Novel photobioreactor for the sustainable production of algal biomass and electricity

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DECLARATION

I declare that this thesis is my own account of my research and that, to the best of my knowledge, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgement has been made in the text. I also acknowledge that the copyright of published manuscripts included in this thesis resides with the copyright holder of those works. I give consent for a copy of my thesis to be deposited in the University Library and on the web, through the University's digital research repository, subjected to provisions of the Copyright Act 1968

Emeka G. Nwoba

December 2020

DEDICATION

To my lovely wife, Fortunate, and my two daughters, Ever and Verity.

ABSTRACT

Mass outdoor microalgal cultures for the production of low priced bio-based commodities (food, feed, fuels) and high-value bioproducts (polyunsaturated fatty acids, pigments, therapeutic agents) require stable and commercially-viable biomass production technologies. The classical open raceway pond, the traditional commercial-scale technology for mass biomass production has significant limitations; low productivity rates due to a long culture depth, high risk of contamination, and lack control of environmental conditions. To produce high biomass density microalgal cultures, closed photobioreactors are preferred due to a better operational control of culture conditions, environmental variables and contamination. However, the operation of solar closed photobioreactors under outdoor scenarios requires sufficient cooling (in summer) and heating (in winter) technologies for guaranteed production of biomass (products) throughout the year. Heating and cooling operations are not only expensive and energy-intensive but require both grid electricity and precious freshwater (already limited) for their effectiveness, thus imposing a sustainability challenge. Therefore, next-generation algal photobioreactor designs must address these challenges of cost, energy and land-use efficiencies, while offering optimum biomass production. To this end, we have developed for the first time a hybrid thermally-insulated photobioreactor that is based on illumination spectral filtering for passive temperature control and integration with photovoltaic panels for electrical energy generation geared towards grid-independent operation. The novel photobioreactor has the illumination surfaces constructed of spectrally-selective low-emissivity film, which reflects >90% of non-photosynthetic photons (ultraviolet and infrared wavelengths) and transmits >70% of photosynthetically-beneficial visible photons (wavelengths spanning 400 to 700 nm) and its

double glass units allow for high thermal insulation. A semi-transparent cadmium telluride photovoltaic cell that transmits 40% of the captured sunlight was glued to the top of the photobioreactor.

To assess the viability and effectiveness of the novel photobioreactor design in thermoregulating microalgal cultures, the growth and photophysiological responses of two microalgae species *Nannochloropsis* sp. MUR 267 and *Arthrospira platensis* MUR 126 were investigated under laboratory conditions. Experimental results show that the maximum culture temperature in the novel photobioreactors was similar to the conventional water jacket system and 23-33% lower than that in the controls without temperature control system. The biomass productivity of *Nannochloropsis* culture in the insulated photobioreactors (112.47±3.36 mg L^{-1} d^{-1}) was only 10% lower than that attained in the water jacket reactor, and no net growth was seen in the control without thermoregulation due to a high temperature. Chlorophyll *a* fluorescence measurements show that both microalgae cultures in the cultivation systems were not thermally stressed. This proof-of-principle study clearly demonstrated that infrared blocking films can significantly reduce heat gain in flat plate photobioreactors without a dramatic reduction in culture performance. At this point, a pilot-scale spectrally-selective insulated-glazed photovoltaic (IGP) flat panel photobioreactor (1.2 m length x 1.5 m height, 10 cm optical depth, 140 L working culture volume) capable of co-producing microalgal biomass and electricity, while eliminating the need of cooling water was developed. The viability of this novel system for culturing *Nannochloropsis* sp. was compared to similar flat panel photobioreactors based on freshwater passive evaporative cooling (PEC), infrared reflecting thin-film coating (IRF), and an open raceway pond (ORP). Maximum culture temperature (33.8 ± 2.9 ˚C) was highest in the IRF reactor while no significant difference was seen between IGP and PEC photobioreactors. Specific growth rate and biomass productivity of *Nannochloropsis* sp. was similar in all closed

photobioreactors; however, ORP showed significantly lower productivity. Algal cultures in these cultivation systems were not thermally stressed. Interestingly, electricity generated from IGP photobioreactor during this period was 2.5-fold higher than the mixing energy requirement.

Investigating the impact of the temperature control strategies on macromolecular content and fatty acid profile of *Nannochloropsis* sp., the normalized biochemical composition of the biomass showed a general trend of lipid > protein > carbohydrate, with no large variation of each across treatments. Besides C16:0, which was 24% higher in the photobioreactors than ORP, no other significant shift in major saturated and monounsaturated fatty acid components of this alga were seen among cultivation systems. The highest eicosapentaenoic acid (EPA, C20:5n-3), 16% and ϒ-linolenic acid (C18:3n-6), 8% of total fatty acid were found in ORP with the lowest average culture temperature and diel temperature variation than photobioreactors. Among all photobioreactors, IGP has the least diel temperature changes with an EPA content that was 21% higher than PEC, indicating that constructing photobioreactors with spectrally-selective materials is a viable strategy for managing the internal temperature, with no significant negative impact on biochemical and fatty acid profiles of microalgae.

When a cold-intolerant microalga, *Arthrospira platensis* was cultured in the thermally-insulated IGP (no heat supplementation) during austral winter and compared with PEC under a cycle of heating (13-hour night) and thermostat-regulated cooling, and a continuously heated ORP, the average temperature in the IGP (21.0±0.03˚C) was similar to the heated PEC. Experimental results indicated that biomass productivity of *Arthrospira* in IGP photobioreactor was 67% higher than ORP and significantly lower than PEC. Phycocyanin productivity (16.3±1.43 mg g^{-1} d^{-1}) showed no variation between photobioreactors but

significantly less in the ORP. During this winter operation, electrical energy output of IGP photobioreactor exceeded mixing energy need by 75%.

Finally, from the energy efficiency perspective, the net energy ratio of a 1-ha IGP facility used to cultivate *Nannochloropsis* sp. without freshwater-based cooling reached 3.0, a value comparable to agricultural bio-oil crops such as *Jatropha* and soybean. The annual biomass productivity was 66.0-tons dry weight ha^{-1}, equivalent to overall energy output of 1,696 GJ ha^{-1}. The integrated semi-transparent photovoltaic panels generated an additional 1,127 GJ ha^{-1} yr^{-1} (313 MWh ha^{-1} yr^{-1}). Energy demands from plant building materials, machinery, fertilizers, plant operations, and biomass harvesting constituted total energy input with a combined value of 707 GJ ha^{-1} yr^{-1}. A comparison with a PEC photobioreactor requiring freshwater-based cooling showed that IGP had a 73% greater net energy ratio using the same plant size and system boundary.

In conclusion, the above results suggest that developed IGP photobioreactor offers a reliable, energy-efficient platform for large-scale production of biomass and high-value chemicals from microalgae, with no requirements for extraneous cooling and heating systems, generating sustainable baseload electrical energy to energize production operations.

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Emeka G. Nwoba. How photovoltaic systems could aid biofuel production – The Electric Energy Society of Australia (EESA) Symposium 28 October 2019

Emeka G. Nwoba. Novel algal photobioreactor eliminates freshwater-related cooling and generates electricity for sustainable production of bio-based products – Murdoch Annual Research Symposium, Australia, 3 June 2019.

Emeka G. Nwoba. Photobioreactor that uses sunlight as fuel to make electricity and generate algal biomass – The Electric Energy Society of Australia (EESA) Symposium, Australia, 29 October 2018

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ABBREVIATIONS

AFDW	ash-free dry weight
C-PC	c-phycocyanin
CWJ	conventional water-jacket
IR	infrared
IGP	insulated-glazed photovoltaic
IRF	infrared reflecting film
NHC	no heat control
OJIP	polyphasic chlorophyll *a* fluorescence transient
PAR	photosynthetic active radiation
PEC	passive evaporative cooling
PBR	photobioreactor
UV	ultraviolet
CO_2	carbon dioxide
PE	photosynthetic efficiency
PSII	photosystem II
CCS	CO2 capture and storage
CCU	carbon capture and utilization
LED	light emitting diode
RGB	red green blue ratio
PV	photovoltaic
WLE	wireless light emitter
EM	electromagnetic
PPV	photosynthetic plasmonic voltaic
OPL	optical path length
RLC	rapid light curve
ETR_{max}	maximum electron transport rate
rETR	relative electron transport rate
RM-ANOVA	repeated measures analysis of variance
PFD	photon flux density
F_q'/F_m'	effective quantum yield under light condition
OJIP	chlorophyll *a* fast transient curve
W_k	heat stress parameter
F_v/F_o	relative activity of water-splitting complex on the donor side of PSII
F_m	maximum fluorescence
I/P	ratio between I and P phase of OJIP
APC	allophycocyanin
SCF	solar control film
S/V	surface area volume ratio

NaCl	sodium chloride
PVC	polyvinylchloride
IGU	insulated glazed unit
CdTe	cadmium telluride
VVM	volume of air per volume of culture per minute
Low-e	low emissivity
SE	standard error
ORP	open raceway pond
NIR	near infrared
VIS	visible
EPA	eicosapentaenoic acid
ARA	arachidonic acid
DHA	docosahexaenoic acid
HCl	hydrochloric acid
GOED	global organization for EPA and DHA
FAME	fatty acid methyl ester
PUFA	polyunsaturated fatty acid
MUFA	monounsaturated fatty acid
SFA	saturated fatty acid
GLA	gamma linolenic acid
MELiSSA	micro-ecological life support system alternative
TiO_2	titanium oxide
CdS	cadmium sulphide
Al	aluminum
ZnO	zinc oxide
ITO	indium tin oxide
$MgCO_3$	magnesium carbonate
PAM	pulse amplitude modulation fluorometer
MC-PAM	microscopy PAM
NER	net energy ratio
$NaNO_3$	sodium nitrate
GWP	green wall panel
Si-PV	silicon PV

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NATURE OF THESIS

The current thesis is prepared and formatted as a thesis by publication where individual chapters consisted of manuscripts which have been published in high impact peer reviewed journals. Each chapter, including the reference style, is presented and formatted strictly in accordance with the journal of publication requirements. However, heading numbering and style, figure, table and equation numbering have been slightly modified where necessary, to allow for harmony and desired flow throughout the thesis. A certain degree of repetition is inevitable due to the nature of the publications and this can be observed more often in the introduction of chapters. Additional text has been added to link chapters together.

CHAPTER 1

Introduction

Microalgae are a diverse group of photosynthetic microorganisms that uses light, CO_2, H_2O, and nutrients to produce valuable biomass. Microalgal biomass constitutes an excellent source of biofuels, food, feed, cosmetics, pharmaceutics, and bulk commodities. Microalgae culture is similar to agriculture, yet its non-reliance on freshwater supply, farmable land and traditional fertilizers makes it attractive as a sustainable and environmentally-friendly bioresource. However, microalgal cultivation is not efficiently conducted at large-scale when compared to soil-based terrestrial crops. Classical open raceway ponds have been recognized as a prime system for easy and economical production of microalgal biomass but have limited control of culture conditions, are susceptible to contamination, have low biomass productivity, and can be unreliable for long term maintenance of cultures. On the other hand, closed photobioreactors offer excellent control of operational cultivation conditions combined with a large surface area to volume ratio that results in significantly high biomass productivity and guaranteed supply of biomass over the year. These merits of photobioreactors come at the cost of high capital investment and energy-intensive operations for temperature control. Flat plate and tubular photobioreactors represent the commonest large-scale closed photobioreactor configurations, although industrial-scale application is only feasible to produce high-value products, whose economic profitability justify the high production cost. Therefore, to produce bulk commodities from microalgae in photobioreactors in a sustainable manner, the operational energy cost most especially for cooling purpose needs to be diminished.

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Review article

Light management technologies for increasing algal photobioreactor efficiency

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ABSTRACT

The ever-increasing demand for food, valuable bio-based compounds and energy has triggered the development of novel and sustainable resources. Microalgae are a promising source of sustainable high-value products. The need for light (suitable intensity and wavelength) and temperature control in microalgal cultures remains the most significant challenge limiting their photosynthetic efficiency and productivity. Appropriate light management has the potential to concurrently maximize photosynthetic productivity and control the temperature of microalgal photobioreactors resulting in a reduction in overall production costs. Here, we review innovations to improve light conversion efficiency and temperature control, such as spectral filtration, plasmonic waveguides, spectral shifting, wireless light emitters and insulated glazing, which typically increase the photosynthetic productivity, while avoiding overheating in photobioreactors. Infrared filtering reduces culture overheating in closed photobioreactors. Spectral shifting, plasmonic waveguiding, switchable glass and insulated glazing technologies can improve light quality received by algal cells. Improving light efficiency and distribution in the algal cultures can significantly enhance biomass productivity when used in open or closed cultivation systems. Based on this background, we illuminate the effectiveness of embedding the above-mentioned technologies into a novel insulated-glazed photovoltaic flat panel photobioreactor for simultaneously increasing the biomass and generating electricity, thus, eliminating the need for cooling systems. This approach opens the way for the development of cost-effective, low-carbon-footprint grid-independent integrated algae-based biorefineries with multi-product yields.

1. Introduction

Dwindling fossil fuel reserves, growing energy demands, and a desire to offset the effects of climate change have become significant global concerns. This has led to the global scientific and engineering community instigating a major effort to develop feedstocks for energy, chemical and materials production derived from renewable sources and can be produced and processed in an eco-friendly manner [1]. While initial efforts have been based around utilizing conventional land-based agriculture, it has become apparent that these practices, particularly for bioenergy crops, are ultimately not sustainable due to (i) the large amounts of arable land and freshwater required, (ii) competition with necessary food production, and (iii) the potentially deleterious effect of climate on traditional agricultural practices. The mass culture of microalgae has emerged as perhaps the most promising source for sustainable and carbon neutral production of biofuels (biodiesel, biogas, bio-crude oil, bioethanol and biohydrogen [2]), high-value bioactive products for nutraceutical and pharmaceutical applications [3,4], and as supplements in human and animal nutrition (Fig. 1). There are even examples of producing biomaterials such as biodegradable and biocompatible bioplastics from some microalgal species [5,6] and other potential applications of microalgal biomass is summarized in Fig. 1.

The case of developing microalgae culture for renewable chemical feedstock production is compelling. These organisms have attractive features, including (i) high photosynthetic conversion competence (10–50 times higher than C4 plants), (ii) capacity for considerable CO_2

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David A. Parlevliet	Conception and design, interpretation of data, critical revision of the manuscript.
Damian W. Laird	Interpretation of data, critical revision of the manuscript.
Kamal Alameh	Interpretation of data, critical revision of the manuscript.
Navid R. Moheimani	Conception and design, interpretation of data, critical revision of the manuscript.

Principal supervisor confirmation

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

Name	Signature	Date
David A. Parlevliet		**8/12/2020**

1.1 Abstract

The ever-increasing demand for food, valuable bio-based compounds and energy has triggered the development of novel and sustainable resources. Microalgae are a promising source of sustainable high-value products. The need for light (suitable intensity and wavelength) and temperature control in microalgal cultures remains the most significant challenge limiting their photosynthetic efficiency and productivity. Appropriate light management has the potential to concurrently maximize photosynthetic productivity and control the temperature of microalgal photobioreactors resulting in a reduction in overall production costs. Here, we review innovations to improve light conversion efficiency and temperature control, such as spectral filtration, plasmonic waveguides, spectral shifting, wireless light emitters and insulated glazing, which typically increase the photosynthetic productivity, while avoiding overheating in photobioreactors. Infrared filtering reduces culture overheating in closed photobioreactors. Spectral shifting, plasmonic waveguiding, switchable glass and insulated glazing technologies can improve light quality received by algal cells. Improving light efficiency and distribution in the algal cultures can significantly enhance biomass productivity when used in open or closed cultivation systems. Based on this background, we illuminate the effectiveness of embedding the above-mentioned technologies into a novel insulated-glazed photovoltaic flat panel photobioreactor for simultaneously increasing the biomass and generating electricity, thus, eliminating the need for cooling systems. This approach opens the way for the development of cost-effective, low-carbon-footprint grid-independent integrated algae-based biorefineries with multi-product yields.

1.2 Introduction

Dwindling fossil fuel reserves, growing energy demands, and a desire to offset the effects of climate change have become significant global concerns. This has led to the global scientific and engineering community instigating a major effort to develop feedstocks for energy, chemical and materials production derived from renewable sources and can be produced and processed in an eco-friendly manner [1]. While initial efforts have been based around utilizing conventional land-based agriculture, it has become apparent that these practices, particularly for bioenergy crops, are ultimately not sustainable due to (i) the large amounts of arable land and freshwater required, (ii) competition with necessary food production, and (iii) the potentially deleterious effect of climate on traditional agricultural practices. The mass culture of microalgae has emerged as perhaps the most promising source for sustainable and carbon neutral production of biofuels (biodiesel, biogas, bio-crude oil, bioethanol and biohydrogen [2]), high-value bioactive products for nutraceutical and pharmaceutical applications [3, 4], and as supplements in human and animal nutrition (Fig. 1–1). There are even examples of producing biomaterials such as biodegradable and biocompatible bioplastics from some microalgal species [5, 6] and other potential applications of microalgal biomass is summarized in Fig. 1–1.

The case of developing microalgae culture for renewable chemical feedstock production is compelling. These organisms have attractive features, including (i) high photosynthetic conversion competence (10-50 times higher than C_4 plants), (ii) capacity for considerable CO_2 sequestration, (iii) the ability to be cultivated in marginal agricultural land using saline and degraded water, and (iv) the ability to recycle nutrients in wastewater and flue gas [7]. The vision of microalgal biorefineries producing multiple products from renewable, or even

waste, feedstocks in an essentially carbon neutral process is alluring. However, significant challenges to large-scale production of many of these products remain to be addressed. For example, it is widely known that biofuel production from microalgae at commercial-scale is currently unfeasible due to high cost of production [8]. At present, only a handful of high-value pigments, e.g., β-carotene, astaxanthin, phycobiliproteins, (from *Dunaliella*, *Haematococcus*, and *Arthrospira* spp.) are being produced at an industrial-scale [9-11]. To meet the growing demand for and realise the potential of microalgae biorefineries as the premier source of these valuable bio-based products, there is a critical need for continuous improvement of the microalgae cultivation technologies.

Microalgae biomass mass production is currently carried out using open ponds and closed photobioreactors (PBRs). Closed PBRs are preferred system providing optimum growth conditions (light, temperature, nutrients) for enhanced biomass production [12]. Furthermore, closed PBRs significantly minimises the likelihood of contamination (by bacteria, protozoa, unwanted algal species) and competition that can occur in the open ponds. This quality is particularly important as products meant for utilization in the pharmaceutical and functional food industries need to be free of significant bacterial contamination. Biomass productivity remains the main driver of commercialization of industrial products from microalgae, as there exist a positive correlation between biomass and algal products productivity, in most cases. High biomass productivity leads to high exploitable biochemical productivity, hence, closed PBRs will be an essential component of the algae industry.

Light, in particular, the portion of the electromagnetic radiation spectrum comprising the ultraviolet (UV), visible and infrared (IR) regions, is the supreme growth-limiting factor that governs cell proliferation and efficiency of microalgal PBRs operating under optimum

temperature and nutrient conditions. Light is the basic energy source for microalgae and its provision in PBRs must be adequate in terms of spectral components, intensity and duration [13]. In practice, light is exponentially attenuated along the optical depth in a PBR due to the significant mutual shading effects that occur among microalgal cells. Due to the difficulty in controlling light reaching the PBR, maintaining optimal light quality in PBRs is a significant constraint for the efficient operation of high-density cultures. Furthermore, a substantial portion of the full spectral bandwidth of light reaching the PBR surface (the UV and IR wavelengths) does not participate in the photosyntetically-driven solar energy conversion process [14]. Only visible light (400 – 700 nm) is considered to be the photosynthetically-active radiation (PAR) that can be harvested and converted to chemical energy in biomass – a function of the selectivity of microalgae light-harvesting pigments. On the other hand, the high-energy UV radiation (<400 nm) results in ionizing effects in absorbing materials and causes cell damage, while the low-energy IR photons (>750 nm) contribute to significant thermal effects and results in overheating of the culture in PBR systems [15]. However, a few oxygenic photosynthetic cyanobacteria (e.g., *Acaryochloris marina*), containing an abundance of chlorophyll *d*, have the selective advantage of utilizing near-infrared (700-750 nm) radiation for photosynthesis [16]. The resultant effect of UV and IR radiation transmitted to the interior of the PBR is a significant decrease in microalgal bioproductivity. Overheating is lethal to algae and this necessitates the deployment of costly measures to minimize these deleterious effects. For example, control of culture temperature in PBRs to avert overheating is carried out either by passive-evaporative cooling systems, such as the spraying of freshwater on the surface of PBRs or use of heat exchange systems. The requirement for a considerable volume of freshwater for cooling PBRs is a severe limitation to their large-scale use. In almost all locations suitable for microalgae farming (i.e., those that have high solar

radiation), freshwater is a limited resource [17]. The need for freshwater temperature control for PBR operation is energy-intensive, expensive, unsustainable, and inefficient. Therefore, selective manipulation of incident insolation, such that the photons harvested by the algal culture consist of the more desirable PAR wavelengths, and less of the harmful UV and heat-inducing IR photons, has the potential to improve PBR efficiency and bioproductivity.

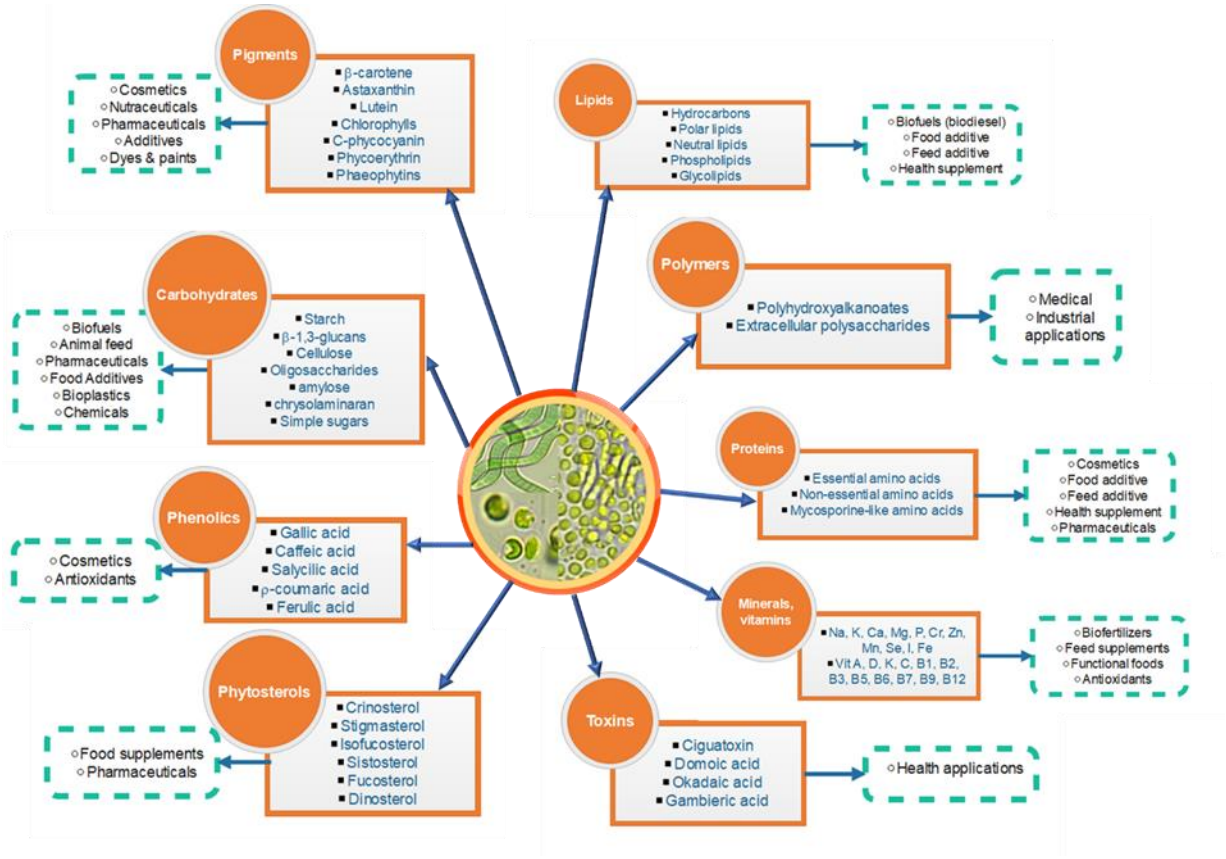

Fig. 1–1. Bio-based products from microalgae and their potential applications [6, 18]

Here, we firstly provide an overview of microalgal light harvesting and transformation, appreciating the significant role that photosynthetic pigments play in this process. We highlight the essential feature of algal growth in closed PBR conditions in relation to the optimization of photosynthetic efficiency (PE). Innovations to improve light use efficiency in microalgae production PBRs (e.g., spectral shifting, conversion, filtration, insulated glazing) to upgrade PE and biomass productivity, as well as control temperature, are then reviewed.

The main aims of applying these management strategies are (i) effective light control in PBRs, (ii) maximization of biomass, and (iii) metabolite productivities. Finally, we suggest what might be achieved by coupling these in light use applications with emerging capabilities in the manufacture of advanced materials.

1.3 Harvesting and transformation of light by microalgae

The energy absorbed by microalgae is, to a great extent, determined by the chemical nature of their native pigments. These pigments have characteristic colours and preferentially absorb specific wavelengths. The pigments have their absorption bands in the visible and near-IR regions of the solar spectrum; these regions correspond to the peak of the solar output. A broad range of pigments evolved in photosynthetic organisms for light capture and processing, including chlorophylls, carotenoids and phycobilins (Table 1–1). Chlorophylls represent the major group, with chlorophyll *a* as the most important. Others, such as chlorophylls *b* and *c (*absorb other light wavelengths and transmit their energy to chlorophyll *a)*, carotenoids, and phycobilins constitute the accessory pigments [19, 20]. Their ability to uptake these wavelengths allows the algal cells to use a wider range of PAR [21]. Carotenoids are strongly involved in chromatic adaptation, where they facilitate the absorption of those light wavelengths inefficiently absorbed by chlorophyll *a*, and transmit the harvested photon energy to the chlorophyll *a* molecule. Carotenoids are also responsible for free-radical scavenging activity in cells, to avoid oxidative damage by conducting dissipation of excess energy as heat [22]. In reality, carotenoids are usually orange, yellow or red coloured, and therefore, do not absorb radiation in these regions, but rather absorb in the violet/blue/green portions of PAR [13]. The phycobilins (e.g., phycocyanin) occur in cyanobacteria and red algae [10].

During photosynthesis, photons are first captured by the chlorophyll *a* and other pigments in association with surrounding proteins (light-harvesting antenna/complexes) and the energy is transferred efficiently to photochemical reaction centers, which consist of pigment-protein complexes [23]. The energy transferred to a photochemical center can be used for photochemistry (biomass formation), fluorescence or dissipated as heat. The size of antennae pigments is species-specific and in line with prevailing light conditions of the particular environment in which the organism is found. In this environment, the photon flux (intensity), spectral distribution (quality) and light uniformity vary. Microalgae can have a large/small number of different pigment complexes and/or they can regulate the amount of each of those pigments that are present at any time. To meet the photosynthetic requirements, species found in environments with limiting light conditions usually have large number of antennae. At saturating irradiances, large antennae pigments are inefficient at absorbing light, resulting in a condition where photon capture is 100-fold faster than the rate at which electrons are funnelled to the reaction centers, ultimately leading to photosaturation, photoinhibition, and photooxidation [24, 25]. This scenario obviously has flow-on effects for the health, photosynthetic efficiency, and productivity of microalgal cultures. In contrast, those species found in high light environments have a smaller number of pigments to avoid photoinhibition [26]. Improving species performance through tailoring of the light-harvesting pigments can increase the spectral sensitivity of the microalga and, consequently, tolerance to photosaturation, photoinhibition, and changing environmental light conditions. It is possible to select those wavelengths from the light spectra that correspond to or are close to, the absorption maxima of microalgae pigments using optically engineered PBRs [27]. These developments will allow for harvesting of solar irradiance with efficiency, lower tendency to reach photoinhibition, and have a net impact on

photosynthetic efficiency, thus, increasing the microalgal biomass and improving the economic viability of industrial-scale photobioreactors.

1.4 Current constraints of culture photobioreactor operations and photosynthetic efficiency

Microalgal biomass production is carried out using open ponds and closed PBRs (Fig. 1–2A-F) [29]. Open ponds have low-cost of operation and production [2, 30, 31]. It is a consensus that the raceway ponds are the most economical technology for mass microalgal production. Hence, worldwide, almost all bulk microalgae biomass is produced in open pond systems. However, improving the performance of cultures in open ponds is usually problematic due to challenges of optimizing confounding environmental variables, such as temperature fluctuation, light limitation, inadequate mixing regimes, and culture contamination [30, 32, 33]. Closed photobioreactors (PBRs) are attractive as they offer better regulation of culture operational factors and conditions that tend to limit microalgal growth, leading to higher photosynthetic efficiency and biomass productivity. Nevertheless, in addition to their high construction cost and operation, closed-PBR-algal cultures face overheating and poor light delivery [34].

1.4.1 Temperature control

The culture temperature affects the intensity of light required by the algae for optimal productivity. At temperatures near optimum, microalgae tend to show tolerance to higher light intensity [13], while overheating diminishes their tolerance to high-intensity light fluxes. The impact of overheating cultures is more significant in closed PBRs than open ponds since the latter have a self-evaporative cooling mechanism. Daily fluctuations and seasonal variations in temperature can drastically change the culture conditions and affect the

microalgal photosynthesis and productivity. In temperate regions, temperature fluctuations of outdoor microalgae cultures in closed PBRs can reach up to 45 ˚C [35]. This temperature is well above the optimal temperature of 20-25 ˚C claimed for most commercial species of microalgae [36]. Photosynthetic activity and cellular metabolism in most species can still occur outside that range (essentially over the extended range of 15-30 ˚C), but there are signifcant decreases in productivity recorded. At temperatures below optimum, there is a positive correlation between temperature increase, photosynthesis, and growth rate, due to the enhancement of Calvin cycle enzymes [36]. However, at temperatures above optimum, photosynthetic activities and cell growth rate rapidly decrease, due to heat stress. This stress results in deactivation of the functional enzymes and proteins of the photosynthetic architecture [37]. For instance, at temperatures above 40 ˚C, the charge-separation function of photosystem (PS) II is inhibited, and the oxygen-evolving activity of PSII is disabled, as Mn^{2+} ions dissociate from the photocatalytic center. The overall effect of this is the production of oxygen free radicals that destabilize the cell's equilibrium, and damage biochemical constituents, leading to lipid peroxidation.

To address the challenge of overheating, passive evaporative cooling systems using freshwater sprays or heat exchangers are used to keep the temperature of the reactor at or below 25 ˚C. A significant amount of heat (up to 18,000 $GJ.ha^{-1}.yr^{-1}$) must be removed to maintain this temperature, in PBRs located in temperate regions (e.g., Western Australia), where the daily culture temperature can easily reach 40 ˚C [38, 39]. Regardless of the cooling efficiency, between 2,400-8,000 $m^3.ha^{-1}.yr^{-1}$ of high-quality freshwater is required to run the evaporative cooling system for a production plant delivering 36.5 tonnes of dry algal biomass annually [35].

On the other hand, heat exchanger systems can utilize seawater [40] but at higher capital and operating expenses. Therefore, evaporative cooling or heat exchange systems are not sustainable in the face of increasing freshwater scarcity. Other solutions, such as direct immersion of PBRs in pools or placement in greenhouses can raise the construction and operating expenses with a negative effect on the environment via excessive energy demand and water footprint. Therefore, cost-effective, energy-efficient, and year-round exploitable solutions for thermal regulation of closed PBRs is still a challenge. As highlighted in Section 1, about 50% of the solar energy incident on the illuminated surface of closed PBRs positioned outdoor is outside of the PAR (i.e., within the infrared regions) and directly participates in culture overheating. Consequently, more than 90% of the captured total solar photons is converted to heat by the culture [41]. Spectral filtration through the removal of IR radiation could form an effective solution at reducing overheating of cultures.

Table 1–1. Microalgae light-harvesting pigments and their photonic characteristics

Light-harvesting pigments class	Major pigment constituents	Absorption spectrum (nm)	Behaviour in solvent	Pigment colour	Algal division
Chlorophylls	*a, b, c_1, c_2, d*	450-475, 630-680, 700-750	Hydrophobic	Green	Cyanophyta, Prochlorophyta, Glaucophyta, Rhodophyta, Cryptophyta, Heterokontophyta, Haptophyta, Dinophyta, Euglenophyta, Chlorarachniophyta, Chlorophyta
Phycobilins	C-phycocyanin, Phycoerythrin, Allophycocyanin	500-650	Hydrophilic	Red, blue	Cyanophyta, Glaucophyta, Rhodophyta, Cryptophyta
Carotenoids	α-, β- & ε-carotene, Lutein, Astaxanthin, Violaxanthin, Fucoxanthin, Zeaxanthin	400-550	Hydrophobic	Red, yellow, orange	Cyanophyta, Prochlorophyta, Glaucophyta, Rhodophyta, Cryptophyta, Heterokontophyta, Haptophyta, Dinophyta, Euglenophyta, Chlorarachniophyta, Chlorophyta

Carvalho et al. [13], Barsanti et al. [28]

1.4.2 Light use efficiency

Efficient use of light in PBRs is a criterion for abundant microalgal biomass production, which is essential for attaining its economic feasibility and large-scale demand. An ultimate pointer to light use efficiency of photosynthetic microbes is the photosynthetic efficiency (PE), i.e., the fraction of the available incident solar energy stored as chemical energy in biomass (lipids, carbohydrates, proteins). PE is essentially a function of light intensity and PBR productivity. In outdoor cultures, typical theoretical maximum values of PE range from 8-12%, based on the total solar spectrum [42]. However, in industrial-scale microaglal production, the cultures rarely attain a practical PE of greater than 1.5 to 2% (Table 1–2), even at optimal culture conditions [13]. The relatively low PE values of current generation algal culture techniques is due to energy and productivity losses encountered in the processes of light energy transfer to the culture (Figure 1–2G). Energy efficiency drastically declines from the sun to the final end-value product. Based on the calculation by Ooms et al. [43], about 17% of the total solar energy is lost due to atmospheric scattering and absorption, which results in attenuation of direct and diffuse light. Latitudinal effects lead to another 30% loss, with a further 65% loss of the transmitted energy due to weather. The positioning of PBRs in relation to sun can decrease the irradiance by up to 50% for horizontally-oriented culture units in contrast to surfaces directly facing the sun. About 57% of the incident photons intercepted by the culture cannot be used in photosynthesis and are deemed wasted (See Figure 1–2G for more details). In sum, between 0.1 and 10% of light becomes the net photosynthetic energy conversion efficiency for carbon fixation utilizing the solar resource [43]. Improving the solar energy to biomass conversion efficiency through strategies to minimize these energy losses (Figure 1–2G) would result in better exploitation of sunlight and increasing PE and maximal productivity.

In addition to strategies that abate solar energy losses, efficient feeding of CO_2 to microalgal cultures enhances maximum biomass productivity and product formation [44]. It has been known that under sufficient light and fertilisers almost all algal cultures are carbon limited, and lack of CO_2 addition can result in up to 80% loss of biomass productivity [44]. Further, under normal atmospheric CO_2, high light intensity negatively impacts photosynthetic performance of microalgae, while tolerance to this intensity occurs at high CO_2 concentration [45]. Towards an environmental protection perspective, the cost inefficiency and high energy consumption of technologies for CO_2 capture and storage (CCS) have promoted microalgae as an environmentally sustainable option for carbon capture and utilization (CCU). The captured CO_2-based inorganic carbon is incorporated into algal cells as valuable biochemicals (Fig. 1–1). Therefore, algal cultivation systems can be integrated with large CO_2 emitters, such as power plants, to reduce their carbon footprint and to generate revenues from algal valuable bioproducts [44].

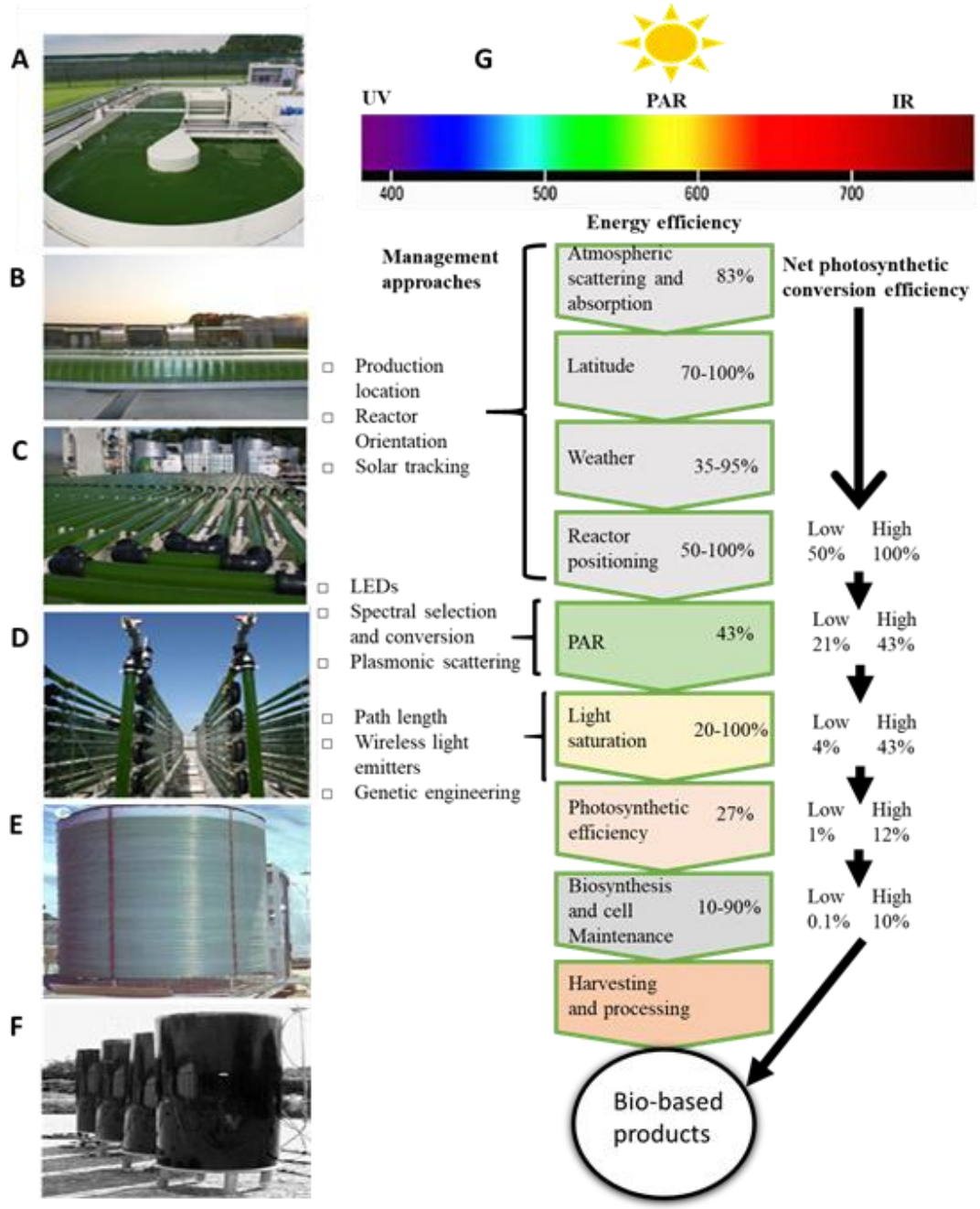

Fig. 1–2. Pilot-scale photobioreactor configurations and conversion efficiency of solar energy to biomass aimed at maximizing light use efficiency. (A) 4,730 L raceway pond [46], (B) 390 L flat plate [46], (C) 560 L horizontal tubular [46], (D) 1,060 L vertical tubular [46], (E) 1000 L Biocoil (Algae R&D Center, Murdoch University), (F) 120 L bubble column [47], (G) Drastic decline of energy efficiency from the sun to the final end-value product [43]. 17% of the solar energy is lost due to atmospheric scattering and absorption which results in attenuation of direct and diffuse light, representing a light availability of 83%. The effect of latitude leads to another 30% loss, with a further 65% loss of the available solar energy due to weather.

Table 1–2. Photosynthetic efficiency, biomass productivity and light intensity of various algal cultivation systems

Photobioreactor	Volume (L)	Location	light path (cm)	Biomass productivity		PE (%)	Microalgae sp.	Purpose	Illuminated surface area (m^2)	Light system	Light intensity ($\mu molphotons m^{-2}s^{-1}$)	Ref.
				$g.m^{-2}.d^{-1}$	$g.L^{-1}.d^{-1}$							
Green Wall panel	315,000	Outdoor	4.5	15	0.02	-	*Tetraselmis suecica*	Biomass	14,000	Sunlight	915	[40]
Near horizontal tubular	98	Outdoor	4.3	13-19.5	0.51-0.76	2.3-3.5	*Nannochloropsis* sp.	High-value EPA	-	Sunlight	857	[48]
Annular Column	120	Outdoor	40	38.2	0.42	9.3	*Tetraselmis suecica*	Biomass	5.3	Sunlight	900	[47]
Horizontal tubular	560	Outdoor	4.6	5.8-15.7	0.3-0.85	1.2-1.8	*Nannochloropsis* sp.	Biomass	27.0	Sunlight	-	[46]
Flat panel	123	Indoor	1.2	5.8-10.2	0.61-1.45	2.0-3.5	*Nannochloropsis* sp.	Biomass	3.4	Artificial light	230	[49]
Vertical tubular	1,060	Outdoor	4.6	10.6-24.4	0.31-0.71	2.4-4.2	*Nannochloropsis* sp.	Biomass	31.0	Sunlight	-	[46]
Raceway pond	100	Outdoor	30	8-20	0.04	0.97 - 0.69	*Muriellopsis* sp.	Lutein	-	Sunlight	1449	[50]
Raceway pond	4730	Outdoor	20	6.2-14.0	0.03-0.08	0.5-1.5	*Nannochloropsis* sp.	Biomass	25.4	Sunlight	-	[46]
Flat panel	390	Outdoor	2	20.5-27.5	0.9-1.2	2.7-3.8	*Nannochloropsis* sp.	Biomass	2.4	Sunlight	-	[46]
Solar tracked flat panel	240	Outdoor	1.5-2.2	-	0.7	-	*S. obliquus* & *Chlorella pyrenoidosa*	-	14	Sunlight	923	[51]
Rotating annular	3.4	Indoor	1.2	103	7.3	7	*Chlorella sorokianiana*	biomass	0.24	Artificial light	1500	[52]
Algae raceway integrated design	7500	outdoor	15	3.5	0.02	1	*Nannochloropsis salina*	Biomass, high value	50	Sunlight	490	[53]
Cylindrical	2	Indoor	5	13	0.7	7	*C. reinhardtii*	Biomass	0.1	Artificial light	200	[54]

Photobioreactor	Volume (L)	Location	light path (cm)	Biomass productivity		PE (%)	Microalgae sp.	Purpose	Illuminated surface area (m^2)	Light system	Light intensity ($\mu molphotons m^{-2}s^{-1}$)	Ref.
				$g.m^{-2}.d^{-1}$	$g.L^{-1}.d^{-1}$							
Glass sponge flat panel	0.28	Indoor	2	28	1.4	6	*Chlamydomonas reinhardtii*	Biomass	0.01	Artificial light	500	[54]
Attached photobioreactor	12	Outdoor	10	65	0.7	15	*Scenedesmus obliquus*	Biomass	0.12	Sunlight	492	[55]
Rotating algal biofilm	8000	Outdoor	0.9	31	0	16	Mixed culture	Biomass		Sunlight	208	[56]
Thin-layer inclined cascades	2200	Outdoor	0.6	19	1.9	4	*Chlorella* spp.	Biomass	224	Sunlight	540	[57]
Inclined bubble column	1.7	Indoor	4	20	0.3	7	*Stichococcus bacillaris*	Biomass and Biofuel	0.002	Artificial light	300	[58]
Tubular with static mixers	883	Outdoor	7.5	10	0.2	3	*Chlorella* sp.	Biomass	15	Sunlight	400	[59]
Flat panel with inclined baffles	12.5	Outdoor	2.5	14	0.6	5	*Chlorella* sp.	Biomass	0.5	Sunlight	333	[60]
Flat panel	45	Outdoor	2.1	420	20	14	*Scenedesmus obliquus*	Biomass	2	Sunlight	1656	[61]
LED-photobioreactor	0.5	Indoor	2	21	2.1	8	*Chlorella vulgaris*	Biomass	0.08	LED	300	[62]
Helical tubular	588	Outdoor	9.7	51	1.8	8	*Nannochloropsis* sp.	Biomass	20.8	Combined natural and artificial light	700	[63]
Thin-layer Flat panel	1.7	Indoor	1.4	18	1.3	11	*Chlorella sorokiniana*	Biomass	0.12	Artificial light	141	[64]

PE, photosynthetic efficiency; $gm^{-2}d^{-1}$, areal productivity; $gL^{-1}d^{-1}$, volumetric productivity; LED, light emitting diode.

