_v/F_m' using red LSCs, blue LSCs, and control (One-way ANOVA, $P > 0.05$). This indicates that using LSCs have neither a negative nor positive effect on the F_v/F_m' of *A. platensis*. Photoinhibition is one of the main issues in outdoor *Arthrospira* cultures indicated by a decrease in the maximum quantum efficiency of PSII photochemistry (F_v/F_m) (Vonshak et al., 2014). The *A. platensis* cultures grown outdoors here clearly were photoinhibited as F_v/F_m reduced by over 40%. They also reported a 35% decrease in F_v/F_m of *A. platensis* grown under outdoor conditions in 2.5 m² ponds at midday (Vonshak et al., 2014). Torzillo et al. (1996) also reported

52% reduction in F_v/F_m of *Arthrospira* cultured outdoors in photobioreactors with the volume of 51 L.

The irradiance of light emitted from red and blue LSCs inside the *Arthrospira* cultures as well as PAR are shown in Figure 3-4A. The irradiance emitted from red LSCs (between 600-700 nm) is significantly higher than that in the blue LSCs (between 400-500 nm). This difference is due to a higher concentration of organic dyes in red commercial LSCs. In this study, blue and red LSCs were designed in such a way to cover about 25% of the total pond surface area meaning that around 75% of the culture was still open to full sunlight exposure. This could be the reason for low values of F_v'/F_m' of *Arthrospira* cells similar to the cultures in control with no LSCs (Figure 3-4B). However, it may be possible to improve F_v'/F_m' of the cells by changing the design of the LSCs used in the ponds. If the surface area of ponds covered by LSCs is changed from 25% to 50% or 100% then there is a possibility of improving F_v'/F_m' of *A. platensis* cells by reducing the exposure of cells to the full spectrum of light. However, further studies are required to investigate the possibility of reducing the overall photoinhibition of the microalgal culture using LSCs.

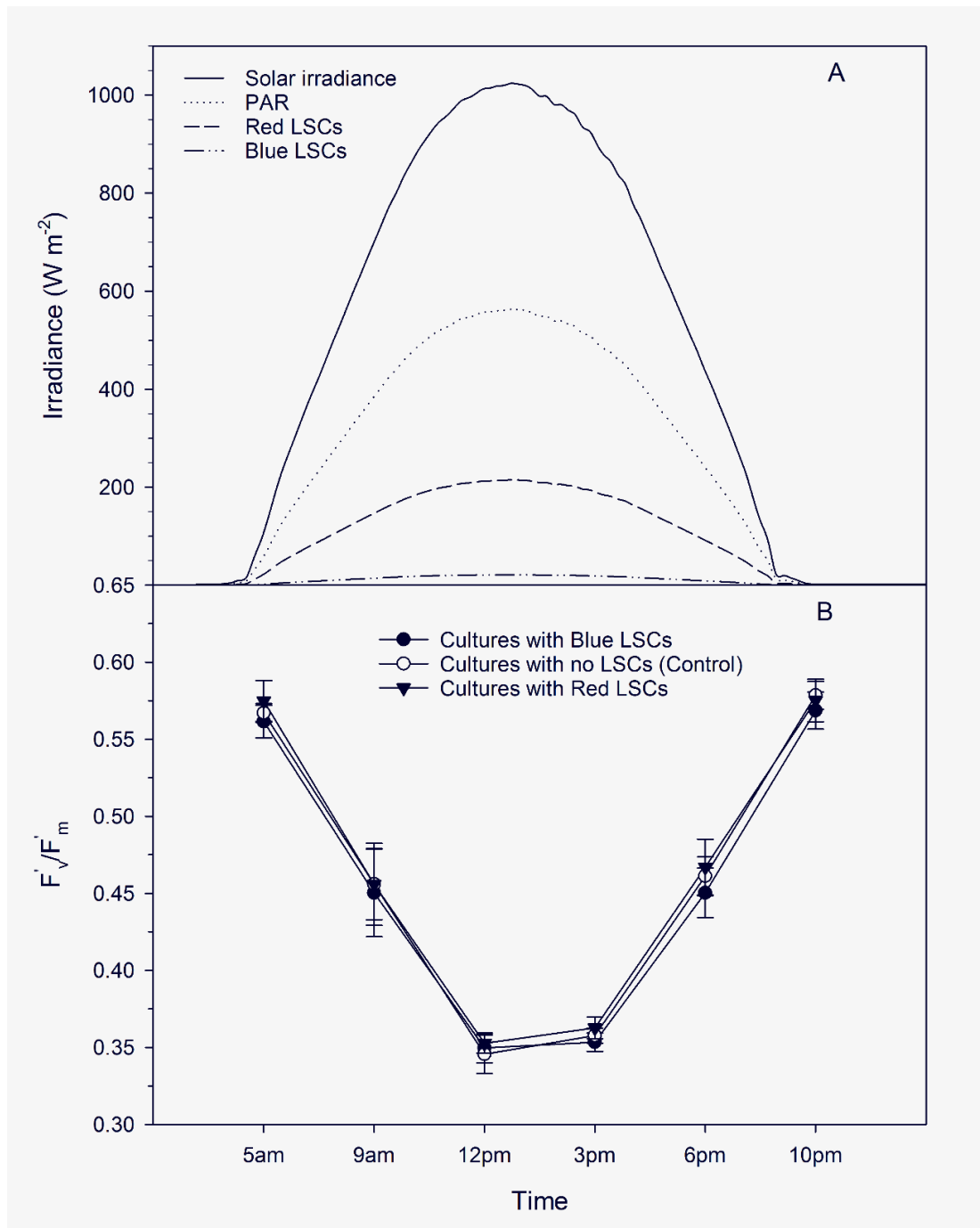


Figure 3-4. A) Solar irradiance, photosynthetic active radiation (PAR), and irradiance of light emitted from blue and red LSCs in the range of 400–500 nm and 600–800 nm, respectively. B) Diurnal changes in the maximal quantum efficiency of PSII photochemistry in *A. platensis* grown outdoors by using red and blue LSCs as a light delivering system. The data were obtained in day 5, 10 and, 14 of the batch cultivations periods.

3.4.4 Chlorophyll 'a' and Biochemical content under different light conditions

Arthrospira is considered as one of the algal species with the highest chlorophyll *a* content in nature (Borowitzka, 2013a). Chlorophyll *a* content of *A. platensis* under conditions tested here was not significantly different in cultures with red LSCs, blue LSCs, and control (One-way ANOVA $P > 0.05$) (Table 2). However, chlorophyll *a* content of *A. platensis* cultures obtained here (11.7 mg gbiomass⁻¹, Table 3-2) is more than 100% higher than that in Detweiler et al. (2015) study which resulted in Chl *a* content of 4.35 mg gbiomass⁻¹ for *A. platensis* cultured in 2 L flask underneath red LSCs which were used as a light shifter. The higher cellular Chl *a* content is likely due to having fewer photons with wavelengths corresponding to Chl *a* absorption peak. It has been shown that growing microalgae in low light lead to higher Chl *a* for delivering the energy needed for photosynthetic reaction centres (Mohanty et al., 1997).

Lipid and protein contents of *A. platensis* cultures in different treatments have also been determined (Figure 3-5). *Arthrospira platensis* lipid contents were not significantly different when using blue LSCs (120 mg gbiomass⁻¹), red LSCs (107 mg gbiomass⁻¹) and control (101 mg gbiomass⁻¹) (One-way ANOVA $P > 0.05$, Figure 3-5). The protein content of *A. platensis* when grown using red and blue LSCs and control, was not also significantly different (One-way ANOVA $P > 0.05$, Figure 3-5) although phycocyanin contents were significantly different between treatments as mentioned in section 3.4.2. Phycobiliproteins are divided into four main groups including 1) phycoerythrin (PE), 2) allophycocyanin (APC), 3) phycocyanin (PC) and 4) phycoerythrocyanin (PEC) (Chakdar & Pabbi, 2015). In this study, phycocyanin was the main aim but the total protein content was also analysed. Considering that the total protein content includes aforementioned four phycobiliproteins, having similar total

protein contents and different phycocyanin contents of *A. platensis* between treatments might be due to having different PE, APC or PEC contents in *A. platensis* cells. In other words, *A. platensis* cells preferably produced other accessory pigments such as phycocyanin under red and blue LSCs. Phycocyanin is an accessory pigment by which the light energy is transferred to chlorophyll *a* and also a nitrogen storage compound (Vonshak, 1997).

The values obtained for protein contents in this study (Figure 3-5) are comparable with the results of Prates et al. (2018) which found 60% protein content in *Arthrospira* when grown under red and blue LED lights and Zhang et al. (2015) that reported protein content of %50 for *Arthrospira* cultured in an outdoor bench-scale bioreactor.

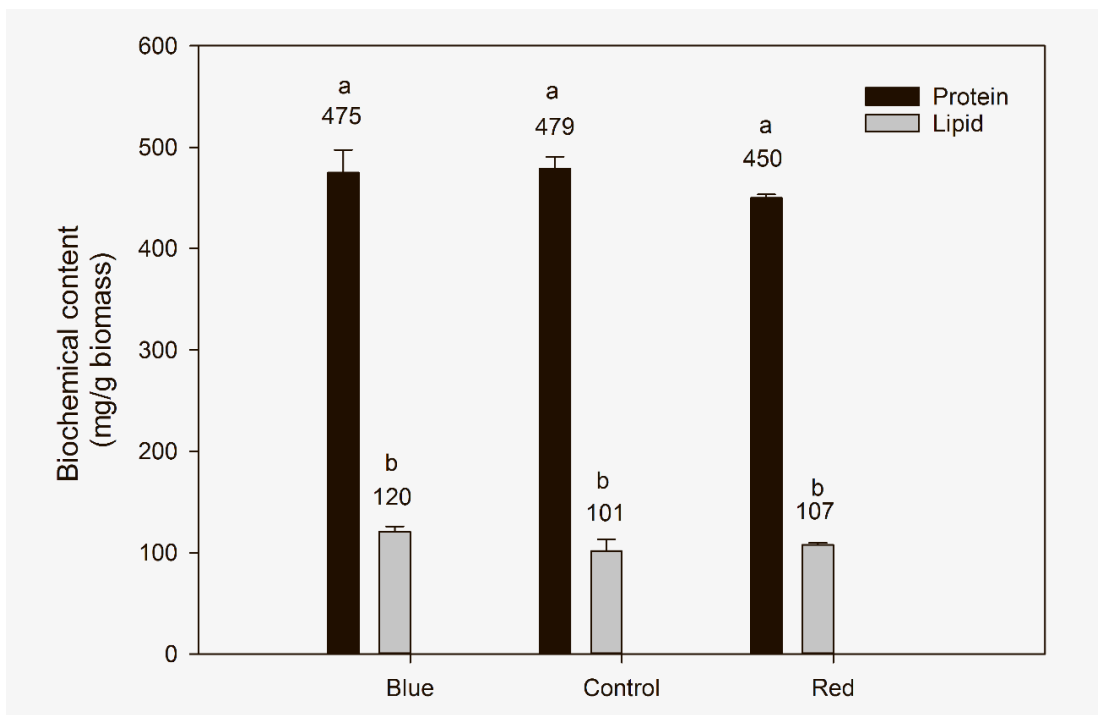


Figure 3-5. Average protein and lipid contents of *A. platensis* under red and blue luminescent solar concentrators. Numbers on bars are the corresponding content values. A capital letter shows a significant difference.

3.4.5 Fouling

Bio-fouling is potentially the greatest disadvantage of using LSCs as a result of the accumulation of *A. platensis* cells on the surfaces and edges of LSCs (Figure 3-6). LSCs emit light from the edges and surfaces and as there is no other light source at the depth of *A. platensis* cultures, the cells are attracted to the surfaces and edges of the LSCs. However, as it is shown in Figure 3-6, fouling was negligible since approximately 5% of the LSCs showed bio-fouling by *A. platensis* cells during the 75 days cultivation period.



Figure 3-6. *Arthrospira platensis* biofouling on a luminescent solar concentrator during 75 days of the cultivation period.

3.4.6 Significance of the study

There is no doubt that paddle wheel driven raceway ponds are the preferred commercial cultivation system for mass production of *Arthrospira*. One of the main obstacles for culturing microalgae in large scale raceway ponds is the availability of light at the depth of the cultures resulting in low biomass productivity. Therefore, increasing light irradiance at the depth of raceway pond cultures by using an efficient light delivering system would enhance the biomass productivity as well as reducing the capital costs. The results of this study clearly showed that the use of red LSCs in raceway ponds with the proposed design (see sections 3.3.2 and 3.3.3) can significantly increase both biomass and phycocyanin productivities of *A. platensis*. The main advantage of using LSCs with such a design is the potential capability of applying them to a large-scale paddle wheel driven raceway pond. As mentioned earlier, using red LSCs increased phycocyanin productivity of *A. platensis* by 44%. This is achieved by delivering a suitable wavelength of light into the depth of algal cultures in raceway ponds. The active light delivering system used in this study has the advantage of an increasing number of photons as well as shifting light to suitable spectra for algal growth. Therefore, for the same phycocyanin productivity achieved in this study, there is an advantage of reducing the cultivation area by 44%. For instance, based on the phycocyanin productivity achieved in this study, there is going to be an annual phycocyanin production of approximately 115 t if the size of the plant is 25 ha with no use of red LSCs. However, if red LSCs are used in the same plant to produce 115 t of phycocyanin per year, then there is going to be 44% less plant size and thus, fewer ponds, equipment, facilities, etc. In other words, using red LSCs could decrease the capital costs or increase phycocyanin productivity of the same size plant by 44%. It

has to be noted that applying red LSCs would increase the capital costs, but it is way lower than the decreased costs by 44%.

3.5 Conclusions

Red LSCs enhanced biomass and phycocyanin productivity of *A. platensis* cultivated in outdoor paddle wheel driven raceway ponds. Chlorophyll *a* and protein contents of *A. platensis* cultures were statistically the same for all treatments. Using LSCs for microalgae cultures in raceway open ponds can significantly increase the light availability to the microalgal cells at the depth of the cultures and bring forward higher biomass and phycocyanin productivity. However, there need to be further studies at a larger scale as well as detailed techno-economics and life cycle analysis to find the true potential of these LSCs for mass cultivation of *Arthrospira*.

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Link to the next Chapter

The results of Chapter 3 showed that red LSCs increased biomass and phycocyanin productivity of *Arthrospira* significantly. *Arthrospira* contains chlorophyll *a* and phycobilins. The main motivation of the next chapter was to investigate the effect of red LSCs on the growth of an alga containing chlorophyll *a* and *b*. Therefore, *Scenedesmus* sp. was chosen as a test species. Not only this alga contains Chl *a* and *b* but also has the biotechnological applications. *Scenedesmus* sp. can be used to treat waste effluents as well as animal feed. This study would give a better indication of red LSCs potential. As blue LSCs did not improve the growth of *Arthrospira*, only red LSCs were used in the next study for *Scenedesmus* sp. cultures.