1.5 Approaches to light use efficiency management in photobioreactors

Efficient conversion of solar energy to valuable bioproducts remains a pressing issue for a wide range of commercial algal biochemicals. Microalgal cells generally absorb all incident photons that fall upon them from across the visible spectrum, due to the high light harvesting efficiency of chlorophyll [13]. Meanwhile, not all the absorbed photons are utilized for photosynthesis, i.e., the broader the light spectrum, the lower the utilization efficiency and consequently, a decrease in maximum growth yield. Improving the light utilization efficiency can enhance the efficiency of reactions leading to carbon fixation. Hence, customizing the incident light spectrum transmitted to algal cultures could ensure efficient utilization of light for the production of specific bioactive compounds. Approaches to modify light is most suitable for PBRs, which might allow for more efficient use of solar energy and would lead to a significantly higher biomass productivity compared to standard outdoor cultivations [27]. Many strategies to improving light use efficiency in microalgae have been trialled. These include strategies to maximise PAR quantity and quality, scattering or guided light delivery within the culture vessel (including cellular engineering), to maximise availability, converting light of low to high photosynthetic utility, and utilizing emerging materials to minimise the transmittance to the culture those parts of the spectra deemed harmful to cell growth.

1.5.1 Spectral selection and filtration

The response of microalgae to different wavelength regions is determined by their action spectra, where absorbed photons are maximally utilized for photosynthesis. Microalgae have a broad range of light-harvesting pigments (Table 1–1) that absorb photon

energy within the PAR range (400-700 nm), but the profile of these pigments to a large extent determine the wavelengths utilized for photosynthesis. The PAR wavelengths represent 28 and 43% of the solar photons and total sunlight energy reaching the earth, respectively [43]. Nevertheless, the red (600-700 nm) and blue (400-525 nm) wavelengths are conventionally absorbed by chlorophylls *a* and *b* and are the most effective in driving photosynthesis. Wavelength filtration technology offers the ability to supply specific light spectra (full range or sections of PAR) for algal photosynthesis (Fig. 1–3A). Given spectral selection, there are two parameters to consider in light absorption by algal cultures: a) the wavelengths that are preferentially absorbed and b) the utilization efficiency of these preferred wavelengths by the algal cells. While photons between 400 and 700 nm are considered sufficient for driving photosynthesis, the photosynthetic rates of microalgae in response to light at distinct wavelengths determine their action spectra.

That is to say, the absorption and the absorbed action spectra of algae differ significantly, revealing that low-value wavelengths due to poor absorption could be highly effective in driving photosynthesis especially in high-density cultures (absorbs all photons) when finally absorbed. While it might be that poorly absorbed wavelengths, such as those in the green range penetrate dense cultures more and are utilized for photosynthesis [65], recent work by Vadiveloo et al. [15] using LEE colour filters has shown that this is not the case, at least for *Nannochloropsis* MUR 266. Fig. 1–3B shows the absorption spectra of *Nannochloropsis* MUR 266. In fact, in this case, the highest biomass growth rate was found with a mix of blue (400-525 nm) and red (600-700 nm) light, highest lipid production under purely blue light (400-525 nm), highest chlorophyll content using a combination of blue and green (450-625 nm), while green light alone yielded no net growth. Therefore, the blue wavelengths are most useful for *Nannochloropsis* MUR 266 for conversion to biomass, as

they have high energy and penetrate the culture best while inclusion of green wavelengths results in increased production of light harvesting pigments implying that green wavelengths are not optimal for the conversion of sunlight into growth or lipid products. Similar findings were reported by Vadiveloo et al. [27] and Tamburic et al. [66], where blue light was identified as the most effective driver of photosynthesis, however, it is important to note that these authors used optically-thin cultures for the experiment. Contrary to the outcomes reported by Vadiveloo and colleagues, other microalgal cultures (*Chlamydomonas reinhardtii* and *Scenedesmus bijuga*), have been shown to exhibit highest biomass productivity for poorly absorbed spectra that contain wavelengths in the predominantly yellow and green regions of the spectrum [65, 67] (Table 1–3). Specifically, the biomass productivity of an indoor flat panel airlift PBR (54 $g.m^{-2}.d^{-1}$ based on illuminated surface area) under yellow (peak 596 nm; spectral half-width 60 nm) light was 1.86 times higher than the productivity under strongly absorbed red (661 nm; 20 nm) and blue (458 nm; 20 nm) spectra (29 $g.m^{-2}.d^{-1}$) [65]. According to Mattos et al. [67], the weakly absorbed green (peak 530 nm) spectrum was more photosynthetically productive in optically-dense culture (2.19 $g.L^{-1}$) as it gave the highest biomass productivity (30 $mg.L^{-1}.d^{-1}$) relative to the strongly absorbed red (655 nm) and blue (470 nm) spectra. Similar findings were reported by Mohsenpour et al. [68] and Mohsenpour and Willoughby [69]. In cyanobacteria, light spectra in the wavelength range of 500-650 nm have been reported to be the most efficient because shorter wavelengths result in photodamage and induction of photoprotection in the organisms [43].

The spectral composition of light also influences metabolite production in microalgae and could be manipulated to enhance production of desired biochemicals (e.g., lipids). Blue wavelengths stimulate photosynthetic growth rates and total lipid content of *Nannochloropsis* spp. [27, 70]. Katsuda et al. [71] showed that blue spectra enhanced the

production of astaxanthin relative to red wavelengths, which stimulated a higher cell proliferation rate in cultures of *Haematococcus pluvialis*. In this case, creating a hybrid wavelength alternating scenario could increase the overall productivity, by first growing under a red spectrum to maximize biomass and later under blue to boost metabolite production (e.g., astaxanthin), and this is easier to manage and achieve in PBRs than open ponds. Wavelength alternation has been shown to have positive effects for maximising algal culture productivity. For example, the highest biomass productivity for a *Chlorella* sp. was achieved by first culturing for 2 days under a blue spectrum, resulting in an increase in cell size, followed by 3 days under red spectral illumination, which lead to increased cell proliferation (Fig. 1–3C) [72]. In a slightly different approach to the use of spectral selection, a recent study has shown that employing light mixtures (e.g., red:green:blue, RGB) can be successful but its optimisation is also species-specific. In a *C. reinhardtii* culture, the maximum biomass productivity (252 $mg.L^{-1}.d^{-1}$) was achieved at a spectral mixture of 80:10:10, RGB, whereas 40:40:20, RGB, gave the highest biomass (321 $mg.L^{-1}.d^{-1}$) and phycoerythrin (16.93 $mg.L^{-1}.d^{-1}$) productivities for *Porphyridium purpureum* [73]. Both microalgae were cultured in a PBR, in order to achieve appropriate spectral control.

It is apparent from the examples above that there is no universal/all-purpose monochromatic wavelength or spectral range that will be optimal for all algal species. These results suggest that hybrid and/or tailor-made light delivery techniques will be required to advance microalgae culture where increases in biomass productivity and/or metabolite production are desired. It is likely that each of the continuous changes or mixtures of specific spectral regions will need to be tested to optimise production outcomes. Thus, the manner in which light can be delivered to cultures becomes of paramount importance to the economic viability of mass culturing using PBRs. Wavelength selection and filtration can be

achieved using a range of technologies, including light colour filters, specially-engineered optics, dyes and luminescent panels [74].

Apart from being able to deliver a more useful amount of PAR to the culture, tailoring the specific wavelengths that can be transmitted to the culture medium should lead to substantially reducing the amount of heat and UV energy absorbed by the culture. This should then result in less issues with UV induced cell damage, and temperature related stress on cultures should decrease, with less reliance on ancillary cooling systems.

1.5.2 Solar tracking devices

Weather, atmospheric scattering, and latitude all affect solar resource availability [43], as microalgal cultures can only utilize photons transmitted by PBRs, the positioning of PBRs become paramount in managing light to maximize biomass productivity. PBR orientation (horizontal, vertical or tilted) is highly significant in cultivation systems with large irradiated surfaces compared to the light path length. On the average for outdoor cultivation systems, PBRs positioned vertically in the east-west direction harvest more solar energy (over 5% more) relative to horizontally placed reactors in the north-south direction [76]. For instance, in winter when cultures are usually photolimited, a vertical east-west oriented PBR intercepted more radiation (26 $MJ.m^{-2}.d^{-1}$) than the north-south orientation in the summer, which intercepted less radiation (16 $MJ.m^{-2}.d^{-1}$) [77]. The suitable orientation of reactors at any location is latitude and longitude dependent [78, 79]. At latitudes ≥ 35˚N, east-west orientation intercepts more solar radiation, whereas north-south direction is perfect for latitudes < 35˚N, as more radiation is intercepted at this position [76]. The orientation of PBRs also determines the type of solar radiation intercepted.

Table 1–3. Effect of different light spectra on the growth rate and productivity of microalgae

Spectrum	Growth rate (d^{-1})	Volumetric productivity ($mgL^{-1}d^{-1}$)	Biochemical components (%AFDW)			Chlorophyll *a* content ($pg\ cell^{-1}$)	Microalgae sp. (Reference)	Light material
			Protein	Lipid	Carbohydrate			
Blue	0.16	28.9	24	59	18	1.38	*Nannochloropsis* MUR 266 [15]	Coloured acetate filters
Red	0.24	86.2	22	48	26	0.3		
Blue-green	0.09	12.9	23	50	25	1.4		
Pink	0.30	101.0	21.5	49	25	0.38		
White	0.29	132.4	20	50	24	0.3		
Blue	/	15	/	/	/	3.8[a]	*Nannochloropsis* MUR 267 [27]	Coloured acetate filters
Red	/	50	/	/	/	2.8[a]		
Blue-green	/	20	/	/	/	3.6[a]		
Pink	/	100	/	/	/	1.5[a]		
White	/	180	/	/	/	2.2[a]		
Blue	0.18	250	/	0.14[b]	/	0.5[c]	*Nannochloropsis* MUR 267 [75]	Coloured acetate filters
Red	0.14	150	/	0.13[b]	/	0.7[c]		
Pink	0.15	200	/	0.15[b]	/	0.9[c]		
White	0.18	280	/	0.12[b]	/	0.5[c]		
Blue	2.2	2,030	/	/	/	/	*Chlamydomonas reinhardtii* [65]	LEDs
Deep-red	2.1	2,240	/	/	/	/		
Yellow	1.6	3,780	/	/	/	/		
White	1.6	3,500	/	/	/	/		
Blue	/	6.67	/	/	/	/	(*Scenedesmus bijuga*) [67]	LEDs
Green	/	30.00	/	/	/	/		
Red	/	15.00	/	/	/	/		
White	/	23.33	/	/	/	/		
Blue	1.64	/	/	/	/	/	*Nannochloropsis* sp. [70]	LEDs
Red	1.61	/	/	/	/	/		
Red-blue	1.61	/	/	/	/	/		
White	1.59	/	/	/	/	/		
Purple	0.62	61.88	/	/	/	/	*Haematococcus pluvialis* [71]	LEDs
Blue-purple	0.65	68.25	/	/	/	/		
Blue	0.62	60.00	/	/	/	/		
Green	0.38	22.25	/	/	/	/		
Red	0.60	58.13	/	/	/	/		
Fluorescent	0.55	34.13	/	/	/	/		

[a]Chlorophyll *a* expressed as %organic biomass, [b]Total lipids expressed as $g.L^{-1}$, [c]Chlorophyll *a* reported as $\mu g.mL^{-1}$, "/", not determined, AFDW, ash-free dry weight, LEDs, light emitting diodes.

Vertically oriented reactors catch more of diffused radiation, while horizontal reactors receive the direct beam of radiation [77, 80]. In this sense, the diffuse light is more photosynthetically-efficient due to its sub-saturating effect, which leads to higher efficiency of photosynthesis.

The application of solar tracking devices to improve the photosynthetic efficiency of systems growing algae to enhance photons collection, is in theory a promising application. Its use in PV (photovoltaic) industry is well established. Solar trackers are turnable devices that direct the microalgae cultivation module to always face the direction of the sun. Solar tracking devices continually angle their orientation to follow the trajectory of the sun throughout the day correctly. Since these monitoring devices track the sun horizontally and vertically, maximum capture and collection of solar energy by PBRs is achieved. Hindersin et al. [51] (Northern Germany, Latitude 53˚N, Longitude 10˚E) have reported on the average, solar-tracked flat-panel PBR (Fig. 1–3D) intercepted up to 79 mol.photons.m^{-2}.d^{-1}, while untracked horizontal PBR only intercepted 55 mol.photons.m^{-2}.d^{-1}. The photons captured by the untracked reactor represent 69% of the irradiance captured by the solar tracked reactor. It is reasonable to mention that maximizing the amount of light intercepted by a reactor is necessary to attain maximum productivity, too long exposure of microalgae to high irradiance usually results in photoinhibition and photooxidation, which negatively impacts PE. Hindersin and colleagues have shown that the solar-tracked PBR overcame light limitation, reduced photoinhibition via reduction in irradiance, controlled overheating by rotating out of direct sunlight when the temperature exceeded a set value, increased productivity, and provided photosynthetic activities indicated by an effective and maximum quantum yield of 0.68 and 0.8, respectively. Coupling solar tracking technology to microalgae cultivation may be very expensive to defeat economies of scale. However, its

application would be much needed in cold temperate regions with unfavorable meteorological conditions, where closed photobioreactors must be used for algae cultivation [51, 61]. If the better performance of cultures and higher biomass productivity could offset the higher cost of construction, this technology would find application in the production of high-value/multi-output microalgal products. Furthermore, combining solar trackers and light filtration technologies for co-generation of biomass and electrical energy would improve the economics of this system. The import of PBR orientation to maximize light collection and improve productivity is more significant in small-scale scenarios. For large-scale production facilities, mutual shading of reactors becomes a challenge, as adjacent PBRs would be light-limited due to shadowing effects and this can have a dramatic impact on the productivity. The vertical orientation of PBRs (e.g., flat panels) is the preferred position for single reactors in isolation, as the effect of shading is less significant, large-scale construction will definitely increase the land area and photosynthetic loss resulting from inter-reactor gaps. This makes PBR spacing, as well as height, a decisive factor for design [78]. Therefore, large-scale installations will experience maximum light exposure and diminish shading effects by horizontal orientation of reactors.

1.5.3 Light guides

Maintaining optimal lighting conditions inside algal PBRs is a critical challenge for culturing microalgae under optically-dense settings. Strong mutual shading among cells results in heterogenous light distribution in PBRs. Microalgae close to the photic zones are subjected to a photoinhibitory light intensity, whereas cells far away from the illumination surfaces are photolimited. These two scenarios are not favourable for biomass accumulation. Externally illuminated PBRs have been designed with short light-paths to ensure adequate illumination and improved photosynthesis by the microalgal cells.

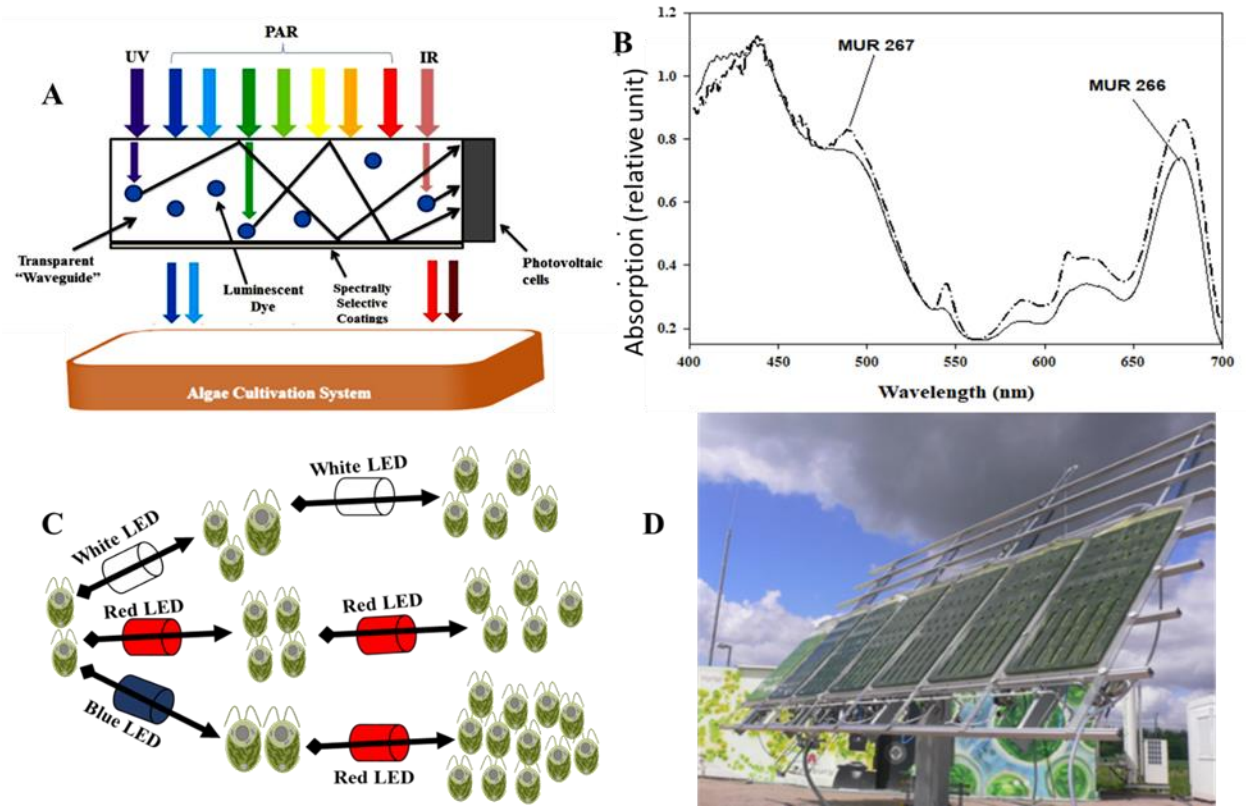

Fig. 1–3. (A) Schematic showing wavelength filtration and selection via a luminescent solar concentrator. (B) Absorption spectrum of *Nannochloropsis* MUR 266 and 267 with two dissimilar pigment concentrations depicting a green spectrum (510-600) that is poorly absorbed. (C) A model showing progressional alternation between blue light to enlarge cell size and red light to induce faster proliferation rate [72]. (D) Solar-tracked 263 L flat panel PBR [51]

However, the short path-length results in a large-surface-area-volume ratio, which makes the implementation at large-scale challenging due to complexity of the configurations. Innovative mixers or baffles have been installed in PBRs to promote sufficient mixing of cells along the light gradient [81], but a strong shear can damage microalgae cells and, therefore, hamper further improvement of PBRs using this strategy.

Internal illumination of PBRs can be used to manage the culture volume. A light source (e.g., LEDs, halogen lamps, sunlight) can be installed inside or guided through optical fibers, light guides, or waveguides to deliver uniform light distribution inside PBRs, especially

the light deficient zones [82, 83]. A novel internally illuminated PBR, comprising fluorescent lamps embedded in submerged glass tubes, was designed for the cultivation of *Chlorella pyrenoidosa* [84]. This PBR exhibited a promising performance for the cultivation of many algal species, since the desired optimal light supply coefficients can be attained by changing the light intensity. Nevertheless, building of the reactor is complicated, and the heat generated by the lamps has a negative impact on the microalgae growth.

Optical fibers can replace the submerged lamps, providing internal illumination without the consequent heat gain. Application of this strategy for the cultivation of *Spirulina platensis* resulted in a 43% increase in biomass productivity over conventional control mechanisms [85]. The use of reflective surfaces inside PBRs favoured significant increase in PAR radiation. A PBR installed with reflective surfaces showed a two- and six-fold increase in PAR distribution inside the PBR and biomass productivity, respectively, compared to PBRs with no reflective surfaces [86]. However, the high cost of optical fibers and reflective surfaces make them impractical for large-scale production.

Recently, an open tank PBR with an in-built transparent rectangular chamber that conducts light deeper into the PBR, and enhances biomass productivity by 56% over conventional control mechanisms, has been reported [87]. Similarly, hollow polymethyl methacrylate tubes inserted in a flat plate PBR for *C. vulgaris* cultivation improved internal illumination 2.0-6.5-fold, by acting as a secondary light source [88]. Its improvement in biomass productivity was 23.4% compared to conventional control mechanisms. However, both of the above designs could result in loss of effective cultivation capacity of the reactor, due to the loss of the space occupied by the internal light sources or radiators. Coupled with probable biofilm formation on these surfaces, the photon availability inside the PBRs may be considerably reduced. Furthermore, microalgae cells close to the incident light surfaces

could be severely photoinhibited because the light distribution qualities around the surfaces were not optimized. Borosilicate glass slides with a chemically etched surface, having an intrinsic property of causing light within the slides to be released, has been applied in a PBR for the production of ethylene from *Synechocystis*. Biomass productivity was raised 8-fold by utilizing this design [89]. But chemical etching of glass slides is a complicated process and difficult to control and scale up.

Recently, a wireless light emitter (WLE) was developed and applied to a PBR to increase the average light intensity and ensure uniform light distribution inside the PBR [90]. In this technology, light emitters are typically freely suspended inside the PBR, and energy is transferred wirelessly by a near-field resonant inductive coupling that illuminates the PBR [90, 91]. Applying this concept, for an intermediate frequency electromagnetic field of 0.95 mT and 178 Hz, the growth rate in the linear phase and the biomass productivity of *Chlamydomonas reinhardtii* in a PBR screening module were 2x and 80% higher, respectively, in comparison with the externally illuminated PBRs [90]. The authors also showed that the WLEs equipped PBR resulted in uniform light distribution and higher average light intensity (Fig. 1–4). Although this drop-in technology could overcome the burden of the massive surface-to-volume ratio in reactors, the viability of large-scale reactors of this type is uncertain.

Generally, many of the designs mentioned above have been applied to PBRs with optical pathlengths < 10 cm, a size unsuitable for industrial-scale PBRs, which have higher optical depths (> 10 cm). In order to address the negative consequences of poor light distribution inside PBRs, a planar waveguide doped with light scattering nanoparticles was recently built in a 25 cm optical pathlength flat panel PBR for cultivation of *C. vulgaris* [92]. This design increases the photic volume by up to 410%, and 220% more biomass was

achieved compared to a PBR with no waveguide. In addition to its structural simplicity, ease of scaling-up, commercial availability and low-cost, this design bypasses secondary processing of light. Considering that the ratio of light emitting to light incident surface areas is large, light concentrating devices, such as Fresnel lenses, would definitely be needed for light-guide-driven PBRs. The use of light concentrating devices would increase initial capital expenditure and operational complexity of the PBR [85, 88]. These designs have only been tested for a few microalgae species and their performance at pilot-scale also needs to be assessed.

Fig. 1–4 has been removed due to copyright restrictions.

1.5.4 Plasmonic light scattering

When an electromagnetic (EM) field interacts with free electrons in metals or metallic films, the free electrons (electron plasma) are excited to have a collective oscillation by the electric portion of light at the metal-dielectric interface [93]. This collective oscillation of the electrons results in bosonic particles called surface plasmons. When the surface plasmons of metals are excited, the EM field is strongly enhanced, leading to increased scattering or absorption of specific wavebands. A photon frequency lower than that of the plasmon is reflected, whereas a photon frequency above that of the plasmon is transmitted. The characteristics and the magnitude of the plasmonic effect depends on the type, shape, size and vicinity of the material [93]. Therefore, tuning the plasmon resonance frequency (by varying the size, concentration, shape, and architecture of plasmons) lends itself to adapting materials to a preferred wavelength-specific application. Certain metals (e.g., copper, gold, silver) used in plasmonic scattering have their electronic interband transitions in the visible portion of the EM spectrum, where absorption of specific energies occur. Furthermore, a high number of metals and semiconductors are reflective in the visible spectrum as their

plasmonic frequency falls in the UV spectrum, and these properties make them useful in plasmonic applications. The plasmonic effect has been used to improve photoconversion in photocells, and recent application has been seen in the excitation of photosynthetic architectures [94, 95]. This means that the plasmonic phenomenon can be used to waveguide photosynthetically useful wavelengths of light into microalgae cultivation reactors, while providing an opportunity to harness the other transmitted wavelengths for other applications.

Resonant interactions of photons and surface plasmons can be used to increase light absorption at a specific wavelength, and can be propagated through surface-plasmon-based light backscattering [96], evanescent light field excitation [94], and light scattering from an internal waveguide [97]. The growth of *Synechococcus elongatus* ATCC 33912 in a PBR equipped with a plasmonic nano-engineered surface (gold nanodisk surfaces) via an evanescent light field confined near the surface of a waveguide was shown to be enhanced by 6.5% relative to the control [93, 94]. The plasmonic nanoparticle achieved 35% backscattering of the red spectrum into the culture in the PBR, while other light frequencies (e.g., blue) were transmitted [93]. Similarly, Ahsan et al. [97] reported that the growth rate of *Synechocystis* S. PCC 6803 increased by 40% in a PBR with the internal surface coated with nano-engineered waveguide light scatterers. The internal waveguide light scattering is based on the Fresnel principle, in which part of the light incident on a plane interface from a medium of lower to higher refractive index will be transmitted and the other reflected. Uniform spatial distribution of light in the PBR was achieved by varying the density of the plasmonics signal. Furthermore, a 30% increase in the growth rate of *Chlamydomonas reinhardtii* cultured in a PBR containing wavelength selective plasmonics (made from silver nanoparticles) was attained by backscattering of the blue portion of light into the culture

[96]. Geometric variation of the reactor and concentration of the nanoparticles were used to control the wavelength and the reflected light flux.

The combination of plasmonic phenomena and biophotovoltaics has resulted in a dual-purpose photosynthetic-plasmonic-voltaic (PPV) cell [98]. Here, bioelectricity production is achieved through a single metal film used for the simultaneous excitation of photosynthesis by plasmonic light delivery to biofilm and collection of photosynthetically-generated current (Fig. 1–5). Applying this concept to excite biofilms of *Synechococcus bacillaris* plasmonically, the PPV cell produced an electrical power of as much as 12 $\mu W.m^{-2}$ with individual cells compared to only 5.7 $\mu W.m^{-2}$ produced by direct irradiation of the biofilms [98].

The plasmonic effect can also be tailored to enhance the production of desired metabolites. Using a polymer film that consisted of spherical nano-engineered silver, up to 35% increase in chlorophyll and carotenoid production was induced in the culture of *Chlamydomonas reinhardtii* by selective transmission of blue light to the culture [99]. Microalgae are known to show a bimodal behavior, requiring blue and red spectra for optimal growth, and this cannot be achieved with single nanoparticle species. Nevertheless, it is practical that backscattering of the blue and red spectra can be achieved for enhanced biomass and metabolite production by suitable mixtures of nanoparticles [96]. Considering that plasmonic nanoparticle suspensions are confined and not dissolved in the medium, they can be recycled many times leading to cost reductions. An essential advantage of plasmonic devices is that they are not bandgap restricted, unlike conventional solar cells. The most significant disadvantages are due to ohmic and electron-core interaction losses resulting from the plasmonic oscillations and high cost. Plasmonic light harvesting presents an exciting future for the delivery of high-intensity wavelength distinct spectra to optically-

dense optofluidic algal PBRs for enhanced production of useful products. However, the technology requires optimization in the areas of metals concentration, electrode spacing, media pH, and device temperature, as well as a robust economic assessment at a pilot-scale level to ascertain environmental safety and implementation feasibility.

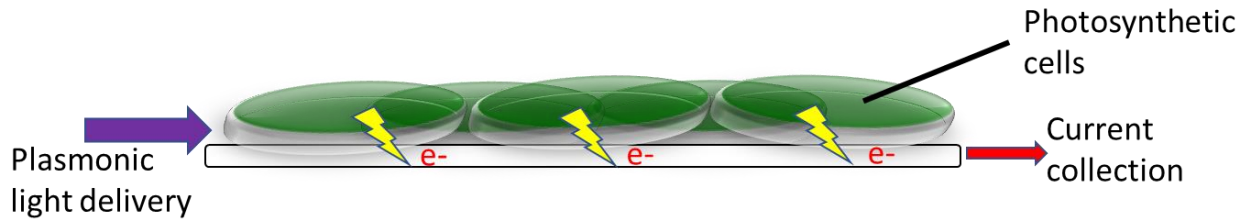

Fig. 1–5. Schematic of a dual-purpose photosynthetic-plasmonic-voltaic cell for combined light delivery and current collection through a plasmonic metal film for the growth of *Synechococcus bacillaris* [98].

1.5.5 Spectral shifting materials

Light wavelenghths of little or no photosynthetic value can be converted to high photosynthetic potential by applying wavelength shifters (or photoluminescent spectral converters), such as fluorescent, semiconducting, and phosphorescent substances. A wide array of dyes, including organic and inorganic, phosphors and quantum dots, have shown promising feasibility for wavelength shifting of light [100, 101]. These materials have distinctive characteristics: (i) a high absorption coefficient that enables them to capture sufficient quantity of photons; (ii) different emission and absorption bands to minimize reabsorption of shifted or converted photons; (iii) high quantum conversion efficiencies; and (iv) low-cost and extended photostability. Interestingly, wavelengths that are unwanted in microalgae farming, such as UV and IR radiation, can be converted to visible light to alleviate their deleterious effects on the cells and improve the light distribution efficiency of the system [102]. Furthermore, conversion of the green spectrum to red wavelengths in optically less-dense cultures would maximize available light for photosynthesis, while conversion of

blue to green in the optically-dense medium will improve light distribution in the culture, as saturation effects caused by high light intensity are avoided. A wavelength shifting dye (photoluminescent phosphor) was used to convert green to red light in a dilute culture of *Haematococcus pluvialis* cultivated using a back-reflecting flat panel PBR. A 36% increase in net biomass was attained due to the spectral conversion [14]. Similarly, Amrei et al. [103] investigated the feasibility of wavelength shifting UV-A to PAR in a UV-stabilized fluorescent polycarbonate coated flat panel PBR and achieved a 10% net increase in biomass productivity.

In silicon photovoltaics (PVs), spectral engineering by modification is among the third-generation strategies proposed to advance their typical efficiency limit [104, 105]. Spectral modification via photoluminescent spectral converters consists of down-conversion (a photon of higher energy converted to two or more photons of lower energy), photoluminescence (shifting photons into desired wavelength region) and up-conversion (two or more photons of lower energy are converted to a photon of higher energy). Spectral conversion is used in applications that require qualitative, rather than quantitative, light delivery. Therefore, the application in microalgae production would be to make the use of solar energy more efficient. However, spectral conversion does not come without a cost. For example, the transduced radiant energy is reduced due to a Stokes-shift (energy difference between incoming and emitted photons) of the photoluminescent spectral converters [106]. In essence, the temporal and spatial distribution of photons should be of the utmost consideration and not just to provide the quality photons. Although spectral conversion/shifting is a promising strategy, much still needs to be done in overcoming the above limitations, especially in the development of high efficiency materials for shifting non-

PAR to PAR wavelengths. Also, practical demonstrations in large-scale PBRs, and life cycle assessment on such systems, need to be carried out.

1.5.6 Artificial illuminators

Artificial light sources for microalgae production are halogen lamps, incandescent bulbs, fluorescent lamps, high-intensity discharge lamps, and light-emitting diodes (LEDs). The use of these artificial lights in horticulture is well established [107]. The suitability of these light sources depends on their qualities, which include their PAR spectrum, power consumption, conversion efficiency, cost, and wavelength distribution (Table 1–4). Fluorescent lamps and LEDs are the most promising artificial lights for algae cultivation, with LEDs usually preferred. The intensive use of LEDs in laboratory-based microalgae culture are due to their beneficial characteristics. They are tailored to output narrow spectra to match the photosynthetic action spectrum of algae, thereby eliminating the emission of ineffective frequencies (Fig. 1–6). Artificial light sources typically have a high conversion efficiency of electricity to light with less energy wasted as heat. Among the common artificial light sources, LEDs have the longest lifespan, lowest heat production, and tolerarance to switch on and off effects [108, 109]. Illumination of PBRs with LEDs are convenient and flexible, making the PBR geometry, orientation, and temperature control easily manageable.

Given that the rate of biological reactions and the gene regulation of the dark reactions of photosynthesis are reliant on temperature, control of temperature becomes a critical factor in optimizing the algal growth [43]. Low outdoor temperatures in cold temperate regions may not be enough to sustain the algal growth, while high irradiance in the tropics, can result in photoinhibition and overheating of cultures. In terms of energetics and economics, active temperature control of algae cultures and PBRs is expensive. Cooling is required in the tropics while the reverse is true for cold temperate regions. Considering

that LEDs do not emit radiation in the infrared range, they may represent an opportunity to manage light quality and PBR temperature in a single technology.

Table 1–4. Common artificial light system for microalgae culture and their photonic characteristics

Illuminator	Intensity (Wm^{-2})	Energy intensity in 400-700 (600-700) nm (%)	Stability over time	Duration (days)
Incandescent bulbs	5.1	4.3 (3.8)	Output degrades	31.3-83.3
Halogen lamps	1.6	3.6 (3.3)	Output stable	125.0-166.7
Fluorescent lamps	5.9	45.7 (20.7)	Output degrades	416.7
Gro-lux fluorescent lamps	3.7	56.8 (37.9)	Output degrades	625
LEDs	14.7-55.5	87.64-98.38 (87.6-98.3)	Output degrades	1458.3-2083.3

Carvalho et al. [13], Blanken et al. [107], Chen et al. [108].

However, the use of LEDs for microalgae culture may be expensive, even though their price is declining, as the electrical energy for its operation must be bought. The cost of electrical energy for an LED cultured microalgae is estimated at $14 per kg^{-1} dry weight (DW), which is around $3,800 per barrel of oil equivalent [43]. This high energy cost makes them economically unviable for the production of low-value biochemicals, such as biofuels. The economics of LED-supported microalgae cultivation improves if coupled to the production

of valuable biomolecules, especially if feedstock such as wastewater and flue gas are used. For instance, the production of astaxanthin at a content of >2.5% DW from *Haematococcus pluvialis* using an LED results in a biomass price of $50-175 kg^{-1}DW. Considering that the price of astaxanthin in the market is around $2,000-7,000 kg^{-1} [43], the cost of LED illumination appears justified. This cost would be much lower if production was supported by combining sunlight and LED illumination, particularly in a cultivation system that is also capable of electricity generation from the solar resource to run production operations, while producing a significant amount of biomass.

The use of LEDs dominates laboratory-scale microalgal research. However, their use in commercial production is scarce. Hence, extrapolation of results obtained from artificial lights to model outdoor scenarios would be misleading, since irradiance profiles and spectral distribution of artificial lights and sunlight do not overlap but the former is usually a poor approximations of the solar irradiance (Fig. 1–6).

1.5.7 Genetic modification

An alternative to the optical engineering of PBRs for increasing the photosynthetic efficiency (PE) is the cellular engineering of the chlorophyll antenna size. Improvement in the conversion efficiency of photosynthesis in transforming solar energy and CO_2 to biomas is key to making microalgae production commercially viable and sustainable. One of the most significant issues with mass microalgal cultivation is the fact that maximum PE cannot be achieved due to mutual shading in dense cultures resulting in light saturation [24]. When microalgae cultures are cultivated under full light, the large number of photon-absorbing chlorophyll antennae contained in the photosystems (PSI and PSII) create a scenario in which the photon capture rate is more efficient than the rate at which electrons are funnelled to the

photosynthetic reaction centers. This constraint can be avoided by reducing the rate at which microalgae absorb photons.

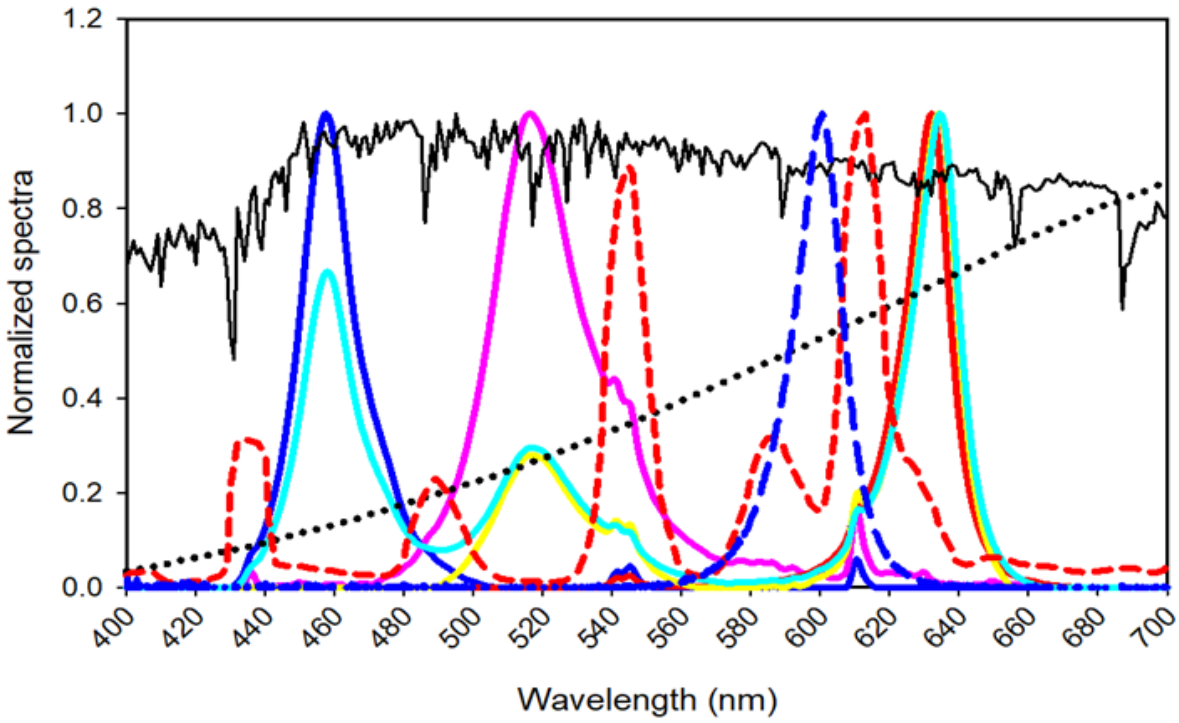

Fig. 1–6. Emission spectra of selected artificial lights: Incandescent bulb 60W (dark, dotted line), Fluorescent lamp (red, short-dash line), Blue LED (blue line), Green LED (pink line), Orange LED (blue, long-dash line), Red LED (red line), white LED (cyan line), Yellow LED (yellow line) and sunlight-ASTM G-173-03, AM1.5 (dark solid line) (irredc.nrel.gov/spectra/am1.5/astmg173/astmg173.html).

A strategy to moderate the photon absorption rate would be to decrease the size and cross-sectional absorption size of the antenna pigments [65]. The light harvesting antenna are the reason for high optical density of microalgae cultures and they are crucial during light-limiting conditions, as they increase in number to optimize photosynthetic efficiency. Reducing the concentration of the light-harvesting complexes of microalgae stabilizes the species to harvest only that proportion of photons that can be efficiently utilized for photochemistry, while increasing light penetration into the culture. In contrast, under

outdoor microalgae mass cultures exposed to intense sunlight, it would be impossible to achieve high photosynthetic efficiency due to heterogenous light distribution in culture systems. Hence, the generated light gradients in the microalgae culture would limit their light use efficiency. Therefore, selective reduction of microalgae photosystems to a small number of functional chlorophyll antenna size would result in photosynthetic saturation at higher irradiance, improved distribution and quantity of light available to each cell, and impact positively on overall biomass productivity [110]. For example, the *Chlamydomonas reinhardtii* mutant tla1 with truncated chlorophyll antenna size proteins engineered through insertional mutagenesis was found to attain photosynthetic saturation at a light intensity of 2,500 μmol.photons.m^{-2}.s^{-1} compared to the wild-type control at 1,000 μmol.photons.m^{-2}.s^{-1}, while the mutant's productivity at 1,500 μmol.photons.m^{-2}.s^{-1} was twice the wild strain [24, 111]. Transgenics of *Chlamydomonas reinhardtii* with reduced antenna sizes have been generated by elimination of chlorophyll *b* synthesis and reduction of the light harvesting genes [24]. Overall, microalgal mutants with truncated antenna size not only allow for better transmission of light in mass cultures (Fig. 1–7) but also harvest high light intensity with less efficiency, thus, mitigating thermal dissipation and photoinhibition of the photosynthetic complex. These two scenarios increase PE and biomass productivity.

Although the genetic modification of the microalgae antenna size has been considered as an efficient light management strategy for improving photosynthetic and productivity yields [110, 112] and has been remarkably successful in model species, trials on some other strains have failed to produce substantial improvement. For example, de Mooij et al. [113] investigated the biomass productivity of four different mutants with genetically modified antenna under simulated mass culture scenarios in a lab-scale flat panel PBR at an irradiance of 1,500 μmol.photons.m^{-2}.s^{-1}. The authors surprisingly reported that the wild-

type algae with unmodified antenna pigments performed better than the mutants in terms of areal productivity. This indicates that their lower pigment concentrations decreased the number of photons absorbed by the cells. The non-performance of the mutants could emanate from their impaired photoprotective mechanism, due to the alteration of their light harvesting molecules [65, 113]. Other significant side effects of genetic modification of the antenna complex could be the reduction in the physical fitness and growth rate of the organisms. To benefit from the potential of reduction in the light-harvesting complex of commercially-relevant microalgae species for maximizing the photosynthetic efficiency and biomass productivity, increased knowledge of photosynthesis and an advanced genetic toolbox for commercial microalgae species are required.

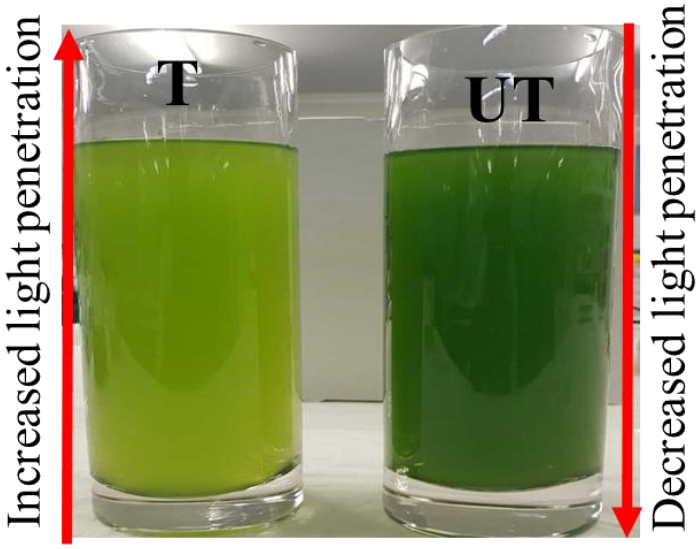

Fig. 1–7. Schematic of light penetration and distribution in a microalgae culture with truncated (T) and untruncated (UT) antennae size. In optically-dense cultures, the external layer of the culture is entirely illuminated and absorbs most of the light energy, while the cells beneath are shaded due to the high concentration of cells pigment. These cells at the external surface are photosynthetically saturated and dissipate much of the absorbed photons as heat. Significant improvement in light penetration and distribution in the culture is enhanced when the cells' photosystems antenna size is truncated by genetic modification [114].

1.5.8 Semi-transparent smart materials

High efficiency semi-transparent photovoltaic structures are being developed for building integrated photovoltaics, and they transmit wavelengths across the PAR spectrum. Perovskites are evolving as one highly promising group of absorber materials for building integrated photovoltaic cells and other semi-transparent applications [115]. These materials have high absorption coefficients, high transparency in the visible spectrum, optimal bandgap of ~1.55 eV (for methylammonium lead iodide), and a power conversion efficiency of more than 15% [115]. To fabricate a semi-transparent perovskite device, the typical opaque metal cathode is usually replaced with transparent conductors such as conductive oxides, thin-metal films, conductive polymers, metal nanowires, carbon nanotubes or graphene. These conductive electrodes may be combined to increase transmittance, decrease resistivity or enhance mechanical properties. Some of these transparent conductors, such as dielectric-metal-dielectric multilayer materials, are already widely exploited in organic photovoltaics, organic light emitting diodes, and optoelectronics as coatings, which can transmit visible wavelengths while absorbing UV and reflecting the IR parts of the incoming spectrum. Microalgae cultivation can benefit from this technology, where the illumination surface of PBRs is built with a perovskite material. The material allows the photosynthetically-useful visible spectrum to be transmitted to support microalgae growth and the remaining portions of the sunlight captured for electrical energy production. By constructing PBRs using such a material, photosynthetic productivity of microalgae could be enhanced and available resources, including sunlight and land, judiciously utilized. Perovskite photovoltaic structures are still in their infancy and their lifetime is still relatively short, and hence they are yet to be commercialized. Even so, application of these materials in microalgae farming seems feasible in the near-future.

While the semi-transparent perovskite type materials are yet to come to market, technologies like solar control films are widely used in commercial architecture [116]. These films are thin, spectrally-selective low-emissivity polyester-based materials that block UV and IR radiation to mitigate excessive solar radiation, while simultaneously allowing unmitigated throughput of visible light depending on the level of transparency [117]. These films are well established as smart windows coatings for residential houses, vehicles, spacecraft, commercial houses, aircraft, and in specialized architectural and marine applications, where control and modification of incident daylight, solar energy gain, and glare is required [118]. The spectral properties of solar control films (Fig. 1–8A) are excellent for tailoring the incoming spectrum for enhancing microalgae growth in PBRs and also allow concomitant control of culture temperature (by excluding IR). They can be easily laminated onto the illumination surface of PBRs and relatively inexpensive. Despite widespread application in architectural settings, this is still an unexploited technology in the field of microalgae cultivation and it is surprising that it has yet to be harnessed for this application.

Another promising smart material technology with potential for microalgal applications is switchable glass. Switchable glass is a polyvinyl butyral-based polymer glass with adjustable light transmittance [119]. It is capable of dynamically varying the visible light and solar energy transmittance by the application of an electrical voltage. It can be fabricated by combining two sheets of conducting glass or sheets of glass and plastic with an off/on feature [120]. When in the off status, the glass is translucently white though still transmitting light diffusely. On the application of electrical voltage (on status), it becomes transparent with the switching effect spanning the entire solar spectrum [118]. The most exciting aspect of switchable glass technologies is that they can be easily integrated with thin-film coatings and automated and tuned (user control), to allow particular wavelengths to be rejected or

transmitted. As switchable glass can be tuned to regulate wavelengths throughput, it also effectively controls heating levels. Applying this technology in microalgae farming, it would be possible to manage photosaturation and photoinhibition in PBRs by automatic or on-demand control of incident light intensity.

1.6. Combining light management technologies to create hybrid PBRs.

Large-scale algal production PBRs depend on incident solar illumination to supply the vital energy required for photosynthesis. Optimal sunlight management is a critical challenge for the design of PBRs as uneven illumination coupled with microalgae spectral selectivity results in light attenuation and incident wavelengths that are not directed into the growth of the culture. In fact, just a few photons of PAR within the entire solar irradiance are utilized for photosynthesis and the remainder (mostly in the UV and IR regions) are wasted or even detrimental to microalgae growth. For enhanced management of the incoming solar resource, the synergy between photovoltaics (PV) and photosynthesis offers a unique approach. The integration of PV with photosynthesis has the potential to allow a single outdoor microalgae production facility to co-generate biomass and electricity, leading to the most efficient use of the available resources (e.g., land and sunlight). Based on this concept, Moheimani and Parlevliet [21] have proposed a PV-microalgae scenario where semi-transparent, spectrally-selective, photovoltaic filters would be positioned above microalgae culture facilities. In that system, a suitable light spectral range is transmitted to the culture, while capturing and redirecting the remaining wavelengths to the integrated PV cells for electricity generation. In other words, the photons that are most efficiently used for conversion to biomass would be directed to the microalgae culture, while the poorly

absorbed and/or harmful spectral components, including UV and IR, are captured and converted to electrical energy to provide the energy required for running the algal production operations.

Excitingly, the successful commercial-scale manufacture of cost-effective spectrally-selective insulated glass units such as the energy-harvesting spectrally-selective glass [74, 121] provides a feasible way to realise this joint PV-PBR hybrid production system. These energy-harvesting spectrally-selective glass are customized low-emissivity clear solar glass created through advanced glazing technology and harvests energy in the transparent glass panel, using nano-engineered particles, microparticles, and specially engineered optics [74]. The micro-particles convert the UV radiation to longer wavelengths, while simultaneously scattering IR and routing them to the edge of the glass panel, with minimal interaction with visible spectrum, for collection and conversion to electricity by conventional PV cells (Fig. 1–8B). The ability of the energy-harvesting spectrally-selective glass to modify the incident light spectrum is yet to be explored for enhancement of photosynthetic productivity and efficiency in outdoor microalgal PBR but its application has been demonstrated in buildings and greenhouses [121]. In addition to the energy harvesting and spectral selectivity properties, this glass is durable (e.g., expected lifetime exceeding 30 years), has high insulation characteristics, and is shatter-proof [74].

A PBR using the energy-harvesting spectrally-selective glass panels would selectively allow >70% of the visible spectrum of sunlight to the microalgae culture, while simultaneously blocking, deflecting and capturing >90% of the UV and IR radiation (Fig. 1–8B, C) and converting that into electricity [121]. It has been estimated that the energy-harvesting spectrally-selective glass panel suitable for a PBR application can generate more than 30 $W.m^{-2}$ of electrical power output for a clear sky scenario [121]. The electricity

generated could promote off-grid production in rural areas, where large-scale microalgae facilities are usually located. Additionally, restricting IR transmission to the culture effectively suppresses heat generation, reducing the need for extraneous cooling of reactors. While utilising this new material for integrated microalgal production and electricity generation via PBR has yet to be rigorously examined, the possibilities such materials present for advancing PBR cultivation technology are compelling.

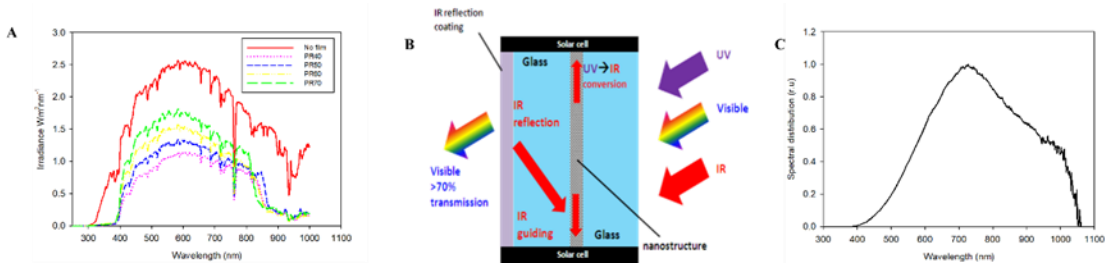

Fig. 1–8. (A) Spectral distribution of various 3M® solar control films Prestige Series at ultrahigh solar irradiance (3022 µmol.photons.m^{-2}.s^{-1}). (B) Schematic of the integrated PV flat panel PBR. (C) Spectral distribution of irradiance emitted from energy-harvesting spectrally-selective glass panel using a 500 W halogen lamp.

1.7. Conclusion

The supply and harvesting of sunlight for efficient conversion to valuable bioproducts is challenging for commercial-scale production of microalgae. As such, much effort has been, and is being, expended on optimising light management strategies in order to improve algal productivity in commercial-scale photobioreactors. Despite this effort, tuning the photosynthetically usable fractions of the solar spectrum is still unfeasible even with the solar resource being effectively free. The practical, large scale application of the light management approaches reviewed here are largely determined by economic feasibility, value of the end-product, system lifetime and ease of implementation. For example, LEDs have a controllable emission spectrum that can match the photosynthetic action spectrum

of various microalgae but they are currently uneconomical for the production of commodity products. Spectral shifting and plasmonic waveguiding are promising technologies for enhancing productivity in microalgae cultivation systems by improving PAR quality, efficiency and distribution in cultures but their practical implementation must overcome reabsorption, scattering and internal losses. Improved and advanced materials such as perovskites, switchable glass and insulated glazing technologies can be included in novel PBRs to customize the incident light spectrum for the production of certain products and advance the current PE limit. Available light control technologies have been tested in only a few microalgae species and there is need for further research to understand the requirements for a wider range of species. The most promising approach to advancing PBR microalgal culture appears to be combining existing and emerging light management technologies synergistically to improve biological productivity, reduce production costs, and minimise environmental impact. An example of this approach is the construction of an insulated-glazed photovoltaic flat panel PBR that uses newly available materials technology, to modify the incident solar spectrum to transmit photosynthetically beneficial PAR to the microalgae culture, whilst harvesting UV and IR wavelengths for electricity production. The development of this type of PBR has advantages for bioproductivity, as well as reducing costs associated with energy consumption and cooling costs. Adoption and fostering of hybrid approaches to microalgae production using PBRs is essential to moving the field forward and adoption of the technologies for commercial production of high-value microalgal products.

1.8. References

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1.9 Aim and outline of this thesis

This thesis was aimed at the development of a self-sustainable photovoltaic powered photobioreactor that generates local electricity and does not require freshwater-based cooling systems for microalgal biomass production. The goal was to maximize photobioreactor productivity, resource and energy efficiency by utilising insulated glazed panels that incorporate spectral filtering to reduce thermal regulation requirements and an integrated capability for renewable electricity generation to allow standalone operation. The objective of this research was to demonstrate the practical potential of the novel photobioreactor for microalgae production at Western Australian latitudes.

In **Chapter 2**, the proof-of-concept for the insulated-glazed photobioreactor is described. Here, I investigated if microalgal cultivation in the reactor is feasible. To characterize the performance of the novel photobioreactor, different cooling designs were assessed and evaluated at lab-scale. Two economically important microalgae species (*Nannochloropsis* sp. MUR 267 and *Arthrospira platensis* MUR 126) with different pigment compositions, morphological structure, and nutrient requirements were trialled for growth in the novel photobioreactor and their performance compared to reactors with different cooling mechanisms.

In C**hapter 3,** a pilot-scale trial of the novel photobioreactor is presented. In that study, the insulated glazed photovoltaic photobioreactor was designed, constructed, and operated outdoors during austral spring (September – November of 2018). The performance of the reactor during *Nannochloropsis* sp. cultivation was compared to a classical open raceway pond and two photobioreactors, one was cooled via freshwater spray and the other by

combined infrared reflection and spectral reduction. Generation of electrical energy was also measured to determine if this was sufficient for grid-independent operation of the reactor.

In **Chapter 4**, the different cooling strategies investigated in Chapter 3 were analysed to ascertain if there was any influence on macromolecular composition and fatty acid profile of microalgal biomass, using *Nannochloropsis* sp. as a model.

In **Chapter 5**, the reliability of microalgal culture in the novel photovoltaic photobioreactor is described. Here, the ability of a cold-intolerant microalga, *Arthrospira platensis* for successful growth in the novel photobioreactor during the austral winter (April – June of 2019) without supplementary heating was demonstrated. The photobioreactor performance was characterized in terms of hydrodynamics, biomass productivity, pigment production and culture health.

In **Chapter 6**, the energy efficiency of the photovoltaic photobioreactor is presented. The net energy ratio of a 1-ha photovoltaic photobioreactor plant situated in Western Australia was modelled based on data obtained from the pilot-scale operation of the reactor. The energetic productivity and thermal needs of the novel photobioreactor was evaluated in detail and compared to a conventional flat plate photobioreactor under passive evaporative cooling system. Embodied energy of plant building materials and fertilizers was considered, as well as the energy consumption for plant operations.

CHAPTER 2

Lab-scale insulated glazed flat plate photobioreactor for microalgal cultivation

Overheating in photobioreactors deleteriously affects the photosynthetic performance of microalgae and can result in complete loss of the culture due to high temperatures. Hence, the operation of photobioreactors requires sufficient, and efficient, cooling technologies to maintain high productivity rates. Passive evaporative cooling system utilizing a freshwater spray on the surface of the photobioreactors is widely used to manage culture temperature. However, freshwater-temperature based cooling system increases operational costs of large-scale photobioreactors, resulting in economic and environmental challenges due to the large energy and water required. The use of spectrally-selective materials that reflects non-photosynthetic photons (i.e., ultraviolet and infrared wavelengths) but transmits photosynthetically-beneficial visible photons (wavelengths spanning 400 to 700 nm), during photobioreactor construction can represent a low-energy-demand thermal solution. To this end, a proof-of-principle was trialled by building photobioreactors with the illumination surfaces made of insulated glass units and low-emissivity films and testing their abilities to support the growth of microalgae under laboratory condition.

Responses of microalgae to growth factors such as changes in temperature and light are species-specific. There is no doubt that microalgal species could behave differently in the novel photobioreactors. As such, the growth, biomass productivity, biochemical composition and photophysiological response of two different microalgae were assessed: *Nannochloropsis* sp. contains chlorophyll *a* only and is of marine origin; *Arthrospira platensis* MUR 126 contains chlorophyll *a* and phycobillins.

Validation and comparison of the growth of *Nannochloropsis* sp. in insulated glazed and thin-film coated photobioreactors is presented in Chapter 2A and that for *A. platensis* in Chapter 2B.

Chapter 2A

Comparison of *Nannochloropsis* sp. growth in insulated glazed and thin-film coated photobioreactors

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Can solar control infrared blocking films be used to replace evaporative cooling for growth of *Nannochloropsis* sp. in plate photobioreactors?

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Can solar control infrared blocking films be used to replace evaporative cooling for growth of *Nannochloropsis* sp. in plate photobioreactors?

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ABSTRACT

Photobioreactor overheating is a significant challenge of microalgal mass production, resulting in low photosynthetic efficiency and poor biomass productivity. Due to cost and performance limitation, passive evaporative cooling systems for managing culture temperature are currently neither economical nor sustainable. In this study, the growth and photophysiology of *Nannochloropsis* sp. MUR 267 in four different flat plate photobioreactors designs, namely, solar control infrared reflecting film (IRF), insulated glazed photovoltaic (IGP), conventional water jacket (CWJ), and no heat control (NHC), were evaluated. Maximum attained culture temperature in the IRF is comparable with CWJ and 22.6% lower than NHC. Biomass productivity in the IRF ($112.47 \pm 3.36\ mg{\cdot}L^{-1}{\cdot}d^{-1}$) is only 10% lower than that attained in the CWJ, and no net growth was seen in the NHC due to a high temperature. The immediate vitality of the cell photosynthetic apparatus monitored diurnally through the effective quantum yield of photosystem II (F_q'/F_m') showed values > 0.6 in IRF, CWJ and IGP. This study showed that clear infrared blocking films can significantly reduce the heat in PBRs without a dramatic reduction in culture performance.

1. Introduction

Microalgae cultivation is an attractive process for sustainable mass production of valuable bio-commodities that can be used as bulk and specialty biochemicals in the pharmaceutical, nutraceutical, cosmetic, aquaculture, and functional food industries [1,2]. While traditional non-microalgae cultivation techniques have been used for the production of some of these biochemicals (e.g., PUFAs from fish oil), they cannot address the growing market demand for sustainable production of these products [3].

Microalgae have the potential to reshape the source of high-value biochemicals for sustainable commercial production. Several microalgae are natural producers of some high demand bioactive compounds (e.g., carotenoids from *Haematococcus pluvialis*, PUFAs from *Nannochloropsis* sp., protein from *Spirulina* sp.) and their fast growth rate combined with all the benefits of eukaryotic expression systems make them ideal candidates for exploitation [4–6]. Microalgae can be grown in open or closed systems with inexpensive nutrients, cultivated using fresh, salt or waste-water, and are biofactories with a phototropic lifestyle [7]. They can also be cultivated all-year-round with no requirement for fertile agricultural land, are easily scaled-up in homogenous cultures, are generally recognized as safe for human consumption, and show less variation in product accumulation, thereby making downstream processing more uniform [8,9]. However, production systems suitable for large-scale cultivation and harvesting of valuable algal products under phototrophic cultures cannot compete cost-effectively with traditional sources due to unresolved technological challenges.

Technologies for microalgae farming are usually based on open pond systems and closed photobioreactors. Open ponds are preferred in the commercial cultivation of microalgae due to lower capital and operating expenses [10] but can only support the growth of a handful of microalgae species in a dilute culture system, resulting in lower biomass productivity [11]. Dilute culture systems also make the recovery of algal specialty biochemicals energy-intensive and too expensive [12]. Due to higher photosynthetic efficiency and, consequently, biomass productivity, closed photobioreactors (PBRs) are the preferred system for the commercialization of high-value microalgal products. The

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Author contribution

Contributor	Statement of contribution
Emeka G. Nwoba **(70%)**	Collection, analysis and interpretation of the data. Drafting of the article and critical revision of the manuscript.
David A. Parlevliet	Conception and design, interpretation of data, critical revision of the manuscript.
Damian W. Laird	Interpretation of data, critical revision of the manuscript.
Ashiwin Vadiveloo	Provision of study material, interpretation of data, critical revision of the manuscript
Kamal Alameh	Interpretation of data, critical revision of the manuscript.
Navid R. Moheimani	Conception and design, interpretation of data, critical revision of the manuscript.

Principal supervisor confirmation

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

Name	Signature	Date
David A. Parlevliet		**8/12/2020**

2A.1 Abstract

Photobioreactor overheating is a significant challenge of microalgal mass production, resulting in low photosynthetic efficiency and poor biomass productivity. Due to cost and performance limitation, passive evaporative cooling systems for managing culture temperature are currently neither economical nor sustainable. In this study, the growth and photophysiology of *Nannochloropsis* sp. MUR 267 in four different flat plate photobioreactors designs, namely, solar control infrared reflecting film (IRF), insulated glazed photovoltaic (IGP), conventional water jacket (CWJ), and no heat control (NHC), were evaluated. Maximum attained culture temperature in the IRF is comparable with CWJ and 22.6% lower than NHC. Biomass productivity in the IRF (112.47±3.36 $mg.L^{-1}.d^{-1}$) is only 10% lower than that attained in the CWJ, and no net growth was seen in the NHC due to a high temperature. The immediate vitality of the cell photosynthetic apparatus monitored diurnally through the effective quantum yield of photosystem II (F_q'/F_m') showed values > 0.6 in IRF, CWJ and IGP. This study showed that clear infrared blocking films can significantly reduce the heat in PBRs without a dramatic reduction in culture performance.

2A.2 Introduction

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Microalgae have the potential to reshape the source of high-value biochemicals for sustainable commercial production. Several microalgae are natural producers of some high demand bioactive compounds (e.g., carotenoids from *Haematoccccus pluvialis*, PUFAs from *Nannochloropsis* sp., protein from *Spirulina* sp.) and their fast growth rate combined with all the benefits of eukaryotic expression systems make them ideal candidates for exploitation [4-6]. Microalgae can be grown in open or closed systems with inexpensive nutrients, cultivated using fresh, salt or waste-water, and are biofactories with a phototropic lifestyle [7]. They can also be cultivated all-year-round with no requirement for fertile agricultural land, are easily scaled-up in homogenous cultures, are generally recognized as safe for human consumption, and show less variation in product accumulation, thereby making downstream processing more uniform [8, 9]. However, production systems suitable for large-scale cultivation and harvesting of valuable algal products under phototrophic cultures cannot compete cost-effectively with traditional sources due to unresolved technological challenges.

Technologies for microalgae farming are usually based on open pond systems and closed photobioreactors. Open ponds are preferred in the commercial cultivation of microalgae due to lower capital and operating expenses [10] but can only support the growth of a handful of microalgae species in a dilute culture system, resulting in lower biomass productivity [11]. Dilute culture systems also make the recovery of algal specialty biochemicals energy-intensive and too expensive [12]. Due to higher photosynthetic efficiency and, consequently, biomass productivity, closed photobioreactors (PBRs) are the preferred system for the commercialization of high-value microalgal products. The higher biomass productivity in closed PBRs makes harvesting of large-scale cultivation a minor economic bottleneck, especially for a multi-product microalgae biorefinery where the entire

biomass would be valorized [13]. Closed PBRs also offer more flexibility in exploiting a wider range of industrial species compared to the open cultivation systems. However, a significant challenge of closed photobioreactors (PBRs) operated in an outdoor environment is the overheating of cultures as a result of the absorption and conversion of excessive solar energy into heat, leading to a reduction in biomass productivity of cultures. In the absence of active temperature control systems, cultures in closed PBRs at favorable locations for microalgae production (e.g., Western Australia) can reach temperatures of more than 55 ˚C [14]. No mesophilic species of commercially-cultured microalgae can thrive at this temperature.

Therefore, active temperature control of closed PBRs is a must to circumvent significant reductions in productivity. Two temperature control methods are currently used commercially [15]: passive evaporative cooling (PEC) utilising a freshwater spray on the surface of the PBR and heat exchangers that distribute cool water to keep the temperature of the reactor at or below 25 ˚C. The PEC is the most economic temperature control method for large-scale cultivation. However, this system requires the distribution and consumption of a considerable volume of freshwater: up to 8,000 $m^3.ha^{-1}.yr^{-1}$ of freshwater to reduce PBR temperature from 40 ˚C to 25 ˚C (equivalent to removing 18,000 $GJ.ha^{-1}.yr^{-1}$ of heat) [16]. In almost all places with high solar radiation ideal for microalgae production, freshwater is a finite resource [17]. Hence, the use of freshwater PEC systems to maintain ideal culture temperatures is not economically or environmentally sustainable. Therefore, an innovative microalgal PBR that requires less, or no, freshwater for cooling purposes would be a game-changer.

Solar energy conversion to biomass through photosynthesis in PBRs is principally limited by microalgae spectral selectivity. Microalgae, due to their unique photosynthetic pigments, harvest energy from the sun in the photosynthetically-active spectrum of 400-700 nm [18,

19]. A significant portion of the full solar spectrum (infrared and ultraviolet regions) is not utilised in the production of biomass. Additionally, the high-energy ultraviolet radiation can cause algal cell damage, while infrared photons chiefly contribute to overheating of cultures [20]. Two recent technologies may provide a solution to these issues without the need for evaporative cooling. Firstly, insulated glazed panels have made it possible to actively block and/or capture these unwanted infrared and ultraviolet photons and convert the unused photons into electrical energy [21, 22]. Secondly, solar control thin films (e.g., infrared blocking films) have been used in commercial and residential houses and cars to control the level of solar heat gain. Solar control infrared (IR) films are low-emissivity thin polyester-based materials that are typically deposited onto the glass surface. They contain particles that are designed to absorb IR energy at specific spectral wavelengths. Clear IR blocking film is used to control temperature by rejecting a significant portion of IR heat energy while allowing the passage of high levels of visible light [23].

Recent studies have shown that microalgae can be successfully cultivated in a PBR constructed of insulated glazed panels. The application of IR selective film technologies have not been applied to microalgae farming. In this study, commercially-available and -inexpensive solar control infrared films are applied to a glass plate PBR and tested for their ability to control the temperature to a range suitable for sustainable *Nannochloropsis* sp. production. *Nannochloropsis* was identified as a suitable test species for PBR production as it is a potential source of high-value lipids (e.g., eicosapentaenoic and docosahexaenoic acids) [22, 24]. The growth, chemical composition and photosynthetic performance of this alga in an infrared reflecting film flat-plate (IRF) were compared to cultivation in insulated glazed photovoltaic flat-plate (IGP), conventional water jacket cooled flat-plate (CWJ), and a control flat-plate with no heat filters (NHC) PBRs.

2A.3 Materials and methods

2A.3.1 Microalgae species and culture medium

The marine Eustigmatophycean, *Nannochloropsis* sp. MUR 267 (size, 3.5 µm) isolated from Swan-Canning Estuary, Western Australia [22] was obtained from the culture collection of the Algae Research and Development Centre, Murdoch University, Western Australia. *Nannochloropsis* sp. was grown using charcoal-filtered (50 µm) natural seawater (Hillary's Beach, Western Australia) enriched with F/2-Si nutrients [25]. The ambient salinity of the seawater was 33‰ (parts per thousand) of sodium chloride. *Nannochloropsis* sp. was sourced from a non-axenic unialgal outdoor raceway pond maintained for more than five months. The initial organic biomass yield of *Nannochloropsis* sp. was 0.230 ± 0.004 $g.L^{-1}$.

2A.3.2 Cultivation setup and growth of microalgae

The cultivation systems used in the experiments comprised vertical flat plate PBRs made of glass (thickness, 5 mm) with dimensions 35 × 25 cm (height × width) and optical path-length (OPL) of 10 cm. The culture temperature of these PBR cultivation systems was controlled by either an insulated-glazed unit (IGP), an infrared blocking film (IRF), or a conventional water jacket system (CWJ – positive control). A PBR with no heat control (NHC) was used as a negative control. The IGP has the illumination surface constructed from spectrally-selective ClearVue PV® glass [21], which blocks and captures greater than 90 % of infrared (IR) and ultraviolet (UV) radiation while transmitting more than 70 % visible wavelengths. The captured IR and UV are converted to electricity through conventional solar cells attached to the edge of the glass panels [22]. The illumination surface of the IRF was laminated with a 3M™ Prestige Series - PR70 sun control residential window film (http://www.3m.com.au). The PR70 solar control film transmits 70 % visible and 30 %

infrared spectra at the ultrahigh solar PAR irradiance (3122 μmolphotonsm^{-2}s^{-1}) measurement at the Murdoch University Algae R&D Centre. The CWJ was cooled using a water-filled glass (thickness, 4 mm) flat plate in front (10 cm gap) as heat filter (10 cm × 40 cm × 40 cm, W×L×H). Water in the heat filter was bubbled with cool air.

The PBRs had a working culture volume of 6.0 L each and were situated in a constant temperature (25 ˚C) room equipped with heating (23 ˚C) and cooling (25 ˚C) systems [26]. Each reactor was mixed continuously (flow rate of 1.5 L.min^{-1}) by uniformly bubbling sterilized-filtered air from the bottom of reactors via spargers with a PondOne O2 Plus 8000 air-pump (4200 L.hr^{-1}, Aquaone). The pH of the culture was not regulated and no additional CO_2, other than that transferred during normal passive gas exchange with the atmosphere, was added to the algal cultures grown at each PBRs. Sufficient airspace was available in each reactor to allow for efficient gas and heat exchange.

Light was provided using a 500 W portable halogen lamp (Arlec HL110 Series 2, Australia), with an emission spectrum approximating the solar spectrum on a 12:12 light-dark cycle. The photon flux density at the center of the IGP PBR surface (285 μmol.photons.m^{-2}.s^{-1}) was used to determine the PAR photon flux density (142.93±0.774 μmol.photons.m^{-2}.s^{-1}) transmitted into the empty system. This transmitted PAR irradiance was normalized inside the empty IRF, CWJ, and NHC PBRs and the spectrum recorded in each PBR using a StellarNet spectrometer (Black-Comet CXR-SR-50, USA) with the sensor positioned at an OPL of 5 cm. Normalisation of PAR ensured that each PBR received similar photon flux densities. The temperature profile of the culture was measured continuously at five-minute intervals using a Pendant underwater temperature data logger (Onset Hobo, USA). The

salinity of the medium was monitored manually by a portable Atago Pal-03S digital refractometer.

Microalgae were acclimated (constancy in effective quantum yield, F_q'/F_m') to each of the reactor conditions at least two weeks in batch mode. Cultures were then switched to a semi-continuous regimen, where 50 % (3 L) of the culture volume was removed and replenished with an equal volume of freshly prepared sterile medium each time late-logarithmic phase was reached.

2A.3.3 Biomass productivity, pigment content and biochemical composition

The harvested cultures were used for measurement of the biomass productivity, biochemical composition, and pigment content. All analyses were performed in triplicate. The growth of algal cultures in the PBRs was monitored by measuring the specific growth rate, biomass yield and productivity using the standard methods detailed in Moheimani et al. [27]. Chlorophyll *a* and total carotenoids were quantified spectrophotometrically in order understand the growth and physiological response of the algae, (Biomate 3S UV-vis spectrophotometer) using the protocol of Jeffrey and Humphrey [28]. Biochemical constituents (total carbohydrate, protein, and lipids) of algal biomass were determined according to the protocols described in Moheimani et al. [27].

2A.3.4 Saturation pulse-based measurement of chlorophyll *a* fluorescence

To determine the health and physiological performance of cultures under the heat control strategies, the effective quantum yield and relative electron transport rate of photosynthesis (indicators of stress or physical fitness of cells) were measured. The effective quantum yield (F_q'/F_m') of photosystem II (PSII) and rapid light curves (RLCs) of *Nannochloropsis* sp. were determined via a pulse amplitude modulation fluorometer (Water

PAM, Walz GmbH, Germany) as described in Vadiveloo et al. [29] from samples taken directly from the PBRs. Relative electron transport rate (rETR) of *Nannochloropsis* sp. were determined according to Szabó et al. [30] for each reactor condition. The maximum electron transport rates (ETR_{max}) and slope (αETR) were obtained from the rETR response curves. To understand the response of photosynthesis to the increasing temperature in the PBRs, a diurnal profile of the photosynthetic parameters was obtained by measurement at hour 0 (before illumination) and two-hour intervals during illumination until hour 13 (one hour after illumination).

2A.3.5 Statistical analysis

To determine if the performance of the cultivation systems differed substantially, a one-way repeated measures analysis of variance (RM-ANOVA) was performed on SigmaPlot (v. 13.0). A *post-hoc* pairwise comparison (Holm-Sidak) was used to group the means. Results are reported as mean ± S.E. (standard error). The null hypothesis of no significant difference between cultivation systems was rejected when $P \leq 0.05$.

2A.4 Results and discussion

2A.4.1 Spectral distribution and temperature profile of cultivation systems

The spectral distribution of the transmitted irradiance at the surface and in the non-cooled PBR (NHC) is composed of all wavelengths in the range 400-1100 nm, with wavelengths in the IR range dominant (Fig. 2A–1(A)). The IGP, CWJ and IRF reactors all transmit a higher amount of PAR wavelengths and reduce the transmission of IR radiation, relative to the external surface and the NHC reactor. Interestingly, the IGP reduces but doesn't completely block, the transmission of wavelengths between ~700-1040 nm (Fig. 2A–1(A)). The only treatments that block the transmission of IR radiation to the interior of the

PBR are the IRF (wavelengths > 860 nm) and the CWJ (wavelengths > 960 nm). All three-temperature controlled PBRs transmit a similar amount of PAR and the level of transmission is considerably higher than for the NHC reactor (Fig. 2A–1(B)). The similarity between the trends for PAR and spectral distribution are due to the normalization of PAR irradiation required by the measurement methodology.

Consistent with the spectral transmission results, the reactor with no heat filter (NHC) transmitted the highest quantity of infrared radiation (700-1100 nm) relative to PAR; 29 % and 33 % more than the CWJ and IRF, respectively (Fig. 2A–2(A)). The spectral selection of the IRF and CWJ are reasonably similar with about 50:50 distribution of PAR and IR. The spectral distribution in the IGP is about 40:60 PAR: IR; a statistically significant difference from IRF but not CWJ (Fig. 2A–2(A)). The total infrared (heat) radiation transmitted into the PBRs was in the order: NHC>IGP>CWJ=IRF (Fig. 2A–2(A)). The similarity of CWJ and IRF is reinforced by looking at a more detailed breakdown of the wavelength composition. Of the transmitted PAR (400-700 nm) wavebands, red light (600-700 nm) was the predominant wavelength range in all the cultivation systems, while blue light (400-500 nm) was the least. A similar trend was recorded in all systems (Fig. 2A–2(B)). Red and blue light is effectively absorbed by *Nannochloropsis* sp. as the typical absorption and action spectra recorded for this species coincide with these wavelengths [31-33]. However, the red spectrum yields a better photosynthetic quantum efficiency [34] than the blue spectrum. Therefore, red light is absorbed by the algae with higher efficiency than other wavelengths, most likely because red photons (e.g., 680 and 700 nm) meet the precise energy requirements of the photosynthetic machinery expressed by this species. In this study, the relatively low content of blue light compared to the red can be explained by the raw spectral characteristics of the halogen light source, which predominantly emits higher proportions of red and infrared

components (Fig. 2A–1(A)). The illumination from the halogen lamps contained both of the spectral regions required for microalgal growth at high cell density, as well as unwanted spectral components. Transmission of wavelengths greater than 750 nm does not have any beneficial effect on photosynthesis (Supplementary Information, Fig. 2A–S1) [35] and merely contributes to an increased heat load for the cultures. Note that halogen lamp spectral distribution and irradiance profile is different to sunlight and care needs to be taken when extrapolating results to outdoor PBRs.

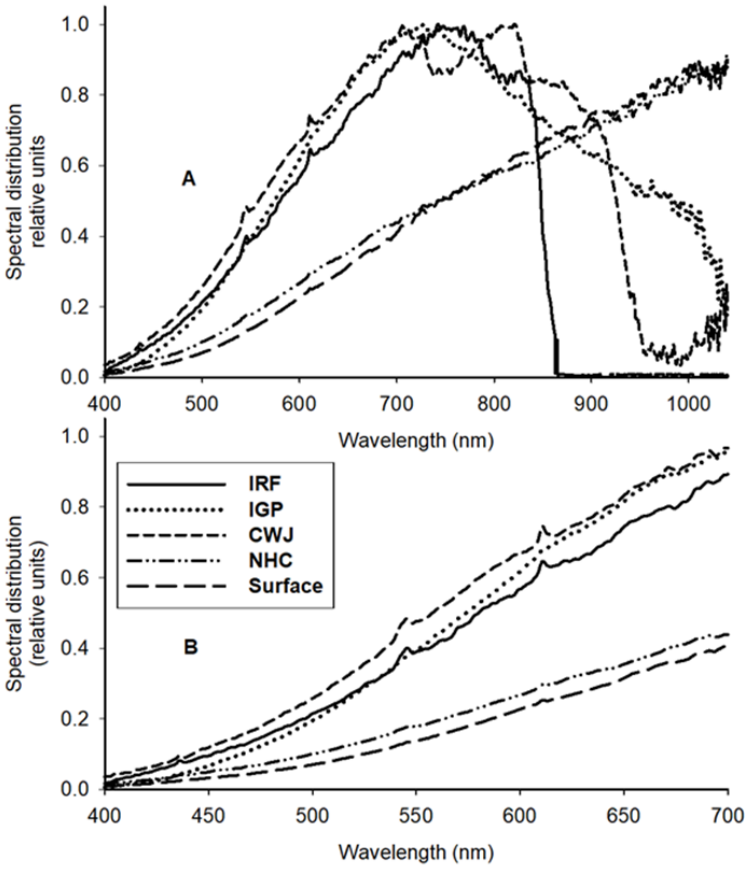

Fig. 2A–1. Normalized relative spectral distribution of transmitted irradiance inside of the empty infrared film flat plate (IRF), insulated glazed photovoltaic flat plate (IGP), conventional water jacket flat plate (CWJ), and no heat control flat plate (NHC) photobioreactors used for the growth of *Nannochloropsis* sp. MUR 267 (A) full and (B) PAR (400-700 nm) spectra of the modified irradiance in each reactor.

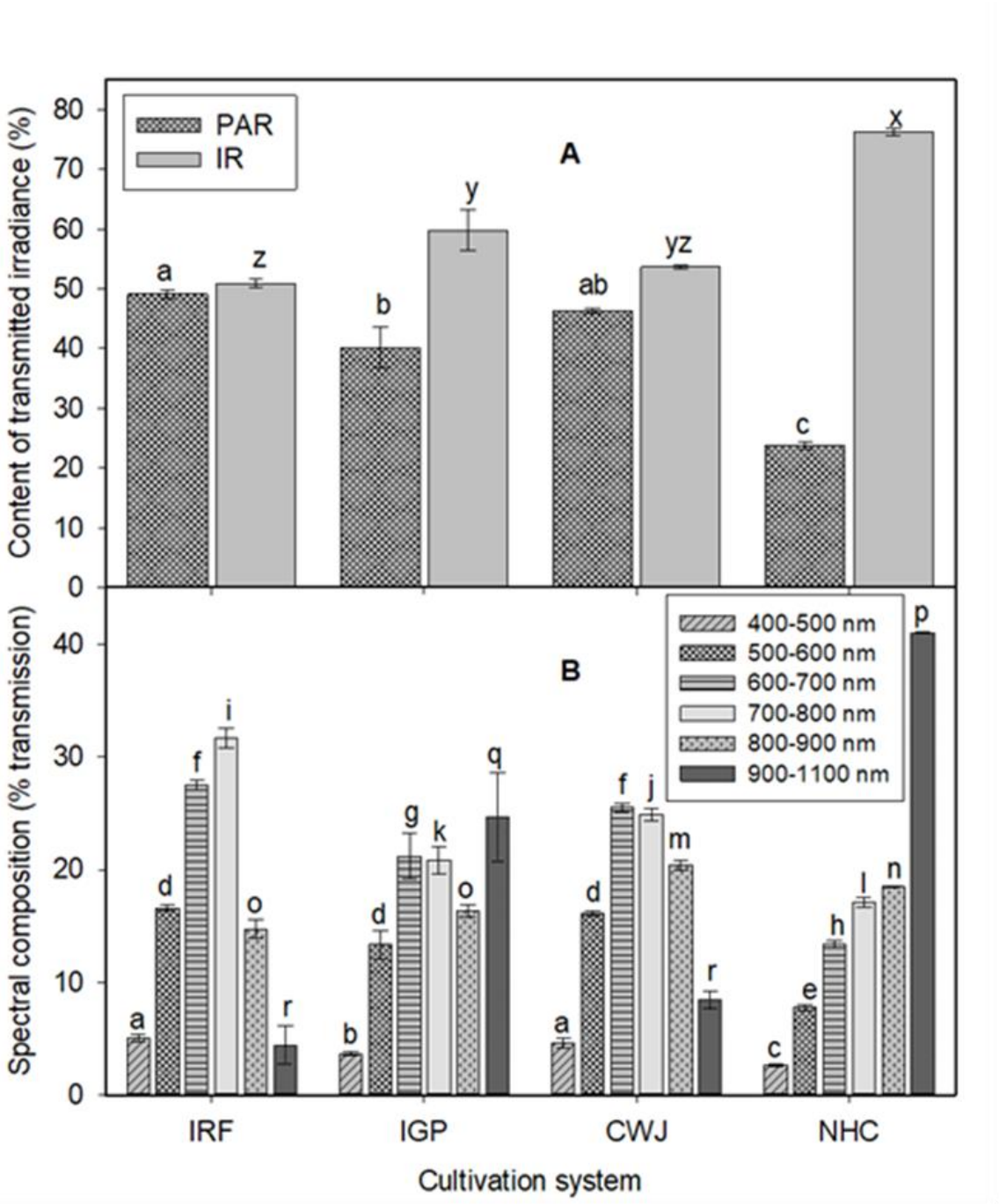

Fig. 2A–2. Spectral content of the transmitted irradiance inside of the cultivation systems showing visible (400-700 nm) and infrared (700-1100 nm) spectra (A) and transmitted photon quality (B) measured at the beginning and each phase of the semi-continuous experiments. Bars with the same letter across groups signify no significant differences (One-Way RM ANOVA, P>0.05, n = 5).

The diurnal temperature profile of the *Nannochloropsis* sp. culture followed the same trend in all the photobioreactors (Fig. 2A–3). Temperature continuously increased during the

hours of illumination and decreased sharply during the dark period. While the minimum temperature observed was the same for all cultivation systems (24.47±0.25 ˚C, effectively room temperature in the controlled environment), the NHC reached the highest maximum temperature of 40.82±0.19 ˚C (Table 2A–1). This temperature proved to be lethal to *Nannochloropsis* sp. MUR 267 resulting in overall negative growth and decline of the culture (Table 2A–1, Supplementary information, Fig. 2A–S2). The maximum temperature of all PBRs was above the reported optimal temperature range of 24-27 ˚C for mesophilic *Nannochloropsis* sp. [33, 36] in general but it is known that *Nannochloropsis* sp. MUR 267 strain can tolerate much higher temperatures (up to 35 ˚C) than this, albeit with a reduction in biomass productivity [22].

The maximum temperature of the IRF photobioreactor was 6.05 % higher than that of the CWJ photobioreactor, but 22.61 % and 5.21 % lower than that recorded in the NHC and IGP photobioreactors, respectively. Although the temperature reaches a maximum just before the lights are turned off, the increase between 10:00 and 18:00 in the CWJ and IRF is well below IGP and NHC (Fig. 2A–3). The reduction in temperature is a significant difference with the IGP which shows a more pronounced temperature increase over this time frame, presumably as a result of the IGP transmitting more light in wavelengths >750 nm (Fig. 2A–2(A)). The maximum temperature for the IGP reactor recorded in this study was lower than that reported by Vadiveloo et al. [22], most likely due to normalization of PAR in the interior of the PBR rather than on the outer surface of the reactor. Also, improvements to the mixing regime in the PBR resulted in improved heat and air exchange.

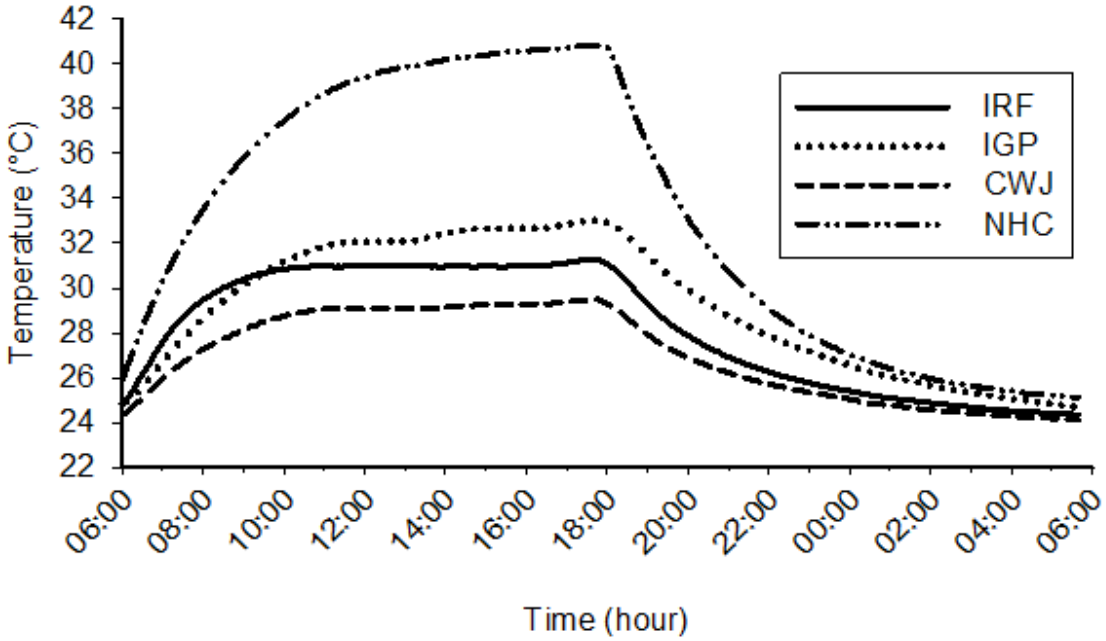

Fig. 2A–3. Diurnal changes in the average temperature of *Nannochloropsis* culture grown in infrared film flat plate (IRF), insulated glazed photovoltaic flat plate (IGP), conventional water jacket flat plate (CWJ), and no heat control flat plate (NHC) photobioreactors.

2A.4.2 Specific growth rate and biomass productivity

Nannochloropsis sp. exhibited remarkable growth in all the PBRs except in the NHC photobioreactor (Supplementary Information, Fig. 2A–(S2 and S3)). The negative growth recorded in the NHC photobioreactor was the result of the collapse of the culture. The high maximum temperature in that reactor was deemed to be the most likely reason for this as the light intensity (142 µmol.photons.$m^{-2}.s^{-1}$) in all cultures was not above the threshold that could have resulted in oversaturation, photoactivation, photoinhibition, or photodamage to the photosystems of this alga [22]. The NHC culture could not be maintained in the semi-continuous regimen and was discontinued after two successive trials without further analysis (Supplementary Information, Fig. 2A–S2). For the remaining three PBRs, the highest specific growth rate (0.199±0.021 d^{-1}) was achieved in the CWJ (Table 2A–1).

Table 2A–1. Specific growth rates, biomass productivity and pigments content of *Nannochloropsis* sp. MUR 267 cultivated semi-continuously in infrared film flat plate (IRF), insulated glazed photovoltaic flat plate (IGP), conventional water jacket flat plate (CWJ) and no heat control flat plate (NHC) photobioreactors.

Parameter	Unit	IRF	IGP	CWJ	NHC
Temperature control strategy	-	Infrared reflecting film	Insulated glazed unit	Cool water as heat filter	None
Maximum temperature attained	˚C	31.59 ± 0.1650^{d}	33.32 ± 0.304^{b}	29.68 ± 0.137^{d}	40.82 ± 0.191^{a}
IR spectrum (700-1100 nm) transmitted into reactor	%	50.85 ± 0.724^{c}	59.85 ± 3.384^{b}	$53.73\pm0.371b^{c}$	76.18 ± 0.676^{a}
PAR spectrum (400-700) transmitted into reactor	%	49.15 ± 0.724^{a}	40.18 ± 3.409^{b}	$46.28\pm0.371a^{b}$	23.78 ± 0.659^{c}
Specific growth rate, μ	d^{-1}	0.192 ± 0.020^{a}	0.176 ± 0.055^{b}	0.199 ± 0.021^{a}	$-0.184\pm0.0909^{*}$
Biomass productivity	$mg.L.^{-1}d^{-1}$	112.47 ± 3.360^{b}	77.34 ± 2.970^{c}	124.97 ± 3.980^{a}	n.a
Chlorophyll *a*	$mg.g^{-1}$ AFDW	8.78 ± 2.140^{a}	8.51 ± 2.005^{a}	9.80 ± 1.782^{a}	n.a
Total carotenoids	$mg.g^{-1}$ AFDW	3.01 ± 0.460^{a}	2.66 ± 0.618^{a}	2.85 ± 0.303^{a}	n.a
Carotenoids/ chlorophyll *a* ratio	%	34.28 ± 2.179^{a}	31.26 ± 1.600^{a}	29.08 ± 2.066^{a}	n.a
Lipid productivity	$mg.L^{-1}.d^{-1}$	75.47 ± 2.570^{a}	60.07 ± 1.998^{b}	63.63 ± 2.957^{b}	n.a

*calculated during batch growth,
AFDW means ash-free dry weight
n.a means not applicable.