Chapter 4

**The effect of red luminescent solar concentrators on the growth of
Scenedesmus sp.**

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**Red luminescent solar concentrators to enhance *Scenedesmus* sp.
Biomass productivity**

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Mohammadjavad Raeisossadati (70%)	Designed and conducted all experiments. Analysed and processed the data and wrote the manuscript.
Navid. R. Moheimani (15%)	Proposed the concept, assisted in the data analysis and revision of the manuscript.
David Parlevliet (15%)	Proposed the concept, assisted in the data analysis and revision of the manuscript.

Supervisors confirmation

I hereby confirm and certify the authorship of this manuscript and the contribution of the first author.

Name	Signature	Date
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4 Red luminescent solar concentrators to enhance *Scenedesmus* sp. biomass productivity

4.1 Abstract

Increasing biomass productivity of an outdoor mass microalgal culture is the main aim of any large-scale algal producer. Under nutrient enriched conditions, light is the primary limits to growth of any microalgae. This normally results in a high level of photolimitation when paddle wheel driven raceway ponds are used as no light can penetrate beyond the top few centimetres of the culture. To achieve high biomass productivity, there is a need to develop an efficient system to deliver light into the depth of microalgal cultures in raceway ponds. We investigated red luminescent solar concentrators (LSCs) in outdoor raceway ponds to downgrade the sunlight, re-emit and, deliver it into the depth of *Scenedesmus* sp. cultures operated at 21 cm depth. Biomass productivity of *Scenedesmus* sp. significantly increased by 18.5% when red LSCs were used ($9.4 \text{ g m}^{-2} \text{ d}^{-1}$). Protein and lipid contents of *Scenedesmus* sp. cultures with red LSCs were also 17.5% and 10% higher than those in control with no LSCs. Therefore, if the aim is biomass production for animal feed, there would be 18.5% less cultivation area for generating the same biomass productivity using red LSCs. This can lead to a significant reduction in the cost of biomass production.

4.2 Introduction

Enhancing microalgal biomass productivity is the main aim of any algal farmer (Benemann, 2008). The top three limiting factors for any mass microalgal cultures are light, temperature and, nutrient (Vonshak et al., 2014). Raceway open ponds are the best cultivation systems for mass production of specific species such as *Scenedesmus* (Borowitzka & Moheimani, 2013) which is a suitable species for wastewater treatment (Nwoba et al., 2017; Raeisossadati et al., 2019b) and can be used as animal feed

(Duong et al., 2015; Vidyashankar et al., 2015). In temperate regions and under nutrient replete conditions, large-scale microalgal cultures in paddle wheel driven raceway ponds are light limited (Vonshak et al., 2014). Raceway ponds are normally operated at the depth higher than 20 cm which only the top five centimeters of a dense algal culture in a raceway pond would receive light (Moheimani & Borowitzka, 2007). This means that the rest of the culture would be in complete darkness (Moheimani & Borowitzka, 2007). Further, a high irradiance received on the surface of the algal culture results in high photoinhibition while the cells at the depth of the pond are photo-limited (Tredici, 2010). Hence, increasing the light irradiance at the depth of a microalgae culture in a raceway pond can significantly increase biomass productivity (Raeisossadati et al., 2019a).

Temporal and spatial light distribution systems are the two main systems to increase light availability to microalgal cells (Raeisossadati et al., 2019a). The temporal light diffusing system provides pulsed photons with high intensity in a short period of time (Laws et al., 1983). Applying a temporal light diffusing system in a microalgal culture requires a turbulent mixing facility to produce pulses with high irradiance which is not practical and feasible (Tredici, 2010). Spatial light distribution system normally requires a system in which light is delivered to microalgal cells (Dye et al., 2011). Different spatial light delivering systems are optical fibers (Xue et al., 2013), trough systems (Fernández-García et al., 2010), parabolic dishes (Chiang et al., 2016), green solar collectors (Zijffers et al., 2008) and, luminescent solar concentrators (LSCs) (Raeisossadati et al., 2019a).

Using LSCs for microalgal cultivation do not require a solar tracking system which results in less cost compared to other systems (Raeisossadati et al., 2019a). Luminescent particles including organic dyes or quantum dots are the main

constituents of a LSC (Debije & Verbunt, 2012). Luminescent particles are normally embedded in polymethyl methacrylate material and absorb light hitting the surface of a LSC. The absorbed light is then reflected internally and emitted at a longer wavelength from the edges of a LSC (Corrado et al., 2013). There have been some small scale studies on using LSCs in closed algal photobioreactors (Delavari Amrei et al., 2014; Mohsenpour & Willoughby, 2013; Sforza et al., 2015; Wondraczek et al., 2013) all of which have used LSCs as a light shifter. However, to date, to the best of our knowledge, no studies have been carried out on using LSCs as light guides for algal growth in a raceway pond.

The estimated cost of biomass production from *Scenedesmus* is around US\$7.52 (based on the inflation rate in 2018) (Borowitzka, 2013). A significant increase in biomass productivity is one way to reduce the cost of *Scenedesmus* production or any other algae. Increasing light availability to algal cells at the depth of a raceway pond by using an efficient light delivering system can be a way to achieve such this goal.

In this study, we investigated the use of red luminescent solar concentrators with a novel design as a way to enhance the biomass productivity of *Scenedesmus* sp. We choose to use *Scenedesmus* for this study as this alga a) can be grown in raceway ponds (Borowitzka & Moheimani, 2013), b) is a suitable candidate to treat various waste streams (Ayre et al., 2017), c) can be sold as a source of animal (Moheimani et al., 2018) or aquaculture (Vizcaíno et al., 2014) feed and, d) is a candidate for mass production of lutein (Sánchez et al., 2008). Our approach is increasing the number of red photons available to the microalgal cells at the depth of outdoor raceway ponds by using commercially available red LSCs. To the best of author's knowledge, this is the first time that the use of LSCs as a light guide was evaluated for growth of any microalga in outdoor paddle wheel driven raceway ponds.

4.3 Materials and methods

4.3.1 Microorganism and culture medium

The freshwater green microalga *Scenedesmus* sp. used in this study was provided by Algal Culture Collection Centre, Murdoch University. This species was cultured in outdoor 0.1 m² paddle wheel driven raceway ponds with the dimensions of 13 × 26 × 80 cm (W×H×L) and final culture volume of 21.5 L (Figure 4-1). The culture was mixed by a four-blade paddle wheel and operated at 21 cm resulted in a mixing rate of 11 cm s⁻¹. The Bold Basal culture medium (Stein et al., 1973) was used for all cultures.

4.3.2 Cultivation design

The raceway ponds used in this study were equipped with red LSCs (Fig 1). Red LSCs were purchased from Evonic company (<https://www.plexiglas-shop.com/Home/>) as PLEXIGLAS fluorescent red sheets with a size of 300 × 200 × 3.00 mm. StellarNet Inc spectrometer, (USA) was used for analyses of the LSCs. The LSCs were installed on the edge of raceway ponds in order to have 200 mm of the panel inside *Scenedesmus* culture and 100 mm of the panel out of the culture facing the sun to collect visible and diffuse light from the sunlight, downgrade and, transfer it to the depth of *Scenedesmus* cultures (Figure 4-1). The bottom part of LSCs inside the *Scenedesmus* cultures was also laser-cut to have sufficient surface area in order to increase the irradiance. The treatments were *Scenedesmus* cultures with red LSCs and control with no LSCs. Each treatment was conducted in five replicates (Figure 4-1C).

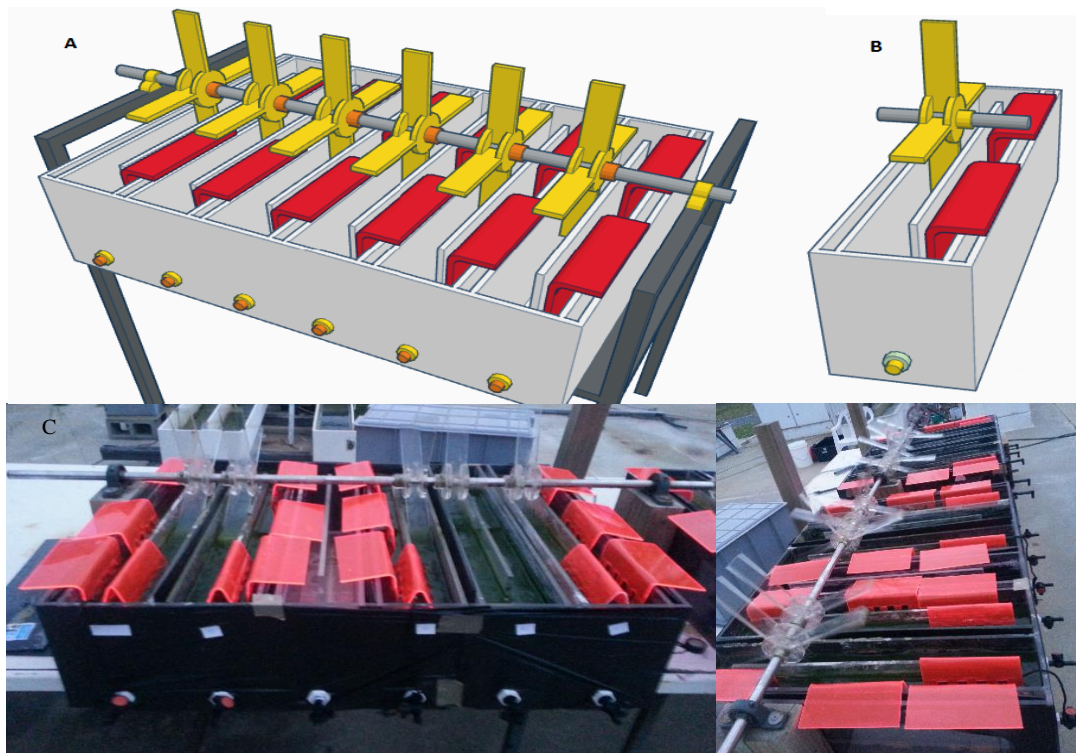


Figure 4-1. A) Schematic, B) A singular and C) structure view of raceway ponds equipped with red luminescent solar concentrators with the culture volume of 21.5L for each pond. In this study ten raceway ponds were used for two treatments; *Scenedesmus* sp. cultures with red LSCs and control with no LSCs in five replicates. The experiment was run for 15 days.

4.3.3 Growth measurement

The experiment was run in a batch mode for 15 days. The harvested biomass during the culture period was used to measure biomass productivity and biochemical extractions. Dry weight was determined using the method previously described by Moheimani et al. (2013). The temperature of the cultures was also measured using a Tinytag TG-4100 (Gemini Data Loggers, UK) temperature loggers during the culture period. The pH and dissolved oxygen concentration of the cultures were also measured using Mettler-Toledo AG (Switzerland).

4.3.4 Protein, lipid, carbohydrate and Chlorophyll determination

The protein and lipid contents of biomass were determined using the methods previously described by (Moheimani et al., 2013). The chlorophyll extraction method of Jeffrey and Humphrey was used for the chlorophyll extraction (Moheimani et al., 2013). Carbohydrate extraction was also carried out using the method of (Kochert, 1978).

4.3.5 Statistical analysis

The statistical analyses were carried out using two tailed t-test by Sigmaplot 14.0 software to determine the significant difference between treatments ($P < 0.05$).

4.4 Results and discussions

As mentioned earlier, this is the first study evaluating the effectiveness of red LSCs to increase light availability into the depth of algal culture. Clearly, the main aim is to enhance the biomass productivity of the algal culture. In this study, we selected *Scenedesmus* sp. for mass production in raceway open ponds as well as assessing the potential effect of increased red light in the culture depth due to LSCs installation on biochemical composition of this alga.

4.4.1 Red luminescent solar concentrator specifications

The spectrometric analysis of red LSCs under sunlight are summarised in Table 4-1 and Figure 4-2. As it is shown in Figure 4-2, red LSCs (27% transmission) reduced the total amount of light transmitted through them and had a peak at 650 nm which is desirable for microalgae growth. Red LSCs converts a lot of the higher energy photons to red photons but is largely transparent to IR.

The number of photons emitting from red LSCs are calculated as the following:
 Considering 17 cm of each red LSC inside the algal culture (Figure 4-3), the corresponding surface area of all edges (S) inside the algal culture is the length of all edges (1900 mm) (10 long edges and 5 small edges, Figure 4-3) multiplied by the thickness of a red LSC edge (3 mm) ($S = 1900 \text{ mm} \times 3 \text{ mm} = 5700 \text{ mm}^2 = 5700 \times 10^{-6} \text{ m}^2$ Equation 4-1):

$$S = 1900 \text{ mm} \times 3 \text{ mm} = 5700 \text{ mm}^2 = 5700 \times 10^{-6} \text{ m}^2 \quad \text{Equation 4-1}$$

Then, the total photosynthetic active radiation (PAR) emitting from all edges (Figure 4-3) of a red LSC ($T_{\text{PAR, Red}}$) equals average PAR emitting from edges of a red LSC (Table 4-1) multiplied by the surface area of all edges inside the algal culture ($T_{\text{PAR, Red}} = 110 \text{ Wm}^{-2} \times 5700 \times 10^{-6} \text{ m}^2 = 0.627 \text{ W}$ Equation 4-2):

$$(T_{\text{PAR, Red}}) = 110 \text{ Wm}^{-2} \times 5700 \times 10^{-6} \text{ m}^2 = 0.627 \text{ W} \quad \text{Equation 4-2}$$

Therefore, having four red LSCs in each pond results in 2.51 W ($0.627 \text{ W} \times 4$) or 11.53 $\mu\text{mole photons s}^{-1}$ (2.51×4.6 (conversion factor for W m^{-2} to $\mu\text{mole photons m}^{-2} \text{ s}^{-1}$)) of additional light inside the algal culture at significant depth. Having the same approach for surfaces (Figure 4-3) of each red LSC (Table 4-1) inside the algal culture would result in 4.90 W / 22.52 $\mu\text{mole photons s}^{-1}$. Thus, the total amount of PAR emitting from four red LSCs in each pond leads to having approximately an addition of 34 $\mu\text{mole photons s}^{-1}$ available to algal cells.