The specific growth rate of *Nannochloropsis* sp. MUR 267 in CWJ was similar to IRF (Table 2A–1) but was significantly higher than the specific growth rate of this alga when

grown in IGP PBR (One-way RM ANOVA, P< 0.05). This result agrees with the findings of Vadiveloo et al. [22], who reported maximum specific growth of *Nannochloropsis* sp. in CWJ when compared to the IGP PBR.

In contrast, the volumetric biomass productivity was significantly different among the cultivation systems with CWJ > IRF > IGP (One-Way RM ANOVA, P <0.001) (Table 2A–1). The volumetric biomass productivity achieved in CWJ and IRF are similar with only a 10 % difference observed. However, the productivity of the IRF photobioreactor was 31 % higher than that of the IGP reactor. Not surprisingly, biomass productivity was inversely related to the maximum temperature recorded in the PBRs, in agreement with the results previously reported by Vadiveloo et al. [22].

Temperature is a critical limit to the growth of any organism as it can impact cellular physiological status. Suboptimal temperature modifies the conformational structure of proteins and affects the functioning of microalgal photosynthetic machinery. Suboptimal high-temperature typically results in the denaturation and deactivation of essential biochemical enzymes as well as changes in the structural integrity of proteins associated with light-harvesting machinery due to heat stress [37-39]. *Nannochloropsis* sp. typically grows most efficiently at temperatures between 24 ˚C and 27 ˚C, and fluctuations from this range have been shown to have a negative influence on the growth and photosynthetic capacity of the alga [33, 36]. However, *Nannochloropsis* sp. MUR 267 is known to grow successfully at elevated temperatures up to 35.2 ˚C, albeit with a 45 % reduction in biomass productivity when compared to growth at 30.6 ˚C [22]. The maximum temperature reached by the CWJ reactor is marginally higher than the optimum 24-27 ˚C range and the IRF only slightly higher again, suggesting that growing *Nannochloropsis* in suitably cooled PBRs is viable. Most exciting is the similarity in the biomass productivity of the IRF and CWJ. These results

strongly suggest that using a readily available IR blocking film on the surface of the PBR gives adequate heat control without the need for active water cooling. Use of the IR film reduces the reliance on precious potable water for the successful cultivation of microalgae for high-value products. However, given that the full photon flux density (PFD) of sunlight (>1000 μmolphotons.$m^{-2}s^{-2}$) is far above the measured light intensity transmitted to the culture in this study, it is possible that the temperature in an outdoor PBR could exceed the maximum growth temperature of selected microalgae. In such a scenario, combining IGP or IRF with a water-cooling system can be an answer. Such a method would certainly result in maintaining a suitable temperature in the PBR. Given this scenario, the evaporative cooling system could be operated only when the temperature exceeds an optimal level.

2A.4.3 Culture health and physiology

Increased culture temperature results in stress to the alga, and this is reflected in a change in physiological status. Such stresses were assessed by monitoring the pigment concentration and photosynthesis characteristics (effective quantum yield and electron transport rates) of *Nannochloropsis* sp. under the different temperature control strategies employed.

2A.4.3.1 Photosynthetic pigment content of *Nannochloropsis* sp.

Photosynthetic pigments are an essential component of the light-harvesting antennae complex responsible for the capture of a photon for microalgal photosynthesis [40]. Chlorophylls and carotenoids participate in light harvesting, processing, and protection of photosynthetic apparatus from oxidative stress-induced damage. Increasing photosynthetic pigment concentration typically enhances the photon capture capability of microalgae and, therefore, results in higher photosynthetic activity and consequently higher

biomass productivity [41, 42]. Chlorophyll concentration was examined for variation as a marker of cell physiology in the different PBRs (Table 2A–1). Chlorophyll *a* concentration of *Nannochloropsis* sp., based on organic biomass, was not significantly different (One-Way RM ANOVA, P >0.05) regardless of the cultivation system (Table 2A–1). Similarly, the total carotenoid content (mg.g^{-1} AFDW) was also not statistically different (One-Way RM ANOVA, P = 0.632) between the treatments (Table 2A–1). Hence, the typical biomass productivity achieved in each of the heat control systems was due to unaffected photosynthetic pigments production by this alga (Table 2A–1). The total carotenoid:chlorophyll *a* ratio, a sensitive marker of oxidative or environmental stress in photosynthetic organisms [29], reveals no significant difference among the cultivation systems (Table 2A–1). The normalization of the spectrum inside of the cultivation systems may be responsible for the lack of difference in the pigment content of the alga in the cultivation systems. Therefore, the slight differences in the proportions of transmitted PAR relative to IR, especially in the IGP photobioreactor, were not significant enough to trigger a variation in the pigment content of *Nannochloropsis* in the various systems. Vadiveloo et al. [22] found no variation in *Nannochloropsis* sp. MUR 267 pigment content (e.g., chlorophyll *a*) between insulated glazed and water jacket photobioreactors despite transmitting different proportions of visible and infrared radiation, suggesting that the spectral normalisation process does not produce experimental artifacts.

2A.4.3.2 Photochemical efficiency of photosystem II

The effective quantum yield (F_q'/F_m') is an immediate measure of the vitality or physical fitness of cells. Strongly declining values would be expected to correlate with thermal stress to the culture, as the photosynthetic apparatus/metabolic enzyme performance are temperature-sensitive. Thus, light energy captured by the photosynthetic pigments of microalgae has three fates; photochemistry, chlorophyll fluorescence, and heat

dissipation. The F_q'/F_m' is a sensitive indicator of the potential effective quantum yield of photochemistry at photosystem II under light condition [43, 44]. The diurnal F_q'/F_m' values for this alga in all the cultivation systems investigated followed a similar trend (Fig. 2A–4(A)): highest at pre-dawn (0.68) before the start of illumination, slight decrease (5.8%) immediately upon illumination, and then remaining steady throughout the hours of illumination before increasing at dusk (i.e., one-hour post-illumination, Fig. 2A–4). The F_q'/F_m' values of the *Nannochloropsis* in the IRF and IGP photobioreactors were not significantly different (One-Way RM ANOVA, $P > 0.05$) and marginally higher than that in the CWJ photobioreactor (Fig. 2A–4(A)). A similar finding was reported for *Nannochloropsis* sp. in IGP and CWJ photobioreactors [22]. The small decrease and steady F_q'/F_m' values in the three reactors during the illumination period, despite the increasing temperature, confirms that these cooling systems can maintain culture temperature at a level that does not induce thermal inhibition of *Nannochloropsis* sp. cells.

2A.4.3.3 Relative electron transport rates (rETR)

The maximum electron transport rate, $rETR_{max}$ characterizes the photosynthetic efficiency and capacity of microalgae. The slope of the rETR curves (αETR) reveals the photon capturing efficiency of photosynthetic organisms. In these experiments, rETR parameters showed a similar trend in the three cultivation systems, suggesting minimal light saturation and maximal transport of electrons to the photosynthetic units [30]. Specifically, the $rETR_{max}$ and αETR were lowest before illumination (hour 0), reached maximum values and remained steady throughout the illumination period, then decreased to the original value one hour after the dark regime was established (hour 13, Fig. 2A–4(B and C)). Overall, this data supports the interpretation that the temperature control strategies in the IRF, IGP,

and CWJ have kept culture temperatures to a range that doesn't result in a breakdown of their photosynthetic capabilities.

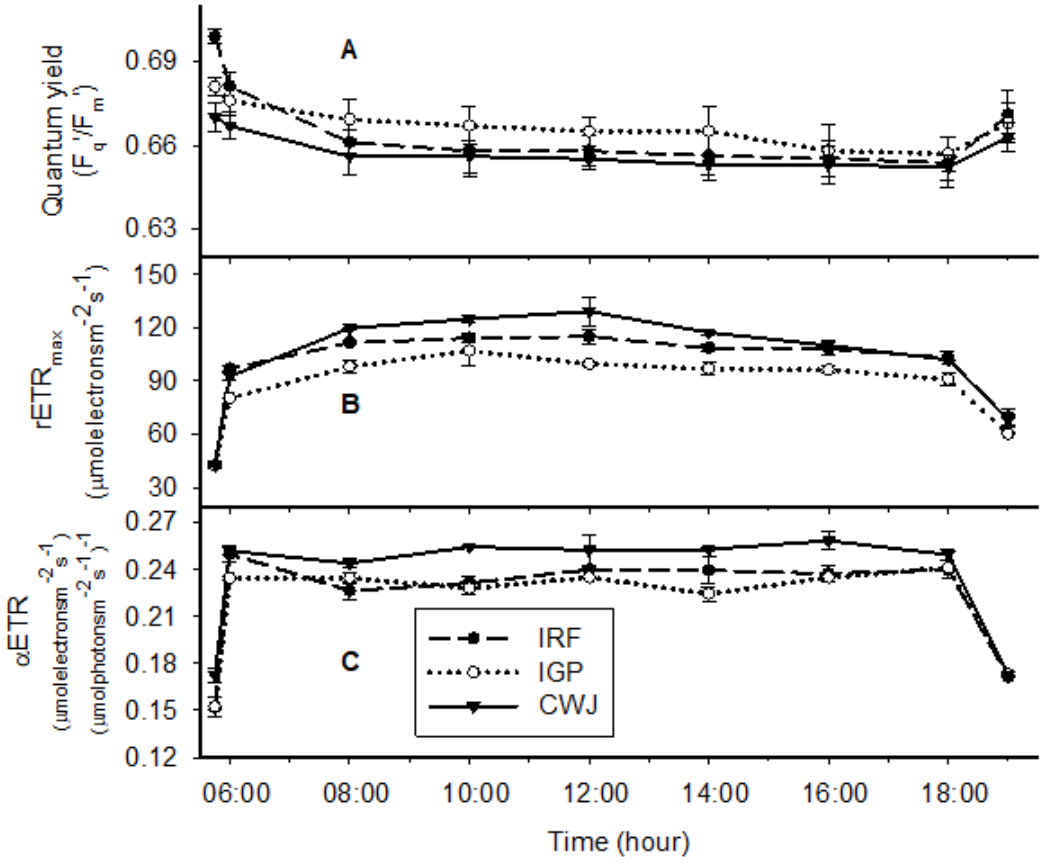

Fig. 2A–4. Diurnal changes in the effective quantum yield of photochemistry at PS II (F_q'/F_m', A), maximum electron transport rate (ETR_{max}, B) and the gradient of the rETR curves (αETR, C) of *Nannochloropsis* sp. cultured in infrared film flat plate (IRF), insulated glazed photovoltaic flat plate (IGP), and conventional water jacket flat plate (CWJ) photobioreactors.

2A.4.4 Biochemical composition of biomass

The biochemical composition (total lipids, carbohydrates, and protein) of the *Nannochloropsis* biomass showed relatively little difference across the three PBRs (Fig. 2A–5). The carbohydrate content did not change at all (One-way RM ANOVA, $P > 0.05$). The protein content was slightly higher in the CWJ, but no statistically significant variation (Holm-Sidak, $P = 0.974$) was observed between the IRF and IGP (Fig. 2A–5). In contrast, the

lipid content was higher in the IRF and IGP photobioreactor compared to that in the CWJ photobioreactor (Fig. 2A–5), although there was no significant variation (Holm-Sidak, P = 0.664) in the lipid content between the IRF and IGP photobioreactors.

Generally, phototropic microalgae with fast growth rates have high protein and low carbohydrate content. Temperature is known to influence microalgal protein biosynthesis, by impacting the synthesis of enzymes, and the high protein and low carbohydrate content of *Nannochloropsis* sp. in these cultivation systems could be explained by the need for individual cells to incorporate more carbon into proteins. For example, the protein and carbohydrate content of *Isochrysis galbana* was found to be enhanced and reduced, respectively, at high temperature [45]: a result similar to the present study. At temperatures above optimum, microalgal cells increase their metabolic activity resulting in the degradation of reserve materials such as carbohydrates for cell maintenance [46]. Nevertheless, total carbohydrate results of the current study are similar to values (7-8 %AFDW) reported in similar systems [22]. Similarly, lipid accumulation by microalgae has been shown to be temperature-dependent. *Nannochloropsis oculata* showed a two-fold increase in lipids as the higher temperature was raised from 20 to 25 ˚C [47]. This trend appears to be the case in the IRF and IGP photobioreactors with higher temperatures correlated to higher cellular lipid content, but not in the CWJ photobioreactor which operated at a lower temperature. These results suggest that the cultures were not stressed enough for changes in the biochemical profile to occur.

The use of solar control infrared blocking films is a smart technology to manage the critical issue of culture temperature in commercial-scale algal PBR operation. This study shows that the physiological health, photosynthetic electron transport activity, and synthesis of biomolecules were not impaired in the *Nannochloropsis* sp. grown PBRs with a

solar control film (IRF) photobioreactor and that similar results were attained with a CWJ photobioreactor. Thus, applying IRF and IGP technologies to enclosed flat plate PBRs offers the potential for significant savings in freshwater and energy consumption associated with PBR cooling. Such savings should increase the economic efficiency of microalgal production processes, especially for those species better suited to PBR conditions than outdoor raceway ponds. Previous studies showed that in many cases, the response of algae to changes in environmental variables is species specific. In here, we tested *Nannochloropsis* sp. MUR 267 and we do no doubt that other species could be behaving differently. Despite the lower volumetric biomass productivity in the IGP compared to CWJ, this design has the potential to be used in microalgal cultivation as it allows for the conversion of part of the incoming solar energy (especially the IR and UV wavelengths, which are useless for photosynthesis) to electricity. Over 30 $W.m^{-2}$ of electrical energy can be generated from the IGP [48] and this power can be used to energize the pumps and mixing, thus reducing overall production costs. Potential improvement of the IGP with the transmission of wavelengths, which are optimal for algal growth (e.g., blue and red spectra) can enable the allocation of other wavelengths not transmitted to the culture be routed to electricity production. While the results from the IRF and IGP PBRs are promising, there remain some questions that need to be addressed to assess these technologies fully. The issue of differences in the solar vs. halogen light spectra and the photon flux has already been mentioned (section 2A.4.1). It may also be envisaged that the spectral selection technology (either IR film or IGP material) may reduce the amount of PAR available to the culture and result in a large drop in culture temperature (especially during winter), negatively affecting growth and, hence, productivity. Given the much variable photon flux the PBR would experience under outdoor conditions, it seems likely that the effect of the reduction in PAR in light-limited cultures produced by the IRF would not

outweigh the increased temperature control throughout of a whole year [23]. However, in the summer when PBRs experience large solar irradiance, productivity losses due to a reduction in PAR available to the cultures can be compensated by temperatures that are more favourable. In the case of the IGP PBR operation during winter, the low emissivity of the thin film will prevent heat in the PBR escaping to the environment, thus maintaining a more constant year-round culture temperature.

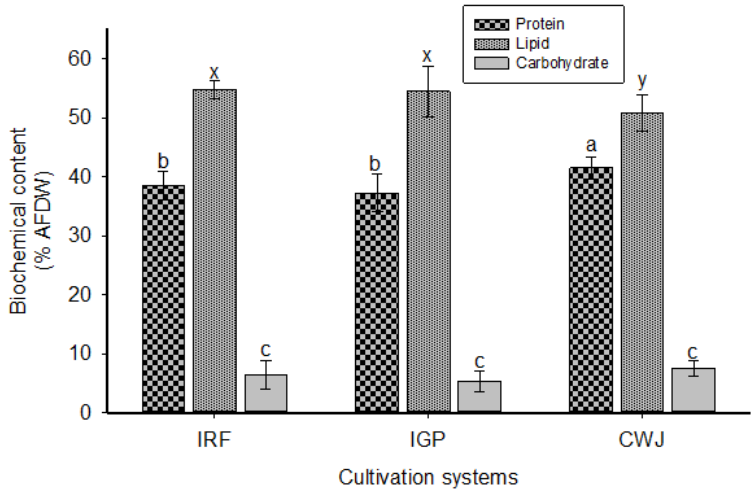

Fig. 2A–5. Biochemical constituents (total lipids, proteins, carbohydrates) of *Nannochloropsis* sp. MUR 267 biomass cultured in infrared film flat plate (IRF), insulated glazed photovoltaic flat plate (IGP), and conventional water jacket flat plate (CWJ) photobioreactors. Bars with the same letter across groups are not significantly different (One-Way RM ANOVA, P>0.05).

2A.5. Conclusions

In here, we demonstrated that coating photobioreactors with spectrally selective materials could be a viable strategy for managing the internal temperature of PBRs for sustainable microalgae cultivation. A solar control film effectively reduced the transmission of IR wavelengths resulting in culture temperatures 22% lower compared to no heat control. No significant difference in average temperatures was observed between the current

industry-norm cooling system (cool water jacketed) and IR reflecting film coated PBRs. No difference in the physiological health of algae grown in the water jacketed and novel IR blocking film PBRs, however, a 10% loss in biomass productivity was recorded in the latter. The use of solar control films on photobioreactors could significantly reduce the water and power requirements for commercial microalgal cultivation. However, further studies need to be carried out under outdoor conditions to test the long-term suitability of proposed systems on the growth and photosynthesis of various microalgal species.

2A.6 References

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2A.7 Supplementary information

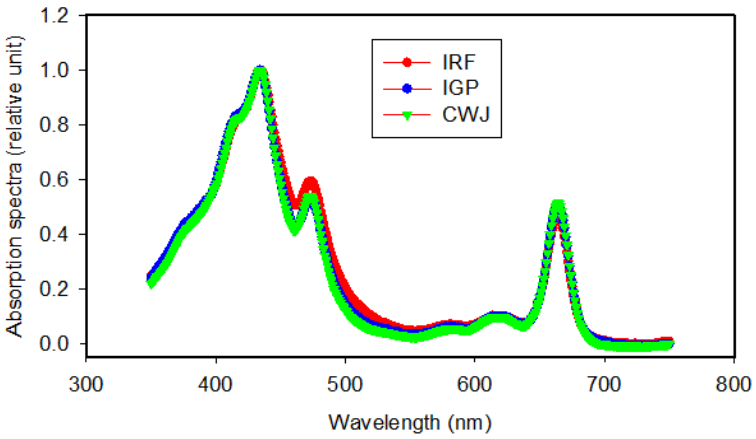

Fig. 2A–S1. Normalized absorption spectra of Nannochloropsis sp. MUR 267 pigments cultured in infrared film flat plate (IRF), insulated glazed photovoltaic flat plate (IGP), and conventional water jacket flat plate (CWJ) photobioreactors. Pigments was extracted using 90% ice cold acetone.

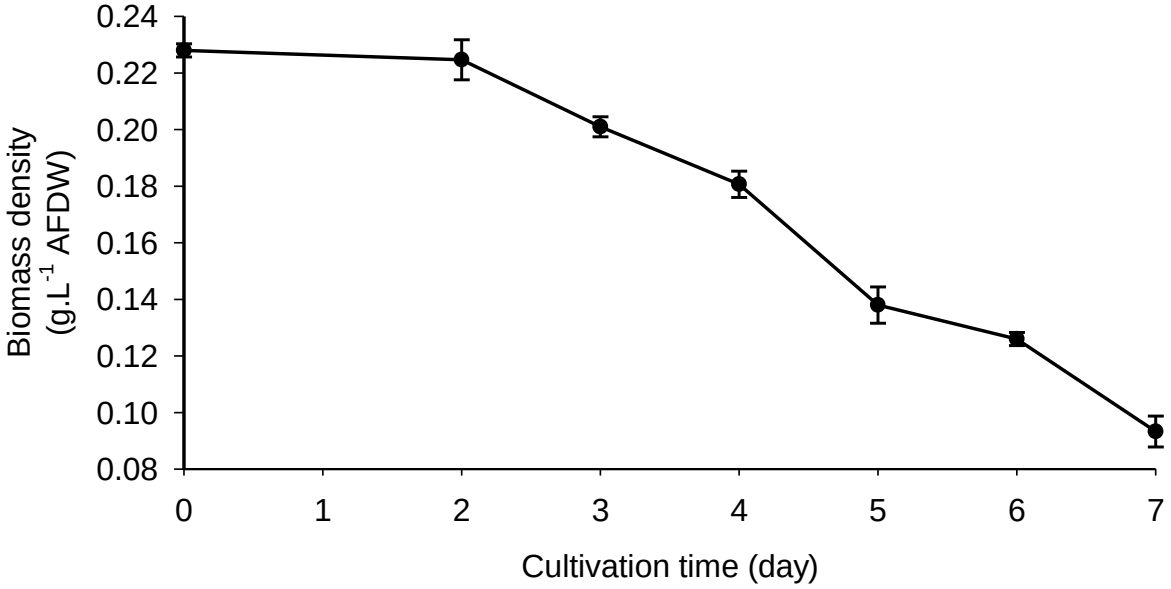

Fig. 2A–S2. Trend of biomass density of *Nannochloropsis* sp. MUR 267 cultured in no heat control flat plate (NHC) photobioreactor. Culture terminated after two successive trials resulted in no net growth due to a supra-optimal temperature.

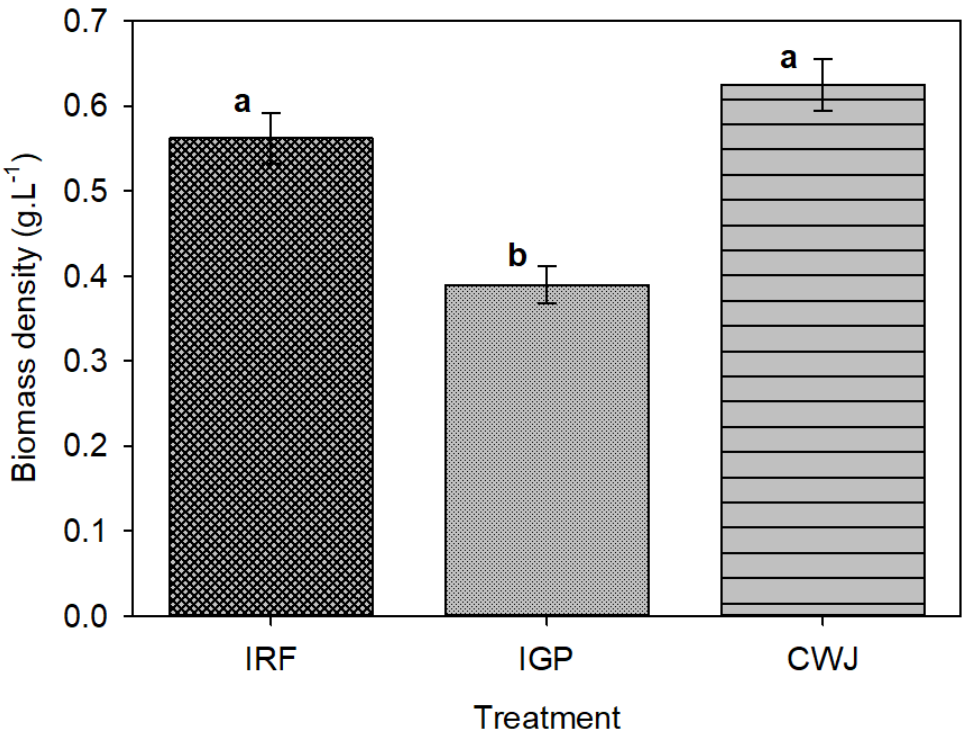

Fig. 2A–S3. Biomass concentration of *Nannochloropsis* sp. MUR 267 cultivated in infrared reflecting film (IRF), insulated glazed photovoltaic (IGP) and conventional water jacket (CWJ) photobioreactors. Different letters indicate significant differences (P < 0.05).

Chapter 2B

Arthrospira platensis cultivation in insulated glazed and thin-film coated photobioreactors

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Regular article

Sustainable phycocyanin production from *Arthrospira platensis* using solar-control thin film coated photobioreactor

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HIGHLIGHTS

- Cooling algal photobioreactors using freshwater is unsustainable.
- Using a solar control film significantly reduced heat gain in a flat plate PBR culturing *Arthrospira*.
- Maximum C-phycocyanin content, 16.8% was achieved in solar control film PBR.
- Solar control films could replace freshwater cooling for sustainable algal production.

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ABSTRACT

Solar irradiance consists of photosynthetically-active photons that can be transformed to valuable biomolecules by microalgae. Light also has undesirable non-photosynthetic photons, such as ultraviolet and infrared wavelengths that heat up algal closed photobioreactors above optimum temperatures for growth. In this study, a solar control infrared blocking film (IRF) is applied to an algal flat plate photobioreactor to block excessive non-photosynthetic photons and regulate the temperature profile of *Arthrospira platensis* cultures for the production of C-phycocyanin (C-PC). The performance of the IRF is compared against other cooling mechanisms such as insulated-glazed photovoltaic (IGP), conventional water-jacket (CWJ) and a no heat control (NHC) photobioreactors. Experimental results show that the maximum temperature (30.94 ± 0.09 °C) in the IRF culture is only 5% higher than that in CWJ culture but 33% lower than that in NHC cultures. No significant differences were found in C-PC content or biomass productivity when *Arthrospira* is grown using IGP, CWJ or IRF but is significantly lower in NHC photobioreactors. Chlorophyll *a* fluorescence probing of *A. platensis* shows that IRF, IGP and CWJ cultures are not thermally stressed, however, NHCs cultures are highly stressed due to supraoptimal temperatures. Our results clearly indicate that solar control film is a potential tool for blocking non-photosynthetic photons and managing culture temperature in flat plate photobioreactors for sustainable C-phycocyanin production from *A. platensis*.

1. Introduction

Bio-based C-phycocyanin (C-PC) is gaining increasing commercial attention as a green chemical in the functional food and pharmaceutical industries due to its hepatoprotective, antioxidant, neuroprotective, and anti-inflammatory functions [1–4]. In the cosmetic industry, C-PC is typically used as a colourant in products such as lipstick and eyeliner [5]. Furthermore, due to its fluorescence-specific properties, high molar extinction coefficient and quantum yield, C-PC finds application in fluorescence microscopy and immunoassays as a fluorescent probe and

Abbreviations: AFDW, ash-free dry weight; C-PC, C-phycocyanin; CWJ, conventional water-jacket; IR, infrared; IGP, insulated-glazed photovoltaic; IRF, infrared blocking film; NHC, no heat control; NHC-41, NHC at 41 °C; NHC-46, NHC at 46 °C; OJIP, polyphasic chlorophyll *a* fluorescence transient; PAR, photosynthetic active radiation; PEC, passive evaporative cooling; PBR, photobioreactor; UV, ultraviolet

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Author contribution

Contributor	**Statement of contribution**
Emeka G. Nwoba **(70%)**	Design, collection, analysis and interpretation of the data. Drafting and critical revision of the manuscript.
David A. Parlevliet	Conception and design, interpretation of data, critical revision of the manuscript.
Damian W. Laird	Interpretation of data, critical revision of the manuscript.
Kamal Alameh	Interpretation of data, critical revision of the manuscript.
Navid R. Moheimani	Conception and design, interpretation of data, critical revision of the manuscript.

Principal supervisor confirmation

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

Name	**Signature**	**Date**
David A. Parlevliet		**8/12/2020**

2B.1 Abstract

Solar irradiance consists of photosynthetically-active photons that can be transformed to valuable biomolecules by microalgae. Light also has undesirable non-photosynthetic photons, such as ultraviolet and infrared wavelengths that heat up algal closed photobioreactors above optimum temperatures for growth. In this study, a solar control infrared blocking film (IRF) is applied to an algal flat plate photobioreactor to block excessive non-photosynthetic photons and regulate the temperature profile of *Arthrospira platensis* cultures for the production of C-phycocyanin (C-PC). The performance of the IRF is compared against other cooling mechanisms such as insulated-glazed photovoltaic (IGP), conventional water-jacket (CWJ) and a no heat control (NHC) photobioreactors. Experimental results show that the maximum temperature (30.94±0.09 ˚C) in the IRF culture is only 5% higher than that in CWJ culture but 33% lower than that in NHC cultures. No significant differences were found in C-PC content or biomass productivity when *Arthrospira* is grown using IGP, CWJ or IRF but is significantly lower in NHC photobioreactors. Chlorophyll *a* fluorescence probing of *A. platensis* shows that IRF, IGP and CWJ cultures are not thermally stressed, however, NHCs cultures are highly stressed due to supraoptimal temperatures. Our results clearly indicate that solar control film is a potential tool for blocking non-photosynthetic photons and managing culture temperature in flat plate photobioreactors for sustainable C-phycocyanin production from *A. platensis*.

2B.2 Introduction

Bio-based C-phycocyanin (C-PC) is gaining increasing commercial attention as a green chemical in the functional food and pharmaceutical industries due to its hepatoprotective, antioxidant, neuroprotective, and anti-inflammatory functions [1-4]. In

the cosmetic industry, C-PC is typically used as a colourant in products such as lipstick and eyeliner [5]. Furthermore, due to its fluorescence-specific properties, high molar extinction coefficient and quantum yield, C-PC finds application in fluorescence microscopy and immunoassays as a fluorescent probe and biomarker for labelling immunoglobulins and receptor proteins [6-8]. The rising demand for naturally-derived C-PC for such a wide array of applications is driven by growing consumer preference for products from environment-friendly and sustainable production sources [9]. Phycocyanin like other phycobiliproteins (allophycocyanin, phycoerythrin) are unique in nature as they are only occurred in algae [10]. Within the microalgae community, *Arthrospira platensis* is the most productive source of C-PC with up to 25 %w/w content in dry biomass [11]. Global production of *Arthrospira* biomass is more than 5000 tons.yr^{-1} with the market for total phycobiliproteins valued at greater than US$ 60 million yearly [7, 10]. Specifically, C-phycocyanin trades for at least US$ 500 kg^{-1} for food-grade [5, 10, 12] and US$ 125 mg^{-1} for analytical grade [13].

Phototropic production of *Arthrospira* biomass for C-PC is generally influenced by light quality particularly intensity, wavelength, source and photoperiodism [14, 15]. Ideal geographical locations suitable for microalgae cultivation generally have solar radiation that is so high it actually impedes microalgae production. A significant issue in microalgae cultivation is that not all of the incoming solar spectrum typically contributes to the photosynthetically-driven energy transformation process – in effect a biological version of the Shockley-Queisser limit in solar cells with a single p-n junction [16]. For example, high-energy ultraviolet radiation (wavelength < 400 nm, UV) results in algal cell damage, and infrared photons (> 750 nm, IR) negatively affect algal productivity by increasing culture temperature potentially leading to algal death. The latter is of a particular problem in photobioreactors [17] used for producing high-value algal-derived products such as C-PC.

Therefore, the spectral selectivity of microalgae limits the scope of cultivable regions, and this would need to be increased if bio-based C-PC is to make the required commercial impact. Hence, it makes sense to selectively manipulate incoming insolation in such a way that the spectrum incident on the algal culture contains less of the harmful UV and IR radiation and more usable PAR photons. This modification has the potential to diminish the average kinetic energy received by the culture, which would result in overall culture temperature reduction.

For bio-based C-PC production, enclosed photobioreactors are the preferred commercial cultivation technology compared to open systems due to lower risk of contamination and higher biomass productivity [18]. For example, the biomass productivity ($mg.L^{-1}.d^{-1}$) of *Arthrospira* achieved in closed PBRs is 5-20 times higher than that attained in open raceway ponds [6]. However, in addition to high capital and operating costs, the required control of culture temperature in PBRs makes these systems energy-intensive and expensive to operate. Commonly employed passive-evaporative cooling (PEC) systems, such as the spraying of freshwater on the surface of PBRs, are generally more economical than heat exchange systems. However, PEC systems are significantly limited by performance and sustainability issues. In particular, in almost all places with high insolation, freshwater is a limited resource [19]. The need for a large volume of water for both cooling and operation of microalgae production processes at large-scale is undeniably a severe limitation. This bulk water requirement could certainly hamper the commercialization of phycocyanin generation from *Arthrospira* sp.

Solar control films (SCFs) are of great potential as a temperature control strategy in algal photobioreactors for economical, sustainable and environment-friendly biomass production. SCFs are spectrally-selective low-emissivity polyester-based materials that are

capable of modifying the incident solar spectrum and blocking UV and IR radiation. In reality, SCFs are widely exploited in cars, residential, and commercial buildings for temperature control [20] but the possible application of SCFs for microalgae farming has not been explored.

In this study, coating the illumination surface of an algal flat-plate PBR with an inexpensive, commercially available clear SCF is evaluated for its ability to modify the incoming light spectrum and temperature of a culture of *Arthrospira platensis* optimised for C-PC production. The efficacy of the clear SCF is assessed by comparing the growth, photosynthesis and C-phycocyanin accumulation of *A. platensis* in the solar control infrared film (IRF) coated flat plate against other PBR configurations such as insulated-glazed photovoltaic flat-plate (IGP), conventional water-jacket cooled flat-plate (CWJ) and no heat control flat-plate (NHC) photobioreactors.

2B.3. Materials and methods

2B.3.1 Microalgae strain and growth medium

The cyanobacterium *Arthrospira platensis* MUR 126 was obtained from the culture collection of the Algae Research and Development Centre, Murdoch University. *Arthrospira platensis* was cultivated using sterilized Zarrouk medium [21]. The inoculum for the experiment was sourced from an outdoor non-axenic unialgal culture maintained over three months in a 25 L open raceway pond. The initial inoculum size was 0.42±0.03 $g.L^{-1}$.

2B.3.2 Cultivation photobioreactors and growth conditions

The cultivation of the *A. platensis* culture was carried out using vertical glass flat plate photobioreactors (10 × 25 × 35 cm, W × L × H) with a working culture volume of 6 L (for more details see [22]). The illumination surface of the photobioreactors was modified using

different heat control strategies. The PBR nomenclatures based on the applied heat control system were as follows: solar control infrared blocking film PBR (IRF), insulated-glazed photovoltaic PBR (IGP), conventional water-jacket PBR (CWJ) and no heat control PBR (NHC). The IRF had its illumination surface coated with a clear solar control infrared film (Prestige Series PR70, http://www.3m.com.au) and the IGP was constructed using a spectrally-selective PV glass panel as its illumination surface [23]. The positive control CWJ had an external water-jacket system as a heat filter while the negative control NHC received direct illumination with no heat filters or cooling mechanism [24]. Sufficient air holes (6 × 2.2 cm diameter) on the lids of the PBRs were provided to allow for the efficient transfer of heat and gases. However, the lid of the NHC was modified to give two variants. One of the NHC systems (designated NHC-41) had the same air hole profile as the previous reactors while the maximum temperature could get to 41 ˚C and the other (NHC-46) had only two air holes (2.2 cm diameter each) at both ends of the lid with maximum temperature up to 46 ˚C.

All the PBRs were situated in a 25 ˚C constant temperature room. Cultures were mixed using aeration with a flow rate of 1.5 $L.min^{-1}$ through air-stone spargers installed at the base of each reactor. A halogen light source (500 W HL110 Series 2, Australia), with comparable emission spectrum to the spectrum of the sun was used to provide illumination for each PBR. The light source was automated to a photoperiod of 12/12 h light/dark diel cycle. The photosynthetic-active radiation (PAR) photon flux density transmitted inside each empty cultivation reactor was standardized to approximately 142.93±0.77 $\mu mol.photons.m^{-2}.s^{-1}$. The spectral characteristics of the transmitted irradiance inside each reactor were recorded using a spectrometer (StellarNet Black-Comet CXR-SR-50, USA). The temperature profile for each cultivation system was continuously measured at five-minute intervals using data loggers (Onset Hobo Temperature logger, USA).

The culture of *A. platensis* was acclimated to the prevailing conditions in each reactor for three weeks based on constancy in the values of effective quantum yield of photochemistry at photosystem II. *Arthrospira* was cultivated using the semi-continuous operation with a culture period of six days, where half of the culture was harvested at the late-exponential growth phase and replaced with the same volume of freshly prepared Zarrouk medium [25]. Harvested biomass was used to determine productivity, pigment content and biochemical composition. Analyses were carried out in triplicate on the culture during semi-continuous operation.

2B.3.3 Measurement of biomass productivity, pigment contents and biochemical composition

Specific growth rate, biomass yield and areal productivity was determined based on the methods described in Moheimani et al. [26]. Chlorophyll *a* concentration was measured using a spectrophotometer (UV-Vis spectrophotometer, BioMate 3S) according to the protocol of Jeffrey and Humphrey as detailed in Moheimani et al. [26]. The C-phycocyanin (C-PC) and allophycocyanin (APC) contents of the *A. platensis* biomass were determined based on the protocol of Bennett and Bogorad [27] using 0.1 M sodium phosphate buffer with repeated freezing and thawing. *A. platensis* biomass was harvested by filtration of 5 mL of the culture through 2.5 cm GF/C filters (0.45 µm, Whatman). The freshly harvested biomass was washed twice with deionized water and resuspended in 5 mL phosphate buffer (pH 6.8). The mixture was subjected to four cycles of freezing at -20 ˚C and thawing at room temperature in the dark. Ruptured cells were crushed and the phycocyanin containing transparent supernatant was collected after centrifugation at 5000 rpm for 10 minutes. Phycocyanin concentration in the sample was determined spectrophotometrically (UV-Vis,

BioMate 3S) at 615 and 652 nm. The chemical composition (total carbohydrates, proteins, and lipids) and total carotenoids of the algal biomass were measured using standard methods described in Moheimani et al. [26].

2B.3.4 Photochemical quantum yield and rapid chlorophyll fluorescence induction kinetics in *A. platensis*

The effective quantum yield (F_q'/F_m') of primary photochemistry at photosystem II (PSII) is typically the best tool used for algal vitality monitoring [28, 29]. The F_q'/F_m' of *A. platensis* in each reactor was measured using cells immediately collected from the cultivation system. To evaluate the response of algal photosynthesis to the changing temperature in the culture vessels during illumination, a diurnal study of the photosynthesis parameters was monitored by measurement at hour zero (i.e., pre-illumination time) and two hours interval throughout the illumination regime up to hour 13 (one-hour post-illumination period). To characterize and evaluate the degree of heat stress to *A. platensis* culture under the PBR conditions, non-invasive polyphasic chlorophyll *a* fluorescence OJIP transients [30] were used as a stress indicator. OJIP transients of cultures in each PBR were measured at the last hour of illumination (before lights switched off), corresponding to the maximum temperature recorded in the systems, using a portable chlorophyll *a* fluorometer (AquaPen-C, Czech Republic). Fluorometer was set at the maximum 3000 µmol.photons.m^{-2}.s^{-1} saturation pulse intensity of blue light (450 nm) and measurements were carried out on the cultures taken directly from the photobioreactors [28]. A double-normalized variable fluorescence was carried out to enable the derivation of the biophysical/phenomenological parameters from the OJIP kinetics curve guiding the description of *A. platensis* physiology under the PBR conditions. While the phenomenological parameter, F_V/F_O represents the

measure of the photosynthetic capacity of PSII reaction centers to evolve oxygen, I/P (ratio of I-step at $F_{30\ ms}$ to P-step at maximum fluorescence intensity, F_M) reflects the electron reception capacity at the acceptor side of PSI. In this JIP-test, the heat stress parameter W_K ($(=F_{0.25\ ms} - F_{0.03\ ms})(F_{2\ ms} – F_{0.03\ ms})^{-1}$) was used to determine the capacity of the PSII donor side to transfer electrons [28, 31].

2B.3.5 Statistical analysis

A one-way repeated measures analysis of variance (RM-ANOVA) was used to evaluate the performance of the photobioreactors. The Holm-Sidak test was used for determining significant differences in means. Results were reported as mean ± S.E. (standard error) over the experiment duration and significant differences between cultivation systems were declared when $P < 0.05$.

2B.4. Results and discussion

2B.4.1 Spectral profile of the cultivation photobioreactors

The spectrum of the transmitted irradiance in the treatment PBRs showed a remarkable difference to that of the no heat control (NHC) flat plate PBR (Fig. 2B–S1). The spectral pattern of the transmitted irradiance inside the NHC and that on the surface of IGP are the same. Similarly, solar control infrared film (IRF), conventional water-jacket (CWJ) and insulated-glazed photovoltaic (IGP) PBRs have the same transmitted spectral profile at wavelengths less than 700 nm (Fig. 2B–S1(A)). Though there was variation in the pattern of modification in each PBR at wavelengths greater than 700 nm, the IRF strongly diminished the infrared component transmitted to the interior of the PBR at wavelengths > 840 nm by 100%. The spectral distribution of PAR inside the PBRs with heat filter systems was similar (Fig. 2B–S1(B)) due to the internal standardization of total PAR in the cultivation systems.

The amount of PAR photons available in the PBRs with heat control systems were relatively higher than recorded in the NHC (Fig. 2B–S1(B)). The PAR spectrum (400-700 nm) typically represents the photons that encourage microalgal growth. However, of the contributing PAR photons, the full energetic potential of all the available photons is not used for biomass formation. Generally, red and blue photons are very efficient in driving photosynthesis in microalgae. In this study, red light (600-700 nm) was found to be most dominant and this outcome trended similarly in the PBRs with heat control systems (Fig. 2B–1).

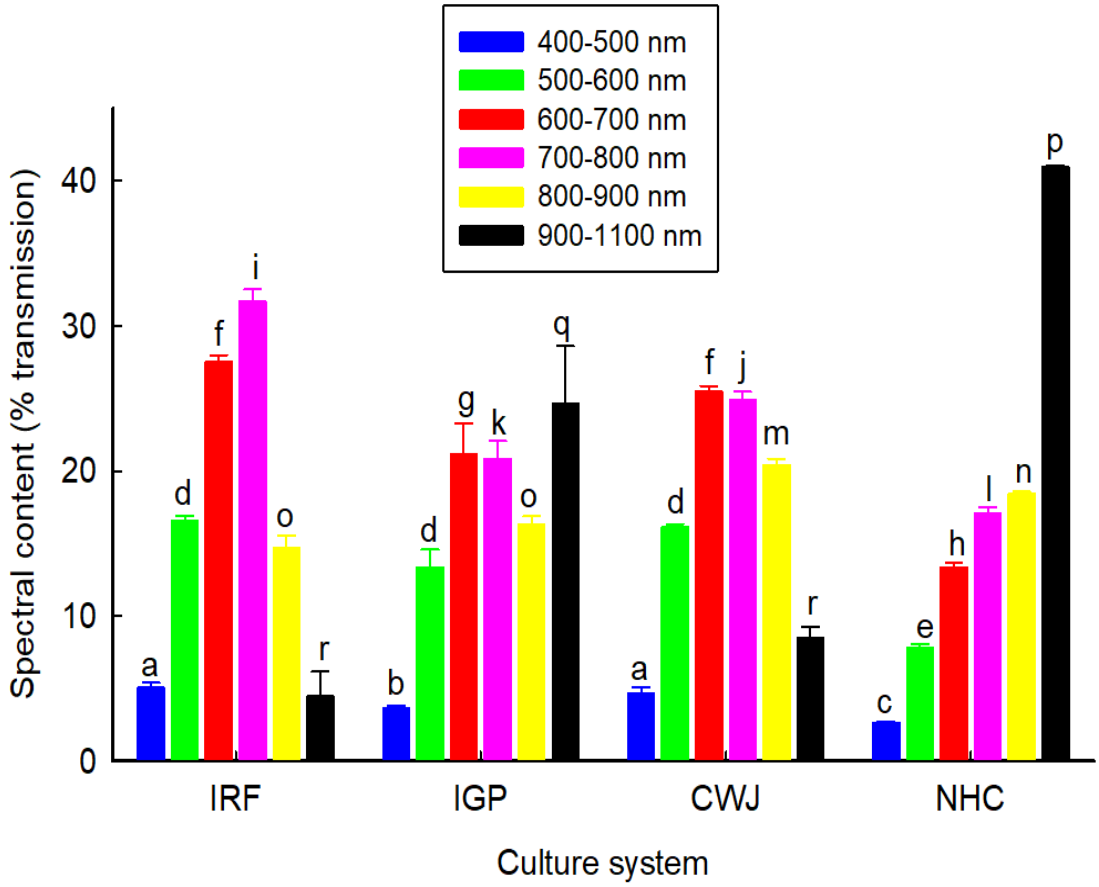

Fig. 2B–1. Spectral quality of the transmitted spectrum inside of the cultivation systems. Bars with the same letter across groups signify no significant differences (One-Way RM ANOVA, *P* > 0.05, n = 5).

2B.4.2 Culture temperature profile and specific growth rate of *A. platensis*

The diurnal temperature profile of *A. platensis* cultures in the PBRs showed substantial variation (Fig. 2B–2). The minimum temperature was similar in all the culture systems. However, the maximum temperature differed significantly (One-way RM-ANOVA, P = <0.001, n = 31) with highest temperature recorded in NHC-46 (Table 2B–1). The maximum temperature in the IRF and IGP were respectively 33 and 31% lower than that of NHC-46. Similarly, the attained maximum temperature in the CWJ was 29 and 37% lower than that of NHC-41 and NHC-46, respectively. The average temperature was lower in the cooled compared to the non-cooled PBRs (Table 2B–1).

The growth of *A. platensis* was negatively affected at temperatures above 41 ˚C with a 29% decrease in specific growth rate in NHC-46 when compared with NHC-41 (Fig. 2B–3(A)). The highest specific growth rate (0.18±0.008 d^{-1}) was achieved in the IRF, which was 44% and 11% higher than in NHC-46 and CWJ, respectively. When the culture temperature was further increased manually to 47.46±0.177 ˚C by adjusting the position of the illuminator closer to the NHC-46 culture, there was a sudden disruption of *A. platensis* growth, and the cells died within 24 hours. The effect of this temperature increase was discernible as the colour of the culture changed from dark-blue to orange.

Non-optimal high temperatures have been reported to result in a significant decline in specific growth rates of microalgae, due to heat stress [24]. The optimal growth temperature reported for *A. platensis* in the literature ranges between 30-37 ˚C. Both Shi et al. [32] and Ogbonda et al. [33] concluded that the optimum growth temperature of *A. platensis* is 30 ˚C after investigating growth rate over a range from 20-40 ˚C. Others have shown that optimal growth can occur at higher temperatures (30-37 ˚C) under laboratory

conditions but temperatures ≥40 ˚C is lethal to this alga [34, 35]. Thermotolerant species of *Arthrospira* are known and can be cultured between 35-40 ˚C [36].

In our study, sustained temperatures >46 ˚C are evidently too high for cell viability of *A. platensis*, resulting in the collapse of the culture. This may be attributed to the denaturation and deactivation of vital biological enzymes, including damage to the structural integrity of the light-harvesting complex integral membrane proteins [37-39]. This phenomenon justifies the long tradition of cooling algal closed photobioreactors with water at locations with high insolation. The result here suggests that PBRs manufactured from IGP or coated with IRF will prevent deleterious heat transfer to cultures at least as effectively as water-cooling. Not having to rely on fresh, clean water for cooling applications has obvious environmental benefits, particularly as many areas suitable for outdoor microalgal production also have limited freshwater resources.

2B.4.3 C-phycocyanin, chlorophyll *a*, and total carotenoid contents

To identify whether the heat control strategies affected the production of C-phycocyanin (C-PC) and other pigments and to what extent, the total content of C-PC, APC, chlorophyll *a* and carotenoid over biomass in the different PBRs were analysed (Fig. 2B–3(A)). The highest C-PC content, 16.5% AFDW, was obtained in IRF while the lowest content, 5.9% AFDW, was obtained in the NHC-46. Except NHC-46, the PBRs IRF, CWJ and IGP showed no variation in C-PC content (One-way RM ANOVA, $P > 0.05$; Fig. 2B–3(A)). C-PC productivity showed no variation between cooled PBRs and significantly different between cooled and uncooled PBRs (Table 2B–1). The APC showed no variation between the heat-controlled systems but differed significantly (One-way RM-ANOVA, $P < 0.05$) between the NHCs and the other insulated PBRs (Fig. 2B–3(A)). The trend of APC in all the cultivation systems was similar to the C-PC: highest under the IRF (66 $mg.g^{-1}$ AFDW) and lowest under

the NHC-46 (38 mg.g^{-1} AFDW). As highlighted in section 3.1, cultures inside the IRF was subjected to higher proportion of red photons, which has been reported to enhance C-PC production in *A. platensis* compared to other wavelengths [40]. The lowest C-PC content found in NHC-46 was due to lower specific growth rates and biomass productivity brought forward by the high temperature (Fig. 2B–3(A)). These findings are consistent with previous reports [41]. Nevertheless, while the C-PC content reported in our current study was not the highest reported for cyanobacteria (18.5%), the achieved content is much greater than many of the reported values (ranging 4.8-18.5%) [42]. Under the conditions of the heat-controlled PBRs, average phycocyanin productivity 1.48±0.11 gAFDW.m^{-2}d^{-1} is higher than 0.82-0.85 g.m^{-2}.d^{-1} typical of outdoor raceway ponds [43, 44]. It is evident from this investigation that the heat control strategies such as the IRF were effective in keeping the culture within temperature optima of *A. platensis*, hence no adverse effect on the C-PC production. In contrast to IRF, C-PC and APC showed 64% and 43% decrease, respectively, in the NHC-46, strongly supporting external cooling systems in algal PBRs is a *sine qua non* for successful C-PC production. IRF proved to be a viable technology for simultaneous modification of incident light spectrum (Fig. 2B–S1) and management of culture temperature (Fig. 2B–2) for the sustainable production of C-PC from *A. platensis*.

Chlorophyll *a* synthesis was affected by temperature stress conditions faced by the *A. platensis* cultures. Among the heat control strategies employing IRF, IGP and CWJ, chlorophyll *a* was significantly lower (Holm-Sidak, P = 0.02) in the CWJ than the IRF cultures, whereas no difference was found between the IRF and IGP (Table 2B–1). However, under very harsh conditions as observed in NHC-46, significantly lower chlorophyll content (One-

way RM ANOVA, P = <0.001) was noticed due to a substantial decrease in chlorophyll *a* synthesis brought forward by supraoptimal temperature.

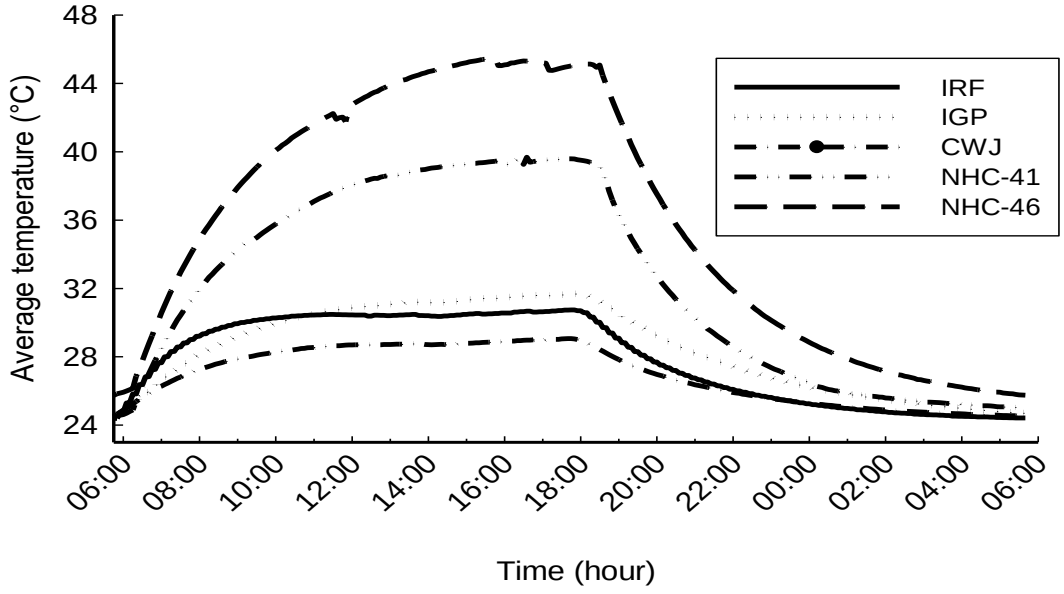

Fig. 2B–2. Diurnal behaviour of the average temperature of *Arthrospira platensis* culture cultivated in solar control infrared film (IRF), insulated glazed photovoltaic (IGP), conventional water-jacket (CWJ), and no heat control (NHCs) flat plate photobioreactors.

The total carotenoid content of *A. platensis* culture did not vary significantly (One-way RM-ANOVA, P = 0.058) among the cultivation systems (Table 2B–1). Total carotenoid was highest in NHC-46 operating at higher temperature and lowest (4.13 $mg.g^{-1}$ AFDW) in CWJ with lower temperature. The insignificant variation of total carotenoids of the *A. platensis* cultures could be attributed to the normalization of the PAR inside the cultivation systems. The carotenoid/chlorophyll *a* ratio presents an interesting picture: highest in NHC-46 (45%), then NHC-41 (38%) and no statistical difference (One-way RM ANOVA, P > 0.05) in PBRs with heat control materials (Table 2B–1). Carotenoids and other accessory pigments in *Arthrospira* such as phycobiliproteins (e.g., phycocyanin and allophycocyanin) are strongly

involved in chromatic adaptation, where they facilitate the absorption of light wavelengths inefficiently absorbed by chlorophyll *a*, transmitting the photon energy to chlorophyll *a* (primary pigment of light-harvesting machinery). In addition, accessory pigments, such as carotenoids, function in free-radical scavenging activity to avoid oxidative damage to the cells by conducting dissipation of excess energy as heat [45]. This particular role is mirrored by the concentration of chlorophyll *a* in the cultures at higher temperatures [46]. The higher carotenoid content relative to chlorophyll *a* in the NHC-46 could be evidence of thermal stress leading non-photochemical quenching activity due to the generation of oxygen free radicals [47]. In contrast, constant values obtained for chlorophyll *a*/total phycobiliproteins ratio in the IRF, CWJ and IGP (Table 2B–1) with heat control systems indicate the close association between the two pigments [46].

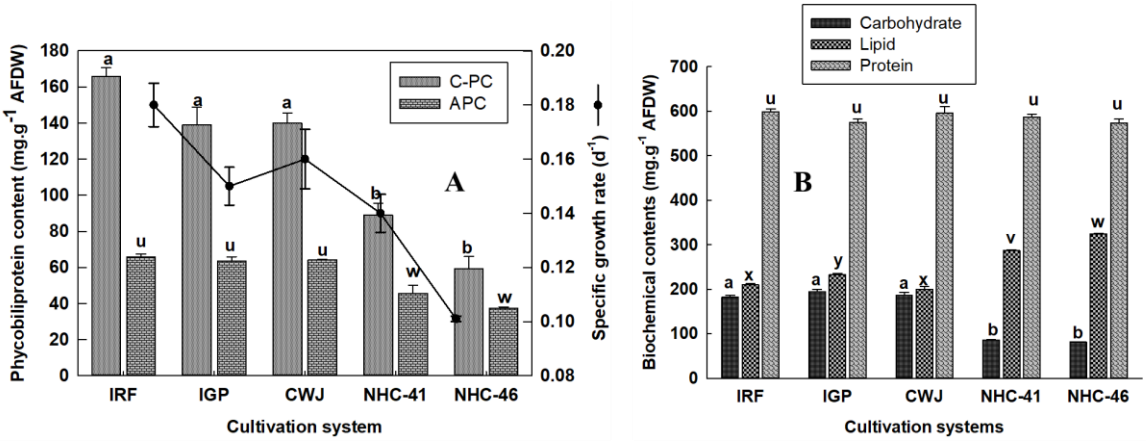

Fig. 2B–3. Specific growth rate and C- phycocyanin (A) and biochemical (B) contents of *A. platensis* biomass cultured in infrared blocking film (IRF), insulated-glazed photovoltaic (IGP), conventional water-jacket (CWJ), and no heat control (NHCs) PBRs. AFDW, ash-free dry weight; C-PC, C-phycocyanin; APC, allophycocyanin. Bars with the same letter across group indicates no significant differences (One-Way Repeated Measures ANOVA, P > 0.05).

2B.4.4 Biomass productivity and biochemical composition of *A. platensis*

The biomass productivity and biochemical composition of *A. platensis* in all the different PBRs was evaluated. Maximum biomass productivity of 10.73±0.68 $g.m^{-2}.d^{-1}$ was found in IRF but was not statistically different from those of IGP and CWJ. In contrast, the productivity obtained in the IRF, IGP and CWJ was significantly higher than that of NHC-46 (One-way RM ANOVA, P = <0.001, Table 2B–1). The slightly higher biomass productivity obtained in the IRF bioreactor may be due to either the optimum temperature profile of the culture or differences in the transmitted wavelengths. It is necessary to point out that IRF had the highest proportion of red light (Fig. 2B–1), which is usually absorbed with higher efficiency by microalgae compared to other wavelengths [40]. The biomass productivity obtained in this study is similar to the 10.4±0.14 $g.m^{-2}d^{-1}$ reported by Kim et al. [48] and higher than 5.2-8.14 $g.m^{-2}.d^{-1}$ obtained by Tredici et al. [49]. Nevertheless, the areal productivity of *Arthrospira* in raceway ponds rarely exceeds 15 $g.m^{-2}.d^{-1}$ even with CO_2 addition [50].

The relative content of total protein in *A. platensis* biomass showed no significant variation (One-way RM-ANOVA, P > 0.05) between the cooled and non-cooled reactors (Fig. 2B–3(B)). On the other hand, the carbohydrate content is significantly higher (One-way RM-ANOVA, P < 0.05) in the cooled PBRs than the NHCs. Total lipid content of cultures increased in a temperature dependent manner with a maximum yield of 324.53±1.47 $mg.g^{-1}$ obtained in NHC-46 (46 ˚C, Fig. 2B–(3B)). Compared to the positive control CWJ, the lipid content of the IRF was 5% higher and not statistically significant (Holm-Sidak, P = 0.18). The values of the biochemical contents (reported in ash-free dry weight, AFDW) in this study are similar to the results by Madkour et al. [51] and Zhang et al. [36], who reported the protein,

carbohydrate, and lipid contents of *A. platensis* biomass at 500-530, 120-130 and 80-100 $mg.g^{-1}$ DW, respectively.

Table 2B–1. Maximum temperatures, biomass productivity and pigment contents of *A. platensis* cultivated in infrared blocking film (IRF), insulated glazed photovoltaic (IGP), conventional water jacket (CWJ), and no heat control (NHCs) flat plate photobioreactors.

Parameter	Unit	IRF	IGP	CWJ	NHC-41	NHC-46
Heat control system	None	Solar control infrared blocking film	Insulated glazed unit	Water jacket	None	None
Maximum temperature	°C	30.94 ±0.09^{d}	32.05 ±0.09^{c}	29.28 ±0.08^{e}	41.07 ±0.18^{b}	46.46 ±0.21^{a}
Average daily temperature	°C	27.92 ±0.15^{d}	28.47 ±0.15^{c}	26.94 ±0.10^{e}	32.29 ±0.33^{b}	35.90 ±0.43^{a}
Biomass productivity	$g.m^{-2}.d^{-1}$	10.73 ±0.68^{a}	10.04 ±0.14^{a}	8.73 ±0.32^{b}	9.98 ±0.47^{ab}	5.20 ±0.40^{c}
Biomass yield	$g.m^{-2}$	66.01 ±5.83^{a}	65.16 ±4.78^{a}	53.51 ±6.15^{b}	61.40 ±3.36^{a}	35.22 ±3.98^{c}
Chlorophyll *a* content	$mg.g^{-1}$	14.64 ±0.20^{a}	12.41 ±0.29^{b}	13.22 ±0.38^{b}	11.92 ±0.79^{bc}	10.37 ±0.29^{c}
Carotenoid content	$mg.g^{-1}$	4.37 ±0.09^{a}	4.35 ±0.24^{a}	4.13 ±0.17^{a}	4.52 ±0.16^{a}	4.70 ±0.22^{a}
C-phycocyanin productivity	$g.m^{-2}.d^{-1}$	1.69 ±0.08^{a}	1.44 ±0.09^{a}	1.32 ±0.07^{a}	0.89 ±0.11^{b}	0.31 ±0.02^{c}
Carotenoid/ chlorophyll *a*	%	29.85 ±0.82^{a}	35.05 ±0.43^{ab}	31.24 ±0.29^{b}	37.92 ±0.64^{ab}	45.32 ±0.57^{a}
Total phyco-biliproteins	$mg.g^{-1}$	231.51 ±0.22^{a}	202.52 ±0.18^{a}	204.19 ±0.36^{a}	134.51 ±0.14^{b}	96.88 ±0.28^{b}
Chlorophyll *a*/phyco-biliproteins	%	6.32 ±0.09^{b}	6.13 ±0.11^{b}	6.47 ±0.16^{b}	8.85 ±0.06^{a}	10.70 ±0.25^{a}

Along the rows, the same letter indicates no significant differences (One-Way Repeated Measures ANOVA, $P > 0.05$).

2B.4.5 Photochemical efficiency and OJIP kinetics of *A. platensis* culture

Changes in the effective quantum yield (F_q'/F_m') of photosystem II (PSII) typically represent the immediate physiological conditions of photosynthetic organisms and the efficiency at which open PSII reaction centers capture excitation energy for metabolic processes [52]. At optimal light and environmental conditions, photosynthetic organisms, such as microalgae, typically have a F_q'/F_m' value between 0.6-0.7 [28, 29]. In the present study, the F_q'/F_m' ratio displayed a similar trend in the three reactors with heat control systems throughout the illumination hours (Fig. 2B–4(A)). This value was highest before and one-hour after the illumination period in the range 0.56-0.58 but remained steady during the illumination period for the insulated PBRs (Fig. 2B–4(A)). Conversely, the NHCs showed a progressive decrease in the F_q'/F_m' values throughout the illumination hour. The values of F_q'/F_m' reported here are higher than those of Torzillo et al. [53] for *S. platensis* under outdoor conditions probably due to the sub-saturating photon flux density cultures were exposed to. Changes in the F_q'/F_m' for the cultures in NHCs during the illumination period were inversely proportional to the increase in temperature and differed remarkably from those of the heat controlled PBRs (Fig. 2B–4(A)). In fact, there was 45% decrease in the F_q'/F_m' values of NHC-46 culture between 05:40 (before lights were switched on) and 18:00 (before lights were switched off) compared to the 9.6% decrease in the IRF over the same period. At 19:00 (one-hour into the dark regime) the recovery potential of the cultures in the insulated PBRs was 33% faster than in the NHCs. The notable reduction in the F_q'/F_m' for cultures in the NHCs could be due to PSII impairment or its long-term downregulation in reaction to thermal stress [54]. This remarkable drop in the photochemical efficiency could negatively affected the biomass productivity and C-phycocyanin production by *A. platensis* in the NHCs, stressing the importance of temperature control in algal PBRs.

OJIP curves for the *A. platensis* grown in the cultivation systems show that the J and I steps in the NHC reactors were higher than cultures in the PBRs with heat control systems (Fig. 2B–4(B)). The heat stress-induced visible changes in the OJIP curve were greater in the NHC-46 compared to the NHC-41. The F_V/F_O ratio (representing the efficiency of the water-splitting complex on the PSII donor side) of *A. platensis* was higher, but not significantly different (One-Way RM ANOVA, $P > 0.05$), in the heat control PBRs compared to the values obtained from the cells cultivated in the NHCs (Fig. 2B–5). This ratio is typically a sensitive measure of the efficiency of photosynthetic electron transport chain and low values of this parameter reveal damage to the electron transport chain [55]. The lowest F_V/F_O (0.36±0.02) of *A. platensis* grown in the NHC-46 (maximum temperature, 46 ˚C) shows that the cells were under thermal stress, hence, the poor growth performance of this alga in the PBR. Although the biomass productivity of this alga at 41˚C (NHC-41) is slightly lower than the productivities in the reactors with heat control strategies, it is interesting to note that the 67% drop in the F_V/F_O in NHC-41 validate the importance of temperature regulation in algal PBRs. This drop is reflected in the production of C-PC by this alga at 41 ˚C (Fig. 2B–3). On the other hand, the heat stress parameter, W_K, was not significantly different (RM-ANOVA, $P > 0.05$) across the cultivation PBRs with temperature control systems (Fig. 2B–5). Higher values in W_K were found in NHC cultures and significantly different ($P < 0.05$) from those of the cultures cultivated in insulated PBRs. The lower value of W_K in the insulated PBRs compared to the NHCs reveals that the cultures were not under stress. Lack of variation in W_K under the no-heat control conditions of the tested PBRs demonstrates the acquired heat tolerance of the donor side of the PSII reaction center [31] in *A. platensis* during culture acclimation. Conversely, the I/P ratio was highly statistically different (One-RM ANOVA, $P = < 0.001$) for all the PBRs with the highest values observed in the NHC-46 and NHC-41 (Fig. 2B–5). In this

study, results show that PBRs with a decrease in effective quantum yield of PSII primary photochemistry resulted in significant changes in the polyphasic OJIP curve and its biophysical parameters. The observed recovery of F_q'/F_m' following dark regime indicates that *A. platensis* photosynthetic apparatus was not irreversibly damaged by heat stress at 41 and 46 ˚C. Hence, our study shows that coating flat plate PBRs with commercial solar control infrared blocking films (IRF) is effective in regulating *Arthrospira* culture temperature in PBRs without any negative impact on the photosynthesis of this alga under the test condition.

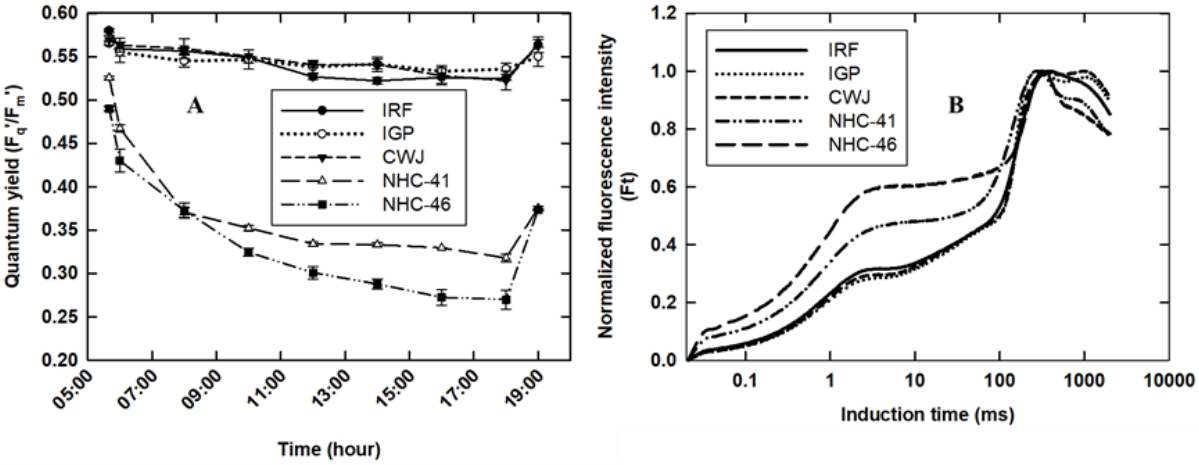

Fig. 2B–4. Effective quantum yield, Fq'/Fm' (A) and double-normalized polyphasic chlorophyll *a* fluorescence rise, OJIP (B) of *A. platensis* cultured in solar control film (IRF), insulated glazed photovoltaic (IGP), conventional water jacket (CWJ), and no heat control (NHCs) PBRs. The OJIP transient is on a logarithmic time-scale ranging between 0.05 ms and 10000 ms. Each curve is a mean of four replicates of the means at semi-continuous phases. Bars with the same letter across group indicates no significant differences (One-Way Repeated Measures ANOVA, P > 0.05).

Excitingly, the results of the PSII photochemical efficiency and phenomenological/biophysical parameters of the polyphasic OJIP transients of *A. platensis* in the IRF, IGP and CWJ corroborated each other, and provide information on the remarkable biomass productivity and phycocyanin content achieved under these PBRs conditions. To

this end, applying IRF and IGP technologies to enclosed PBRs (e.g. flat plates) offers the potential for significant savings on the freshwater and energy consumption associated with PBR cooling. Based on the mechanistic model of Béchet et al. [56] 2,400 $m^3.ha^{-1}.yr^{-1}$ of high quality freshwater is required for evaporative cooling of *A. platensis* culture operating at a temperature of 35 ˚C. Use of IRF or IGP technology could eliminate this cost entirely. There would also be additional cost savings as the energy and infrastructure required for cooling the PBRs would no longer be needed.

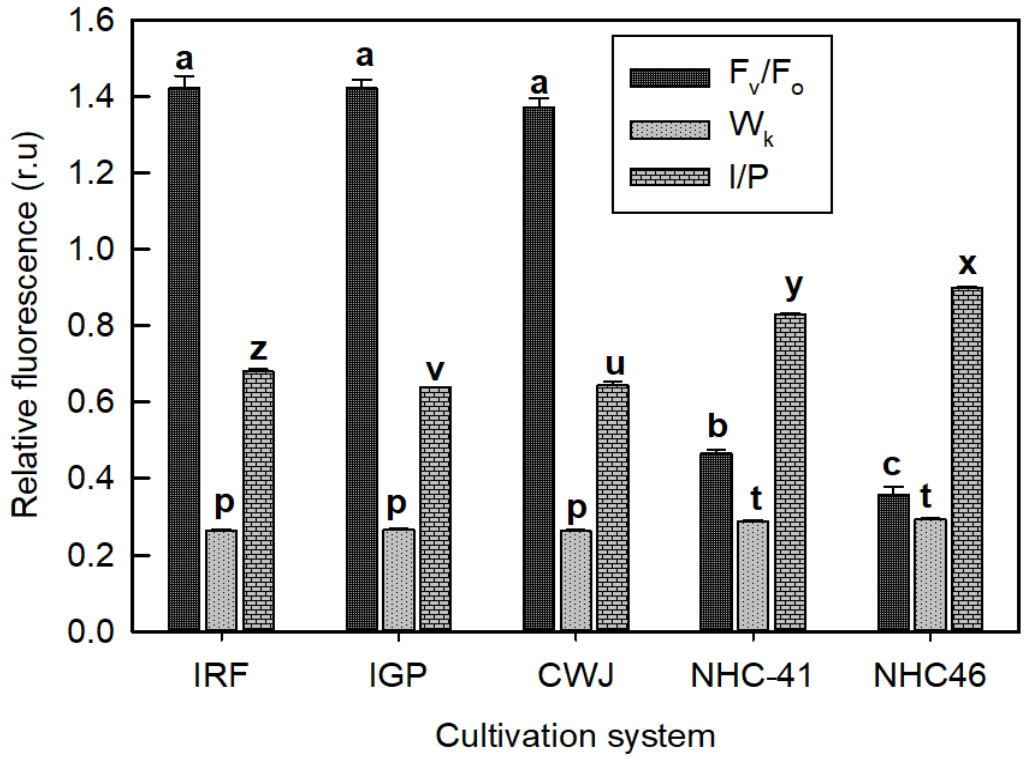

Fig. 2B–5. Phenomenological parameters derived from OJIP transient curves of *A. platensis* cultivated in infrared blocking film (IRF), insulated glazed photovoltaic (IGP), conventional water jacket (CWJ), and no heat control (NHCs) PBRs. F_V/F_O, relative activity of the water-splitting complex on the donor side of PSII; I/P, ratio of the I to P steps of the OJIP test; W_K, heat stress parameter. Bars with the same letter across the group indicate no significant differences (One-Way Repeated Measures ANOVA, P > 0.05).

2B.5. Conclusions

Experimental results have demonstrated that the application of solar control infrared blocking film to an algal flat plate PBR (IRF) growing *A. platensis* reduces the maximum temperature attained in the culture by 33%, compared to PBRs with no heat control (NHC). Specifically, the effect of this temperature reduction results in 52% more biomass and 64% more C-phycocyanin in the IRF versus NHC. Biomass and phycocyanin productivity of IRF culture are similar to the conventional water-jacket (CWJ) and insulated-glazed photovoltaic (IGP) PBRs. Results from chlorophyll *a* fluorescence measurements show that *A. platensis* cultures in the IRF, IGP and CWJ are not thermally stressed, however, NHCs cultures are heat stressed. Therefore, utilizing solar control film in algal PBRs growing *A. platensis* for C-PC production would be a smart strategy for cooling with apparent environmental benefits as suitable locations for outdoor microalgal production usually have freshwater limitation.

2B.6 References

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2B.7 Supplementary information

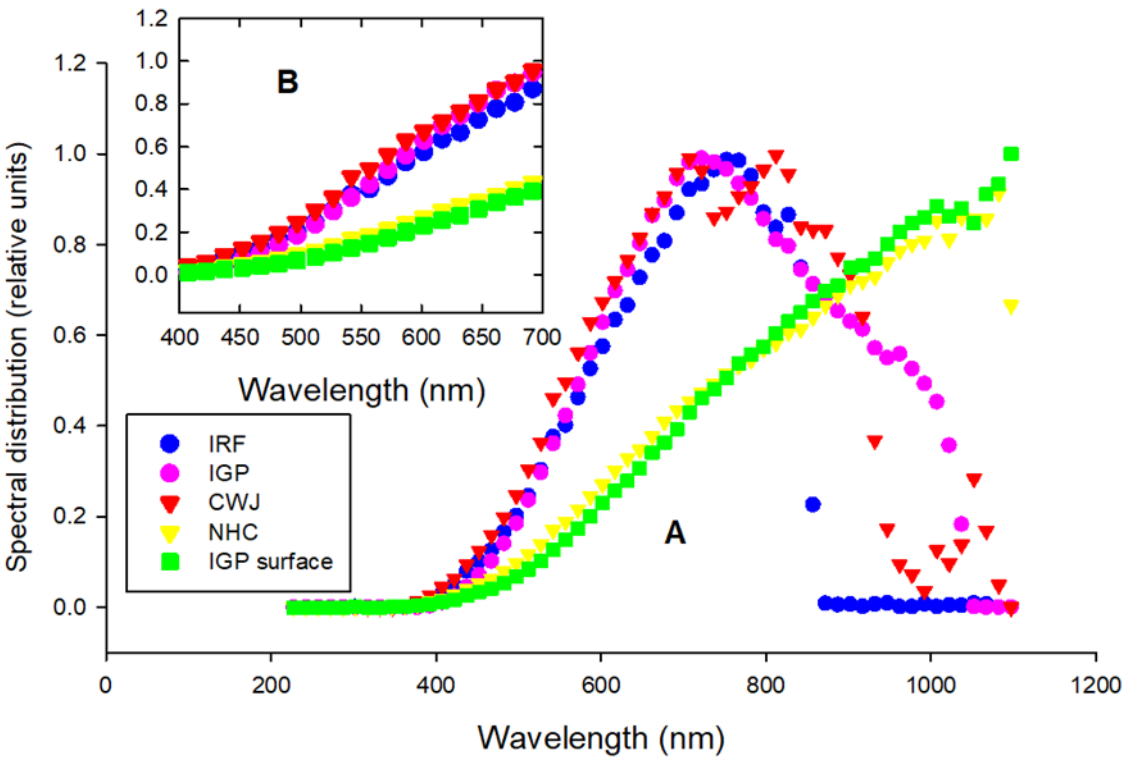

Fig. 2B–S1. Normalized relative spectra of transmitted irradiance inside of the empty infrared film (IRF), insulated glazed photovoltaic (IGP), conventional water jacket (CWJ), and no heat control (NHC) flat plate photobioreactors used for the growth of *Arthrospira platensis*. (A) full and (B) PAR (400-700 nm) spectra of the modified irradiance.

Link to the next chapter

Photobioreactors based on insulated glazed panels and low-emissivity films were developed for microalgae culture and their ability to support the growth of *Nannochloropsis* sp. and *A. platensis* were validated under laboratory conditions (Chapter 2). To truly harness the potential of microalgal cultivation at scale using the photobioreactors, it needs to be undertaken under outdoor conditions utilising freely available solar energy. Thus, the results from the indoor experiments described in Chapter 2 were used to design, develop and trial an outdoor version of the photobioreactors with similar spectrally-selective coatings and photovoltaic integration for electricity generation. In this chapter, the spectral characteristics, thermal behavior and biological performance of a spectrally-selective insulated-glazed flat panel photovoltaic photobioreactor co-producing microalgal (*Nannochloropsis* sp.) biomass and electricity while eliminating the need of cooling water, under outdoor condition was studied. This novel photobioreactor was compared with (a) a classical open raceway pond and (b) two other flat plate reactor designs, one coated with low-emissivity film and the other using a passive evaporative cooling system.

Chapter 3

Outdoor pilot-scale production of *Nannochloropsis* sp. biomass in self-cooling standalone photovoltaic photobioreactors

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Pilot-scale self-cooling microalgal closed photobioreactor for biomass production and electricity generation

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ABSTRACT

Excessive cooling and energy requirements limit microalgal culture in closed photobioreactors. Here, the thermal behavior and biological performance of a spectrally-selective insulated-glazed photovoltaic (IGP) flat panel photobioreactor capable of co-producing microalgal biomass and electricity, while eliminating the need of cooling water was evaluated. The viability of this novel system for culturing *Nannochloropsis* sp. was compared to flat panel photobioreactors based on passive evaporative cooling (PEC), infrared reflecting thin-film coating (IRF), and open raceway pond. Maximum temperature (33.8 ± 2.9 °C) was highest in the IRF reactor while no significant difference was seen between IGP and PEC photobioreactors. Specific growth rate and biomass productivity of *Nannochloropsis* sp. was similar in all closed photobioreactors; however, raceway pond showed significantly lower productivity. Algal cultures in these cultivation systems were not thermally stressed. Electricity generated from IGP photobioreactor was 2.5-fold higher than the mixing energy requirement. Experimental results demonstrate a stand-alone IGP photobioreactor co-producing algal biomass and electricity, requiring no cooling water and grid electricity for operation.

1. Introduction

Continued human population growth and CO_2-related changes in climate have increased the interest in developing renewable and sustainable sources of food, energy, and valuable bio-based chemicals. Microalgae are a promising technology in this regard as they have evolved to tap into the enormous solar energy resource available to mankind and convert the same into sustainable and environment-friendly renewable energy [1], high-value products [2], and effective CO_2 bioremediation [3]. Large amounts of microalgal biomass must be produced to make the process economically feasible and this is an essential requirement for meeting large-scale demand.

Microalgae can be grown in open ponds or closed photobioreactors. The bulk of microalgal biomass produced worldwide is currently cultivated in open ponds [4,5]. However, only handful of species can grow in open ponds [6], limiting exploitable bio-products from microalgae. Closed photobioreactors (PBRs) can represent an attractive approach when compared to open ponds due to their significantly higher biomass productivity and lower culture contamination [7]. The closed system allows a greater range of microalgal species to be successfully cultured, expanding the potential products that can be harvested [6]. Despite the development of a variety of PBRs, commercial exploitation of these prototypes for the production of bioproducts is restricted by the product value (e.g., the cost of fossil fuels is many orders of magnitude lower than microalgal biofuel), operational factors, a negative energy balance, and the design feasibility of such PBRs [7,8]. For these reasons, nearly 90% of existing global microalgal biomass production comes from open ponds [9,10]. Solar microalgal cultivation is deemed to be one of the most feasible pathways for the production of valuable bio-based products, including biofuels, due to the abundance of solar energy resources [2,11]. Consequently, the quantity and quality of light intercepted by an algal culture are the most influential factors on productivity and overall PBR efficiency [12,13]. In this context, closed photobioreactors with large illuminated surface area to volume ratios (S/V) have been designed and developed to improve algal productivity rates [14,15]. This large S/V, coupled with the closed geometry of

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Author contribution

Contributor	Statement of contribution
Emeka G. Nwoba (70%)	Design of the study, acquisition of data, analysis and interpretation of data, and drafting the article.
David A. Parlevliet	Conception and design of the paper as well as critical review of the article for important intellectual content.
Damian W. Laird	Concept and design of the experiment, analysis and interpretation of the data, critical revision of the article for important intellectual content, and final approval of the article.
Kamal Alameh	Conception and design of the paper, provision of study material as well as critical revision of the article for important intellectual content.
Navid R. Moheimani	Conception and design of the study, provision of study material as well as critical revision of the article for important intellectual content.

Principal supervisor confirmation

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

Name	Signature	Date
David A. Parlevliet		**8/12/2020**

3.1 Abstract

Excessive cooling and energy requirements limit microalgal culture in closed photobioreactors. Here, the thermal behavior and biological performance of a spectrally-selective insulated-glazed photovoltaic (IGP) flat panel photobioreactor capable of co-producing microalgal biomass and electricity, while eliminating the need of cooling water was evaluated. The viability of this novel system for culturing *Nannochloropsis* sp. was compared to flat panel photobioreactors based on passive evaporative cooling (PEC), infrared reflecting thin-film coating (IRF), and open raceway pond. Maximum temperature (33.8 ± 2.9 ˚C) was highest in the IRF reactor while no significant difference was seen between IGP and PEC photobioreactors. Specific growth rate and biomass productivity of *Nannochloropsis* sp. was similar in all closed photobioreactors; however, raceway pond showed significantly lower productivity. Algal cultures in these cultivation systems were not thermally stressed. Electricity generated from IGP photobioreactor was 2.5-fold higher than the mixing energy requirement. Experimental results demonstrate a stand-alone IGP photobioreactor co-producing algal biomass and electricity, requiring no cooling water and grid electricity for operation.

3.2 Introduction

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biomass must be produced to make the process economically feasible and this is an essential requirement for meeting large-scale demand.

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likely to be exploited commercially), are temperature-sensitive, with narrow optimum values ranging between 20 and 25 ˚C [6]. In some locations, supra-optimal temperatures, up to 55 ˚C, are easily attained at noon on sunny, clear sky days under outdoor conditions [16]. Such high temperatures can result in the complete deterioration of the culture, making the need for cooling systems in PBRs vital to maintain viability and bioproductivity.

Several solutions are available for cooling photobioreactors, such as the passive-evaporative cooling system, direct immersion in thermo-regulated pools, use of heat exchangers, dark sheet shading of photobioreactors, and placement in greenhouses [6]. However, these methods are severely limited by sustainability concerns due to high capital and operating costs, and also result in an undesirable impact on the environmental footprint due to very high energy and water demand. The compelling need for cooling photobioreactors could also lead to a negative energy balance, invalidating the high productivity integrity of this system.

Therefore, there is a need for cost-effective and low-energy-demand solutions for the thermal regulation of solar illuminated closed photobioreactors. Infrared filtration of solar radiation has been suggested as an effective thermal solution by a number of authors [6, 9, 17]. Sunlight is composed of a wide range of different wavelengths but only the range between 0.4 – 0.7 μm is suitable photosynthetically-active radiation (PAR) that can be absorbed for microalgal photosynthesis. Not all of the solar spectrum reaches a culture in a closed photobioreactor. The ultraviolet (UV, < 0.4 μm) portion (~5 %, AM1.5 spectrum) of the sunlight at the surface of the earth is reflected by glass optical surfaces [17]. Of the incident light, approximately 55 % of the total solar energy available at ground level is within the infrared spectrum (IR, > 0.7 μm) and directly contributes to the overheating of cultures in photobioreactors. Overall, it is estimated that about 90 % of the total energy available from

the solar spectrum is transformed to heat by the culture based on 5 % photosynthetic efficiency [9]. Thus, ensuring that wavelengths > 0.7 μm do not enter the interior of the photobioreactor, dramatically reduces the heat gain of the culture. An efficient mechanism to remove IR wavelengths from incoming radiation and thus, reduce culture temperatures on clear sunny days is to employ spectrally-selective glass that reflects or absorbs near-infrared wavelengths while transmitting visible photons when constructing the reactor. Using such a material restricts transmission of solar infrared radiation into the culture. Photobioreactors with heat-reflecting surfaces are particularly attractive as they will result in reducing the use of extraneous freshwater cooling systems for thermal regulation. This concept has been recently demonstrated for the successful cultivation of different microalgae species under controlled laboratory conditions by incorporating insulated glazed panels and solar control thin films as temperature control mechanisms [18]. Building on these studies, we have developed a pilot-scale fully functional energy-harvesting insulated glazed solar photobioreactor for microalgae farming under outdoor conditions.

In this study, we provide detailed information on the design of a pilot-scale insulated glazed photovoltaic (IGP) photobioreactor for the continuous culture of *Nannochloropsis* sp. and its performance during the austral spring season. *Nannochloropsis* sp. is a favored candidate for large-scale production of biofuel, aquaculture feed, and valuable biochemicals (e.g., ω-3 fatty acids) because of its fast growth, tolerance to biotic pollution and high energy conversion efficiency [19, 20]. The thermal behavior and biological performance of IGP photobioreactor were compared against the industry-norm open raceway pond, a solar control thin film-coated flat plate PBR, and a flat panel PBR utilizing a passive-evaporative cooling mechanism.

3.3 Materials and methods

3.3.1 Microalgae and culture medium

The marine Eustigmatophyte, *Nannochloropsis* sp. MUR 267, isolated from Swan-Canning Estuary, Western Australia [21], was obtained from Murdoch University Algal Culture Collection, Australia. *Nannochloropsis* sp. was propagated using unsterilized (but filtered, 50 µm) natural seawater (Hillary's Beach, Western Australia) enriched with sterilized F/2-Si nutrients [22]. The growth medium was maintained at the ambient salinity of the seawater, (33‰ NaCl). The *Nannochloropsis* sp. used for this study was obtained from a non-axenic unialgal culture maintained in the late logarithmic growth phase in a 2 m^2 outdoor raceway pond for ≥ 12 months. The experiment was carried out during the austral spring (October to November).

3.3.2 Experimental set-up

Flat-plate geometry is a widely used photobioreactor configuration for microalgae cultivation, due to high S/V, efficient light delivery to the culture, and scalability. The plate PBRs used in this study have an optical path length of 10 cm (internal depth).

3.3.2.1 Evaporatively cooled and infrared reflecting thin-film-coated PBRs

The passive-evaporative cooled, PEC, and solar-control infrared reflecting thin-film coated, IRF, photobioreactors had their base and sides constructed of 19 mm thick clear float glass. The illuminated and back surfaces were built with 12 mm thick glass in clear acidic cure silicone. These PBRs have the dimensions, 126 cm x 125 cm (length x height), with a front-illuminated surface area of 16,000 cm^2 and a maximum filling capacity of 160,000 cm^3.

The internal temperature of the PEC PBR was regulated by a passive-evaporative cooling mechanism involving freshwater sprayed onto the illuminated surface [3]. The

cooling system was automated (temperature set point of 27 ˚C) and comprised of a freshwater reservoir tank (350 L) containing a submersible pump that delivered water through PVC pipes across a solenoid valve to a sprinkler system fitted on the surface of the reactor. A gutter system was provided at the bottom of the PBR for the efficient collection of the cooling water in a lossless scenario back to the reservoir for reuse (only losses encountered was from evaporation from the photobioreactor and reservoir surfaces). Recycling of cooling water results in a significant decrease in the amount of freshwater needed for cooling (water loss of 20 L vs. 5,400 L m^{-2} d^{-1} for recycling vs. non-recycling, respectively). The use of seawater for cooling purpose was not considered because the crystallization of salt on the PBR surface makes cleaning difficult and laborious in addition to reducing the penetration of sunlight. The reservoir (working volume of 300 L) was shaded to reduce warming by direct sunlight. The need for a chiller to regulate the culture temperature was not considered as it would increase system complications, cost and energy demands.

For the IRF PBR, the temperature was managed by lamination of a solar-control (low-emissivity) thin-film on the illuminated surface including the sides and not the back surface. The transmission characteristics of the low-e film used for the experiment is shown in Supplementary Information Fig. 3–S1.

3.3.2.2 Geometry of the IGP photobioreactor

The third customized plate photobioreactor was constructed of insulated glass units (IGUs) and coupled to an energy-generating photovoltaic (PV) panel (Fig. 3–1). The IGP PBR comprised 5 mm thick IGUs, each having two glass panes sealed together with an airspace between them (distance between the inner and outer panes is 2.6 cm) to ensure high thermal insulation properties. The front 120 cm x 150 cm (length x height) IGU consists of a low-emissivity (low-e) thin film deposited on the outer surface. The low-e film was spectrally-

selective, allowing > 75 % of visible light to pass through while reflecting > 90 % of the ultraviolet (UV) and infrared (IR) spectral components. The low-e film reduces heat loss in winter by reflecting heat back into the reactor and reduces the heat gain during summer via spectral selection/reduction. The IGUs that comprise the back and bottom of the PBR did not contain the low-e film but those on the sides do. A 120 cm x 60 cm (length x height) semi-transparent solar glass (CdTe PV panel, 40 % transmission) was externally attached (glued) to the upper part of the front glass, allowing 40 % of the sunlight through to the interior, while converting the rest to electricity, which is typically stored in a battery and used for powering electrical systems (e.g., air pump for mixing, light-emitting diodes, LED lights). The PV panel was placed 90 cm above the base of the reactor. All the plate reactors were inclined at a tilt angle of 32˚, with a north-south orientation of all cultivation systems to maximize light capture [6].

3.3.2.3 Open Raceway pond (ORP)

The fourth cultivation system is a 10,000 cm^2 (200 cm x 50 cm) north-south oriented paddlewheel-driven open raceway pond (fiber-glass) operated at a mixing speed of 0.22 m s^{-1} [23] and effective culture volume of 200 L.