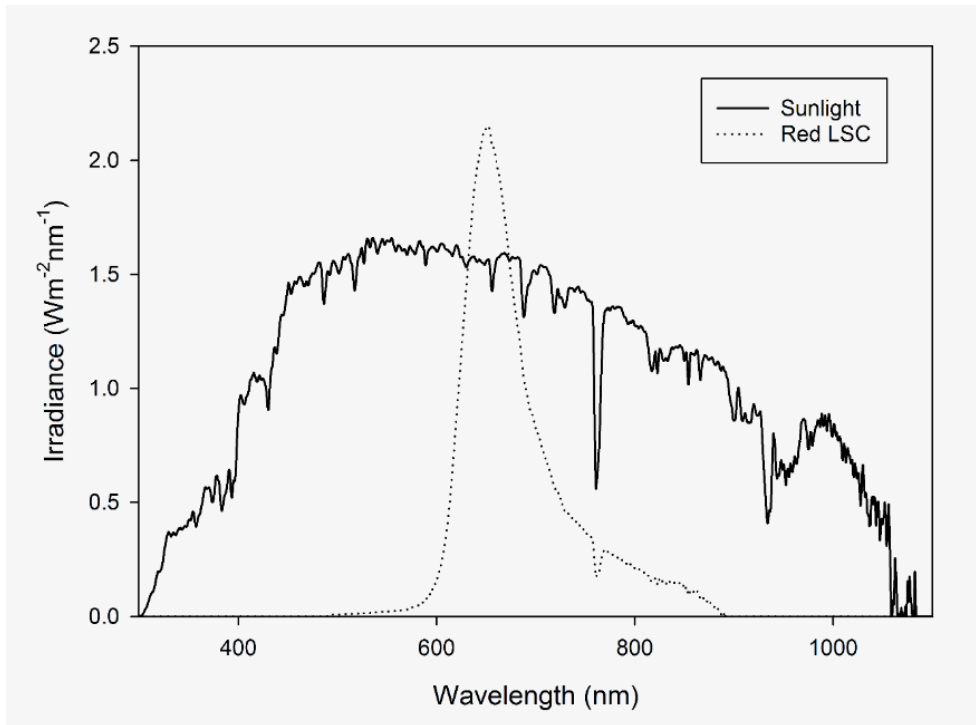


Figure 4-2. Solar spectrum and the spectrum of red LSCs (PAR region is in the range of 400-700 nm).

The spectrum of red LSCs was obtained under the open sun at midday.

Table 4-1. Solar radiation and red LSCs specifications. The spectrometric analysis was done at midday under the open sun (The estimation is that the solar irradiance variation was negligible during the period of the study, Fig 4A).

Parameter	Solar irradiance (Wm ⁻²)	*Red LSC (Wm ⁻²)	
		Edge	Surface
Total	803	188	92
PAR	443	110	60
400-500nm	128	-	-
500-600nm	159	-	-
600-700nm	155	104	45
700-800nm	134	30	21

*The light irradiance emitted from a red LSC was negligible in the range of 400-600 nm.

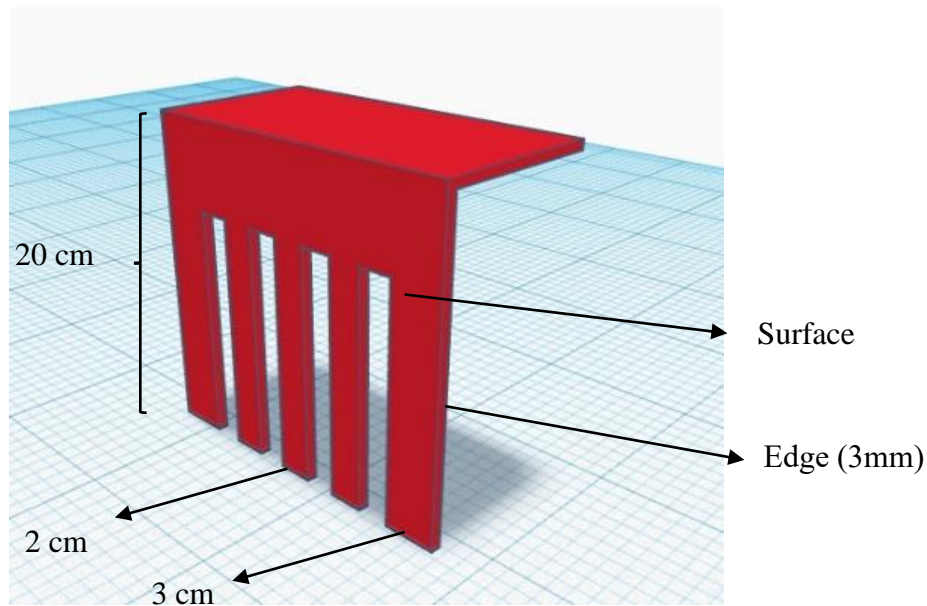


Figure 4-3. Schematic design of a red LSC inside *Scenedesmus sp.* cultures. 17 cm of each LSC was inside the algal culture.

4.4.2 Growth rate and biomass productivity

The outdoor cultivation of *Scenedesmus sp.* was carried out in five replicates as a batch between 29/10/2018 and 14/11/2018. The maximum irradiance of up to 1300 W m^{-2} was observed in day 2 which was approximately 60% higher than the lowest irradiance in day 4 with the intensity of about 800 W m^{-2} (Figure 4-4A). The daily variations in air and pond temperatures were from 35°C to 7°C , and 30°C to 5°C , respectively (Figure 4-4B&C). The highest daily evaporation was up to 0.55 mm in day 12, while the highest average rainfall was 0.5 mm in day 7 (Figure 4-4D&E). The pH of cultures in both treatments, with and without red LSCs, was in the range of 9-11 during the cultivation period. Dissolved oxygen concentrations were not also significantly different between *Scenedesmus sp.* cultures with red LSCs and control (data are not shown).

The main aim of the study was understanding the effect of red LSCs on maximizing biomass productivity and growth rate of *Scenedesmus* sp. as a result of providing more irradiance to the *Scenedesmus* cells. When grown with red LSCs, *Scenedesmus* had a higher number of cells during the cultivation period compared to the control (Figure 4-4F). Also, the highest biomass yield obtained for *Scenedesmus* sp. cultures with red LSCs and control were 1.18 ± 0.014 and 1.13 ± 0.038 g L⁻¹, respectively (Table 4-2). Furthermore, the maximum specific growth rate (μ) of *Scenedesmus* sp. cultures with red LSCs was 16% significantly higher than that compared to control with no LSCs during the cultivation period (Figure 4-4G, Table 4-2) (Two-tailed t-test, $P < 0.05$). The maximum specific growth rate obtained for *Scenedesmus* sp. in this study under red LSCs (0.101 d⁻¹) is in accordance with Gupta and Pawar (2018) study which used red and white LED light to grow *Scenedesmus abundans* in a 10 L airlift PBR and reported the specific growth rate of 0.119 and 0.102 d⁻¹ for red and white LEDs respectively. Li et al. (2010) also resulted in a 15.8% higher specific growth rate culturing *Scenedesmus* sp. under red LEDs compared to white light.

Table 4-2. Maximum biomass yields, biomass productivity, maximum specific growth rate (μ) and, chlorophyll content of *Scenedesmus* sp. cultures with red LSCs and control. Data are value \pm SE (Two-tailed t-test, $P < 0.05$, $n = 5$). Different letters show a significant difference in each row.

Parameter	Red LSCs	Control
Max biomass yield (g L^{-1})	1.18 \pm 0.014 ^a	1.13 \pm 0.038 ^a
Max specific growth rate (d^{-1})	0.101 \pm 0.003 ^a	0.087 \pm 0.003 ^b
Max volumetric productivity($\text{mg L}^{-1} \text{d}^{-1}$)	43.6 \pm 1.3 ^a	36.8 \pm 0.73 ^b
Max areal productivity ($\text{g m}^{-2} \text{d}^{-1}$)	9.37 \pm 1.3 ^a	7.89 \pm 0.73 ^b
Chl a (mg / g biomass)	1.35 \pm 0.032 ^a	1.24 \pm 0.096 ^a
Chl b (mg / g biomass)	0.369 \pm 0.037 ^a	0.28 \pm 0.026 ^a
Lipid (mg / g biomass)	133 \pm 2.9 ^a	121 \pm 2.4 ^b
Protein (mg / g biomass)	436 \pm 8.8 ^a	371 \pm 15 ^b
Carbohydrate (mg / g biomass)	335 \pm 11 ^a	333 \pm 7.1 ^a

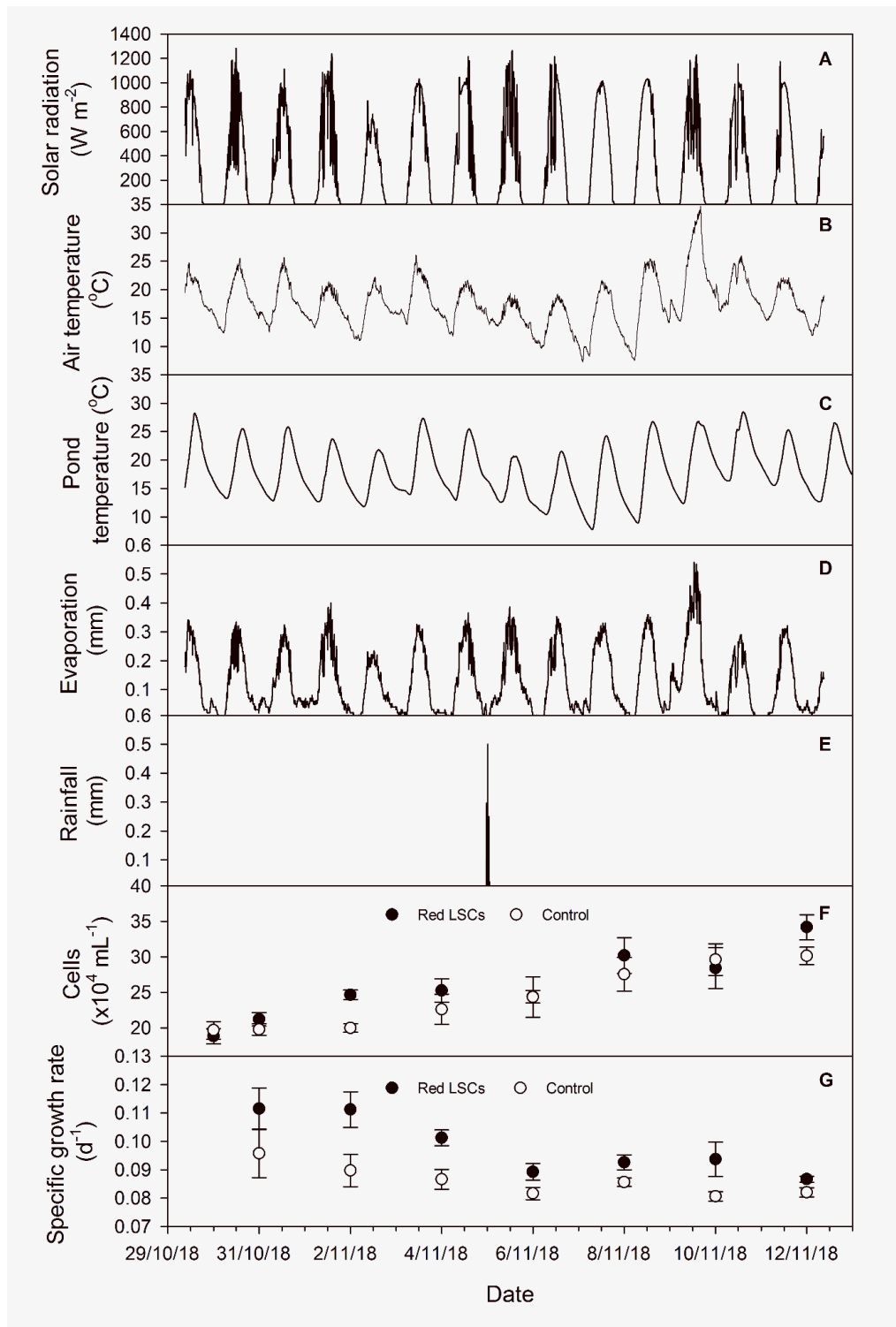


Figure 4-4. Solar irradiance (average of 10 min) (A), changes in air temperature B), pond temperature C), evaporation D), rainfall E), cell concentration F) and specific growth rate G) during the 15 days of *Scenedesmus* sp. cultivation from 29/10/2018 to 14/11/2018 in outdoor raceway ponds under red LSCs and control ponds with no panels. Data are shown as value \pm SE in F) and G).

The most important algal growth measurement tool is biomass productivity (Borowitzka & Moheimani, 2013). The biomass productivity of $43.6 \pm 1.3 \text{ mg L}^{-1} \text{ d}^{-1}$ ($9.4 \text{ g m}^{-2} \text{ d}^{-1}$) was obtained for *Scenedesmus* sp. cultures equipped with red LSCs (Table 4-2) which was 18.5% higher than that for *Scenedesmus* sp. cultures when grown in raceway ponds with no LSCs (Two-tailed t-test, $P < 0.05$, Table 4-2). The most likely reason for having higher productivity in *Scenedesmus* sp. cultures with red LSCs is that there is more light irradiance available to microalgal cells into the depth of the cultures. The spectrum of red light (600-700nm) has the longest wavelength band and the lowest energy level meaning that it cannot penetrate into the depth of dense microalgal cultures (Mohsenpour & Willoughby, 2013). It has been reported that 90% of sunlight would be absorbed by the first 10 mm of a dense outdoor microalgal culture in a raceway open pond and the rest of the culture experience a severe light limitation and virtually are in total darkness (Beardall & Raven, 2013). The spectrometric analyses of red LSCs under sunlight are summarised in section 3.1. Red LSCs had a peak at 650 nm (Figure 4-2) which is suitable for *Scenedesmus* sp. cells growth as this alga contains Chl *a* and *b* which have two major absorption peaks at blue (450-475 nm) and red (630-675 nm) light (Masojídek et al., 2013).

There are two ways of expressing the amount of light entering the system through red LSCs; 1) From an energy perspective which gives an overall picture of the total amount of light entering the system, 2) From the peak intensity view that tells how often the microalgal cells will pass the edge of LSCs receiving brief bursts of light by taking mixing rate into account.

From the energy perspective, the total amount of PAR available for *Scenedesmus* sp. cells at the depth of each pond, inside the algal culture, emitting from four red LSCs is $34 \mu\text{mol photons s}^{-1}$ (see section 4.4.1 for detailed calculations). In other words,

using red LSCs in each pond bring about $34 \mu\text{mol photons s}^{-1}$ more light as energy to the depth of *Scenedesmus sp.* cultures. That means injecting $34 \mu\text{mol photons s}^{-1}$ deep into the *Scenedesmus sp.* cultures where it would otherwise be in darkness. This helps move the light from the photo-saturated surface to the depth of the cultures.

Moreover, mixing the cultures moves algal cells where they pass the edges and surfaces of LSCs. Based on the mixing rate (11 cm s^{-1}) and the thickness of the LSCs (3 mm), *Scenedesmus sp.* cells pass an edge of a red LSC in 27 ms. Considering PAR emitting from an edge of a red LSC ($110 \text{ W m}^{-2} / 506 \mu\text{mol m}^{-2} \text{ s}^{-1}$, Table 4-1), *Scenedesmus sp.* cells receive around $506 \mu\text{mol m}^{-2} \text{ s}^{-1}$ in 27 ms from each edge (it should be noted that there are 10 edges in each LSC). A surface of each red LSC also emits $60 \text{ W m}^{-2} / 276 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and considering 2.4 cm as the average width of the surface (the width of one piece of a forked LSC,

Figure 4-3) and mixing rate of 11 cm s^{-1} , *Scenedesmus sp.* cells received $276 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in 218 ms when they pass each surface of a red LSC. In other words, it can be said that *Scenedesmus sp.* cells with red LSCs received brief bursts of light with different intensities for durations less than a second inside the cultures while there was total darkness for the cultures without LSCs (control).

The P_{max} and I_k levels of *Scenedesmus sp.* are highly dependent on high or low light intensities that also affects the photoadaptation of the cells (Tredici, 2010). Sforza et al. (2014) reported the *Scenedesmus obliquus* growth was limited at the light intensity higher than $150 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Having approximately further $34 \mu\text{mol photons s}^{-1}$ at the depth of *Scenedesmus sp.* cultures with red LSCs means that algal cells received more PAR light as well as having pulses from red LSCs edges inside the culture which led to 18.5% higher biomass productivity in cultures with red LSCs.

The biomass productivity obtained in this study is comparable to the productivity achieved by Gupta and Pawar (2018) which reported $43 \text{ mg L}^{-1} \text{ d}^{-1}$ as biomass productivity of *Scenedesmus abundans* in a 10 L airlift PBR under red LED light compared to $25 \text{ mg L}^{-1} \text{ d}^{-1}$ under the white LED light. Further, Eustance et al. (2016a) grew *Scenedesmus acutus* in an outdoor raceway open pond with the volume of 2300 L and resulted in $6.62 \text{ g m}^{-2} \text{ d}^{-1}$ (Table 4-3). Demura et al. (2018) also reported maximum biomass productivity of $7.5 \text{ g m}^{-2} \text{ d}^{-1}$ for growing *Scenedesmus acuminatus* and *Desmodesmus* sp. in 1 m^2 outdoor raceway open pond with a volume of 120 L (Table 4-3). However, it has been reported using inclined thin layer ponds (Doucha & Lívanský, 2009) and aerating microalgal cultures with CO_2 (Raesossadati et al., 2014) could increase biomass productivity. Dilov et al. (1985) and Vendlova (1969) reported biomass productivity of 19 and $12 \text{ g m}^{-2} \text{ d}^{-1}$ for cultivating *Scenedesmus obliquus* and *Scenedesmus* sp. in inclined thin layer ponds. Having higher biomass productivity in an inclined thin layer reactor is due to more availability of light to the microalgal cells (Raesossadati et al., 2019b).

Table 4-3. Biomass productivities of *Scenedesmus* grown outdoors in raceway and inclined thin layer ponds.

Culture volume (L)/Pond area (m ²)	Cultivation system	Culture period (months)	Areal productivity (g m ⁻² d ⁻¹)	Volumetric productivity (g L ⁻¹ d ⁻¹)	Species	Location	References
-	Raceway pond	12	15	-	<i>Scenedesmus obliquus</i>	Bangkok, Thailand	(Payer et al., 1978)
2300/10	Raceway pond	2	6.62±2.3	-	<i>Scenedesmus acutus</i>	Mesa, AZ, USA.	(Eustance et al., 2016a)
120/1	Raceway pond	11	4.1±0.231	26.98±1.91	<i>Scenedesmus acuminatus</i> , <i>Desmodesmus</i> sp.,	Fukushima, Japan	(Demura et al., 2018)
21.5/0.1	Raceway pond	0.5	9.37	43.6 ± 1.3	<i>Scenedesmus</i> sp.	Perth, Western Australia	Current study
2500/-	Inclined thin layer	+2	12	-	<i>Scenedesmus</i> sp.	Tylitz, Poland	(Vendlova, 1969)
2500/-	Inclined thin layer	7	19	-	<i>Scenedesmus obliquus</i>	Rupite, Bulgaria	(Dilov et al., 1985)

4.4.3 Chlorophyll and biochemical contents under red luminescent solar concentrators

Chlorophyll *a* and *b* contents of *Scenedesmus* have been measured. The Chl *a* and *b* contents of *Scenedesmus* under red LSCs were 1.35 and 0.369 mg gbiomass⁻¹, respectively which were not significantly different with the cultures with no LSCs (Two-tailed t-test, P>0.05) (Table 4-2). However, the Chl *a* content was higher than Chl *b* in both treatments (Table 4-2). Apart from chlorophyll, biochemical contents of *Scenedesmus* have been also measured. Recently, there has been a great need to explore new protein sources as a food supplement to respond to future protein demand. *Scenedesmus* is considered as one of the species having a high amount of chemical composition including protein (50-56%), carbohydrate (10-17%) and lipid (12-14%) in their algal biomass (Becker, 2007). Amino acids are the main constituents of a protein which are the benchmark to determine the nutritional quality of a protein based on the amino acids content and availability (Becker, 2007). Based on the quality program recommended by (Noack, 1974), the amino acids pattern of biomass protein of *Scenedesmus* is in accordance with the food protein reference (Becker, 2007). The more recent application of microalgal biomass is to be used as animal feed. It has been shown that 30% of world algal biomass is sold for animal feed (Becker, 2007) and has the standard quality to be used as animal feed such as poultry (Vidyashankar et al., 2015) and aquaculture (Vizcaíno et al., 2014).

As microalgal biomass is used for food supplements, the importance of other components of biomass such as carbohydrate and lipid are taken into account. In this study, the protein content of *Scenedesmus* sp. under red LSCs was 436 mg gbiomass⁻¹

(43.6%) which was 17.5% higher than that in control (Two-tailed t-test, $P < 0.05$) (Figure 4-5, Table 4-2). However, the carbohydrate content of *Scenedesmus* sp. cultures with red LSCs and control was not significantly different (Figure 4-5, Table 4-2).

The protein and carbohydrate contents achieved in this study is comparable with literature. Vidyashankar et al. (2015) reported protein and carbohydrate contents of 21.5 and 49% for growing *Scenedesmus dimorphus* in an a raceway pond with working volume of 1000 L. Eustance et al. (2016b) also reported 30% and 28% protein and carbohydrate content for *Scenedesmus acutus* grown in outdoor raceway ponds with the volume of 2300 L. Vidyashankar et al. (2015) showed that defatted biomass of *Scenedesmus dimorphus* was safe to feed rats in both short-term (14 days) with single-dose feeding (20 % (w/w) feed) and long-term (90 days) repeated-dose feeding (at 5 and 10 % (w/w) feed).

Scenedesmus also has high lipid content that could be used for biofuel, chemicals, and nutraceuticals (Becker, 2004). In this study, *Scenedesmus* cultures under red LSCs (133 mg gbiomass⁻¹) showed 10% higher lipid content compared to control with no LSCs (Two-tailed t-test, $P < 0.05$) (Figure 4-5, Table 4-2). The higher lipid content of *Scenedesmus* cultures under red LSCs might be due to more photons available in cultures with red LSCs. It has been believed that higher light irradiance leads to more lipid accumulation in microalgal cells (Guschina & Harwood, 2013). The lipid content of *Scenedesmus* sp. cultures in this study is in accordance with Ho et al. (2012) study which reported maximum lipid content of 10.3% for *Scenedesmus obliquus* cultivated in a 1 L glass vessel under aeration of 2.5% CO₂. Based on the results of protein and lipid contents of *Scenedesmus* sp. achieved in this study (Figure 4-5), it can be concluded that the biomass has a good potential to be used for animal feed or biofuel

production. Obviously, there needs to be more analysis on biomass to find out the true potential of protein and lipid for further applications.

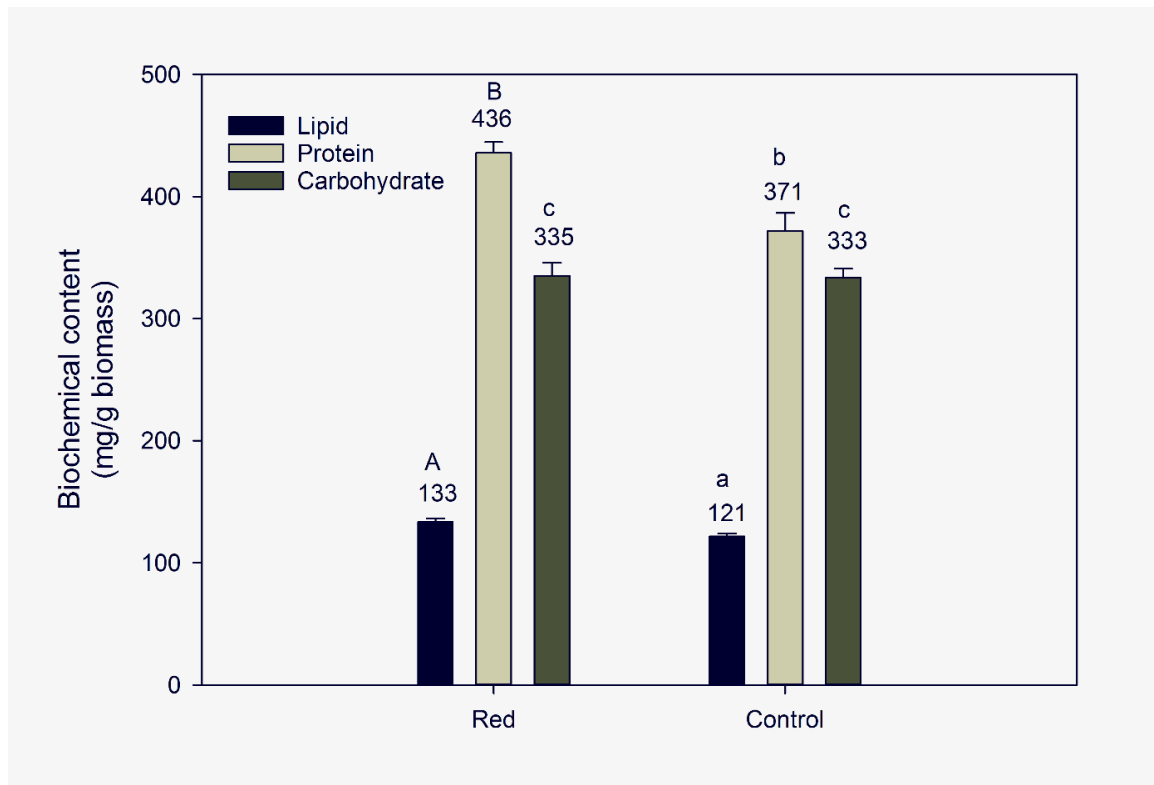


Figure 4-5. Lipid, protein and carbohydrate content of *Scenedesmus* sp cultures with red LSCs and control with no panels. Data are value \pm SE. Numbers on bars are the corresponding content value. The capital letter shows a significant difference (Two-tailed t-test, $P < 0.05$, $n = 5$).

4.4.4 Fouling

Biofouling is potentially the greatest disadvantage of using LSCs as a result of the accumulation of *Scenedesmus* sp cells on LSCs (Figure 4-6). LSCs emit light from the edges and surfaces and as there is no other light source at the depth of *Scenedesmus* sp cultures, the cells are attracted to the edges and surfaces of LSCs. However, as it is shown in Figure 4-6, fouling was negligible since approximately a small part of a red LSC showed bio-fouling by *Scenedesmus* sp. cells during 15 days of the cultivation period.



Figure 4-6. *Scenedesmus* sp. biofouling on a luminescent solar concentrator during 15 days of the cultivation period.

4.4.5 Significance of the study

There is no doubt that paddle wheel driven raceway ponds are the preferred commercial cultivation system for mass production of *Scenedesmus* sp. One of the main obstacles for culturing microalgae in large scale raceway ponds is the availability of light at the depth of the cultures resulting in low biomass productivity. Therefore, increasing light irradiance at the depth of raceway pond cultures by using an efficient light delivering system would enhance the biomass productivity as well as reducing the capital costs. Our study clearly showed that the use of red LSCs in raceway ponds with the proposed design (see sections 4.3.2 and 4.4.1) can significantly increase biomass productivity, protein and lipid contents of *Scenedesmus* sp. The main advantage of using LSCs with such a design is the potential capability of applying them to a large-scale paddle wheel driven raceway pond. The significant portion of the cost of biomass production is capital expenses which up to 50% of it accounts for

building the ponds. Therefore, for the same biomass productivity achieved in this study, there is an advantage of reducing the cultivation area by 18.5%. This is achieved by delivering a suitable wavelength of light into the depth of algal cultures in raceway ponds. The active light delivering system used in this study has the advantage of an increasing number of photons as well as shifting light to microalgal cells.

4.5 Conclusions

Red LSCs enhanced biomass productivity and specific growth rate of *Scenedesmus* sp. cultivated in outdoor paddle wheel driven raceway ponds. Protein and lipid contents of *Scenedesmus* sp. cultures were also significantly higher in cultures with red LSCs compared to control. Using LSCs for *Scenedesmus* sp. cultures in raceway open ponds can significantly increase the light availability to the cells at the depth of the cultures and bring forward a higher growth rate for *Scenedesmus* sp. cells. However, there need to be further studies at a larger scale as well as detailed techno-economics and life cycle analysis to find the true potential of these LSCs for mass cultivation of *Scenedesmus* sp.

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Link to the next Chapter

The results of previous Chapters showed that red LSCs could significantly improve biomass productivity of *Arthrospira* and *Scenedesmus* sp. cultures as well as phycocyanin productivity of *Arthrospira* cells. To find out the true potential of LSCs, a cost analysis was carried out on a large-scale raceway pond *Arthrospira* cultivation system. The production cost of biomass and phycocyanin of *Arthrospira* grown in a raceway pond with red LSCs were compared with those in a conventional raceway pond with no LSCs. In this study, CAPEXs and OPEXs of *Arthrospira* biomass and phycocyanin production have been considered for the production costs analysis.

5 Luminescent solar concentrators can reduce the cost of raceway ponds grown *Arthrospira*

5.1 Abstract

Increasing *Arthrospira*'s biomass and phycocyanin productivities are potential ways to reduce overall production cost. One of the main challenges that limits the growth of microalga in a large scale outdoor open pond cultivation is the low light availability to the cells. Therefore, increasing number of photons available to algal cells using a light delivering system will increase biomass and phycocyanin productivities and potentially reduce the production costs. In here, the economic feasibility of using luminescent solar concentrators as a light delivering system on an *Arthrospira*'s production scale raceway pond plant was assessed. The biomass and phycocyanin production costs were also calculated. Using red luminescent solar concentrators would result in a biomass and phycocyanin production costs of AU\$ 3.16 and AU\$ 125 per kg. These are 14% and 34% lower biomass and phycocyanin production costs compared to when conventional raceway ponds were used. This clearly shows that using LSCs can significantly lower the cost of biomass and phycocyanin production if the same size production facility is used.

5.2 Introduction

Arthrospira's large-scale cultivations began in 1960's in Mexico (Borowitzka, 2013b). There are various commercial *Arthrospira* producers in the world such as Earthrise Farms (California, USA), Sosa Texcoco (Lake Texcoco, Mexico), Siam Algae Company (Thailand) and Cyanotech (USA) (Suizu, 1998). It has been believed that *Arthrospira* can improve the immune system, promote calcium absorption, prevent aging and can be used as a source of high value products (Ye et al., 2018). On the other hand, this alga can be used as a source of protein for areas with low animal protein

production. For instance, naturally grown *Arthrospira* has been used as food in Myanmar for the last few decades (Habib, 2008). The mass cultivation of this alga for Africa has been also proposed (Grobbelaar & Bornman, 2004).