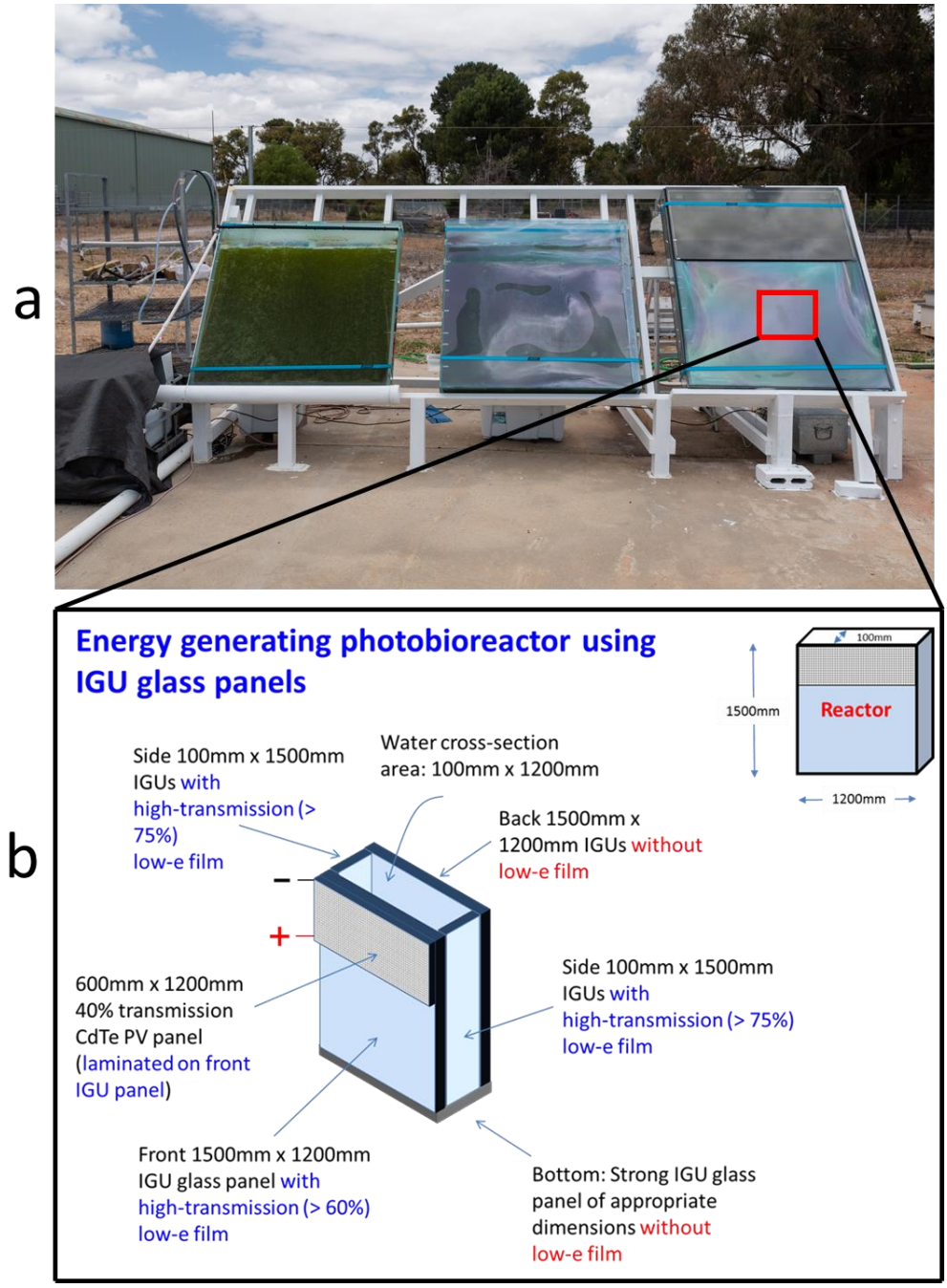

Fig. 3–1. Microalgae cultivation flat plate PBRs. (a) Image of the PBRs in operation at the Algae R&D Centre, Murdoch University, Australia. Left to right: passive evaporative cooling (PEC), infrared reflecting film (IRF) and insulated-glazed photovoltaic (IGP) photobioreactors. (b) Schematic showing construction details of the IGP photobioreactor. IGU means insulated-glazed unit.

3.3.3 Cultivation of microalgae and analytical measurements.

All photobioreactors were subjected to identical operational parameters with a working volume of 140 L. The culture volume of the raceway pond was maintained at the optimum operational depth of 20 cm (200 L) [23, 24] to ensure efficient mixing by the paddlewheels. For a 1 m^2 raceway pond at this operational depth, the flow pattern is normally turbulent with a Reynolds number above 3,200 and light:dark frequency of cells of 1.6 s in the first 1 – 2 m. The remaining section of the pond exhibits a laminar flow configuration with a Reynolds number less than 2,000. Such conditions have previously been shown to maximise biomass productivity [24]. Due to evaporation loss in the pond, daily top-up was required using tap water.

The microalgae suspension was mixed continuously by feeding filter-sterilized ambient air to both ends of 1.20 m long ceramic diffusers installed at the base of each photobioreactor. Airflow was provided by a PondOne O2 Plus 8000 air pump (4,200 L h^{-1}) at an aeration rate of 0.21 vvm (volume of air per volume of culture per min, a more useful expression in microalgal biotechnology) and airflow pressure was regulated with a flowmeter. This aeration rate corresponded to a superficial gas velocity of 0.0039 m s^{-1}, mixing time of 106.3±3.2 s, and gas-hold of 0.017±0.0002 in the photobioreactors under a biphasic system composed of air and tap water [25, 26]. Culture pH was unregulated, and no CO_2 gas was infused into the cultures. The culture temperature profile was logged continuously at five-minute intervals via an underwater temperature logger (Pendant Onset Hobo, USA). The salinity of the microalgae suspension was measured manually using a digital refractometer (Atago Pal-03S). Spectral distribution and analysis of the transmitted solar radiation was measured using a StellarNet spectrometer (Black-Comet CXR-SR-50, USA) with the sensor positioned 5 cm from the inner illuminated surface of each empty

photobioreactor. The spectrometer utilizes dual blazed 40 mm diameter concave grating optics to perform high performance spectral analysis in the UV-VIS-NIR wavelength range covering 200 – 1,100 nm. Spectral measurements were carried out at noon.

Nannochloropsis sp. was cultivated in batch mode for two weeks, after which, cultures were subjected to semi-continuous harvest based on their specific growth rate (maximum growth reached every three days). Specific for the IGP photobioreactor, cells were first grown in the plate reactor with IGU and with no low-e film and thereafter, cultivated in the IGP photobioreactor (contained IGU and low-e film) to assess viability of the system. Cell density was measured using an Improved Neubauer Chamber Haemocytometer [27]. Biomass productivity, specific growth rate, and chlorophyll *a* content of the biomass were determined based on the protocols detailed in Moheimani et al. [27]. During cultivation, microalgae were acclimated to the systems' conditions through measurement of the operating efficiency of photosystem II photochemistry (F_q'/F_m') [18, 19]. *Nannochloropsis* sp. F_q'/F_m' was measured between sunrise and sunset using a chlorophyll *a* fluorescence fluorometer (Portable AquaPen-C, Photon Systems Instruments, Czech Republic). The fluorometer was set at the maximum 3000 µmol photons m^{-2} s^{-1} saturation pulse intensity of red light (620 nm) and measurements were carried out on cultures sourced directly from the cultivation systems [12].

3.3.4 Statistical analysis

All experimental analyses were performed in triplicate. The data obtained were explored and analyzed by a One-Way Repeated Measures Analysis of Variance (RM-ANOVA) following a post-hoc Holm-Sidak multiple comparison test. A significant difference between treatments was declared at $P < 0.05$. The SigmaPlot v13.0 program was used for all statistical

analyses and measurements reported as means ± standard error (SE, except stated otherwise) over the cultivation period (n = 6).

3.4 Results and discussion

3.4.1 Spectral characterization of the photobioreactors

Light intensity and spectral quality are the most important factors affecting microalgal growth and photosynthetic performance [28]. The productivity of any microalgal cultivation technology is directly dependent on the overall radiation intercepted. Hence, integrating spectrally-selective technologies to solar photobioreactors will visibly modify the intensity and quality of the spectrum inside the reactor [29]. For all the photobioreactors evaluated in this study, remarkable differences in the nature of the transmitted light spectrum were observed (Fig. 3–2). The spectrally-selective insulated-glazed photovoltaic flat plate (IGP) and infrared reflecting film-coated flat plate (IRF) photobioreactors both transmitted wavelengths in the PAR-IR range of 400 – 900 nm, while the PEC's light transmission of 340 – 1,100 nm included UV, PAR, and IR wavelengths (Fig. 3–2). For the low-e film itself, the transmitted spectral pattern did not markedly change when compared to the photobioreactors based on infrared reflection (Fig. 3–2a). The semi-transparent PV panel transmitted wavelengths between 400 and 960 nm but at a lower intensity (Fig. 3–2a). However, the spectral characteristics of the IGU was similar to unmodified solar irradiance, but with a decreased intensity (Fig. 3–2). It is therefore important to state that all three thermal control mechanisms transmitted different degrees of heat (infrared radiation) into the respective flat panel photobioreactors. Analysis of the composition of the light transmitted indicated that photons in the wavelength range of 500 – 600 nm is slightly higher (2 – 5 %) than other photons in all the photobioreactors with blue wavelengths trending

higher than red (Fig. 3–2b). In terms of the PAR photons which are crucial for microalgal photosynthetic biomass formation, the light wavelengths trended similarly, 500 – 600 (blue-green) > 400 – 500 (blue) > 600 – 700 (red) nm in all the photobioreactors (Fig. 3–2). Transmission of wavelengths associated with heat transfer with little or no beneficial effect on photosynthesis (700 – 1,100 nm) were minimal in the IGP and IRF photobioreactors but much more significant in the PEC. In fact, wavelengths between 900 – 1,100 nm were completely removed in the former compared to the latter (Fig. 3–2b). It is interesting to note that the wavelength distribution in the IGP and IRF photobioreactors are essentially the same, even though the former has multiple panes in its design (Fig. 3–2b). However, the intensity of light transmitted by the IRF is higher than that transmitted by the IGP (Fig. 3–2a). The dominance of blue-green wavelengths in the light transmitted into the photobioreactors is likely a result of the nature of the solar spectrum. In contrast, our previous studies ([18, 19]) found red photon (600 – 700 nm) as the most dominant in the evaporative cooling, infrared reflecting film, and insulated glazed photovoltaic photobioreactors under laboratory-controlled conditions using a 500 W halogen illumination source. These differences are brought forward by the different light sources (sunlight vs. artificial halogen), which can affect the response of photosynthetic organisms in these systems and require carefulness when extrapolating indoor results to real outdoor cases. Analysis of the amount of energy available in each reactor shows that the spectral power (W m^{-2}) of the transmitted photons (Table 3–1) correlated with the photon quality (Fig. 3–2). Hence, the total spectral energy available in the cultivation reactors trended IRF > PEC > IGP, with the IRF showing a 68 % higher energy transmission than the IGP (Table 3–1, Fig. 3–2a). The ORP was exposed to full sunlight (Table 3–1) and the magnitude of the solar radiation on the culture surface was over-saturating due to a small area of concentration. This level of

sunlight increases the evaporation rate in the open raceway pond resulting in loss of water and need for evaporation make up. Nevertheless, high evaporation results in self-cooling of cultures in open ponds.

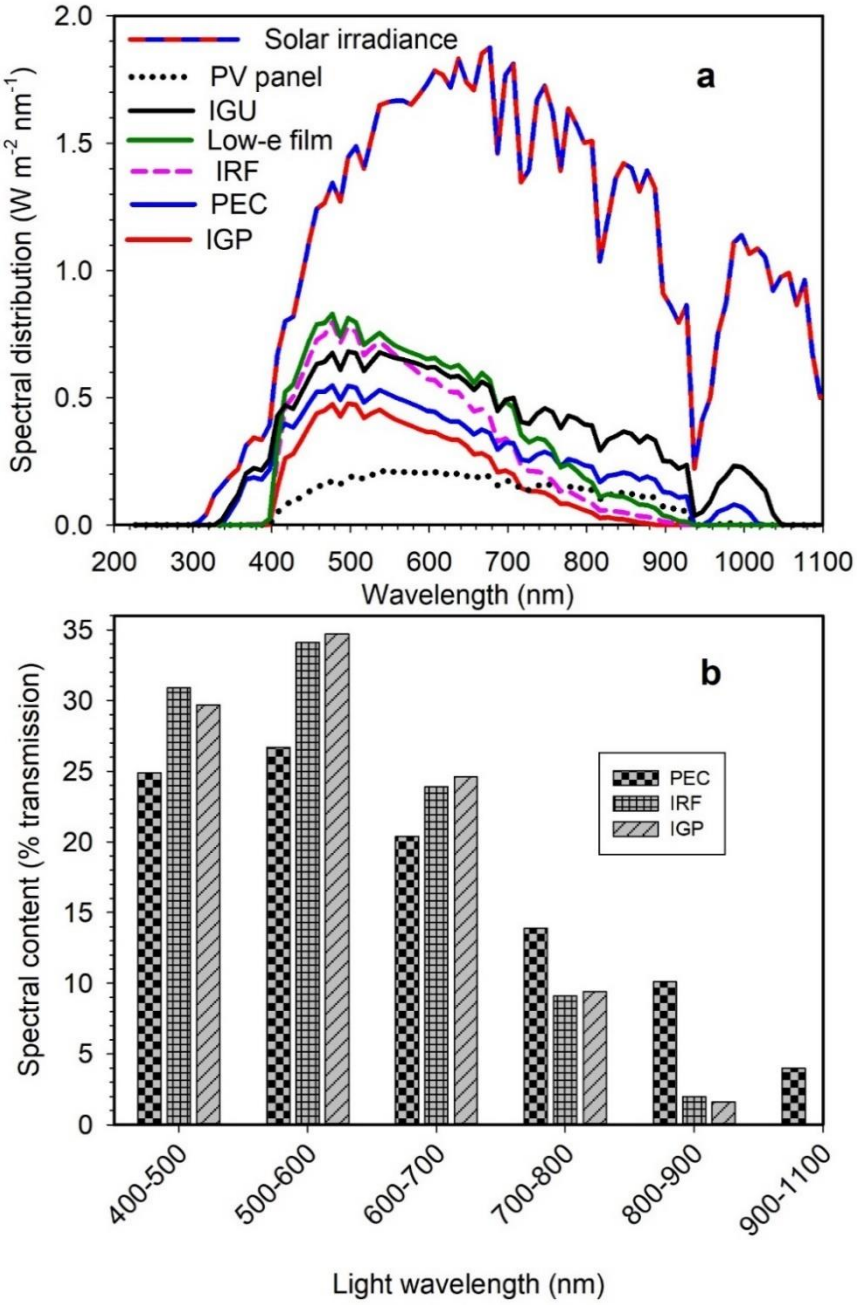

Fig. 3–2. Spectral distribution (a) and composition (b) of irradiance transmitted in the flat plate PBRs. IRF, infrared reflecting film; PEC, passive evaporative cooling, and IGP, insulated-glazed photovoltaic (PV) photobioreactors. IGU (insulated-glazed unit) has no low-e film on the illumination surface.

3.4.2 Temperature profiles of the cultivation systems

For the comparison of the thermal behavior of the photobioreactors, the change in temperature (ΔT) was calculated based on the difference of the culture temperature (T_c) and ambient temperature (T_a) at a given time (i.e., $\Delta T = T_c - T_a$) (Fig. 3–3). Maximal temperature variation was found in the IRF photobioreactor, while a more uniform (less change over time) temperature profile was observed in the ORP (Supplementary information Fig. 3–S2). Variation from ambient temperature is most pronounced in the photobioreactors ($-1<\Delta T<16$) and minimal for the raceway pond varied narrowly ($-4<\Delta T<4$). Highest minimum temperature was maintained in the IGP photobioreactor, while the lowest minimum temperature was found in the ORP (Fig. 3–3a). Maximum temperature attained in the cultivation systems were, IRF > PEC = IGP > ORP (One-way Repeated Measures, ANOVA, $P < 0.05$) (Fig. 3–3b). The diurnal pattern of temperature variation shows that warming of air at sunrise does not typically result in an immediate increase in the culture temperature (Fig. 3–3c). In fact, the air temperature is either similar to or slightly higher than the culture temperature up to mid-morning (≤ 09:00 am). A closer look at Fig. 3–3b shows that IGP photobioreactor has both a lower heat dissipating tendency (retains more heat energy) and slower temperature increase when compared to the other two photobioreactors. In terms of daily average temperature in each reactor, maximum temperature (25.0±4.3 ˚C, ±SD) in the IGP photobioreactor was significantly ($P < 0.05$) higher than that attained with IRF, PEC and in ORP, by 5.8, 14.4, and 26.4 %, respectively (Table 3–1). Relating these results (i.e., average temperatures) to the spectral power of photons transmitted in each photobioreactors, a non-significantly positive correlation (Pearson Product Moment) were found; IRF ($r = 0.91$, $P = 0.09$), IGP ($r = 0.92$, $P = 0.08$) and PEC ($r = 0.28$, $P = 0.72$). The lower spectral power of the IGP photobioreactor should typically result in a lower average temperature. However, its average

minimum temperature was significantly higher (One-Way ANOVA, $P < 0.05$) than those of the other reactors, while the average maximum temperatures trended as IRF > PEC = IGP > ORP (One-Way ANOVA, $P < 0.05$; Table 3–1). The higher average temperature vs. lower total spectral power is due to the reduction of heat loss at night (resulting in a higher minimum temperature) when compared to the trend of these parameters with the IRF. The thermal retention (minimizes heat loss) at night and the gradual increase in temperature (minimizes heat gain) in the day for the IGP design are attributed to the double glazing which was coupled together with the IR reflecting film provided in the reactor. This innovative feature results in the internal culture temperatures that are more favorable for growth for a longer period of time and hence, could solve the problem of large diurnal temperature fluctuations typical of conventional photobioreactors. These essential characteristics of the IGP prototype are advantageous given the deleterious impact of high or low temperature on the photosynthetic performance of microalgae. Average temperatures of the photobioreactors over the cultivation period were equal or lower than the optima, 24 - 27 ˚C [6], for culturing *Nannochloropsis* sp. Unlike conventional photobioreactors (e.g., flat panel, Biocoil) that usually experience a rapid temperature increase during the day and equally rapid decline at night [23, 24], the ability of the spectrally-selective cultivation systems to keep the culture temperature at or near the optimal for the cultivation of photosynthetic cells shows great promise for microalgal production in outdoor solar conditions.

As highlighted in Section 3.2, an efficient strategy to reduce the temperature increase in PBRs in the summer period could be through the use of a special filter glass (with high selective transmission of only PAR wavelengths) to decrease the quantity of infrared radiation transmitted to culture mass. Note that the quantity of solar radiation received by the culture can be decreased by increasing the glass absorbance; however, this is inefficient

(Goetz et al 2011). Hence, the majority (about 90 %) of the total solar energy absorbed by the glass surface is transmitted to the culture [9], and this could promote photobioreactor overheating.

Tropical and warm temperate countries are favored for the mass production of microalgae due to higher overall solar radiation and suitable weather conditions almost all year round [30]. However, these regions are prone to elevated temperature, which is a critical challenge for outdoor microalgae culture in closed photobioreactors. Based on the 55 ˚C maximum temperature recorded in the control photobioreactor with no cooling system, a flat panel photobioreactor inclined at 32˚ and located at Murdoch in Western Australia needs to evacuate 17.6 MJ of heat energy m^{-2} of reactor d^{-1} to keep the culture temperature at 25 ˚C. Maintaining this culture temperature using an evaporative cooling system with no recycling of the freshwater would require 5,200 ± 180 L m^{-2} of reactor d^{-1} of high-quality water. This figure is based on calculations from data obtained during the experiment on sunny, cloudy and windy days (n = 6). If freshwater could be recycled, evaporative loss still reaches as much as 20 ± 4 L m^{-2} d^{-1}. A previous report shows that a column photobioreactor sited at Merced, California would require the removal of 18,000 GJ ha^{-1} yr^{-1} of heat energy to keep the culture at 25 ˚C given a density of one photobioreactor per m^2 [31]. This situation would require 8,000 m^3 ha^{-1} yr^{-1} of quality freshwater for evaporative cooling of the photobioreactor for the period of operation. For photobioreactors located at Murdoch University, Murdoch, Western Australia (32.067˚ S, 115.84˚ E), thermal cooling is achieved by passive evaporative cooling throughout the year, while heating is provided at night using aquarium heaters during winter period only. However, our study demonstrates that by embedding IR reflective systems on the illumination surfaces of flat plate photobioreactors

(as per the IGP design) a substantial amount of heat gain could be eliminated reducing the need for additional cooling.

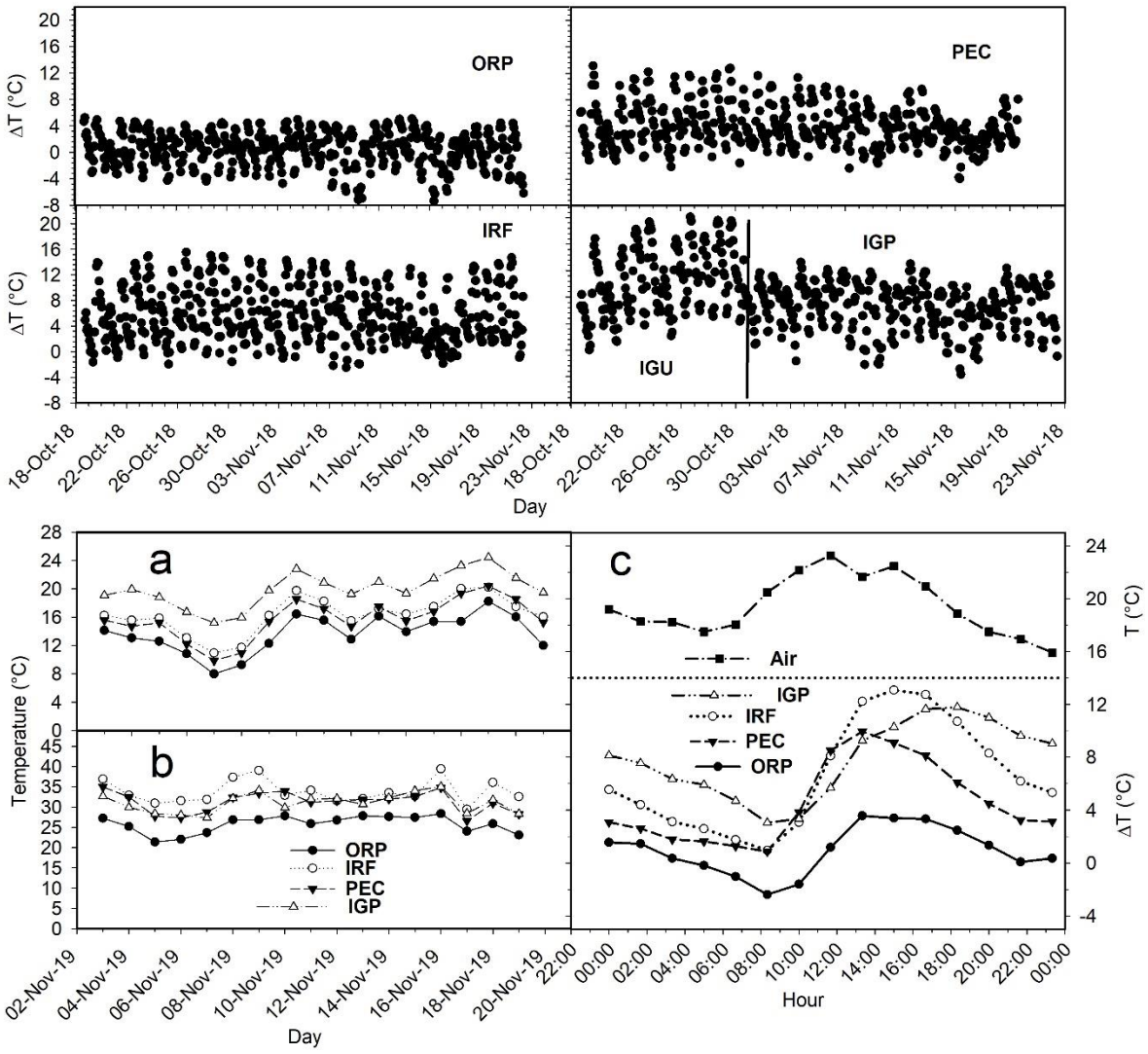

Fig. 3–3. Variation of culture temperature compared to air temperature (top and middle panels), minimum (a), maximum (b) and diurnal (c) culture temperatures over air temperature (ΔT, culture temperature – air temperature) during the cultivation of *Nannochloropsis* sp. MUR 267 in raceway pond (ORP), passive evaporative cooling (PEC), infrared reflecting film (IRF), and insulated glazed photovoltaic (IGP) photobioreactors on 05 Nov. 2019. IGU means an insulated glass unit photobioreactor without a low-e film on the illumination surface.

Table 3–1. Average temperature and spectral power of transmitted wavelengths in the cultivation systems during the growth of *Nannochloropsis* sp. in the austral spring season.

Parameter	Unit	ORP	IRF	PEC	IGP
Heat control technology	**None**	**None**	**Thin film**	**Evaporative cooling**	**Filter glass unit**
$T_{minimum}$	˚C	13.7±2.7^{c}	16.4±2.6^{b}	15.7±2.8^{bc}	20.0±2.5^{a}
$T_{maximum}$	˚C	25.8±2.2^{c}	33.8±2.9^{a}	31.2±2.6^{b}	31.0±2.4^{b}
$T_{average}$	˚C	18.4±4.7^{d}	23.6±6.6^{b}	21.9±5.9^{c}	25.0±4.3^{a}
Wavelength			**Spectral power (W m^{-2})**		
400-500	nm	106.6	62.2	47.9	35.9
500-600	nm	159.9	68.7	51.3	42.1
600-700	nm	175.0	48.0	39.3	29.8
700-800	nm	151.9	18.3	26.7	11.5
800-900	nm	128.8	4.0	19.5	1.9
900-1100	nm	164.3	0.0	7.6	0.0
Total power	**W m^{-2}**	**886.5**	**201.2**	**192.3**	**121.2**

ORP, open raceway pond; IRF, infrared reflecting film; PEC, passive evaporative and IGP, insulated glazed photovoltaic. * Values with the same letter along the rows are not significantly different (P >0.05). Error values for daily temperature indicate standard errors, n = 30.

3.4.3 Comparison of microalgae growth (cell density, specific growth rate and biomass productivity) in the cultivation systems

To investigate the suitability of the IGP photobioreactor for mass production of microalgae under outdoor real-life conditions, experiments were conducted during the

austral spring at Perth, Western Australia (-32° 03' 59.47" S 115° 50' 6.29" E, solar radiation ranging between 200 and 1,300 W m^{-2}, Supplementary information Fig. 3–3). When *Nannochloropsis* sp. was cultivated in the insulated glass unit photobioreactor without low-e film on the illumination surface (IGU), the viability of the culture could not be maintained as seen by the rapid decrease in cell density beginning on 25 October (Fig. 3–4). It was found that the maximum temperature of the reactor at this period was 43 ˚C (Supplementary information Fig. 3–2). At this point, a low-e film was embedded (made an integral component of the system) on the illumination surface of the IGP photobioreactor, and the culture temperature was substantially reduced and *Nannochloropsis* sp. culture remained viable (Fig. 3–4, 29 October onward). Overall, higher cell density was observed in the IGP photobioreactor compared to the other cultivation systems, demonstrating that the growth of *Nannochloropsis* sp. in the reactor was viable. No statistically significant difference (One-Way RM ANOVA, $P > 0.05$) in specific growth rate (μ) and biomass productivities was found between any of the closed photobioreactors (Fig. 3–4a, b). However, the ORP recorded significantly lower μ and biomass productivities when compared to the closed photobioreactors tested here (One-Way RM ANOVA, $P < 0.05$). Productivity of microalgae in a culture vessel can be assessed based on surface area or culture volume. Specifically, the areal productivity of *Nannochloropsis* sp. in the IGP photobioreactor was 14 %, 14 % and 46 % higher than that recorded in those with PEC, IRF and in ORP, respectively. The higher areal biomass productivity achieved in the IGP photobioreactor compared to others is likely due to a combination of effects, e.g., sub-saturating PAR photon flux density (Fig. 3–2a) and a more favorable temperature (25 ˚C) provided in it. In flat panel photobioreactors, higher biomass productivity is achievable because the large surface areas tend to dilute the light impinging on the illumination surface. In other words, the sunlight falling on a given ground

area is spread over a larger surface area of the reactor (unlike the raceway pond with a small areal concentration) and for the spectrally-selective surfaces, the solar intensity is decreased substantially. Hence, microalgal cells are exposed to lower (sub-saturating) light intensities, maximizing their photosynthetic efficiency. The resultant effect of this light dilution is a lower tendency of microalgae to dissipate the absorbed photons into heat energy [32], increasing photosynthetic rate and biomass productivity. Volumetric productivities obtained in this study (0.12 – 0.14 g L^{-1} d^{-1} for closed photobioreactors, Fig. 3–4c) was lower than values (0.16 – 1.7 g L^{-1} d^{-1}) reported for *Nannochloropsis* in flat plate photobioreactors by others (Table 3–2). The lower volumetric productivity could be due to a larger optical pathlength, 10 cm vs. 1.3 – 5 cm used in similar studies (Table 3–2). This large pathlength could lead to light deficient zones in the photobioreactor, lowering the volumetric productivity. Culture depth is of crucial importance in the design of photobioreactors because of its inverse relationship with photobioreactor's productivity. For this reason, closed photobioreactors usually have depths ≤ 10 cm with ultra-thin photobioreactors having the highest volumetric and areal productivities [6]. However, in current case of using reflective IR glass surfaces, culture depth < 10 cm formed part of the exclusion criteria due to a highly favorable tendency of the photobioreactor heating up faster brought forward by small inertia. Furthermore, flat panel photobioreactors with depths ≥ 10 cm are ideal for large-scale production of microalgal bioproducts [6, 33]. Unlike the present study, CO_2 addition also contributed to the higher volumetric productivities achieved by these researchers as previous studies showed up to 80 % less biomass productivity when algae are grown with no CO_2 addition [34]. The areal productivity (15 – 18 g m^{-2} d^{-1}) achieved in the current study are similar to the values reported in the literature for *Nannochloropsis* sp. (Table 3–2). The tilted position of the flat panel

photobioreactors (Fig. 3–1a) ensured maximum exposure to photon flux density, which contributed to the reasonable footprint productivity.

The raceway pond has a longer optical path length (20 cm) relative to the illuminated surface area of the PBRs (10 cm) and this leads to cells spending more time in the dark due to inefficient mixing regime. The consequences are significantly lower biomass productivity. Under a long light-dark condition, respiration outpaces photosynthesis, culture growth and net productivity are dwarfed due to a large dark zone [35]. In the dark zone, microalgae respire energy that otherwise could be used for growth. The presence of a dark zone in a cultivation system will reduce the net productivity of the culture, as part of the culture in the dark has negative growth. Furthermore, lower culture temperature was recorded in the raceway pond creating suboptimal photosynthetic conditions for a significant portion of the day during the cultivation period. A similar finding has been reported for *Nannochloropsis* sp. CCAP 211/78 cultivated in flat panel photobioreactors (optical pathlength of 2 cm) and raceway pond (optical path of 20 cm [32]). Cultivation of *Nannochloropsis* sp. in raceway pond (especially for biofuel application) is currently considered a low-cost option when compared to photobioreactors, however, this approach requires a large landmass and the cultures are susceptible to contamination by grazers and other microalgae. Further, raceway pond is impractical for year-round sustainable productivity in many places in the world due to unstable weather conditions (e.g., high rain in winter). Closed flat panel photobioreactors are known to overcome this challenge of open ponds microalgal production processes, and the developed insulated glazed photovoltaic photobioreactor possesses compelling attributes critical to advancing microalgae technology.

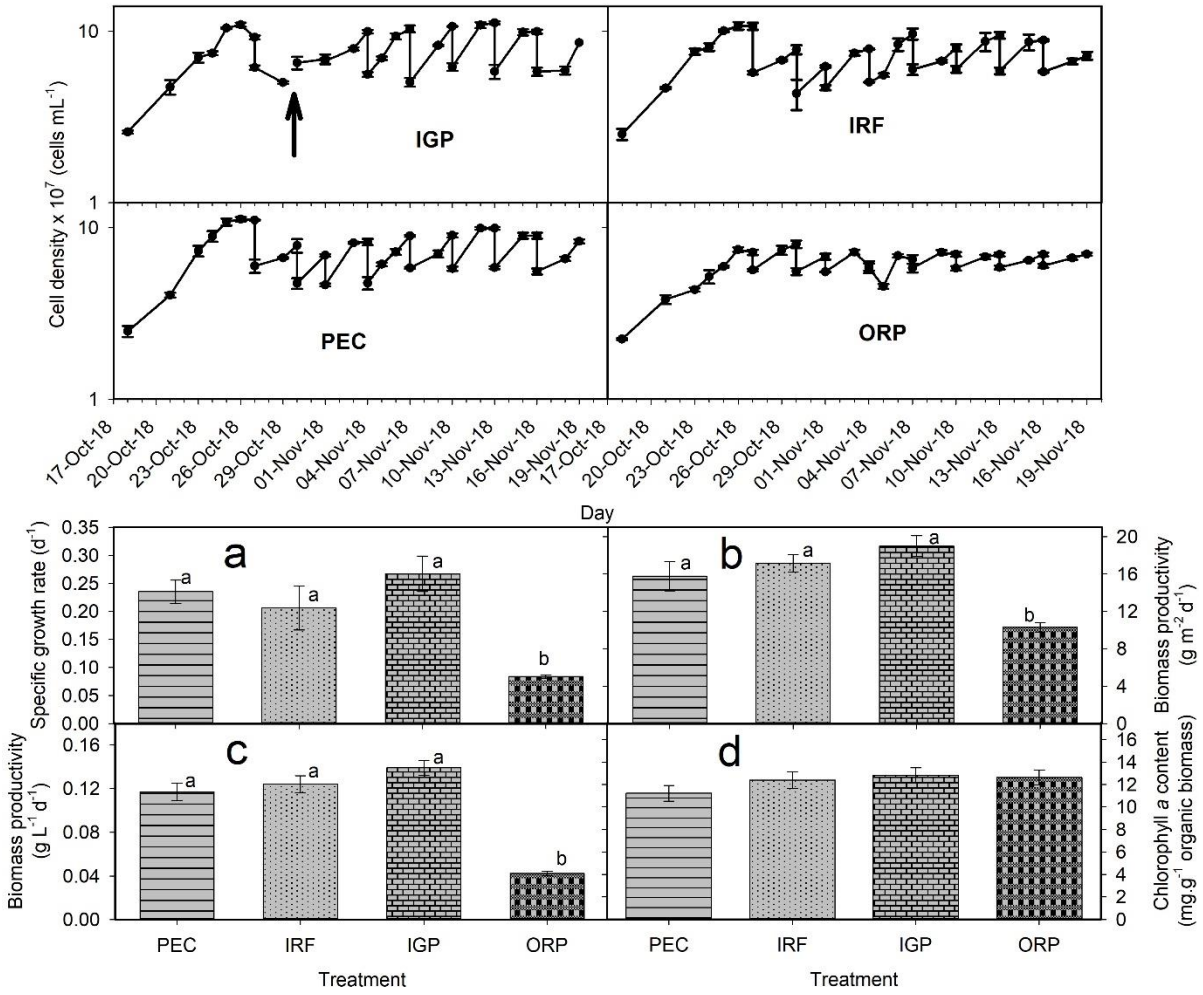

Fig. 3–4. Log-transformed cell density (top panel) of *Nannochloropsis* sp. MUR 267 cultivated semi-continuously in insulated glazed photovoltaic (IGP), infra-red film reflecting (IRF), evaporative cooled (PEC) photobioreactors and raceway pond (ORP) during austral spring. Specific growth rate (a), ground areal biomass productivity, g m^{-2} d^{-1} (b), and volumetric biomass productivity, g L^{-1} d^{-1} (c) and chlorophyll *a* content (d) of the *Nannochloropsis* sp. Error bar indicates standard error, n = 6.

Table 3–2. Ground areal and volumetric productivities for flat plate photobioreactors and raceway ponds under outdoor conditions.

Reference	Location	Light path (cm)	Microalgae species	Areal productivity (g m^{-2} d^{-1})	Volumetric productivity (g L^{-1} d^{-1})
			Flat plate photobioreactors		
[36]	Israel	1.3 – 17	*Nannochloropsis* sp.	11 – 22	1.7 – 0.25
[37]	Israel	10	*Nannochloropsis* sp.	14.2	0.27
[38]	Almeria Spain	5	*Nannochloropsis gaditana*	8 – 18	0.16 – 0.36
[39]	Italy	4.5	*Nannochloropsis* sp.	15.8	0.36
[40]	Colorado, USA	5	*Nannochloropsis oculata*	/	0.15 – 0.37
[32]	The Netherlands	2	*Nannochloropsis* sp.	20.5 – 27.5	0.9 – 1.2
This study	Murdoch, Australia	10	*Nannochloropsis* sp. MUR 267	14.6 – 18.9	0.12 – 0.14
			Raceway pond		
[41]	South Spain	30	*Scenedesmus obliquus* SAG 276-10	8.3	0.03
[42]	South Spain	30	*Muriellopsis* sp.	8 – 20	0.04
[43]	Israel	12	*Nannochloropsis salina*	24.5	0.2
[44]	Almeria Spain	11	*Nannochloropsis gaditana*	22.4	0.09 – 0.19
[32]	The Netherlands	20	*Nannochloropsis* sp.	6.2 – 14.2	0.03 – 0.09
This study	Murdoch, Australia	20	*Nannochloropsis* sp. MUR 267	10.3	0.04

* Areal productivity calculated based on ground area. Table modified from Vree, Bosma, Janssen, Barbosa and Wijffels [32], "/" not available.

3.4.4 Chlorophyll *a* content and operating efficiency of PSII photochemistry

The effective quantum yield of primary PSII photochemistry (F_q'/F_m') in addition to the pigments content (e.g., Chlorophyll *a*) can be used to shed light on the immediate health (physical fitness) or vitality of cultures, with low values indicative of physiological stress [12].

Nannochloropsis chlorophyll *a* content per unit cell mass (mg g^{-1} organic biomass) did not vary significantly between culture systems (One-Way RM ANOVA, $P > 0.05$) (Fig. 3–4d). This is likely due to the fact that the transmitted PAR wavelengths composition trended similarly in all the PBRs (Section 3.4.1, Fig. 3–2b). These results from outdoor solar irradiated photobioreactors are in agreement with our previous findings on the incorporation of IR reflecting film on the illumination surface of the PBR when culturing *Nannochloropsis* sp. indoors with halogen lamps [19]. The chlorophyll *a* values found in this study are well within values reported in previous studies for this species [12, 21]. The result demonstrates that the use of reflective materials on the surface of photobioreactors to limit the input of IR heat-inducing wavelengths to the culture does not have any negative consequence on the chlorophyll *a* production in the systems.

Measurement of the F_q'/F_m' in a sinusoidal fashion shows a trend that is similar for all the cultivation systems (Fig. 3–5). However, a large decrease was observed at noon, the time of maximum solar radiation, and its magnitude was not the same for the photobioreactors tested here. The result shows that the F_q'/F_m' values were highest at pre-dawn and dusk after sunset, with values ranging between 0.62 vs. 0.58 for IGP and 0.54 vs. 0.51 for ORP (Fig. 3–4c). At 09:00, the F_q'/F_m' values at pre-dawn decreased by 24, 13, 9, and 7 % for PEC, IRF, IGP, and ORP, respectively. At about 13:00 (noon sunshine), these values decreased further, a

magnitude of 31, 23, 16, and 22 % for the PEC, IRF, IGP, and ORP, respectively from the original values. However, values recovered to be high at dusk corresponding to a strong decrease in solar radiation and temperature (Fig. 3–5). Hence, the diurnal pattern of the F_q'/F_m' replicated the same trend and behavior as that of the temperature and incident solar radiation. The significant dip observed in the PEC was due to a higher input of solar radiation into the culture. It is very important to note that the PEC photobioreactor (with a pronounced decrease) received wavelengths spanning the whole solar spectrum before the automated cooling system was activated. The decrease in the photochemical efficiency during the noon solar radiation could be due to a regular photoprotective mechanism to protect the photosynthetic machinery from solar-driven photodamage [45]. This decrease could also be attributed to photoinhibition of the photosynthetic apparatus, however, the recovery of the culture at dusk reveals no permanent damage to this critical system. Given the fragility of the PSII reaction center to thermal damage, it takes a combination of high irradiance and temperature to rapidly cause irreversible damage [46]. Our data demonstrate that by modifying the illuminated surface to effectively exclude the entry of IR radiation into the photobioreactor, as demonstrated by the IGP system, the magnitude of the photoinhibition observed when culturing *Nannochloropsis* sp., can be reduced by 23 % compared with the conventional PEC mechanism, and consequently leads to 11 % improvement in biomass production. This data supports the results of our previous work on indoor cultivation [18, 19] and demonstrates successful application of IR reflecting technologies in microalgal biomass mass production under outdoor conditions.

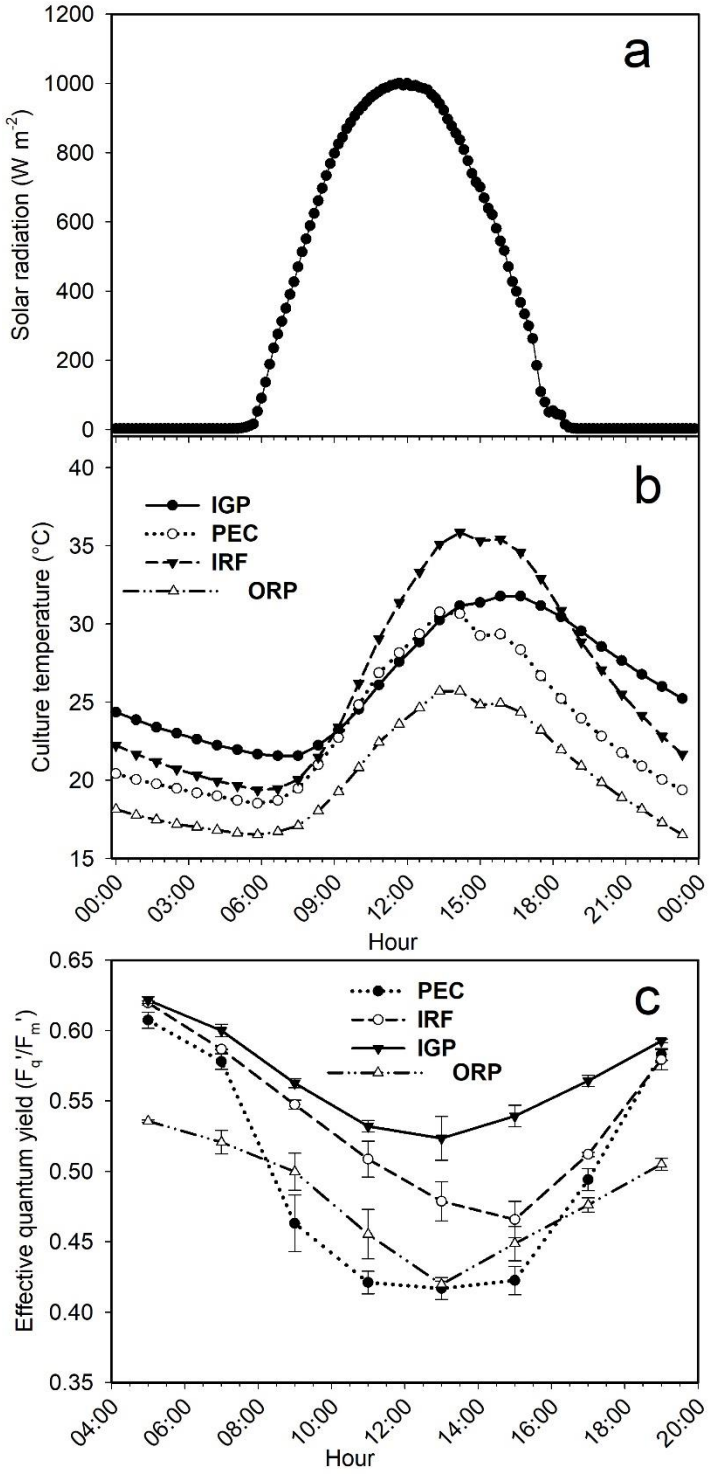

Fig. 3–5. Diurnal pattern of (a) solar radiation, (b) culture temperatures and (c) effective quantum yield of *Nannochloropsis* sp. on any typical day (18 November), cultivated in evaporative cooled (PEC), infrared reflecting (IRF), and insulated glazed (IGP) photobioreactors, and raceway pond (ORP). Error bar indicates standard error, n = 3.

3.4.5 Electrical energy generation from the IGP

While the biomass productivity of the IGP and IRF photobioreactors are statistically similar, the former configuration does allow harvesting of solar energy via the transparent PV panel and this could be used to energize production operations. This power can be used to operate the microalgal photobioreactor or supplied to light-emitting diodes (LEDs) at night, extending the illumination period and thus leading to improvements in the productivity of the culture. Photovoltaic integration into spectrally-selective photobioreactors is anticipated to create stand-alone energy-efficient photobioreactors, and eliminate/decrease their operational energy requirements from grid electricity. Basic energy measurement shows that up to 67 W_p m^{-2} of electrical power (Supplementary Information, Table 3–S1) is generated from the transparent PV (13 % efficiency) attached to the reactor. During photobioreactor operation for microalgal biomass production and not considering downstream processing (which requires added energy), energy consumption for mixing represents the most significant cost to the total operational energy and at the same time varies as the culture volume [47].