The main biochemical composition of *Arthrospira* biomass consists of 55-70% protein (including phycobiliproteins), 6-8% lipids and 15-25% carbohydrate (Belay, 1997). Phycobilins such as phycocyanin are highly fluorescent, coloured and water soluble and, have been widely used in health food, nutraceutical and pharmaceutical applications (Becker, 2007). Indeed, phycocyanin is of great importance as one of the high value products from microalgae and has attracted an attention due to its natural colour as well as non-toxic characteristic. Phycocyanin has also been used as fluorescence marker for flow-cytometry and biochemistry. Due to commercial sensitivity and low availability of data in literature, it is rather difficult to estimate the exact market value and price for phycocyanin. However, Borowitzka (2013b) reported the phycocyanin market value of US\$60 million in 2013 with the price of US\$500-10,000 kg⁻¹ based on purity of the product. Further, market value for phycocyanin in 2018 was reported to be over US\$112 million (Pagels et al., 2019). This clearly indicates a trend in the higher demand for this product in market.

To become a commercial reality and increase profitability, reducing *Arthrospira*'s phycocyanin and biomass production cost is a critical target of any commercial producer. Main limiting factors in mass cultivation of *Arthrospira* are nutrient, temperature and, light (Vonshak et al., 2014). Cultivation of *Arthrospira* in a temperate region where there is optimum temperature and enough nutrient is provided to the cultures, would leave light as the main limiting factor. Considering a large scale cultivation of *Arthrospira* in a raceway pond as the most economical algal cultivation system, *Arthrospira* cells would be photoinhibited on the surface of the culture and

photonlimited at the depth of the culture (Tredici, 2010). One way to overcome light limitation of a large scale *Arthrospira* culture is to increase the number of photons to the depth of the culture (Raeisossadati et al., 2019). Increasing number of available photons to *Arthrospira* cells which are in total darkness at the depth of the cultures can increase biomass and phycocyanin productivities and reduce biomass and phycocyanin production costs. It was shown that using red luminescent solar concentrators (LSCs) can significantly increase biomass (26%) and phycocyanin (44%) productivities of *Arthrospira* (MUR 129) when grown in an outdoor raceway pond (Raeisossadati et al., 2019).

In this study, the economics of applying LSCs in mass production of *Arthrospira* cultivation was assessed. The focus in this economic assessment is to evaluate the phycocyanin and biomass production costs as the main products of *Arthrospira* using LSCs in an industrial scale production plant. We also compare these costs with the conventional cost of *Arthrospira* when grown using conventional raceway pond cultivation system with the same size plant.

5.3 Resources

5.3.1 Location of plant

The two important parameters in a large scale cultivation of *Arthrospira* are light and temperature which are uncontrollable in raceway open ponds (Borowitzka & Vonshak, 2017). These factors determine the suitability of a location for mass culture of *Arthrospira*. Location is of highest importance to the productivity of the *Arthrospira* and any other algal cultures because of the climate as well as affecting the production cost in terms of land availability and cost (Borowitzka & Vonshak, 2017). A suitable location for mass algal culture is also where there is an easy access to water and CO₂

(Borowitzka, 2013b). Further, seasonal variations can have a significant effect on the algal mass production as well as economic viability which arises when selecting a site for a production plant (Borowitzka & Vonshak, 2017). It has been shown that Western Australia could be an ideal location for large scale microalgal cultivation (Boruff et al., 2015). One of the Western Australian appropriate location for mass production of *Arthrospira* is Bindoon (31.3735° S, 116.0953° E) (Suizu, 1998). The climate conditions and average solar irradiance of Bindoon is shown in Table 5-1 and Figure 5-1. The location has appropriate solar irradiance as well as suitable temperature for *Arthrospira* cultivation (Table 5-1 and Figure 5-1). Australian *Spirulina* Farms Pty. Ltd commenced a field study for mass production of *Arthrospira* in Bindoon (31.3735° S, 116.0953° E), Western Australia (Suizu, 1998). Currently, there is also a commercial *Spirulina* production company based in Darwin (12.4634° S, 130.8456° E), Northern Territory, Australia (<http://www.australianspirulina.com.au/>).

Table 5-1. Climate conditions of Bindoon, WA.

Average daily maximum temperature	27 °C
Average daily minimum temperature	12 °C
Average daily mean temperature	18 °C
Average rainfall	800mm
Average daily sunshine hours	9 h

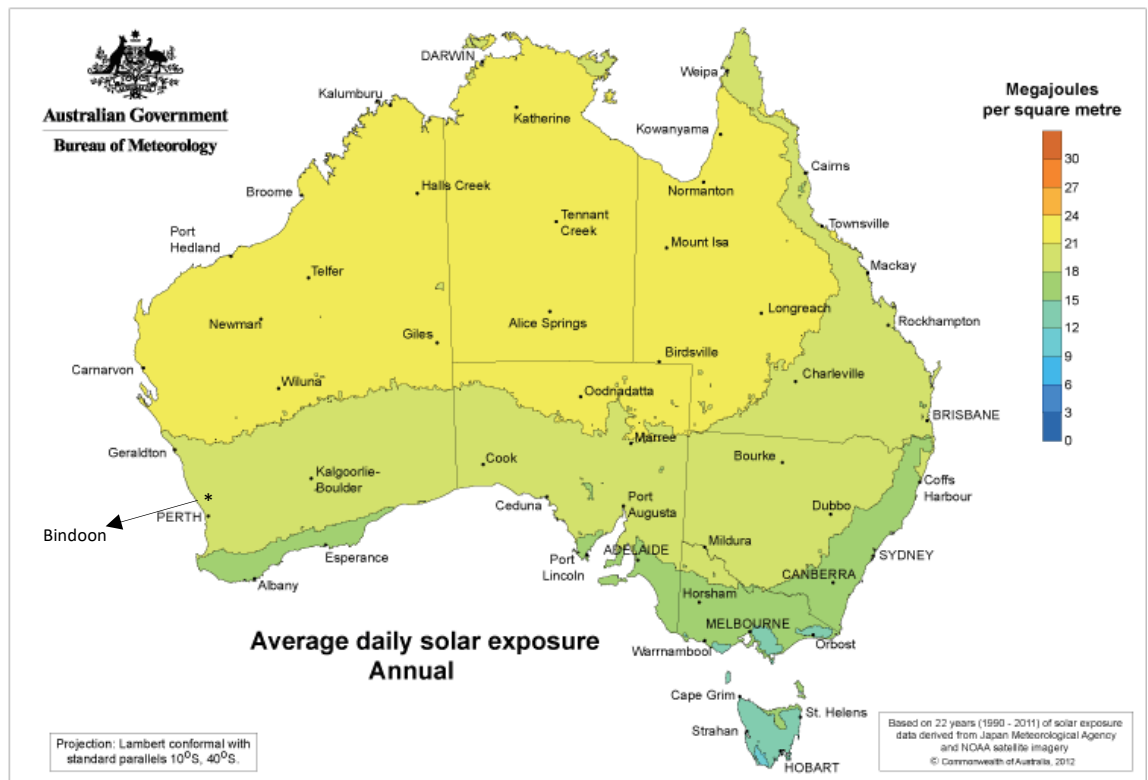


Figure 5-1. Average daily solar irradiance in Australia in a year (http://www.bom.gov.au/jsp/ncc/climate_averages/solar-exposure/index.jsp).
* Bindoon, WA.

5.3.2 Strain selection

One of the most important factors for having a successful commercial microalgal production plant is to select a suitable species. *Arthrospira* is a cyanobacteria which can grow in an optimum temperature in the range of 30-35 °C. It means in a temperate regions selecting a strain of *Arthrospira* being able to grow in low temperature in winter is a key factor to operate the production in cold weather since it is almost impossible to fully control the temperature in large scale raceway ponds and cultures deteriorate fast in cold weather (Borowitzka & Vonshak, 2017). Further, a significant reduction in production cost can be achieved by selecting a low temperature tolerant species to extend the duration of production for a further two months. For instance,

Belay (1997) reported the seasonal growth of *Arthrospira* for only 8 months of a year at Earthrise Farm where there is an optimum temperature for the growth while the cold weather stopped the production plant in the winter season. Growth rate, biochemical composition and resistance to mechanical and physiological stress are also other major determinants to select a strain for commercial production (Borowitzka, 1999). Accumulation of oxygen as a result of photosynthesis is also inevitable in *Arthrospira* culture especially in a large scale cultivation where it reaches up to 500% of saturation when the photosynthesis rate is high (Suizu, 1998). The high level of oxygen may be detrimental to *Arthrospira* culture and lead to a total crash of the culture, thus, one would need to isolate strains of *Arthrospira* capable of growth at high oxygen concentration. Suizu (1998) showed that *Arthrospira platensis* (MUR 129) tested by Raeisossadati et al. (2019) is a suitable strain for mass production cultivations to grow at Bindoon, WA.

5.3.3 Nutrients and Carbon dioxide

Nutrients are a major expense accounting for about 15-25% of the production cost for a large scale cultivation of *Arthrospira* (Belay, 1997). Culture medium must be recycled after harvesting for economic and environmental reason (Richmond & Hu, 2013). Recycling the medium reduces the water cost as well as cost of nutrients as there are still considerable amount of nutrients left in the culture medium post-harvest. Further, recycling reduces the risk of environmental contamination related to releasing a huge amount of a culture medium to the environment (Borowitzka & Vonshak, 2017).

The essential nutrients for *Arthrospira* growth are sodium bicarbonate, nitrate, phosphate, sulphur, Na⁺ and, K⁺ that are available in the Zarrouk medium (Zarrouk,

1966). The high alkalinity and buffered of the medium are provided by high concentration of sodium bicarbonate (16.8 g L^{-1}) to maintain the pH of the culture in the range of 8.5 - 11. A pH lower than 8 increases the risk of growth of other microalgae such as *Chlorella* and a higher pH of 11 may inhibits the growth of *Arthrospira* (Belay, 1997). Furthermore, CO_2 may also be pumped in a large scale *Arthrospira* cultivation to increase the algal productivity (Raeesossadati et al., 2014; Raeesossadati et al., 2015).

5.3.4 Fresh water supply

Arthrospira is a freshwater cyanobacteria and freshwater is needed for a large-scale cultivation of *Arthrospira*. A potential freshwater source for a mass cultivation of *Arthrospira* is groundwater (Kim et al., 2007). However, groundwater sources may contain high level of Ca^{2+} which can inhibit *Arthrospira* cultivation (Kim et al., 2007). *Arthrospira* cultivation require a high alkalinity made by addition of sodium bicarbonate. High level of calcium can lead to CaCO_3 precipitation when the culture is topped up daily replace evaporated water (Taylor & Brownlee, 2016). High calcium content can also result in loss of medium iron and phosphorus (Taylor & Brownlee, 2016). Pre-treatment of raw water and monitoring the chemistry of the cultures during the growth period can be considered as a solution to solve these problems (Belay, 1997). On the other hand, using groundwater has the advantage of maintaining the culture temperature in winter (Borowitzka & Vonshak, 2017). Considering Bindoon, WA, as a potential location for a large scale cultivation of *Arthrospira*, there are groundwater sources available that can be used as a fresh water source for the *Arthrospira* cultures (<http://www.water.wa.gov.au/maps-and-data/maps/perth-groundwater-atlas>).

5.4 Methodology

The process of biomass and phycocyanin production from *Arthrospira* is shown in Figure 5-2. Paddle wheel driven raceway ponds are used for a mass cultivation of *Arthrospira* (See section 5.5.1 and Figure 5-3 for details). In the first step, *Arthrospira* cultures are grown in raceway ponds. The culture is harvested/dewatered in the second step and in the third step, the harvested biomass is processed for the desired products. The culture medium is recycled after harvesting/dewatering and downstream processes (Figure 5-2).

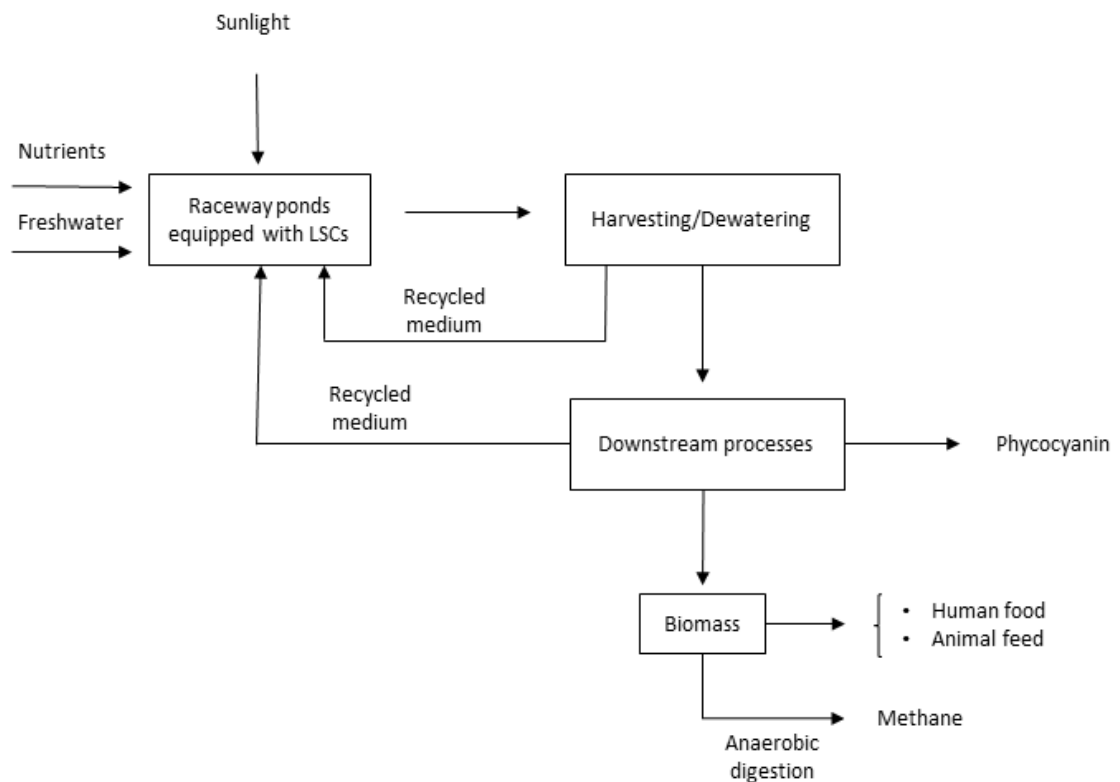


Figure 5-2. The flow process of biomass and phycocyanin production from *Arthrospira*.

5.4.1 Harvesting and dewatering

Stepwise filtration is the best harvesting method for *Arthrospira* (Belay, 1997). The efficiency of harvesting depends on trichome and mesh size of the filters at each stage.

Using filters with smaller mesh size results in higher harvest efficiency but takes longer time and the flow rate is significantly lower. However, increasing the flow rate may lead to damaging the *Arthrospira* cells and thus, reducing the efficiency of harvesting (Belay, 2013). When grown a large scale, *Arthrospira* is generally harvested semi-continuously (only a portion of the culture is harvested, and the harvested medium is replaced with either fresh or recycled medium). The percentage and interval time of the harvested culture depends on the growth rate and the desired biomass concentration for a harvest (Borowitzka & Vonshak, 2017).

After harvesting, a proper drying method should be applied to *Arthrospira* biomass to ensure the high quality of the product. Various drying methods such as spray drying, drum drying, freeze drying, and sun drying are proposed to dry algal biomass (Grima et al., 2004). To date spray drying is found to be the most economical method for drying the *Arthrospira* (Belay, 2013). The spray drying method includes a drying chamber into which *Arthrospira* droplets are sprayed to evaporate the water. In the following step, the powder is exposed to 60 °C heat to evaporate the left-over of moisture (Belay, 1997). The quality of the product is significantly depending on the moisture content. If the moisture content is greater than 8%, high contamination by moulds and bacteria can be seen (Belay, 2013). However, over-drying of the powder may can lead to loss of vitamins, chlorophyll and carotenoids (Belay, 1997).

5.4.2 Phycocyanin extraction

An efficient extraction method must be applied to extract phycocyanin from the cells efficiently. The extraction of phycocyanin from *Arthrospira* is normally carried out in two steps by mechanical and chemical methods (Chaiklahan et al., 2011). In the mechanical extraction, the cells are disrupted by mechanical methods such as

ultrasonication, bead mill or high-pressure systems (Pagels et al., 2019). The extraction of phycocyanin is then carried out by a chemical extraction method using solvents such as phosphate buffer (Pagels et al., 2019). Purification of the final product, using chromatography methods, can also be taken into consideration if the aim is to use phycocyanin for pharmaceutical purposes. Chaiklahan et al. (2018) used chemical extraction method by using the phosphate buffer as a solvent for the extraction of phycocyanin from *Arthrospira* in a pilot scale plant. The biomass was added to phosphate buffer in an agitation tank to obtain the mixture ratio of 1:100 (w/v) followed by centrifugation to remove the cell residue. In the final step, the crude extract was filtered using ultrafiltration membrane (Chaiklahan et al., 2018).

5.4.3 Luminescent solar concentrators

Using luminescent solar concentrators (LSCs) has been reported as an effective light delivering system to increase biomass productivity of microalgae cultures in raceway ponds (Raeisossadati et al., 2019). Raeisossadati et al. (2019) showed that using raceway ponds with red LSCs can results in 26% and 44% higher biomass and phycocyanin productivities of *Arthrospira* when compared to conventional system. This study was carried out under outdoor climatic conditions of Perth (31.9505° S, 115.8605° E), Western Australia between 15/12/2017 and 03/03/2018. Raeisossadati et al. (2019) showed that using red LSCs was an efficient method to not only downgrade sunlight to the desired wavelength for microalgae but also deliver it to the depth of the cultures. Higher biomass and phycocyanin production rates of a large scale *Arthrospira* production plant because of using LSCs can have a significant impact on reducing the production costs of biomass and phycocyanin. In here, the cost of *Arthrospira* biomass and phycocyanin production when applying LSCs has been

assessed. The production costs has been also compared with those in a conventional cultivation of this alga.

5.5 Economic assessment

As highlighted earlier, using LSCs showed to significantly increase *Arthrospira*'s biomass and phycocyanin productivities. Higher productivity can lead to a lower cost of production. However, to the best of author's knowledge, there yet to be any study analysing the economic viability of using LSC's to reduce the overall cost of *Arthrospira* biomass and phycocyanin production.

5.5.1 Model description and assumptions

As required in any economic assessment, several assumptions have been taken into consideration for production of biomass and phycocyanin from *Arthrospira*. The main assumptions are:

- 1) The baseline annual *Arthrospira* biomass productivity in an outdoor raceway pond is $9.7 \text{ g m}^{-2} \text{ d}^{-1}$ (300 t year^{-1}) based on the results reported by (Belay, 2013; Raeisossadati et al., 2019).
- 2) The operating days in each year is set at 330 days. This should be manageable based on the suitable climatic condition of Bindoon, Western Australia as the selected site (e.g. high solar irradiance (Figure 5-1) and required temperature profile).
- 3) The size of each pond is 5000 m^2 (0.5 ha) with a depth of 30 cm (Figure 5-3) and the mixing rate of 30 cm s^{-1} (Borowitzka & Moheimani, 2013) Zarrouk medium is used with the main nutrients of N, P (Zarrouk, 1966). CO_2 is added to supply as an inorganic carbon source (Raeisossadati et al., 2015).

- 4) The baseline for phycocyanin (C-PC) content of *Arthrospira* is considered 15% (Chaiklahan et al., 2018).
- 5) The capital expenditures (CAPEXs) and operational expenses (OPEXs) for the biomass and phycocyanin productions from *Arthrospira* are summarized in Table 5-2 and Table 5-3.
- 6) The amount of LSCs required (~ 2.1 kg per m^2) is based on the results obtained by Raeisossadati et al. (2019), and the associated cost of LSCs is calculated based on the market price of LSCs (AU\$ 3 per kg) (Zhejiang Huashuaite New Material Technology Co.).

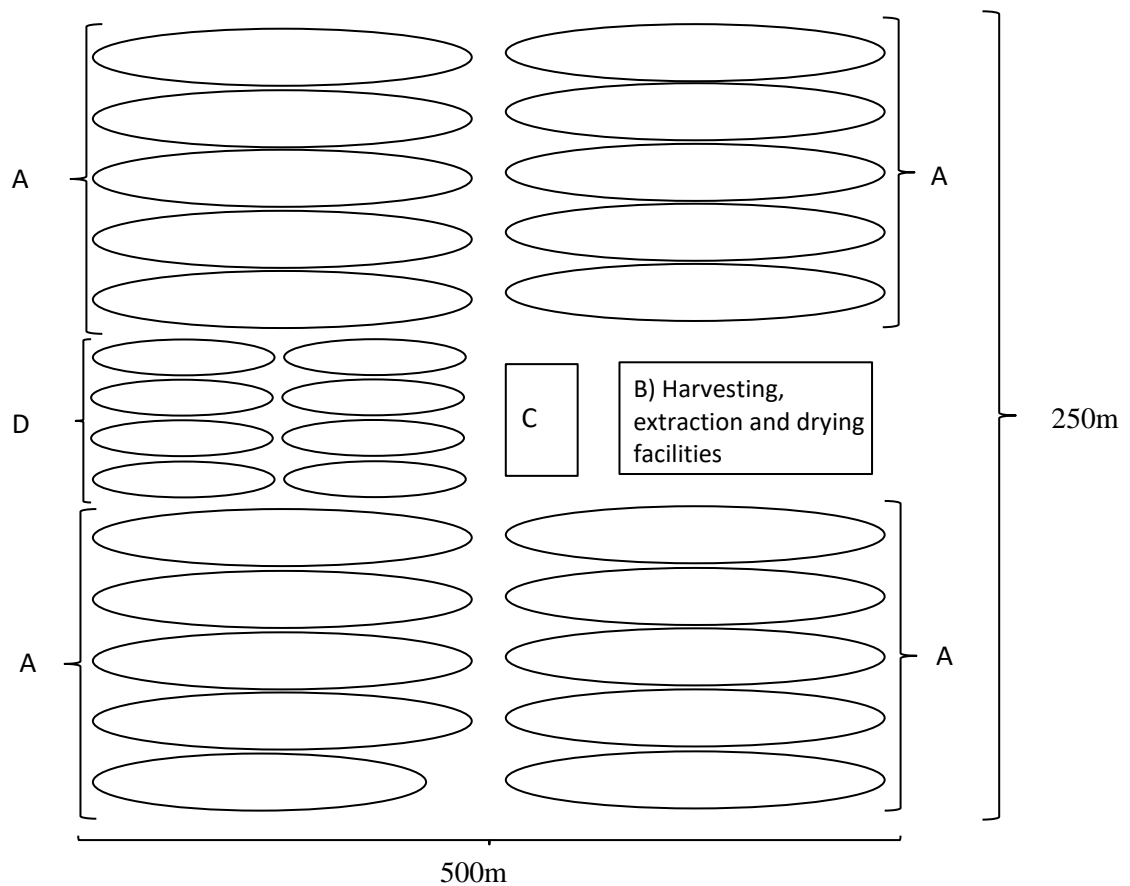


Figure 5-3. The *Arthrospira* production plant layout. A) The cultivation raceway ponds, B) harvesting, extraction and, drying facilities, C) administration building and, D) inoculum ponds. The size of each cultivation raceway pond is 200×25 m except one with the size of 160×25 m and the total cultivation ponds area is 9.4 ha.

5.5.2 Accuracy of estimates

The level of accuracy of cost estimates highly depends on the actual details and conditions of a project based on capital and operational expenses (Lundquist et al., 2010). Lundquist et al. (2010) reported a guideline in which the accuracy level of a cost estimate is determined based on the economic details provided. They showed that an economic assessment which reports the budgeting and authorization estimate, including process diagrams, production plant layout and almost all major equipment and facilities costs would result in the cost accuracy of -20% to +30% (Lundquist et al., 2010). Therefore, the cost accuracy of the current study lies in the range of -20 to +30%. Potentially, the cost accuracy can be improved to -15% to +20% if more details on the final suite of processes and technologies to be used in the facilities are provided in the economic assessment (Lundquist et al., 2010).

5.5.3 Cost estimation method of biomass and phycocyanin production

The costs of biomass and phycocyanin production have been calculated based on the CAPEXs and OPEXs and the amount of biomass and phycocyanin produced in a year. The annual instalment equation was used to calculate the annual capital cost of production plant based on bank interest and loan term and the CAPEXs (Ishika, 2017).

$$\text{Annual instalment cost} = A \times \frac{r(1+r)^n}{(1+r)^n - 1} \quad \text{Equation 5-1}$$

where A is capital cost, r is bank interest and, n is loan term. In this study a loan term of 10 years with interest of 6.35% has been considered.

For the cost of biomass and phycocyanin production in a conventional system with no LSCs, the corresponding OPEXs is added to cost of annual instalment (Equation 5-1)

and then divided by the amount of biomass and phycocyanin produced in a year. For instance, the production cost of biomass is calculated as Equation 5-2 (Ishika, 2017):

$$\text{Biomass production cost (AU\$ kg}^{-1}\text{)} = \frac{\text{Eq.1+Annual OPEXs}}{\text{Annual biomass production (kg)}} \quad \text{Equation 5-2}$$

When LSCs applied to the system, the capital cost of LSCs system was added to the total CAPEXs (A in Equation 5-1) to calculate the annual instalment cost and the corresponding OPEXs of LSCs was also considered to estimate the biomass and phycocyanin production costs.

5.6 Results

The economic assessment here is based solely on calculating the *Arthrospira*'s biomass and C-PC production costs using the total capital and operational costs of cultivation and extraction systems with and without applying LSCs to the cultivation system. The size of the production plant is estimated based on producing 30% of phycocyanin world production (45 t year⁻¹) (Borowitzka, 2013a). Thus, considering the phycocyanin content of 15% (assumption 4), 9.4 ha of cultivation area is required to produce 300 t year⁻¹ of *Arthrospira* biomass. Therefore, the amount of LSCs required for 9.4 ha is 200 t with the corresponding cost of AU\$ 31,503 per 0.5 ha pond (Table 5-2).

5.6.1 Biomass and C-Phycocyanin production costs

The capital and operational expenditures of biomass production and C-PC extraction facilities are summarized in Table 5-2 and Table 5-3. The estimation of the total CAPEXs of the raceway pond cultivation system for biomass and phycocyanin production from *Arthrospira* is AU\$ 285,732 ha⁻¹ and AU\$ 173,144 ton⁻¹. y⁻¹. Furthermore, the OPEXs estimated for the cultivation system is AU\$ 77,827 ha⁻¹ and

the operational cost for phycocyanin production is AU\$ 130,153 ton⁻¹. y⁻¹. Based on Equation 5-1 and 5-2, *Arthrospira* biomass production cost would be AU\$ 3.67 kg⁻¹ considering the biomass production of 300 t per year (assumption 1) while the phycocyanin production cost would result in AU\$ 178 kg⁻¹.

Table 5-2. Capital expenditures for the productions of *Arthrospira* biomass and phycocyanin.

CAPEXs	
Cultivation system*	
Parameter	Cost (AU\$/ha)
Site preparation, grading, compaction	5,868
Raceway ponds & mixing	112,678
CO ₂ supply & distribution	10,092
Harvesting & Dewatering	16,430
Water & nutrient supply	26,370
Building & roads & drainage	4,694
Electrical supply & distribution	31,464
Instrumental & machinery	1,173
Land cost**	18,000
Subtotal	226,772
Engineering (15% of subtotal)	34,015
Contingency (5% of subtotal)	11,338
Total Fixed Capital	272,126
Working capital (5% of total fixed capital)	13,606
Total CAPEXs	285,732
Total CAPEXs for 9.4 ha	2,686,216
Phycocyanin production*** (45 t/y)	
Parameter/quantity	Cost (AU\$)

Mixing tanks (5000 L) / 6	60,000
Continuous centrifuge (5000 L/h)/2	448,000
Storage tank (500 L) / 20	199,360
Cooling system (5000 L/h) / 2	17,334
Freeze Drier (40 kg/day) / 23	5,084,173
Filtration system / 5	1,657,890
Maintenance	324,741
Total CAPEXs	7,791,498
Luminescent solar concentrators (LSCs)	
2.1 kg m ⁻²	AU\$ 3 kg ⁻¹
Cost per ha	AU\$ 63,000
Cost for 9.4 ha	AU\$ 592,272
Engineering, shipping, etc. (15%)	AU\$ 88,840
Total cost for 9.4 ha	AU\$ 681,113

* Numbers are based on (Benemann & Oswald, 1996) and (Lundquist et al., 2010).

** The land cost is based on <https://www.commercialrealestate.com.au/for-sale/bindoon-wa-6502/>.

*** Data are based on (Chaiklahan et al., 2018).

Table 5-3. Operational expenditures for *Arthrospira* cultivation system and phycocyanin extraction.

OPEXs	
Cultivation system*	
Parameter	Cost (AU\$/ha)
Power	4,678
Nutrient N and P	820
NaHCO ₃	14,112
CO ₂	16,886
Labour	6,846
Maintenance and insurance (5% of CAPEX)	13,606
Depreciation (10% of CAPEX)	20,877
Total annual OPEXs	77,827
Total OPEXs for 9.4 ha	731,663
Phycocyanin production** (45 t/y)	
K ₂ HPO ₄ (478.5 ton/year)	3,523,650
KH ₂ PO ₄ (237.7 ton/year)	1,288,125
Water (45000 m ³ /year)	25,200
Electricity (kWh/year)	33,260
Labour (4 persons/year)	240,000
Depreciation	746,675
Total annual OPEXs	5,856,910

* Values are based on (Benemann & Oswald, 1996).