To assess the capacity of the IGP photobioreactor to supply the energy required for its operation, an estimate of both the mixing energy and power output from the PV are calculated for a 1-ha (100 m x 100 m) microalgal plant located in Perth, Western Australia and operated for 11 sunniest months (330 days) in a year. Given a density of one reactor per square metre at a gap of 100 cm x 20 cm (L x W, illumination surface facing North = W), a total of 2,840 IGP photobioreactor units (total culture volume = 397.6 m^3) is required. At biomass productivity of 18 g m^{-2} d^{-1} (this study) and energy content of 25.7 MJ kg^{-1} (7.14 kWh kg^{-1}) for *Nannochloropsis* sp. [48], annual biomass productivity and expected energy yield are 59.4 tons ha^{-1} and 424.1 MWh ha^{-1}, respectively. To produce this amount of biomass, two

blowers will be required [47] to provide compressed air at a daily flow rate of 0.21 vvm to the plant. The specific energy consumption for the blowers is estimated as 38 W m^{-3} (based on the equation of Chisti [49]), which is equivalent to 912 Wh m^{-3} d^{-1} and 362.6 kWh d^{-1}. Therefore, the total annual energy cost required for mixing is 119.7 MWh ha^{-1}. In terms of the electrical energy output generated from the semi-transparent photovoltaic panel based on the data available from the photobioreactor operation and material information sheet, the average energy output is simulated for this location to be 9.6±0.96 kWh $month^{-1}$ per module. This energy output from the photovoltaic is equivalent to 299.9 MWh ha^{-1} yr^{-1}, which is 2.5-fold higher than the energy required for mixing operation.

Therefore, PV-integrated flat plate photobioreactors (e.g., the IGP design described here) show promise for the production of cultures devoid of contaminants at high cell densities, effectively control culture temperature with no requirement for extraneous cooling systems, minimizes water loss by evaporation and generates sustainable baseload electrical energy to power production operations. This photobioreactor represents an excellent energy-efficient technology particularly suitable for the production of high-quality microalgal feedstocks for aquaculture, value-added pigments, pharmaceuticals, nutraceuticals, and bioactive compounds, as well as supplying quality inocula for commercial-scale biofuel activities. The insulated glazed photovoltaic technology also unfolds a reliable experimental platform for studying microalgal performance on a large-scale especially in remote areas away from grid electricity. However, this grid-independent PV-microalgal photobioreactor could allow for the operation of the photobioreactor (e.g., mixing) during the daytime period only, and this strategy is shown to significantly reduce biomass productivity and photosynthetic performance of cultures [50]. The coupling of solar battery storage systems to the IGP design results in the redirection and storage of additional

electricity produced from the photovoltaic during the daylight hours for application at night to mix photobioreactors. This battery system is a critical component of this innovative design as it provides seamless voltage supply to the mixing apparatus irrespective of the variation in solar irradiance and the presence of cloud cover. Nevertheless, the combination of technologies in the IGP photobioreactor provides a suitable system for outdoor solar based cultivation of microalgae with high biomass productivity that are cooling water and grid energy independent.

3.5 Conclusions

Maximizing photosynthetic solar energy capture by photobioreactors correlates with high biomass productivity; however, the culture could overheat due to a high flux of sunlight. This study has demonstrated an insulated glazed photovoltaic photobioreactor based on spectral filtration of solar energy to maintain optimum temperature for the growth of *Nannochloropsis* sp. without requiring costly and extraneous cooling systems. An unrivaled attribute of the IGP photobioreactor is its ability to generate up to 67 W m^{-2} electrical energy while supporting >14%, 14% and 71% higher biomass productivity than passive evaporative cooling, infrared reflecting thin-film photobioreactors, and raceway pond, respectively, offering grid electricity and cooling water independent platforms for microalgal biomass production.

3.6 References

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3.7 Supplementary data

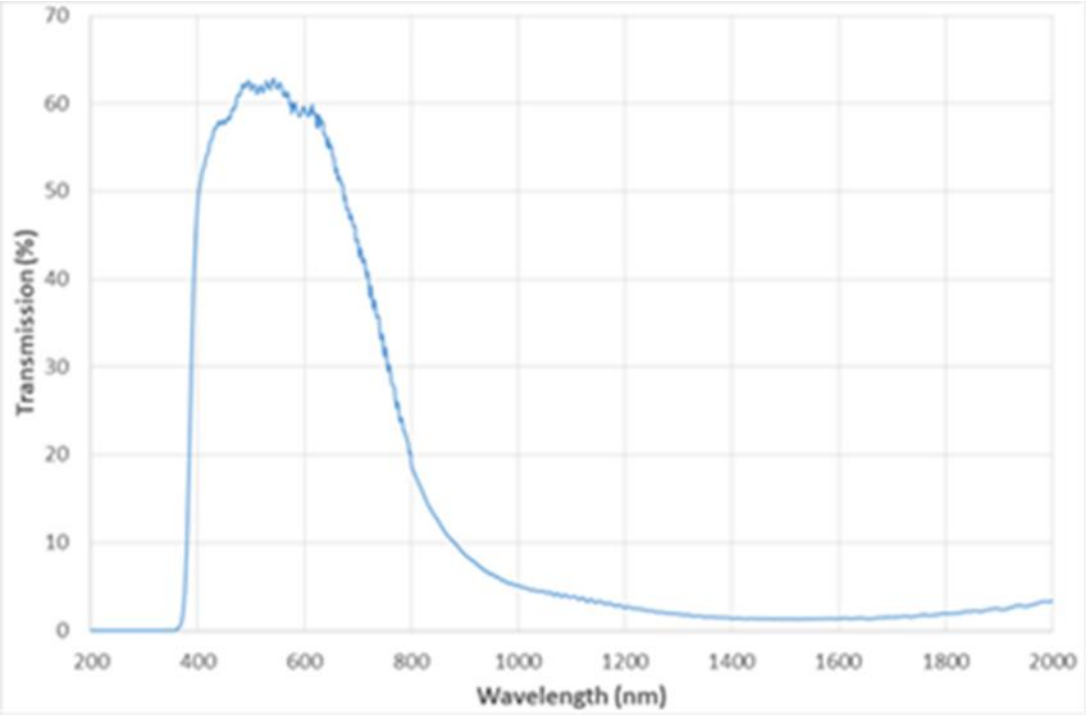

Fig. 3–S1. Transmission spectrum of the solar control infrared reflecting thin film deposited on the illumination surfaces of the infrared reflecting film and insulated-glazed photobioreactors.

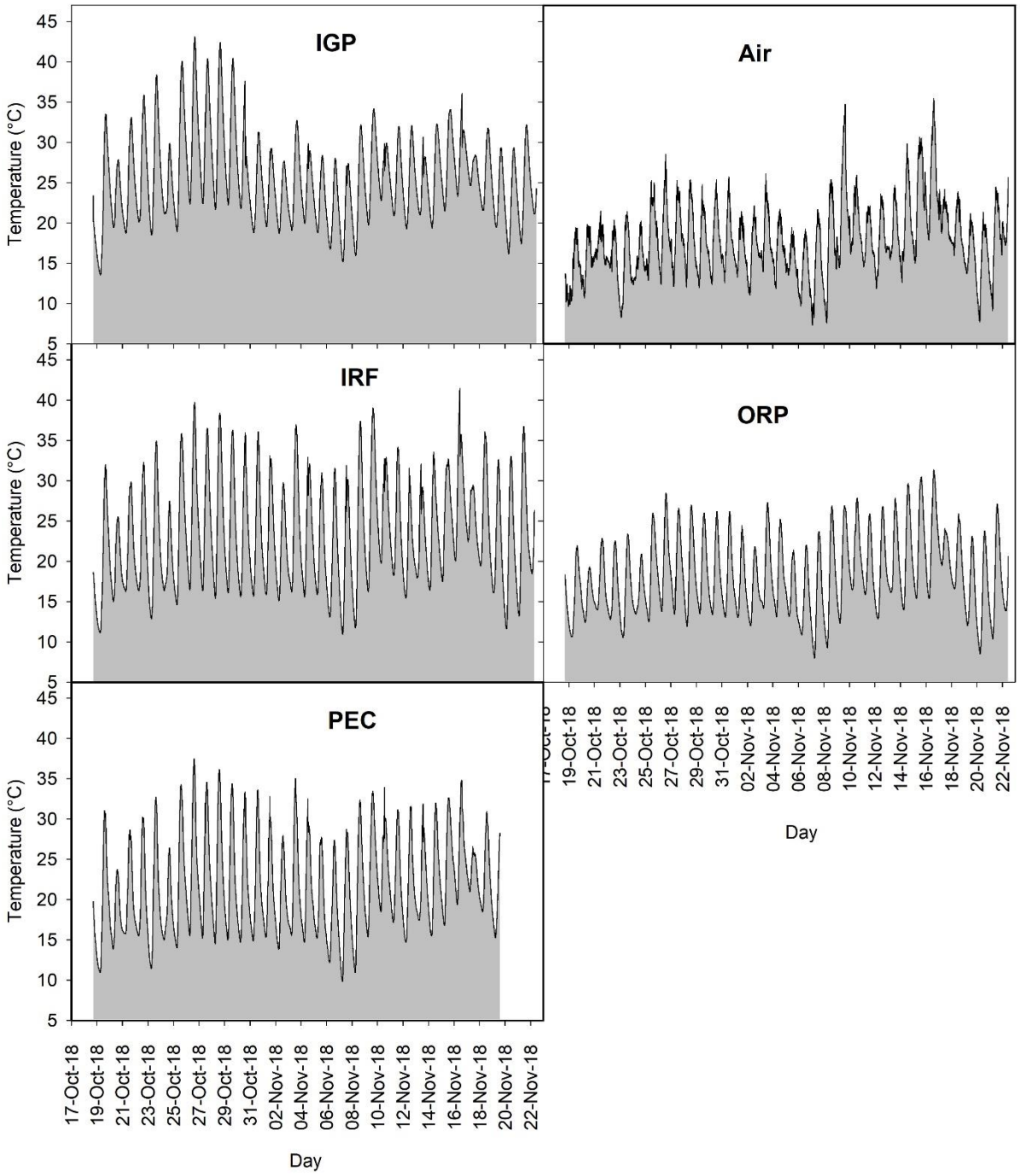

Fig. 3–S2. Temperature variation in the cultivation systems during the culture of *Nannochloropsis* sp. MUR 267 in evaporative cooled (PEC), infrared reflecting film (IRF), and insulated glazed photovoltaic (IGP) PBRs, and raceway pond (ORP).

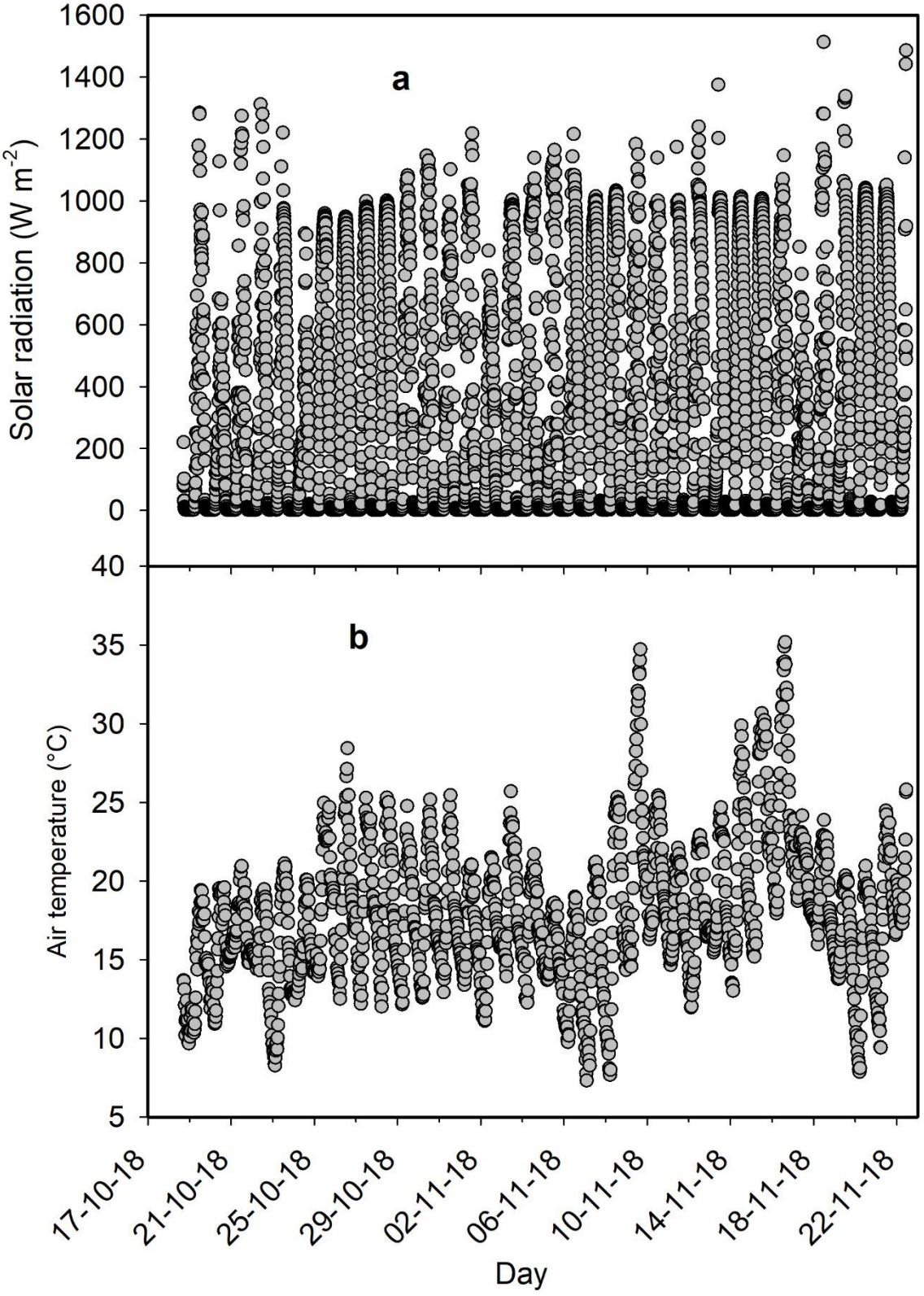

Fig. 3–S3. Solar radiation (a) and air temperature (b) variation during the growth of *Nannochloropsis* sp. under outdoor conditions.

Table 3–S1. Outdoor performance of the 1.2 m x 0.6 m 40% transparency photovoltaic glass

Parameter	Symbol	Unit	Value
Open circuit voltage	V_{OC}	V	116
Short circuit current	I_{SC}	A	0.59
Voltage at maximum power	V_{MP}	V	87
Current at maximum power	I_{MP}	A	0.55
Peak power	MPP	W_p	48

Link to the next chapter

With the outdoor performance of the novel photobioreactor proven, it was then necessary to determine if there was any significant differences to the production of intracellular algal products that might affect the use of the technology for larger scale operation. As noted previously, the physiological responses of microalgal cultures can vary depending on temperature and solar energy and wavelength variations. This in turn can be reflected in the production of metabolites such as pigments, protein composition, and high-value fatty acid profile. The results obtained suggest that photobioreactors constructed of insulated glazed panels and coated with low-emissivity films blocked harmful heat transfer to cultures and performed just as well as the freshwater-based cooling with additional advantages such as limiting daily temperature fluctuation and generating electrical energy for operational need of the reactor. Not having to rely on fresh clean water for cooling has obvious environmental benefits, particularly as many areas suitable for outdoor microalgal production also have limited freshwater resources. However, physiological responses of microalgae cultures to temperature and solar light variations could induce a species-specific alteration in their metabolites and high-value fatty acids production.

In the following chapter, I evaluate the impacts of the thermoregulation in photobioreactors constructed with insulated glazed panels and solar control low-emissivity films on macromolecular composition and fatty acid profile of *Nannochloropsis* sp. cultures under outdoor conditions.

Chapter 4

Effect of cooling strategies on the macromolecular composition and fatty acid profile of *Nannochloropsis* sp.

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Does growing *Nannochloropsis* sp. in innovative flat plate photobioreactors result in changes to fatty acid and protein composition?

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Abstract

Solar cultivation of microalgae in photobioreactors is a valuable bioprocess for the sustainable production of commercially useful metabolites. However, the conventional culture temperature control method in solar closed photobioreactors of evaporative cooling is neither economical nor sustainable. In this study, a novel spectrally selective, insulated glazed flat plate (IGP) photobioreactor employing an infrared reflecting system embedded in the illumination surface was used for cultivation of *Nannochloropsis* sp. The impact of the temperature control technology on protein, lipid, carbohydrate content and fatty acid profile of *Nannochloropsis* sp. was investigated and compared to closed photobioreactors using passive evaporative cooling (PEC) and an infrared reflecting film (IRF) on the surface as well as an open raceway pond (ORP). Among all cultivation systems tested, the biochemical composition of biomass (mg g^{-1} organic biomass) showed a general trend of lipid > protein > carbohydrate, with no large variation of each across treatments. However, the areal and volumetric productivities of these constituents were significantly higher in the photobioreactors than in the ORP; results consistent with biomass productivity data. Of the major saturated and monounsaturated fatty acids present, only the proportion of C16:0, which is 24% higher in the photobioreactors than in the ORP, changed significantly among cultivation systems. The highest content of high-value dietary fatty acids, eicosapentaenoic acid (EPA, C20:5n-3; 15.5%) and ϒ-linolenic acid (C18:3n-6; 8%) were found in the ORP but were similar to that produced in the IGP (15.9 and 3.4%, respectively). Among all photobioreactors, the IGP had the least diel temperature changes and an EPA content that was 21% higher than PEC. Photobioreactors constructed with spectrally selective materials effectively allow management of internal reactor temperature with no significant negative impact on biochemical and fatty acid profiles of microalgae.

Keywords Eicosapentaenoic acid · Polyunsaturated fatty acids · *Nannochloropsis* · Photobioreactors · Spectrally selective materials · Temperature control

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Introduction

Microalgae are emerging as a reliable source of valuable exploitable bio-feedstocks as they are rich in carbohydrates, lipids, proteins, and pigments that can be utilized in the production of energy, feed, chemicals, biomaterials, pharmaceuticals, personal care products and food (Borowitzka 2013; Barsanti and Gualtieri 2018). Exploitation of microalgae for use in the functional food sector is particularly attractive as the microalgae show promise for sustainable cultivation using free solar resources. In this context, a number of species and strains are known to produce essential long-chain polyunsaturated fatty acids (LC-PUFA), such as arachidonic acid (ARA, C20:4n-6), eicosapentaenoic acid (EPA, C20:5n-3), and docosahexaenoic acid (DHA, C22:6n-3), that are critical dietary ingredients in human and animal nutrition (Suzuki

Author contribution

Contributor	Statement of contribution
Emeka G. Nwoba (70%)	Collection, analysis and interpretation of the data. Drafting of the article and critical revision of the manuscript.
David A. Parlevliet	Conception and design, interpretation of data, critical revision of the manuscript.
Damian W. Laird	Interpretation of data, critical revision of the manuscript.
Ashiwin Vadiveloo	Provision of study material, interpretation of data, critical revision of the manuscript
Kamal Alameh	Interpretation of data, critical revision of the manuscript.
Navid R. Moheimani	Conception and design, interpretation of data, critical revision of the manuscript.

Principal supervisor confirmation

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

Name	Signature	Date
David A. Parlevliet		**8/12/2020**

4.1 Abstract

Solar cultivation of microalgae in photobioreactors is a valuable bioprocess for the sustainable production of commercially useful metabolites. However, the conventional culture temperature control method in solar closed photobioreactors of evaporative cooling is neither economical nor sustainable. In this study, a novel spectrally-selective, insulated-glazed flat plate (IGP) photobioreactor employing an infrared reflecting system embedded in the illumination surface was used for cultivation of *Nannochloropsis* sp. The impact of the temperature control technology on protein, lipid, carbohydrate content and fatty acid profile of *Nannochloropsis* sp. was investigated and compared to closed photobioreactors using passive evaporative cooling (PEC) and an infrared reflecting film (IRF) on the surface as well as an open raceway pond (ORP). Among all cultivation systems tested, the biochemical composition of biomass (mg g^{-1} organic biomass) showed a general trend of lipid > protein > carbohydrate, with no large variation of each across treatments. However, the areal and volumetric productivities of these constituents were significantly higher in the photobioreactors than ORP; results consistent with biomass productivity data. Of the major saturated and monounsaturated fatty acids present, only the proportion of C16:0, which is 24% higher in the photobioreactors than ORP, changed significantly among cultivation systems. The highest content of high value dietary fatty acids, eicosapentaenoic acid (EPA, C20:5n-3;15.5%) and ϒ-linolenic acid (C18:3n-6; 8%) were found in the ORP but were similar to that produced in the IGP (15.9 and 3.4%, respectively). Among all photobioreactors, the IGP had the least diel temperature changes and an EPA content that was 21% higher than PEC. Photobioreactors constructed with spectrally-selective materials effectively allow

management of internal reactor temperature with no significant negative impact on biochemical and fatty acid profiles of microalgae.

4.2 Introduction

Microalgae are emerging as a reliable source of valuable exploitable bio-feedstocks as they are rich in carbohydrates, lipids, proteins, and pigments that can be utilized in the production of energy, feed, chemicals, biomaterials, pharmaceuticals, personal care products and food (Barsanti and Gualtieri 2018; Borowitzka 2013). Exploitation of microalgae for use in the functional food sector is particularly attractive as the microalgae show promise for sustainable cultivation using free solar resources. In this context, a number of species and strains are known to produce essential long-chain polyunsaturated fatty acids (LC-PUFA), such as arachidonic acid (ARA, C20:4n-6), eicosapentaenoic acid (EPA, C20:5n-3), and docosahexaenoic acid (DHA, C22:6n-3), that are critical dietary ingredients in human and animal nutrition (Suzuki et al. 2019). Effective culture of suitable microalgae for LC-PUFA represents an opportunity to provide a natural, healthy sustainable alternative to the exploitation of fish oils; a practice that is already under environmental scrutiny due to negative impacts on fish stocks and marine food webs.

The most consistent factor linking any microalgal bio-feedstock production and economically viable exploitation is the extent of actual biomass production. There are relatively few cases where the biosynthesis of a particular bio-feedstock compound and generic biomass productivity are completely decoupled. Thus, the economically viable use of microalgae for producing LC-PUFA requires stable and commercially-viable biomass production technology. Mass outdoor microalgal biomass production can be achieved using open ponds and, in effect approximately 90% of global bulk microalgal production is based

on this culturing method (Pruvost et al. 2016). Classical open raceway ponds are generally recognized as inexpensive and simple cultivation systems to scale-up (Borowitzka 1999) but do come with some serious drawbacks, such as, a high risk of biological contamination limits applicability to the cultivation of extremophiles; the inability to control culture temperature impacts on maintenance of optimal growth conditions; and a long optical depth of culture results in low productivity rates. The combination of these factors means that the open raceway pond is typically unable to meet the essential requirements that guarantee sustained year-round biomass productivity under outdoor conditions.

Closed photobioreactors, while more capital expensive to set-up, offer the benefits of finer control of culture conditions and environmental variables as well as reduced contamination risk. The result is higher biomass productivity, greater potential to culture a wider range of microalgal species and strains, and less downstream processing for bio-feedstocks destined for functional foods markets. But use of photobioreactors is not all upside. Despite the emergence of solar photobioreactors as suitable platforms for culture of numerous microalgae species (Vo et al. 2018), they are susceptible to overheating because of >50% of the radiation impinging the photobioreactor surface is within the infrared (IR) range, i.e., wavelengths above 700 nm (Hindersin et al. 2013; Goetz et al. 2011) and the strongly exoenergetic nature of photosynthetic microalgae growth. In fact, Pruvost et al. (2016) have suggested that up to 95% of overall solar spectral energy collected by a photobioreactor is transformed to heat by the culture. Culture overheating is problematic as it negatively impacts photosynthesis resulting in decreased growth and biomass productivity.

In temperate regions, sustained year-round productivity in photobioreactors can only be achieved by both cooling and heating of the cultures as intense solar irradiance in summer

results in supra-optimal temperatures (Nwoba et al. 2019a) and suboptimal low temperatures during winter (Torzillo et al. 1991). Both high and low extreme temperatures can lead to complete deterioration of growth and loss of productivity and heating and cooling regimes need to be optimised for algal strain and location.

We have recently reported the development and performance of a photobioreactor that seeks to overcome the issues with culture over and under heating (Nwoba et al. 2020b). The photobioreactor uses insulated glazed panels and a spectrally-selective material and has been utilised to culture both *Arthrospira platensis* and *Nannochlorpsis* sp. under outdoor conditions during the austral spring and autumn without the need for additional cooling or heating (Nwoba et al. 2020b, a).

That work focussed on the temperature control of this novel photobioreactor and only reported general growth of the cultures. However, microalgae are now being cultured in order to exploit their high-value intracellular components rather than just biomass. It is possible that physiological responses of microalgae cultures to temperature and solar light variation could induce alteration in the expression of high-value metabolites (e.g., high-value fatty acids) that could negatively affect the overall productivity of those metabolites, even if biomass production is maintained. Culture of *Nannochloropsis* sp. has been widely investigated due to its potential for commercial-scale biofuel production. Some strains have also been shown to accumulate high-value lipids, such as ω-3 fatty acids (EPA) and valuable carotenoids (violaxanthin, zeaxanthin, β-carotene, vaucheriaxanthin-ester) (Shene et al. 2016), that could be utilised in functional food applications or as an aquaculture feed additives for that improves the nutritional profile of the farmed product.

As such, this study addresses the question of whether the improved thermoregulation of this novel, spectrally selective photobioreactor can maintain or improve biomass productivity without negatively impacting the accumulation of high-value intracellular components such as fatty acids. This information is essential to ascertain the economic viability of using photobioreactors for large scale microalgal culture applications.

4.3 Materials and methods

4.3.1 Experimental set-up

The cultivation systems consisted of three rectangular flat plate photobioreactors and a raceway pond. The first plate reactor is a custom designed photobioreactor made of insulated glazed units (IGUs) and integrated with a low-e film (from now on known as insulated-glazed low-emissivity photobioreactor, IGP). The IGP consisted of five 5 mm thick IGUs, each with double glass panes sealed together with an airspace between them to give high thermal insulation properties. The front IGU panel (1.2 m x 1.5 m, L x H) has a low-emissivity (low-e) thin-film embedded on the outer surface. This low-e film was spectrally-selective and transmitted >75% of visible light to the culture and concomitantly reflected >90% of ultraviolet (UV) and IR wavelengths. The back and the bottom IGUs lacked the low-e film, whereas the sides contained low-e films (Nwoba et al. 2020b). A 1.2 m x 0.60 m thin film-based semi-transparent CdTe photovoltaic panel was laminated to the top of the IGP, 0.9 m above the base (Nwoba et al. 2020b). This semi-transparent PV panel transmitted 40% of the captured sunlight to the interior and generated electricity from the remainder. Both the second and third photobioreactors were built with clear float glass, in which the base and sides were 19 mm thick. The illuminated and back surfaces were constructed of 12 mm thick glass in clear acidic cure silicone. The dimensions of these two photobioreactors were 1.26 m

x 1.25 m (L x H), the illuminated surface area was 1.58 m^2 and the maximum filling capacity was 0.16 m^3 (Table 4–1). One of these photobioreactors had the illumination surface and sides laminated with the solar-control low-e film (designated as infrared reflecting film photobioreactor, IRF) as a temperature management mechanism. The back surface and the bottom of the IRF photobioreactor did not contain low-e film. The remaining plate photobioreactor had the illumination surface sprayed with freshwater to control its internal temperature and is herein designated as the passive-evaporative cooled photobioreactor (PEC). The cooling system of the PEC photobioreactor consisted of a thermostat system comprised of a freshwater reservoir tank (working volume of 0.30 m^3) with a submersible pump that delivered water through PVC pipes across a solenoid valve to a sprinkler system fitted on the surface of the reactor. The cooling system was activated when the culture temperature reached the temperature set point of 27 ˚C and deactivated when the temperature dropped by 2 ˚C. A gutter system was provided at the bottom of the photobioreactor for the efficient collection and recycling of the cooling water. The reservoir was shaded to reduce warming by direct sunlight. These three photobioreactors were tilted at an angle of 32˚ (Fig. 4–1) to the vertical and the illuminated surface oriented to the north to maximize the quantity of light intercepted (Nwoba et al. 2019a). Each photobioreactor had a culture depth (light-path, internal diameter) of 0.10 m (Table 4–1). The fourth cultivation system was a 1 m^2 (0.20 m x 0.50 m) fiberglass open raceway pond (designated as ORP) driven by a paddlewheel system (Raes et al. 2014). The ORP had a similar orientation to the photobioreactors (i.e., north-south direction).

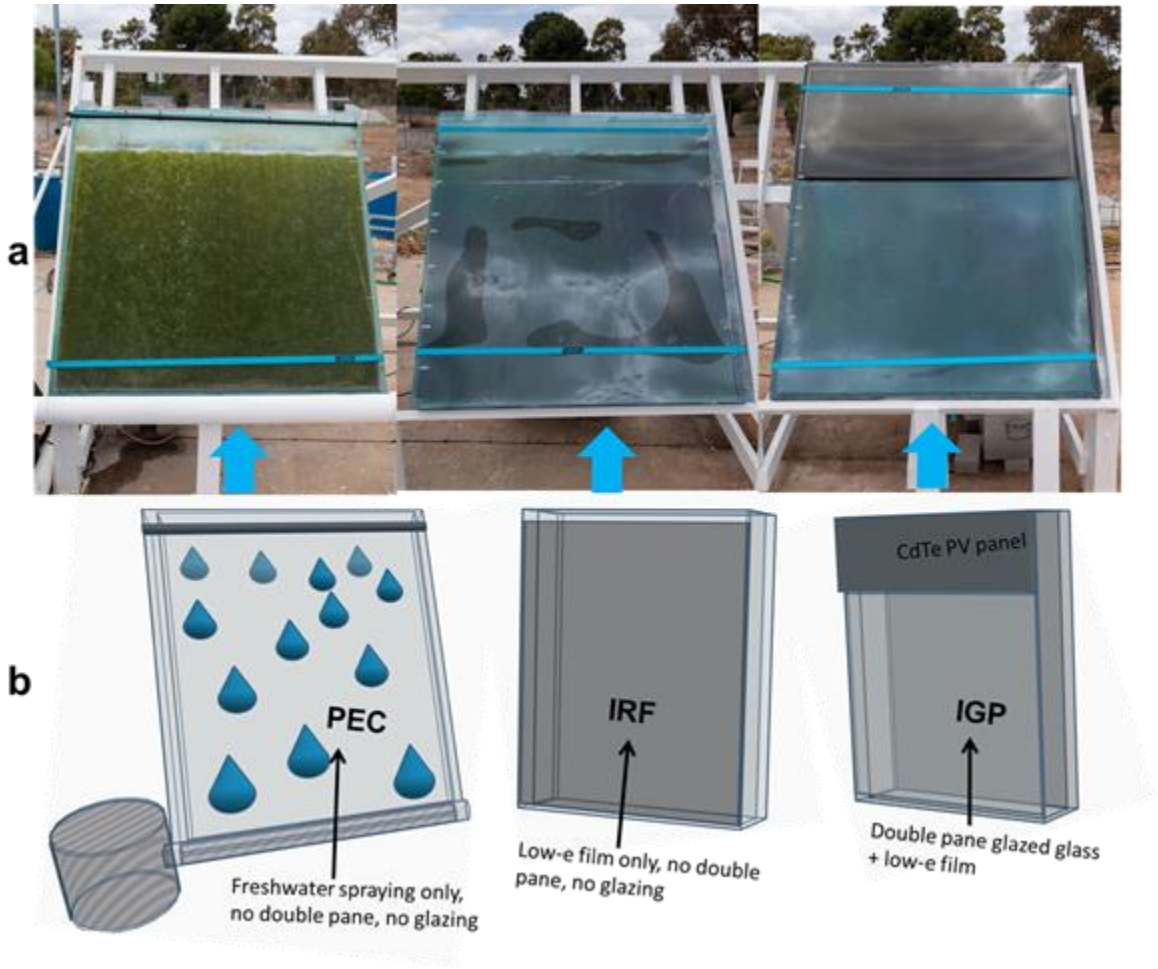

Fig. 4–1. Microalgae cultivation flat plate photobioreactors. (a) Image of the photobioreactors in operation at the Algae R&D Centre, Murdoch University, Australia. Left to right: passive evaporative cooling (PEC), infrared reflecting film (IRF) and insulated-glazed photovoltaic (IGP) photobioreactors. (b) Schematic of the illumination surface of the photobioreactors.

4.3.2 Microalgae strain, culture medium and cultivation conditions

Nannochloropsis sp. MUR 267, a marine Eustigmatophyte, originally isolated from Swan-Canning Estuary, Western Australia (Nwoba et al. 2019b), was obtained from the Murdoch University Culture Collection, Australia. Preparation of the culture medium was carried out using charcoal filtered (but untreated) natural seawater (Hillary's Beach, WA) supplemented with sterilized F/2 – Si nutrient stocks (Guillard and Ryther 1962) and the ambient salinity of the seawater, 33‰ NaCl was maintained. The inoculum for the

experiment was sourced from a unialgal, but non-axenic, culture that had been maintained in exponential growth phase in 2 m^2 raceway ponds under outdoor climatic conditions in Perth, WA. For more than 12 months, no other photosynthetic microorganisms, including diatoms, were observed by microscopic evaluation of the inoculum. The experiment was conducted from October to November of 2018, the austral spring season.

Mixing of the microalgal culture in the photobioreactors was achieved by air bubbling using an air pump (PondOne O2 Plus 8000, 4,200 L h^{-1}) coupled to a silicone tube connected to both ends of ceramic diffusers (length, 1.2 m) fixed at the bottom of the cultivation systems. The air provided by the pump was filter-sterilized and the pressure controlled using a flowmeter. The mixing was optimized at an airflow rate of 0.21 L L^{-1} min^{-1} (volume of air per volume culture per minute). The hydrodynamic parameters of the fluid in a biphasic system comprised of air and tap water at the optimised aeration rate were calculated to be: superficial gas velocity, 0.0039 m s^{-1}, mixing time, 106.3±3.2 s, and gas-hold, 0.017±0.0002. The ORP was operated at a mixing speed of 0.22 m s^{-1} (Raes et al. 2014). All the plate photobioreactors (IGP, IRF and PEC) were subjected to identical operational parameters with an effective culture volume of 0.14 m^3 (Table 4–1). The ORP had the culture volume maintained at the optimum operational depth of 0.20 m (0.20 m^3) (Raes et al. 2014) that allowed for efficient mixing by the paddle wheel. Evaporation volume make-up was required for ORP using tap water. The pH of the cultures were not controlled and no carbon dioxide was added to the cultures. Cultivation systems operation, growth measurements and conditions followed those reported in Nwoba et al. (2020b).

The performance of *Nannochloropsis* sp. in each cultivation systems in terms of growth and biomass productivity is summarized in Table 4–1.

Table 4–1. Average culture temperatures, areal biomass productivity, and specific growth rate of *Nannochloropsis* sp. MUR 267 cultured in flat plate photobioreactors and raceway pond during the austral spring season. The same superscript letters along rows indicate no significant difference (RM ANOVA, P > 0.05). Values represent mean±SE.

Parameter	PEC	IRF	IGP	ORP
Temperature regulation mechanism	Water-assisted evaporative cooling	Low-e film	Glazed glass+low-e film	Natural evaporative cooling
Dimension ((L x W or H, m)	1.26 x 1.25	1.26 x 1.25	1.20 x 1.5	2 x 0.5
Optical depth (m)	0.1	0.1	0.1	0.2
Working volume (m^3)	0.14	0.14	0.14	0.2
Average temperature (˚C)	21.9 ± 5.9^{c}	23.6 ± 6.6^{b}	25.0 ± 4.3^{a}	18.4 ± 4.7^{d}
Maximum temperature (˚C)	31.2 ± 2.6^{b}	33.8 ± 2.9^{a}	31.0 ± 2.4^{b}	25.8 ± 2.2^{c}
Biomass productivity (g m^{-2} d^{-1})	15.8 ± 1.1^{a}	16.9 ± 1.1^{a}	19.0 ± 0.9^{a}	10.3 ± 0.5^{b}
Biomass productivity (g L^{-1} d^{-1})	0.12 ± 0.008^{a}	0.12 ± 0.008^{a}	0.14 ± 0.007^{a}	0.04 ± 0.002^{b}
μ d^{-1}	0.24 ± 0.02^{a}	0.20 ± 0.03^{a}	0.27 ± 0.03^{a}	0.08 ± 0.0^{b}
Effective photochemical quantum yield	0.50 ± 0.03^{c}	0.54 ± 0.02^{a}	0.57 ± 0.01^{a}	0.48 ± 0.01^{c}

4.3.3 Determination of carbohydrate, lipid, and protein content

Total lipid, carbohydrate and protein content of the biomass were determined as described in Moheimani et al. (2013). Total lipid extraction and estimation was performed using the method of Bligh and Dyer (1959) with 5 mL microalgae sample filtered with GF/C filters and then treated with liquid N_2 to lyse the cells. The ruptured cells were homogenized to a smooth paste in a plastic centrifuge tube containing 5.7 mL of chloroform:methanol:deionized water 1:2:0.8 (v/v/v) as a solvent. The tubes were centrifuged at 3000 x g for 10 minutes and the supernatants decanted into 10 mL screw top glass tubes and the lid tightly sealed. The biomass pellets in the plastic tubes were redissolved in the solvent, centrifuged and supernatant dispensed into the same glass tube and vortexed. Then 3 mL each of deionized water and chloroform were added to the mixture which was mixed by vortexing separating into a methanol-water top phase (non-lipid layer) and chloroform bottom phase (lipid layer). The top layer was carefully separated from the bottom layer using a fine Pasteur pipette and the chloroform layer transferred to pre-weighed vials. The content of the vials was evaporated at 38 ˚C under a stream of nitrogen gas. The mass of the lipid in the vial was determined gravimetrically. Lipid productivity was calculated as the product of lipid content in biomass and biomass productivity.

The total carbohydrate content of the biomass was estimated using the phenol-sulphuric acid method (Moheimani et al. 2013). Filters of microalgae samples (5 mL) were treated with 2 mL of 1 M sulphuric acid in plastic centrifuge tubes, homogenized to a smooth paste and the volume made up to 5 mL using the same solvent. The tightly capped tubes were incubated in a 100 ˚C water bath for 60 minutes. These samples were cooled, centrifuged and 1 mL of 5% w/v phenol solution was added to the 2 mL of the supernatant, rapidly vortexed and followed by the addition of 5 mL concentrated sulphuric acid. The tubes

were cooled to room temperature over 30 minutes and the absorbance measured spectrophotometrically at 485 nm. The carbohydrate content determined using a glucose standard curve and productivity determined as a product of carbohydrate content and biomass productivity.

The protein content of the biomass was determined using the Biuret method (Lowry et al. 1951). GF/C filters containing 5 mL of microalgae culture were homogenized with 2 mL Biuret reagent in a 10 mL centrifuge tube and made up to 5 mL. The tubes were incubated in a water bath heated to 100 ˚C for 1 hour. The tubes were removed and 0.5 mL Folin-phenol reagent was added and mixed well by vortexing. The tubes were allowed to cool, centrifuged at 3000 x g for 10 minutes and the absorbance of the supernatant measured at 660 nm. The protein content of the samples was determined from a standard curve prepared using bovine serum albumin protein standard and expressed as organic weight of dry biomass.

4.3.4 Fatty acid profile of *Nannochloropsis* sp. biomass

For fatty acid analysis of *Nannochloropsis* sp. biomass, the Global Organization for EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) omega-3 (GOED) fatty acid method (GOED 2019) was used. In brief, dried lipid samples obtained from the Bligh and Dyer protocol (Bligh and Dyer 1959) were dissolved in 0.05 g L^{-1} butylhydroxytoluene in trimethylpentane and diluted to 10 mL with the same solution. A 2 mL aliquot of the solution was evaporated at 50 ˚C under a stream of N_2. 1.5 mL of 20 g L^{-1} NaOH in methanol was added to the dried aliquot, blanketed with N_2, capped tightly and heated at 70 ˚C using a heating block for 7 minutes. After cooling down to 40 ˚C, derivatization of the extracts to their fatty acid methyl esters (FAMEs) was conducted by adding 2 mL of 14% boron trichloride-methanol, covered with N_2, heated at 70 ˚C for 30 minutes and cooled to 40 ˚C. All chemicals were sourced from SigmaAldrich and of analytical grade. The FAMEs were analyzed on a

Shimadzu gas chromatograph mass spectrometer (GCMS-QP2010S) using splitless injection and helium carrier gas. A DB-WAX UI narrow bore column (30 m x 0.25 mm x 0.25 µm, length x internal diameter x thickness, Agilent) was used for separation. A 0.2 µL aliquot of Supelco 37 Component FAME Mix (1:10 dilution in trimethylpentane) was added to each sample before being injected at 250 ˚C at a constant pressure of 7.5 psi, resulting in a GC linear velocity of ~36 cm s^{-1}. The carrier gas flow rate was 0.98 mL min^{-1}. Separation was achieved with the following temperature gradient: 50 ˚C, held for 1 min, ramped to 200 ˚C at 25 ˚C min^{-1}, then ramped at 3 ˚C min^{-1} to 230 ˚C and held for 18 mins. Data collection by the mass spectrometer was set to scan from m/z of 50 – 600, beginning 4 mins post injection.

4.3.5 Statistical analysis

Significant differences in treatments were analyzed by a One-Way Repeated Measures Analysis of Variance (RM-ANOVA). A post-hoc, Holm-Sidak multiple comparison test was used to separate the means. A significant difference was considered when *p*-value < 0.05. The SigmaPlot v13.0 program was used for all statistical analyses and data is reported as means ± standard error (SE, except stated otherwise) over the cultivation period (sample size, n = 6).

4.4 Results

4.4.1 Environmental conditions, growth and productivity

While the focus of this work is on potential changes in the biochemical content of the alga based on cultivation technology, it is important to briefly describe the environmental conditions experienced by the cultures and review the biomass productivity achieved in each of the cultivation technologies. Detailed interpretation and discussion on the temperature profiles, spectral selection and biomass productivity achieved in the various

photobioreactors and the ORP can be found in Nwoba et al. (2020b). Maximum temperatures, biomass productivity, specific growth rates, and effective photochemical quantum yields from that work are summarised in Table 4–1.

Physiological responses of microalgae cultures to solar light and temperature variations could induce a species-specific alteration in their metabolites and high-value fatty acids production. The maximum daily solar irradiance during the cultivation period was almost stable at 1,000 W m^{-2} , with only slight variation on a few occasions (Fig. 4–2a). The daily air temperature ranged between 7.3 ˚C and 35.4 ˚C in November (Fig. 4–2b). The maximum culture temperature in the PEC and IGP were similar, below 30 ˚C, for at least 74% of the cultivation days (Figs. 4–2c and 4–2d). It is important to note that the culture temperature in the IGP reached 42 ˚C in October prior to integration of the low-e film (Fig. 4–2d), indicating that double glazing only was not sufficient for temperature control. For the IRF photobioreactor, the maximum temperature was essentially stable at 33 – 34 ˚C throughout the cultivation, whereas that for the ORP was highest (28 ˚C) in November (Figs. 4–2e and 4–2f). The average maximum temperature in the cultivation systems trended as IRF > PEC = IGP > ORP (Table 4–1). The average temperature over the cultivation period was significantly different among cultivation systems (RM ANOVA, $F_{4,22383} = 1432$, $p = <0.001$), with the highest temperature in the IGP photobioreactor and lowest in the ORP (Table 4–1).

The specific growth rate and the ground areal biomass productivity were significantly higher in the photobioreactors than the ORP (RM ANOVA, $F_{3,15} = 23.7$, $p = <0.001$ for μ and $F_{3,15} = 16.4$, $p = <0.001$ for productivity) (Table 4–1). It is important to note that despite the lack of a statistically significant difference in productivity between the photobioreactors, the

areal biomass productivity (18.97 ± 0.92 (SE) g m^{-2} d^{-1}, Table 4–1) achieved in the IGP photobioreactor was 17% higher than that in the freshwater cooled PEC photobioreactor.

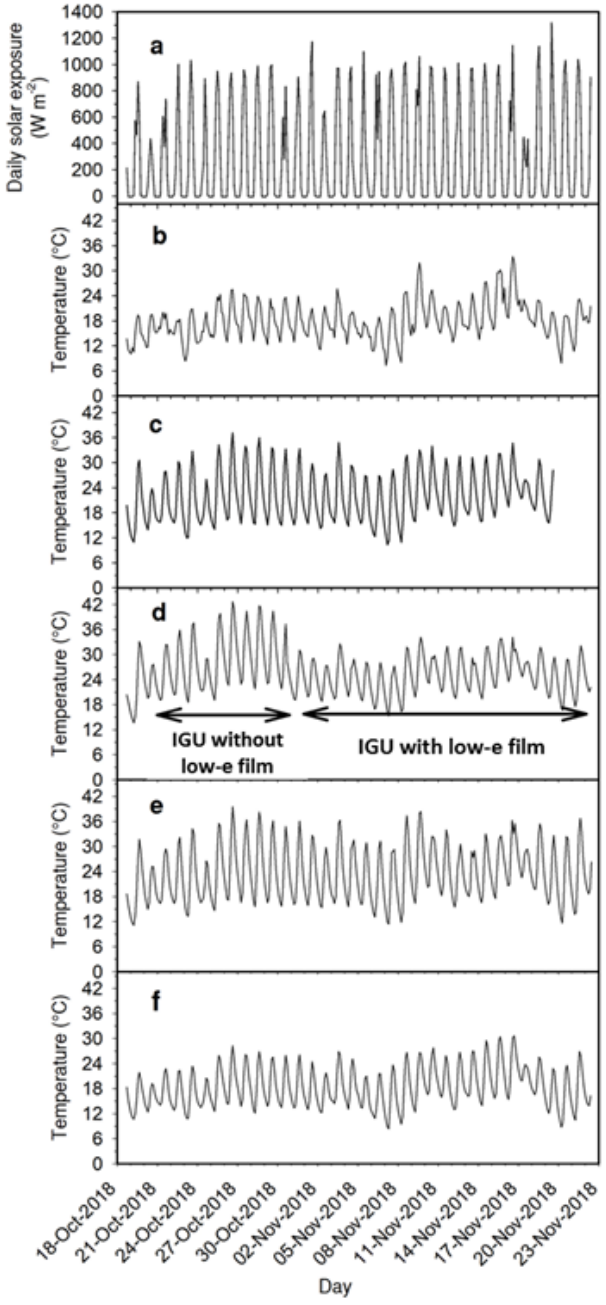

Fig. 4–2. (a) Daily solar exposure, (b) air temperature, and (c–f) culture temperatures of *Nannochloropsis* sp. cultured in (c) evaporative cooled photobioreactor, (d) insulated glazed photobioreactor, (e) infrared reflecting film photobioreactor, and (f) raceway pond during the austral spring season (October – November 2018). IGU means insulated glass unit.