** Data are on based on (Chaiklahan et al., 2018).

The production costs of *Arthrospira* biomass and phycocyanin were calculated for a conventional industrial scale raceway pond system. Using LSCs in such a production system using raceway ponds would make a significant difference in the production costs. As mentioned earlier, Raeisossadati et al. (2019) reported that biomass and phycocyanin productivity of *Arthrospira* increased for 26% and 44% by using red LSCs on a raceway pond. As it is shown in Table 5-2, using red LSCs would increase

the total capital cost of *Arthrospira* production by AU\$ 63,000 per hectare. In other words, using LSCs would increase the capital cost of cultivation system by 22% and the total capital costs of biomass and phycocyanin systems by 6.5% while improves biomass and phycocyanin productivity of *Arthrospira* by 26% and 44%. Consequently, using LSCs in the 9.4 ha production facility could increase biomass production of *Arthrospira* from 300 to 378 t year⁻¹ and phycocyanin production from 45 to 64.8 t year⁻¹. This will result in reducing the biomass production cost by 14% to 3.16 kg⁻¹. Such a system will also reduce phycocyanin production cost by 34% to 125 kg⁻¹. This clearly indicate that that using LSCs for growing *Arthrospira* can significantly lower the costs of biomass and phycocyanin production if the same size production facility is used.

5.6.1.1 The contribution of various cost elements to CAPEXs and OPEXs

CAPEXs and OPEXs of biomass and C-PC production of *Arthrospira* are summarised in Figure 5-4 to Figure 5-7. When LSCs are used, the highest contribution to CAPEX (31%) is the cost associated with the raceway pond and mixing system. This is then followed by the LSCs cost (20%) and engineering (9.5%) as the second highest contribution elements (Figure 5-4). The CAPEXs analysis of C-PC extraction system also shows that freeze-drying system has the highest contribution (65%) followed by filtration system (21%) as the second highest contribution element (Figure 5-5).

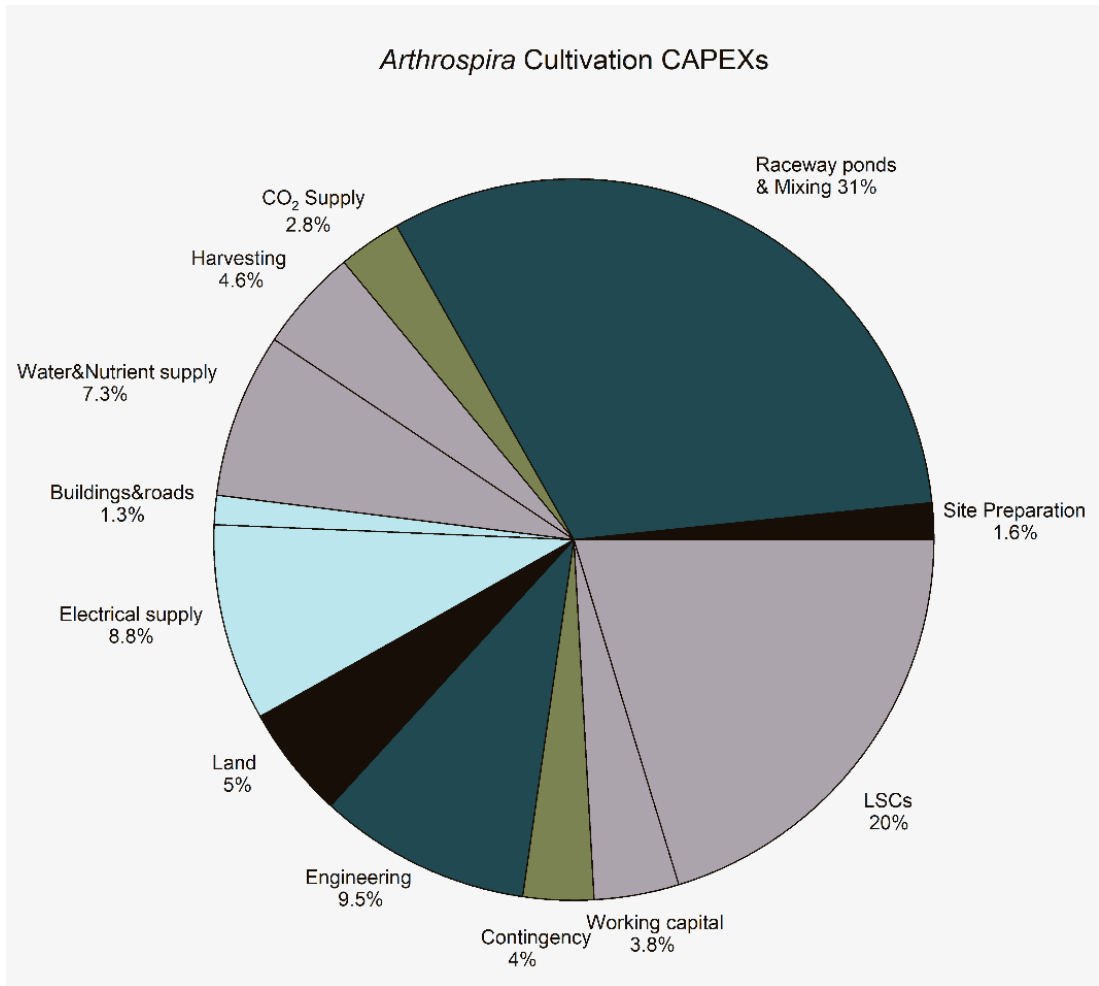


Figure 5-4. The contribution of different elements to *Arthrospira* cultivation CAPEXs.

C-Phycocyanin Extraction CAPEXs

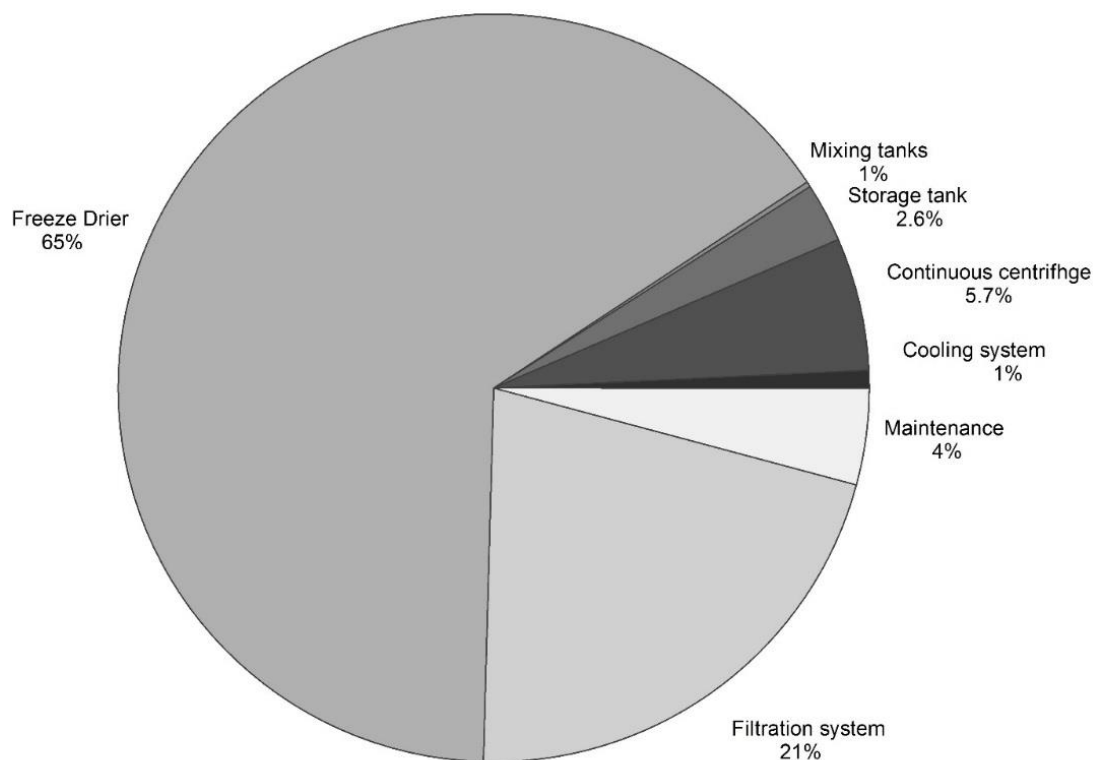


Figure 5-5. The contribution of different elements to C-Phycocyanin extraction CAPEXs.

Furthermore, OPEXs of biomass production showed that depreciation (27%) has the highest percentages while solvents (82%) has the highest cost contribution in OPEXs of C-PC extraction systems (Figure 5-6Figure 5-7). CO₂ (22%), NaHCO₃ (18%), maintenance (17%), labour (9%), power (6%) and, nutrient (1%) are other elements in OPEXs of biomass production (Figure 5-6) while depreciation (12%), labour (4%), water (1%) and, power (1%) are the elements contributing in OPEXs of C-PC extraction system (Figure 5-7).

Arthrospira Cultivation OPEXs

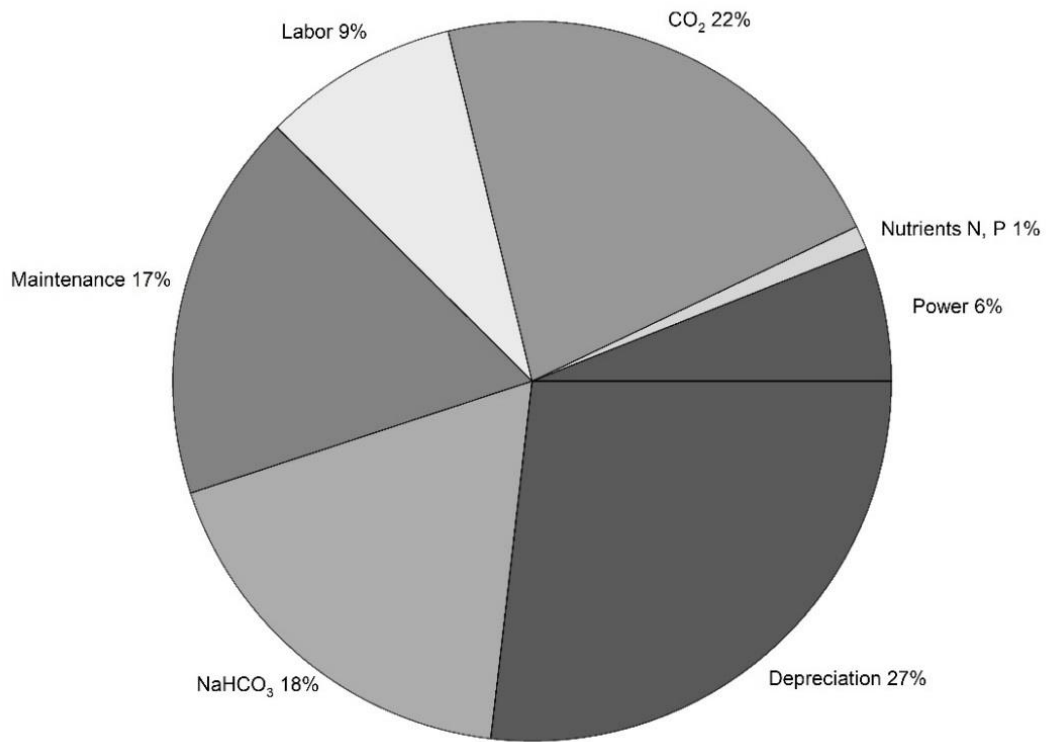


Figure 5-6. The contribution of different elements to *Arthrospira* cultivation OPEXs.

C-Phycocyanin Extraction OPEXs

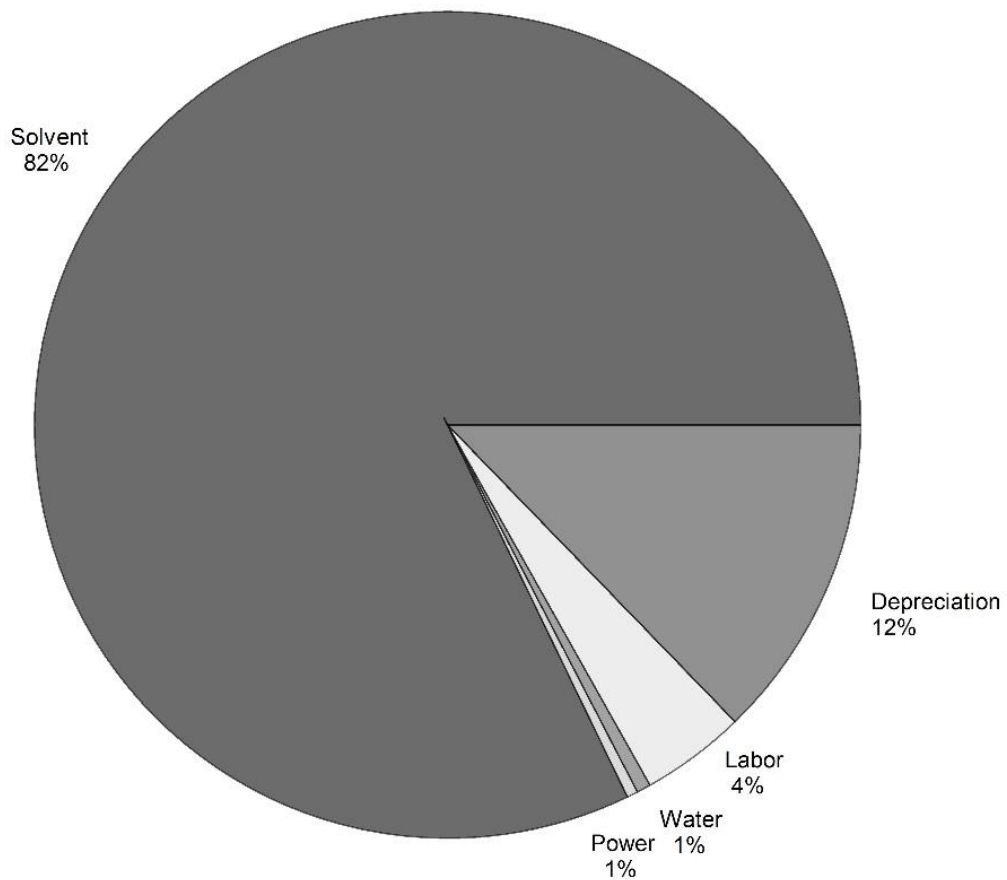


Figure 5-7. The contribution of different elements to C-Phycocyanin extraction OPEXs.

5.6.1.2 Sensitivity Analysis

A sensitivity analysis was carried out to assess the effect of each variable on the overall cost of C-PC production. The sensitivity analysis was done based on changing one parameter at the time while other parameters were constant. The percentage change was based on having 50% higher or lower on the corresponding assessed parameter. As it is shown in Figure 5-8, the changes in the C-PC production cost is based on changing variables costs which have the most significant impact on the C-PC production cost including C-PC OPEXs, C-PC CAPEXs, harvesting/dewatering,

power, solvent, nutrients, LSCs and, phycocyanin content in biomass. It is to be noted that power is the total electricity consumption in OPEXs of both biomass production and C-PC extraction systems. Amongst all variables, the phycocyanin content in biomass and biomass productivity have the highest impact on the C-PC final production cost (Figure 5-8). Increasing the percentage of C-PC in biomass by 50%, from 15% to 22.5%, would result in lowering the C-PC production cost by 33%, while decreasing phycocyanin content of biomass by 50% would double the cost of C-PC production (Figure 5-8). It is noteworthy that the effect of increasing biomass productivity for 50% to about $14.5 \text{ g m}^{-2} \text{ d}^{-1}$ was the same as the effect of increasing the phycocyanin content (Figure 5-8). In other words, increasing biomass productivity by 50% would also decrease the C-PC production cost by 33% to AU\$ 84 kg^{-1} (Figure 5-8).

The next important variable was OPEXs of C-PC extraction. Reducing OPEXs of C-PC production by 50% would reduce the cost of C-PC production by 36% while a 50% increase in OPEXs of C-PC production would increase the cost of C-PC production by 37% (Figure 5-8). The effect of a 50% increase or decrease in solvent cost of C-PC production was also similar to the effect of OPEXs of C-PC production (Figure 5-8).

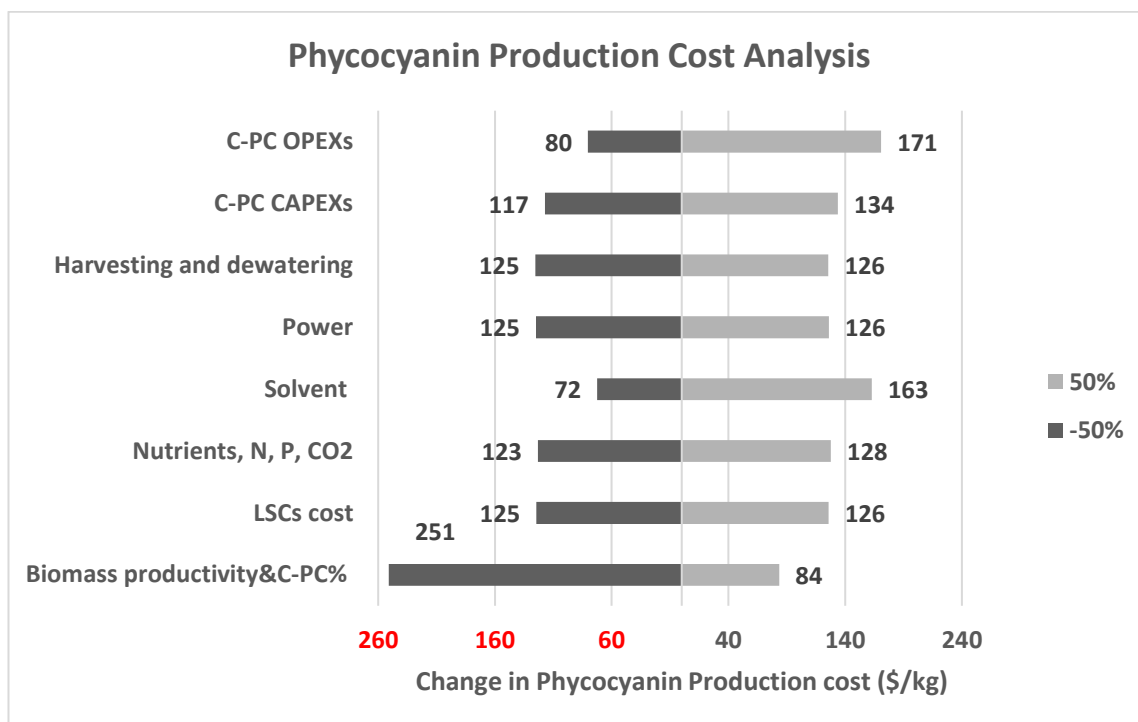


Figure 5-8. Sensitivity analysis for C-PC production cost.

5.6.2 Co- or by product

Using the left over *Arthrospira* biomass from C-PC extraction can be also used for co- or by-product to reduce the overall production cost. Methane production as a result of fermenting the algal biomass is considered as a major by-product (Borowitzka, 2013b). Alternatively, the left-over of *Arthrospira* biomass can also be used as a high grade animal feed (protein) with the value of around US\$ 1000 per ton (Borowitzka, 2013b). While the bulk of revenue will potentially be generated from the CPC production. The co-products can also generate a significant revenue.

5.7 Discussion

There is a very limited information on the production cost of *Arthrospira* biomass and phycocyanin due to commercial purposes. The production costs of *Arthrospira* biomass and phycocyanin obtained in this study is lower to the values reported in the

literature. For instance, Borowitzka (2013b) reported the *Arthrospira* biomass production cost of US\$ 10-20 kg⁻¹ for a different large scale plants. Chaiklahan et al. (2018) also reported a phycocyanin production cost of about US\$ 250 kg⁻¹ while Borowitzka (2013a) reported a selling cost of US\$ 500-10000 depending on purity of phycocyanin.

There is no doubt that increasing biomass and phycocyanin productivity of *Arthrospira* is the main aim of any large-scale cultivation system to reduce the cost of biomass production or targeted high value product such as phycocyanin. Increasing availability of light to the microalgal cells by a light diffusing system would improve the growth rate. However, to date, the most challenging part is the economic feasibility of such a system when a light distribution system is applied. In this study, the economic assessment aiming at evaluating the costs of biomass and C-PC production using red LSCs showed a significant decrease in the final production costs. Using a traditional large-scale raceway pond cultivation of *Arthrospira* results in a C-PC production cost of AU\$ 187 kg⁻¹ while using LSCs in the raceway ponds would bring the cost down to AU\$ 125 kg⁻¹. The results in the current economic analysis for the phycocyanin production cost (AU\$ 187 kg⁻¹) is less than the phycocyanin production cost of about US\$ 250 kg⁻¹ reported by Chaiklahan et al. (2018).

The sensitivity analysis in the current study showed that using LSCs with the C-PC content of 22.5% in biomass would lower the C-PC cost to AU\$ 84. As it is shown in Figure 5-8, C-PC content or biomass productivity, C-PC OPEXs and solvent are the most important variables which have the most significant effect on the final cost of C-PC production. It means that there is still a chance of further decrease in the cost of C-PC production by improving the variables. For instance, Chaiklahan et al. (2018) showed that the phosphate buffer used for phycocyanin extraction can be recycled in

the process. Thus, considering a buffer recycling efficiency of 50% would result in 50% less cost for solvent which would reduce the C-PC production cost from AU\$ 125 to AU\$ 72 kg⁻¹ by 42% (Figure 5-8).

Co- or by-product can also make an *Arthrospira* production plant more profitable. In this study, the amount of left-over biomass from phycocyanin extraction will be 313 t and 255 t with and without LSCs in the system. Considering that the left-over biomass from phycocyanin extraction has still high protein content, it can be used for animal feed. Considering the biomass market price of US\$ 1000 t⁻¹ as animal feed (Borowitzka, 2013b), it means that the left over biomass would generate revenue of around US\$ 313,000 and US\$ 255,000 in cultivation system with and without LSCs.

5.8 Conclusions

This is the first economic analysis to evaluate the use LSCs as light guides to increase light availability to microalgae cells in a large-scale cultivation system. This study showed that using LSCs in a large scale *Arthrospira* cultivation to produce phycocyanin decreased the cost of C-PC production by 34%. This analysis indicated that using LSCs for a large scale *Arthrospira* cultivation can be a promising method to not only improve biomass and phycocyanin productivity but also lower the cost of C-PC production.

5.9 References

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6 General conclusion

6.1 Outcome of the study

In this study three microalgae, *Arthrospira platensis* (MUR 129), *Scenedesmus* sp. (MUR 268) and *Chlorella* sp., (MUR 269) were tested under red and blue luminescent solar concentrators. The feasibility experiment showed that *Arthrospira platensis* (MUR 129), *Scenedesmus* sp. (MUR 268) grew better under red and blue LSCs and thus, they were selected for outdoor experiment in raceway ponds.

Arthrospira platensis showed significantly higher biomass and phycocyanin productivity under red LSCs when grown in raceway ponds outdoors compared to control and blue panels. The phycocyanin content of *Arthrospira platensis* were also significantly higher under red and blue LSCs than that in control. However, maximum quantum efficiency of *Arthrospira platensis* were not significantly different in all treatments. *A. platensis* cultures grown outdoors in this study clearly were photoinhibited as F_v'/F_m' reduced by over 40%. Moreover, biochemical analysis also resulted in no significant difference in protein and lipid content of outdoor *Arthrospira* cultures under red and blue LSCs and, control.

Biomass productivity of *Scenedesmus* sp. significantly increased by 18.5% when red LSCs were used. Specific growth rate of *Scenedesmus* sp. was also significantly higher under red LSCs compared to control. Protein and lipid contents of *Scenedesmus* sp. cultures with red LSCs also increased 17.5% and 10% under red LSCs.

In this study, the biomass productivity of both *Arthrospira platensis* (MUR 129), *Scenedesmus* sp. (MUR 268) grown outdoors increased significantly under red LSCs. The reason can be viewed from two different perspectives; 1) The total energy delivered by red LSCs to the system, 2) The instant light emitted from the red LSCs to

the system. Using four red LSCs in each raceway pond showed delivering of $34 \mu\text{mol photons s}^{-1}$ to the depth of microalgal cultures. This means injecting $34 \mu\text{mol photons s}^{-1}$ deep into the microalgal cultures where it would otherwise be in full darkness. This helps move the light from the photosaturated surface to the depth of the cultures. Furthermore, taking mixing rate, thickness of the red LSCs and, PAR emitting from an edge of a red LSC would provide $506 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ to microalgal cells in 27 ms. In other words, microalgal cells received around $506 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in 27 ms when they pass each edge of a red LSC. Thus, it can be said that microalgal cells with red LSCs received brief bursts of light with different intensities for durations less than a second inside the cultures while there was total darkness for the cultures without LSCs (control cultures). It also should be noted that considering the light saturation of most microalgae is around $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, delivering $34 \mu\text{mol photons s}^{-1}$ into the depth of microalgae cultures could make a huge difference on the growth of cultures.

Besides, the outdoor raceway ponds in this study had a more uniform and turbulent mixing pattern (more vertical mixing) compared to large scale raceway ponds and thus, more microalgal cells could be exposed to sunlight. But even with such a turbulent mixing, *Arthrospira* and *Scenedesmus* sp. cultures with red LSCs had a significantly higher biomass productivity compared to control with no LSCs. It clearly demonstrated that there is a high possibility of higher biomass productivity when using red LSCs in a larger raceway pond where the uniform mixing is almost impossible. In large raceway ponds, there is only vertical and turbulent mixing near paddle wheels followed by a long laminar flow along the channel which leaves the most of microalgal cells in darkness. The region of laminar flow increases with longer channels where

there is little vertical mixing. That means cells at a lower depth receive very little light while cells on the surface are photoinhibited.

In this study, the preliminary economic feasibility of using luminescent solar concentrators as a light delivering system on an *Arthrospira*'s production scale raceway pond plant was also assessed. Using red luminescent solar concentrators resulted in a biomass and phycocyanin production costs of AU\$ 3.16 and AU\$ 125 per kg. These are 14% and 34% lower than the production costs of biomass and phycocyanin compared to conventional raceway ponds. The sensitivity analysis also showed that biomass productivity, C-PC content and, C-PC OPEXs are the most important parameters influencing the final production cost of phycocyanin. The production cost analysis clearly showed that using LSCs can significantly lower the cost of biomass and phycocyanin production if the same size production facility is used.

6.2 Significance of the study

Without a doubt, paddle wheel driven raceway ponds are the preferred commercial cultivation system for mass production of microalgae. One of the main challenges for culturing microalgae in large scale raceway ponds is the low availability of light at the depth of the cultures resulting in low biomass productivity. Therefore, increasing light irradiance at the depth of raceway pond cultures by using an efficient light delivering system would enhance the biomass productivity as well as reducing the capital costs. The results of this study clearly showed that the use of red LSCs in raceway ponds with the proposed design (see Chapters 3 and 4) can significantly increase biomass productivity of *A. platensis* and *Scenedesmus* sp. The main advantage of using LSCs with such a design is the potential capability of applying them to a large-scale paddle

wheel driven raceway pond. As mentioned earlier, using red LSCs also increased phycocyanin productivity of *A. platensis* by 44%. This is achieved by delivering a suitable wavelength of light into the depth of algal cultures in raceway ponds. The active light delivering system used in this study has the advantage of an increasing number of photons as well as shifting light to a suitable spectrum for the microalgal growth. Therefore, for the same phycocyanin productivity achieved in this study, there is an advantage of reducing the cultivation area by 44% potentially. The cost analyses also indicated that the overall cost of biomass and phycocyanin production can be significantly lowered if LSCs are used.

6.3 Future direction

The results of this study clearly showed that using commercially available red LSCs can improve biomass productivity of at least two microalgal species grown in raceway ponds. The red LSCs worked well for both *Arthrospira* and *Scenedesmus* grown in raceway ponds but the blue LSCs did not show significant improvement in growth. As mentioned in Chapter 3, the reason is because of a lower blue pigment concentration in the blue LSCs compared to red pigments in red LSCs. Thus, one of the potential optimization studies can be made on the use of blue LSCs having a higher concentration of blue pigments and assess the effect of them on the growth of different microalgae species. Theoretically, blue photons are desired in the process of photosynthesis. Therefore, injecting higher blue light into the depth of microalgae cultures can potentially result in higher biomass productivity.

Secondly, the commercially available LSCs were used in this study. LSCs are made of a polymer, polymethyl methacrylate, and pigments are embedded inside panels that

corresponds to the colour of the panel. Using the lab-made LSCs can potentially result in a lower cost compared to the commercial LSCs.

More importantly, there is a need to test the suitability of the process at the scale. The scale tested in this study was limited. Scaling up is indeed very important. Such studies can give a better indication of the effects of LSCs and capability of using them in a larger scale microalgae cultivation. Optimization on a large-scale installation of these LSCs is also a must. The LSCs will need to be designed in a way to have more surface area at the part of panels inside the microalgal cultures. A modelling analysis can help to optimize such a design of LSCs to increase the efficiency of LSCs and thus, achieving highest possible biomass productivity for microalgal cultures.

In conclusion, this study was the first study of its kind to use LSCs for microalgal cultivations in raceway ponds to not only shift the sunlight to the desired wavelength but also deliver the light to depth of the cultures. This study showed that using LSCs can be a promising method to increase light availability to microalgal cells at the depth of the cultures in raceway pond.