4.4.2 Total lipid, carbohydrate and protein composition of biomass

The biochemical components (total lipid, carbohydrate and protein), expressed in terms of unit cell mass, were investigated for variation among the cultivation systems (Fig. 4–3). The organic mass normalized total lipid and carbohydrate contents showed no significant difference ($P > 0.05$) across treatments. However, the protein content in the PEC photobioreactor (329.9 ± 24.8 (SE) mg g^{-1} ash-free dry weight) was significantly lower than in the IGP photobioreactor (RM ANOVA, $F_{3,15} = 3.68$, $p = 0.038$) (Fig. 4–3a) but not significantly different from that recorded for the IRF photobioreactor or the ORP. Lipid and carbohydrate productivities both in mg L^{-1} d^{-1} and g m^{-2} d^{-1} were substantially higher in the photobioreactors than in the raceway pond, with no variation in the former (Fig. 4–3b,c). In contrast, the protein productivity in mg L^{-1} d^{-1} was significantly different among the cultivation systems ($F = 45.59$, $p < 0.001$), and the areal protein productivity exhibited a similar trend as that for the carbohydrate and lipid productivities (Fig. 4–3b,c). The volumetric protein productivity was 28% higher in the IGP photobioreactor than in the PEC and the general trend was IGP > PEC = IRF > RWP (Fig. 4–3b).

4.4.3 Fatty acid composition

In all treatments, the dominant fatty acids found in *Nannochloropsis* sp. MUR 267 biomass were C16:0 (palmitic acid), C16:1 (palmitoleic acid), C20:5n–3 (eicosapentaenoic acid, EPA) and C18:0 (stearic acid) (Table 4–2). These four compounds accounted for 68–80% of total fatty acid content. Palmitic acid was the largest component of the total fatty acid mixture at 28–37%, followed closely by palmitoleic acid (18–22%). Generally, no large changes in major fatty acid composition was observed between cultivation methods.

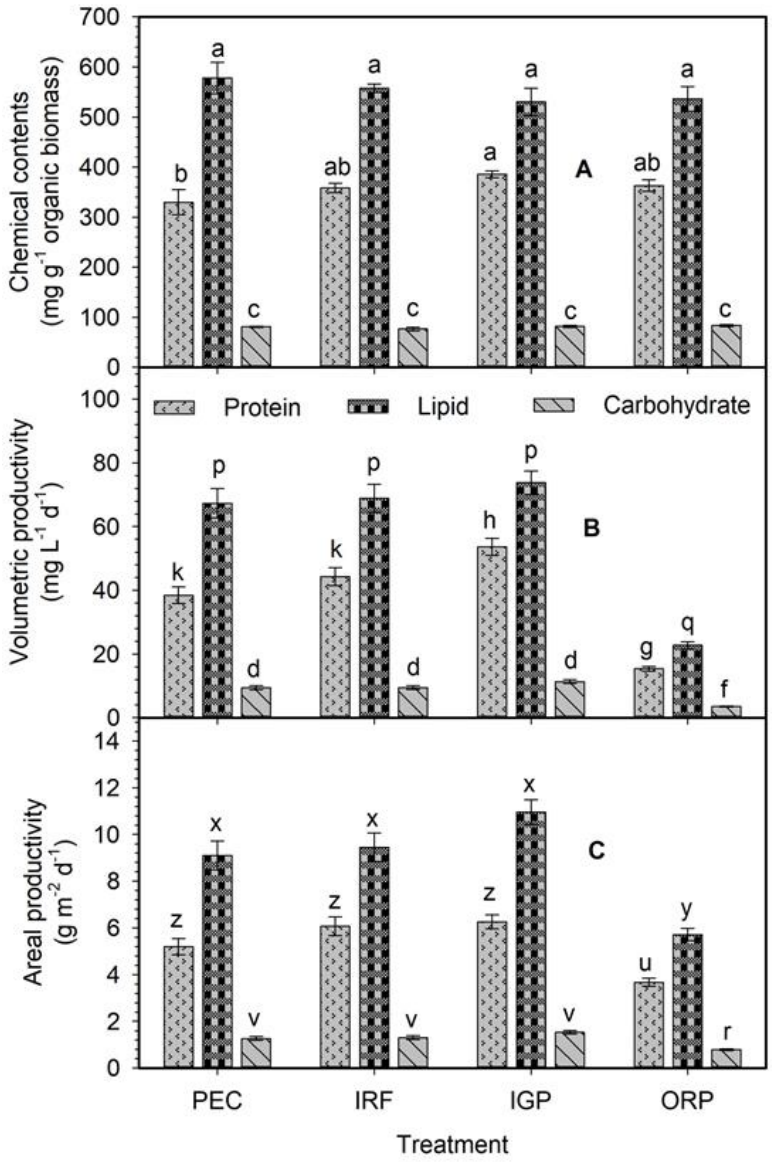

Fig. 4–3. Macromolecular composition (total protein, carbohydrate and lipid) of *Nannochloropsis* sp. MUR 267 biomass cultivated in insulated-glazed low-e (IGP), infrared reflecting film (IRF), passive evaporative cooled (PEC) photobioreactors and open raceway pond (ORP) during the austral spring season. Chemical contents based on (a) organic biomass, (b) volumetric productivity and (c) areal productivity. The same letter across bar indicates no significant difference (p > 0.05). Error bar indicates standard error.

Among the predominant fatty acids, only C16:0 showed a statistically significant difference in the photobioreactors (22.6 ± 1.15 % (SD)) compared to the raceway pond (one-way ANOVA, F = 5.4, p = 0.009). Among the lesser fatty acid components, lauric acid (C12:0)

and eicosanoic acid (C20:0) were significantly higher (47% for C12:0 and 56% for C20:0) in the ORP than the photobioreactors.

Saturated fatty acids (ΣSFAs) ranged from 46.2% of total fatty acids in the ORP to 51.7% in the PEC, with similar values in the other photobioreactors. Total polyunsaturated fatty acids (ΣPUFA) tended to be lower in the photobioreactors (19-25%) compared to the ORP (31%), with the IRF producing biomass with the lowest PUFA content (Table 4–2). Total monounsaturated fatty acids (ΣMUFA) were similar in all cultivation systems (23–27% of total fatty acids). Eicosapentaenoic acid (EPA) content was statistically significantly higher (Holm-Sidak, P = 0.003) in IGP and the ORP compared to the PEC and IRF systems. Another of the nutritionally important minor fatty acids, arachidonic acid (ARA, 20:4ω6), was found at higher content in the biomass obtained from the ORP, IGP and IRF systems compared to the PEC. The arachidonic acid content in the ORP was 31% higher than that in the biomass cultivated in PEC. Biomass from the ORP was relatively enriched in gamma-linolenic acid (GLA, 18:3ω6) with a 50% higher content compared to the photobioreactors (Table 4–2).

Table 4–2. Fatty acid composition (% total fatty acid) of *Nannochloropsis* sp. MUR 267 cultivated in flat plate photobioreactors and an open raceway pond under outdoor conditions.

Fatty acid	12:0	14:0	15:0	16:0	16:1	18:0	18:1	18:2ω6	18:3ω6	20:0	20:4ω6	20:5ω3	ΣSFA	ΣMUFA	ΣPUFA
Common name	Lauric acid	Myristic acid	Pentadecylic acid	Palmitic acid	Palmi-toleic acid	Stearic acid	Oleic acid	Linoleic acid	ϒ-linolenic acid	Eicosanoic acid	Arachidonic acid	Eicosapen-taenoic acid			
PEC	0.90 ±0.01^{b}	5.23 ±0.29^{a}	1.00 ±0.01^{a}	35.98 ±1.54^{a}	21.88 ±1.55^{a}	7.05 ±0.73^{a}	4.01 ±0.34^{b}	1.52 ±0.28^{a}	4.94 ±0.71^{b}	1.53 ±0.28^{ab}	3.93 ±0.33^{b}	12.58 ±0.56^{b}	51.69	25.89	22.97
IGP	0.70 ±0.04^{b}	5.04 ±19^{a}	0.97 ±0.04^{a}	36.62 ±2.61^{a}	20.44 ±1.55^{a}	6.59 ±0.27^{a}	4.45 ±0.32^{a}	1.27 ±0.06^{a}	3.38 ±0.45^{b}	1.03 ±0.12^{b}	4.87 ±0.49^{a}	15.89 ±0.45^{a}	50.95	24.89	25.41
IRF	0.74 ±0.03^{b}	5.48 ±0.32^{a}	0.95 ±0.02^{a}	35.55 ±1.18^{a}	21.80 ±1.55^{a}	7.39 ±0.18^{a}	5.56 ±0.22^{a}	1.39 ±0.15^{a}	3.80 ±0.61^{b}	1.23 ±0.28^{b}	4.53 ±0.29^{a}	9.18 ±0.38^{c}	51.34	27.36	18.90
ORP	1.46 ±0.18^{a}	5.69 ±24^{a}	1.19 ±0.10^{a}	27.90 ±1.89^{b}	17.56 ±0.53^{a}	7.09 ±0.40^{a}	5.29 ±0.37^{a}	1.83 ±0.13^{a}	8.09 ±0.88^{a}	2.88 ±0.53^{a}	5.64 ±0.29^{a}	15.49 ±0.82^{a}	46.21	22.85	31.05

PEC passive evaporative cooled, IGP insulated glazed photovoltaic, IRF infrared reflecting film photobioreactors, and ORP open raceway pond. SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids. Values reported in the table are means (±standard error) per treatment (n = 6). Same superscript letters along table column indicate no significant difference (P > 0.05).

4.5 Discussion

Large-scale microalgal production photobioreactors rely on incident sunlight to provide the energy required for algal photosynthesis. Hence, a positive correlation of biomass productivity with available solar radiation and air temperature typically favors tropical and warm temperate regions for mass production of microalgae, due to the higher average solar radiation and consistency of climatic conditions throughout the year (Boruff et al. 2015). In this context, locations in Australia such as the Pilbara, Karratha and Geraldton, have favourable site selection criteria for microalgal mass culture (Boruff et al. 2015).

It is well-recognized that light intensity impacts cell growth resulting in photophysiological changes that can then alter the biochemical content of photosynthetic cells (He et al. 2015). However, little is known about the impact of light modification of lipid, protein, and carbohydrate content in microalgae due to spectrally-selective cooling of photobioreactors. In order to support the case for large scale use of this type of technology in outdoor photobioreactors, it is essential to ascertain what effect the use of spectrally selective technologies will have on not just biomass productivity but the expression/accumulation of intracellular components of commercial interest.

Lipid was the most abundant macromolecule in *Nannochloropsis* sp. MUR 267 biomass with carbohydrate content being lowest. This is consistent with previous work conducted by our group (Nwoba et al. 2019b; Vadiveloo et al. 2016). Despite the significantly higher ground areal biomass productivity achieved in the IGP photobioreactor, lipid content based on the organic weight of that biomass was similar to other cultivation systems. This result suggests that the use of IR reflective technologies on the illumination surfaces of photobioreactors neither enhances nor reduces lipid production in individual

Nannochloropsis sp. MUR 267 cells. Increase in biomass density is usually expected to result in higher total lipid accumulation. This result is consistent with that presented by Estime et al. (2015), who have found no significant difference in the total lipid per unit cell mass between plasmon-enhanced and control cultures. Therefore, the higher total lipid content is not as a result of the induction of lipid accumulation in microalgal cells.

When normalized based on ash-free dry weight (organic biomass), the protein content of the biomass was similar in all cultivation systems. Statistical analysis suggests a difference between the protein content of the PEC and IGP reactors but neither of these are significantly different from the IRF or ORP systems. There was an approximately 14% higher protein content attained in the IGP compared to the PEC photobioreactor that may be a result of the greater time that the culture is under optimum temperature conditions and sub-saturating light intensity in the former photobioreactor set-up (Nwoba et al. 2020b). Under sub-saturating light conditions, photosynthetic efficiency is enhanced and the non-photochemical quenching pathway of the chlorophyll *a* fluorescence is diminished, resulting in increased biomass production (Müller et al. 2001).

In terms of product produced, the areal and volumetric lipid, protein and carbohydrate productivities responded in a similar direction as the areal and volumetric biomass productivity in all treatments, with the lipid values usually higher than the protein content, corroborating the findings on biochemical yield. In other words, both areal and volumetric productivities of lipids, carbohydrates and proteins in the ORP were substantially lower than the photobioreactors due to the lower biomass accumulation in the open ponds. The lower biomass productivity of the ORP is most likely due to culture depth. Culture depth is of critical significance in the design of microalgal cultivation systems as shorter depth tends to achieve higher productivity as a result of mutual shading of cells which increases

dark zones (non-photosynthetic zones) for individual cells yielding significantly lower biomass productivity. Under these conditions, respiration takes precedence over photosynthesis, thus reducing the culture growth and net productivity, especially when cells are in the dark zone (Yang et al. 2016). The 20 cm optical depth of the ORP was twice that of flat plate photobioreactors which leads to a much greater volume of the culture residing within the non-photosynthetic dark zone.

Flat plate photobioreactors are generally known to attain higher biomass productivities than ORPs because of their large surface area to volume ratio, shorter light path, and better mixing. In these circumstances, algal photosynthesis is improved and thereby increasing photosynthetic rates and productivity (Vree et al. 2015). A similar finding has been reported for *Nannochloropsis* sp. CCAP 211/78 cultivated in flat panel photobioreactors (optical pathlength of 2 cm) and raceway ponds (optical path of 20 cm) (Vree et al. 2015). These results show that building photobioreactors with spectrally selective infrared reflecting materials does not lead to the alteration of total lipid, protein, or carbohydrate levels, or their productivities, in *Nannochloropsis* sp.

To investigate the impact of the different cultivation strategies on the fatty acid composition of *Nannochloropsis* sp., total lipids were derivatized to their individual FAMEs. No significant shift in major saturated fatty acid components was observed among the cultivation systems, with palmitic acid (16:0) constituting the most abundant fatty acid: consistent with other work on *Nannochlorposis* reported in the open literature (Suzuki et al. 2019). There was no variation in the presence or amount of major fatty acids between the conventional cooling system (PEC) and IGP photobioreactors, a result that is consistent with that for the total lipid content. Generally, C14:0, C15:0, C16:1, C18:0, and C18:2n-6 were similar in all cultivation systems. Within the saturated fatty acid family, only C12:0 (lauric

acid) is significantly higher in the raceway pond than photobioreactors. However, C20:0 (eicosanoic acid) was considerably higher in the ORP, IRF and IGP than the PEC photobioreactor. The saturated and monounsaturated fatty acids were higher in the photobioreactors than in the raceway pond.

Only a handful of microalgal species have the requisite metabolic pathways to synthesize long-chain (>C18) PUFAs such as α-linolenic acid (ALA, C18:3n-3), ϒ-linolenic acid (GLA, C18:3n-6), arachidonic acid (AA, C20:4n-6), eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) (Li et al. 2019). Certain strains of *Nannochloropsis* spp. are potentially valuable biofactories for GLA, ARA and EPA production. Our results on fatty acid analysis are in agreement with the report by Ma et al. (2014), who identified GLA, ARA and EPA production in different *Nannochloropsis* spp. Previously, Wen and Chen (2003) have indicated that higher PUFA concentrations are related to stress caused by lower than optimal culture temperature, although they do note a report on *Porphyridium purpureum* that suggests, for that alga, growth at optimal temperature resulted in biomass with the highest EPA content. Data from this work is somewhat equivocal on the effect of temperature on the accumulation of PUFA vs. SFA vs. MUFA. The ORP certainly has the lowest maximum temperature and the highest PUFA content (31% of total fatty acids) but there doesn't appear to be a statistically significant difference between the ORP or the photobioreactors in this regard. Admittedly we haven't explicitly tested sub-optimal culturing temperatures within the photobioreactors and this is something that could be looked at in future. While the maximum temperature experienced by the alga in PEC and IGP photobioreactors was the same, total PUFA content in the IGP was 10% higher than in the PEC. Similarly, EPA and ARA content in the IGP system were significantly higher than in the PEC, probably due to reduced temperature variation in the former. However, all LC-PUFAs identified in this alga

were higher in the raceway pond than in the photobioreactors, though EPA content in ORP vs. IGP was similar.

The fatty acid identification results show that *Nannochloropsis* sp. MUR 267 produces a considerable amount of EPA, up to 16% of the total fatty acids. The proportion of EPA produced by this alga is consistent with previous reports for *Nannochloropsis* sp. (Li et al. 2019; Ma et al. 2014; Cai et al. 2013) and 5% (of total fatty acids) higher than that reported for *Tetraselmis chuii* and *Koliella artarctica* (Suzuki et al. 2019; Lang et al. 2011). The biosynthetic pathway for EPA production usually occurs either through ω-3 or ω-6 route in photosynthetic microorganisms (Guschina 2006). The ω-6 biosynthetic route is the primary metabolic pathway of EPA synthesis in eustigmatophytes such as *Nannochloropsis* sp. (Shene et al. 2016) and the presence of arachidonic acid (ARA) is a pointer to this pathway in MUR 267.

While the ORP appears to be a better cultivation system to produce LC-PUFAs than photobioreactors, its low biomass productivity presents a severe limitation for large scale exploitation. Moreover, the ORP consumes a large amount of landmass, supports the growth of extremophiles because of susceptibility to contamination, and rarely guarantees sustained year round biomass production in many regions of the world as a result of unstable weather conditions (e.g., high rainfalls in winter). Closed flat panel photobioreactors overcome this challenge of open pond microalgal production and the IGP photobioreactor used in this study possesses compelling attributes critical to advancing microalgae cultivation technology. However, closed photobioreactors are liable to overheating, thus, it requires sufficient and efficient cooling technologies to maintain high productivity rates.

Passive evaporative cooling systems utilizing a freshwater spray on the surface of the photobioreactor is the most widely used strategy to manage high culture temperatures.

Despite their reasonable efficiencies in thermoregulation, cooling systems increase the complexity, capital, and operational costs of large-scale photobioreactors. Hence, these strategies are currently economically and environmentally challenging due to their large energy and water requirements. For instance, to maintain the culture temperature of a column photobioreactor plant situated in Merced, California at the optimal temperature for most commercial microalgal species (25 ˚C), at least 18,000 GJ ha^{-1} yr^{-1} of heat energy must be evacuated, assuming a density of one photobioreactor per m^2 (Bechet et al. 2010). Providing cooling power of this degree by the evaporative cooling mechanism entails the consumption of 8,000 m^3 of quality freshwater, with a substantial increase in capital and operational costs, and consequently, a significant environmental footprint. The use of mineral-laden water for the cooling operation is usually not a choice due to the build-up of dissolved solids on the photobioreactor illuminated surface, resulting in light limitation for the culture and a need for a regular cleaning regime. For a flat panel photobioreactor inclined at 32˚ and located at Murdoch in Western Australia, 17.6 MJ of heat energy per square metre of reactor per day (based on 55 ˚C maximum temperature in a control photobioreactor with no cooling system) must be evacuated to keep the culture at 25 ˚C. To maintain this culture temperature by an evaporative cooling system with(out) recycling of the freshwater, approximately 20 (5,200) L m^{-2} of photobioreactor day^{-1} of high-quality water was required (Nwoba et al. 2020b). Applying IRF and IGP technologies to enclosed photobioreactors (e.g., flat plates) offers the potential for significant savings on the freshwater and energy consumption associated with photobioreactor cooling.

Our previous studies (Nwoba et al. 2020b; Nwoba et al. 2019b) have established that the use of IR reflective materials on the illumination surfaces of photobioreactors, to limit the input of heat-inducing wavelengths to the culture, eliminates a substantial quantity of

solar-derived heat energy. The result of removing that injection of heat into the culture medium was that the IGP photobioreactor sustained the growth of *Nannochloropsis* sp. keeping the culture temperature within the optimum range with no loss of biomass productivity. This work in this study now extends that to show that the IGP photobioreactor design provides culture conditions that have no deleterious effects on the synthesis of high-value products such as EPA. Hence, photobioreactors integrated with spectrally-selective technologies perform just as well as the freshwater-based cooling and have additional advantages such as limiting daily temperature fluctuation, which is good for stability in macromolecular composition. Not having to rely on fresh clean water for cooling has obvious environmental benefits, particularly as many areas suitable for outdoor microalgal production also have limited freshwater resources.

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Link to the next chapter

The reliability of culture is a critical requirement for meeting the economic viability and large-scale demand of microalgal biomass, and it is often more significant than high productivity. The operation of solar closed photobioreactors under outdoor scenarios will require energy-intensive cooling (during summer) and heating (during winter) technologies for guaranteed production of biomass (products) throughout the year. Heating and cooling are expensive and require both grid electricity and precious freshwater (already limited) for their effectiveness, thus imposing a sustainability challenge. The reliability of culture in an insulated-glazed flat panel photobioreactor integrated with CdTe photovoltaic cell with no cooling and heating was investigated during austral winter using a cold-intolerant microalga, *A. platensis*. Its growth performance and photophysiological response in the novel photobioreactor was compared to other cultivation systems with varying degrees of heating.

Chapter 5

Reliability of microalgal culture in photovoltaic photobioreactor

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Outdoor phycocyanin production in a standalone thermally-insulated photobioreactor

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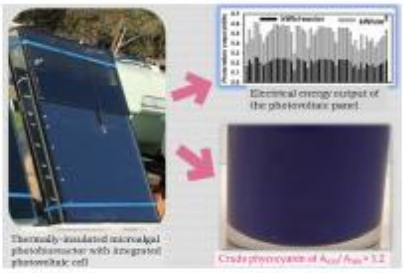
GRAPHICAL ABSTRACT



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ABSTRACT

The operation of solar microalgal photobioreactors requires sufficient cooling and heating to maintain reliable high productivity year-round. These operations are energy-intensive and expensive. Growth characteristics and phycocyanin production of *Arthrospira platensis* were investigated during the austral winter using a thermally-insulated photobioreactor with photovoltaic panel integration for electricity generation. This was compared with a control photobioreactor under a cycle of heating (13-hour night) and thermostat-regulated cooling, and continuously heated raceway pond. Average temperature in the photovoltaic photobioreactor (21.0 ± 0.03 °C) was similar to that in the heated control. Biomass productivity of *Arthrospira* in the novel photobioreactor was 67% higher than in the raceway pond but significantly lower than the control. Phycocyanin productivity (16.3 ± 1.43 $mgg^{-1}d^{-1}$ and purity (1.2 ± 0.03) showed no variation between photobioreactors but was significantly lower in the raceway pond. Electrical energy output of the photovoltaic photobioreactor exceeded mixing energy needs by 75%. These results indicate that the novel photobioreactor offers a reliable, energy-efficient platform for large-scale production of high-value chemicals from microalgae.

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Author contribution

Contributor	Statement of contribution
Emeka G. Nwoba (70%)	Methodology, Investigation, Formal analysis, Validation, Writing – original draft, Writing – review and editing.
David A. Parlevliet	Conceptualization, Formal analysis, Resources, Methodology, Validation, Supervision, Writing – review and editing.
Damian W. Laird	Conceptualization, Resources, Supervision, Writing – review and editing.
Kamal Alameh	Conceptualization, Resources, Methodology, Validation, Supervision, Writing – review and editing.
Navid R. Moheimani	Conceptualization, Resources, Methodology, Supervision, Writing – review and editing.

Principal supervisor confirmation

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

Name	Signature	Date
David A. Parlevliet		**8/12/2020**

5.1 Abstract

The operation of solar microalgal photobioreactors requires sufficient cooling and heating to maintain reliable high productivity year-round. These operations are energy-intensive and expensive. Growth characteristics and phycocyanin production of *Arthrospira platensis* were investigated during the austral winter using a thermally-insulated photobioreactor with photovoltaic panel integration for electricity generation. This was compared with a control photobioreactor under a cycle of heating (13-hour night) and thermostat-regulated cooling, and continuously heated raceway pond. Average temperature in the photovoltaic photobioreactor (21.0±0.03˚C) was similar to that in the heated control. Biomass productivity of *Arthrospira* in the novel photobioreactor was 67% higher than in the raceway pond but significantly lower than the control. Phycocyanin productivity (16.3±1.43 $mgg^{-1}d^{-1}$ and purity (1.2±0.03) showed no variation between photobioreactors but was significantly lower in the raceway pond. Electrical energy output of the photovoltaic photobioreactor exceeded mixing energy needs by 75%. These results indicate that the novel photobioreactor offers a reliable, energy-efficient platform for large-scale production of high-value chemicals from microalgae.

5.2 Introduction

Microalgae are increasingly recognized as novel farmable bioresources suitable for the production of many naturally-derived biochemicals. *Arthrospira platensis* (*A. platensis*, marketed as *Spirulina*) is acknowledged as one of the most economically exploitable microalgal species and is commercially cultivated on a large-scale with strong applications in the nutraceutical, medical, cosmetic, aquaculture, and animal feed industries (Xie et al., 2015). Due to its high protein content, this microalga has been used as edible food and food additive (Borowitzka, 2018a) for several decades. It has even been successfully tested as an auto-regenerative biological life support system for astronauts in space (the famous MELiSSA – Micro-Ecological Life Support System Alternative – project) where O_2 and edible foods (*Spirulina* juice and cakes) are recovered from the metabolic waste products (CO_2, urine, faeces) generated by the crew. For these reasons, *A. platensis* has massive economic value, and increasing the efficiency of production is of great biotechnological interest globally.

While microalgae can be readily exploited as a foodstuff, in recent years, there has been great interest in extracting and utilizing high-value pigments from this biological resource (Borowitzka, 2018a). Of particular interest is the essential luminescent protein biomolecule, C-phycocyanin, a high-value biopigment that is exclusively found in cyanobacteria and known for its potency as an antioxidative, anticarcinogenic, antiviral, therapeutic, and fluorescent agent as well as a natural blue food colorant (Nwoba et al., 2020a; Nwoba et al., 2019b).

At a global level, commercial-scale technologies for mass production of *Arthrospira* are currently dominated by classical open raceway ponds. Fundamentally, these ponds,

which typically have a land area in the range of 0.1 – 5 ha and optical depths of 0.15 – 0.3 m, are driven by low-cost paddle wheels, making them inexpensive to build and easily scalable (Borowitzka and Moheimani, 2012). Despite their utility in algal cultivation, classical raceways have significant drawbacks and have almost reached their technical limits for further improvement. Among these weaknesses is the inability to regulate environmental and operating variables, which makes sustained year-round production difficult and limits use in many regions of the world. These limitations have triggered the development of closed photobioreactors. Using closed photobioreactor results in better operational control of culture conditions that allows opportunities for the production of biomass with a more stable macromolecular composition and higher annual biomass productivity (Carlozzi, 2003). As such, the use of enclosed photobioreactors in the production of *Spirulina* biomass for food (in the context of 'farm to fork'), feed, and pharmaceutical applications is compelling due to culture reliability and guaranteed supply of biomass.

However, photobioreactors are expensive to build, are energy-inefficient, and prone to overheating of the culture, particularly when used in outdoor cultivation. If the true potential of mass microalgal cultivation is to be realized, the efficient conversion of free solar radiation to biomass and desired biochemical products is essential. In this context, temperature and thermoregulation of algal cultures in photobioreactors are critical challenges for solar microalgal farming (Hindersin et al., 2014; Pruvost et al., 2019; Pruvost et al., 2016; Sforza et al., 2015; Wondraczek et al., 2015).

Each species/strain of microalgae has a specific optimal temperature window for growth, and hence bioproductivity, generally in the range 10 – 35 ˚C (Chaumont, 1993; Ras et al., 2013). In real-world outdoor operating scenarios, such temperature ranges are easily surpassed in summer (even in temperate regions) with cultures reaching sustained

temperatures that can induce cell mortality, thus, requiring efficient cooling of photobioreactors. In temperate climates, most especially in winter, extremely subnormal temperatures can lead to loss of growth stimulation and bioproductivity, and this is strongly the case for thermo-tolerant microalgal species, such as *A. platensis*. In this context, culture heating could be beneficial. For these reasons, sustained year-round production in closed microalgal systems can only be achieved by thermal regulation involving both heating and cooling. However, the use of cooling and heating systems to manage culture temperature in photobioreactors have significant energy and environmental costs. Thus, low-energy use, low-cost, and year-round practicable solutions are critical factors for the thermoregulation of photobioreactors.

A solution to these biological, engineering, and economic constraints is the development of thermally-insulated photobioreactors based on passive temperature control and integration with photovoltaic panels for electrical energy generation (Nwoba et al., 2019a, Nwoba et al., 2020b). The combination of these two approaches presents numerous advantages for the thermal regulation of a photobioreactor and may allow standalone application (Nwoba et al., 2020b, 2020c). A double-glazed photobioreactor with low-emissivity film embedded in the illumination surfaces can filter incoming sunlight by reflecting heat-inducing infrared radiation from the system and transmitting photosynthetically-beneficial visible photons to the culture. As a result, the system warms up slowly during the day and minimises heat dissipation at night, thereby reducing diel temperature fluctuations. Incorporating an integrated photovoltaic panel into the bioreactor design supplies local electricity for grid-independent operation. The combination of passive temperature control and photovoltaic energy generation forms a crucial design criterion for

conjoint production of the desired chemical product, e.g., high-value pigment, and electrical energy for operational need from a single photobioreactor.

The overarching aim of this study was to investigate the biological performance of *A. platensis* culture in a thermally-insulated flat plate photobioreactor with integrated photovoltaic panel under the outdoor climatic conditions of Perth, Australia during winter. The biomass productivity, phycocyanin production, and photophysiological performance of the culture in this system were compared with the performances of a photobioreactor under a cycle of passive evaporative cooling in the day and heating at night, and a raceway pond that required 24-hour heating to maintain the culture in the optimal growth temperature range.

5.3 Materials and methods

5.3.1. Microalgal strain and culture medium

The alkaline- and thermo-tolerant cyanobacterium, *Arthrospira platensis* (*A. platensis* MUR 126), was sourced from the culture collection of the Algae Research and Development Centre, Murdoch University, Australia. *A. platensis* culture was first grown indoors from a culture maintained in the laboratory in 50 mL flasks. This was scaled up through successive volumes to 20 L in carboys using a sterilized Zarrouk medium (Zarrouk, 1966), in which the pH was increased to 9 using 1N NaOH. At this point, the culture was taken outdoors and cultivated in a fresh tapwater-based unsterilized Zarrouk medium in flat-plate photobioreactors to raise the inoculum for the main experiment. In practice, *Arthrospira* grows in a highly alkaline (400 meq L^{-1}, pH 11) culture medium and an optimum temperature of 35 – 38 ˚C (Borowitzka, 2018a). These relatively extreme culture conditions result in little to no contamination issues during mass outdoor cultivation of this alga. During growth, no

other photosynthetic microorganisms, including diatoms, chlorophytes and even *Oocystis* were observed under microscopic evaluation of the inoculum. *A. platensis* is a heat-tolerant, cold-intolerant microalga, and loss of growth happens at temperatures < 15 ˚C (Borowitzka, 2018a); hence photobioreactors were heated at night to ensure cell viability. The main experiment was run for three months (April – June) of austral winter (Perth).

5.3.2. Cultivation systems design

The cultivation systems used in this work comprised flat plate photobioreactors and a raceway pond located at the experimental facility of Algae Research and Development Centre, Murdoch University, Western Australia, Australia (Fig. 5–1). The closed flat plate photobioreactors, each with 10 cm optical depth, consisted of the following: (a) a custom-designed insulated glazed photovoltaic (IGP) photobioreactor with double glass panes (5 mm x 120 cm (length) x 150 cm (height)) sealed with an airspace (to provide high thermal insulation) and coupled to a photovoltaic panel. The illumination surfaces (front and side panels) had a spectrally-selective low-emissivity thin-film applied that transmits at least 70 % of photosynthetically-active wavelengths (400 – 700 nm) and reflects more than 90 % of infrared and ultraviolet radiation. The low-e film was made of seven layers including two Ag layers and five dielectric – TiO_2 and ZnO. A semi-transparent CdTe photovoltaic panel (120 cm x 60 cm) was glued to the top of the reactor, 90 cm above the base. The CdTe solar film was made of four main layers consisting of ITO, CdS, CdTe, and Al, which transmits 40 % of the captured solar energy into the culture system (Nwoba et al. 2020c) and converts the remainder to electrical energy, (b) a single glazed infrared reflecting film (IRF) photobioreactor constructed of clear float glass in clear acidic cure silicone. The base and sides, and front and back panels of this system were built with 19 mm and 12 mm thick glass, respectively, and the dimensions of the reactor were 126 cm by 125 cm (L x H). The front and

side panels were laminated with the low-e film (made of seven layers including two Ag layers and five dielectric – TiO_2 and ZnO) with 70 % and 10 % transmittances of visible light and infrared radiation, respectively, (c) a control single glazed flat-plate photobioreactor without a low-emissivity film and with a thermostat-based passive evaporative cooling (PEC) system operating during the day. The dimensions of the PEC reactor were exactly the same as that of the IRF photobioreactor. Further details on the photobioreactor design and spectral characteristics of light transmitted by each configuration can be found in Nwoba et al. (2020b, 2020c), (d) a paddle wheel-driven open raceway pond (ORP). This was made of fiberglass and was 20 cm x 50 cm x 30 cm (length x width x depth) (Raes et al., 2014).

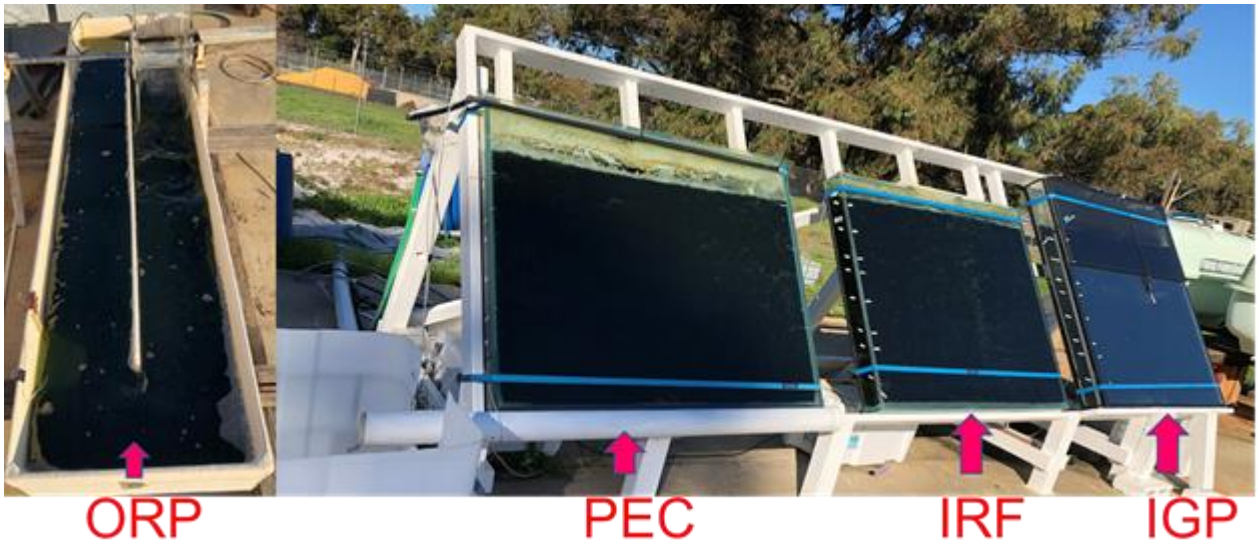

Fig. 5–1. Microalgae cultivation systems in operation at the experimental facility of Algae R&D Centre, Murdoch University, Australia. Insulated-glazed photovoltaic (IGP), infrared reflecting film (IRF), passive evaporative cooling (PEC) flat-plate photobioreactors, and open raceway pond (ORP). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

5.3.3. Cultivation system operation and conditions

All cultivation systems had the same culture volume (140 L) and orientation (north-south). The PEC photobioreactor, with a freshwater based cooling system, had a semi-buried reservoir tank (working capacity of 1000 L) containing a submersible sump pump (10 m^3 h^{-1}, Aquapro) that pumped water through a polyvinyl chloride pipe fitted with a solenoid valve to sprinklers fixed above the illumination surface. A thermostat activated and deactivated the cooling system when the culture reached a temperature set point of 37±2 ˚C. Cooling water was recycled through a gutter provided at the base of the reactor. Heating of the culture at night (19:00 – 08:00) was supplied in the PEC (300 W aquarium heater, set at 30 ˚C) and IRF (50 W aquarium heater, set at 30 ˚C) photobioreactors. No heating was employed for the thermally-insulated IGP system. The ORP was maintained under 24-hour continuous heating (305 W aquarium heater, set at 30 ˚C).

Mixing in the ORP (22 cm s^{-1}) was achieved using a paddle wheel system. For the closed photobioreactors, mixing was provided by air bubbling, delivered using a PondOne O2 Plus 8000 air pump (4,200 L h^{-1}) through silicone tubes to 120 cm long rectangular ceramic diffusers fitted at the base of the reactors. The air was filter-sterilized, and a uniform airflow pressure regulated by flowmeter. Photobioreactors were tilted at 32˚ to the vertical with the illumination surface facing north to maximize solar radiation capture (Nwoba et al., 2019a).

A. platensis was cultivated in the culture systems for two weeks to ensure acclimatization to each condition and operated as a batch for a further two weeks. At this point, a semi-continuous harvest system was implemented at a culture residence time of eight days for six periodic harvests. The harvesting frequency and ratio were based on the specific growth rate determined at the late-exponential phase of the batch culture. At each harvest, the standing biomass in the culture systems was similar (returned to similar biomass

density) and harvested volume replaced with the same quantity of fresh Zarrouk medium. The harvested biomass was used for growth measurements. The culture temperature profiles were tracked uninterruptedly at five-minute intervals using an underwater temperature logger (Pendant Onset Hobo, USA). A 10-minute recorded data of solar radiation, air temperature, and rainfall over the cultivation period were sourced from the Murdoch University Weather Station (wwwmet.murdoch.edu.au). Addition of fresh tapwater to counter evaporative loss was required for the ORP only.

5.3.4 Hydrodynamics

The hydrodynamic parameters (mixing time, gas holdup, superficial velocity, and Reynolds number) of the photobioreactors were measured at the optimal airflow rate. The flow rate was varied daily, and the *Arthrospira* trichomes (cell filaments) were observed microscopically for damage after 24 hours. The optimal airflow rate, which was determined experimentally, was the flow rate that produced minimal shear to the filaments with no settling of cells or dead zones in the reactors.

5.3.4.1 Mixing time

The mixing time, which is the time required to achieve 95 % of complete mixing of liquid, was determined by the acid tracer pulse-response method using 35 % HCl (Camacho et al., 2000; Contreras et al., 1998). The photobioreactor was filled with tap water to the required operational volume of 140 L, followed by air bubbling for 30 min to expel carbonates, especially CO_2. A 50 mL aliquot of the acid solution was injected at the center of the reactor, and the change in pH was monitored (pH electrode positioned at the center) using a digital pH meter (SevenGo Duo Pro, Mettler Toledo). The time taken for the pH of the liquid to stabilize (attain a constant value) was recorded, and the mean of at least four

measurements was obtained. The mixing time was determined only in the biphasic system consisting of air and tap water, and not in the triphasic system of air, culture medium, and *A. platensis* cells to avoid negative impact on the microalgal cells.

5.3.4.2 Gas holdup

Gas holdup refers to the fractional volume of the dissolved gas phase. The volumetric expansion method (Reyna-Velarde et al., 2010) was used to determine the culture gas holdup (Eq. (5–1))

$$Gas\ holdup = (H_G - H_L)/H_G \quad (5–1)$$

where, H_L and H_G were the free-surface of liquid (before aeration) and liquid+gas heights (m) in the photobioreactor, respectively.

5.3.4.3 Superficial gas velocity

Superficial gas velocity (U_G, m s^{-1}) was derived from the product of airflow rate (volume of air per volume of liquid per second) and total culture volume (m^3) divided by the cross-section area of the aerated zone (m^2) (Hernández-Melchor et al., 2017).

5.3.4.4 Reynolds number

Reynolds number (Re) for rectangular photobioreactors (non-circular) was calculated from Eq. (5–2) as described in Hernández-Melchor et al. (2017).

$$Re = [\rho v(4R)]/\mu \quad (5–2)$$

where ρ was the liquid density (kg m^{-3}); v was the liquid average velocity (m s^{-1}); μ was the dynamic liquid viscosity (kg m^{-1} s^{-1}); R was the hydraulic radius obtained from Eq. (5–3)

$$R = La/(2L + 2a) \quad (5–3)$$

where L was the length (m) and a was the width (m) of the photobioreactor.

5.3.4.5 Volumetric power input

The volumetric power input (P/V, W m^{-3}) as a result of the sparging was determined as the product of the culture density (1008.9 kg m^{-3}) (Reyna-Velarde et al., 2010), the acceleration due to gravity (m s^{-2}), and the superficial gas velocity (m s^{-1}) in the aeration zone (Sierra et al., 2008) (Eq. (5–4)). The rheological characteristics of the culture were assumed to be the same as of water (Reyna-Velarde et al., 2010).

$$P/V = \rho g U_G/(1 + (A_d/A_r)) \quad (5–4)$$

where ρ is the liquid density (kg m^{-3}); g is the acceleration due to gravity (m s^{-2}); U_G is the superficial gas velocity (m s^{-1}); A_d is the cross-sectional area of downcomer (m^2); A_r is the cross-sectional area of the riser (m^2).

5.3.5 Growth kinetics and measurements

Biomass dry weight (expressed as ash-free dry weight, AFDW) was determined by filtering 5 mL of culture through a pre-combusted, pre-weighed glass fibre filter (Whatman GF-C, diameter 2.5 cm, pore size 0.45 µm). The filter with concentrated algal biomass was dried overnight in an oven set at 90 ˚C, cooled in a desiccator, weighed, dried in a furnace at 450 ˚C overnight, and cooled in the desiccator before a final mass was recorded. The biomass density (g L^{-1} AFDW) was determined gravimetrically, and the specific growth rate calculated from the biomass density taken at different times (Moheimani et al., 2013). *Arthrospira* growth was modeled using a first-order equation (Eq. (5–5) and (5–6)). The volumetric and ground areal biomass productivities of *A. platensis* in the cultivation systems were calculated using Eqs. (5–7) and (5–8), respectively (Vree et al., 2015)

$$\delta x/\delta t = \mu X \quad (5–5)$$

$$\mu = \ln(X_f/X_i)/t \quad (5–6)$$

$$P_v = (X_f - X_i)/t \quad (5\text{–}7)$$

$$P_g = (P_v\, x\, V)/A \quad (5\text{–}8)$$

where μ is the specific growth rate (day^{-1}); X_f is the final biomass density at harvest ($g\ L^{-1}$); X_i is the initial biomass density ($g\ L^{-1}$); t is the cultivation duration (days); P_V is the volumetric biomass productivity ($g\ L^{-1}\ d^{-1}$); P_g is the ground areal productivity ($g\ m^{-2}\ d^{-1}$); V is the cultivation system volume (L); and A is the occupied footprint area of the cultivation system (m^2).

5.3.6 Chlorophyll *a*, C-phycocyanin, total carotenoids, and total proteins measurements

Chlorophyll *a* and total carotenoids were extracted by homogenizing samples in 90 % ice-chilled acetone spiked with a pinch of $MgCO_3$ under dim light based on the methodology of Jeffrey and Humphrey (1975). Cell debris was pelleted by centrifugation at 4000 x g for 10 min, and the absorbance of the supernatants measured at 452 nm, 647 nm, and 664 nm wavelengths using a UV-VIS spectrophotometer (Biomates 3S). The concentrations of chlorophyll *a* and total carotenoids in the extracted pigment samples were calculated as presented in Moheimani et al. (2013).

The C-phycocyanin content of the algal biomass was measured as reported in Bennett and Bogorad (1973) using repeated freezing and thawing in 0.1 M sodium phosphate buffer (pH 6.8). Freshly harvested cell mass (5 mL) was rinsed in deionized water and suspended in the buffer. The suspension was treated to four cycles of freezing (-60 ˚C) and defrosting (to room temperature). The defrosted mixture was homogenized and centrifuged at 5000 rpm for 10 minutes. The concentration of C-phycocyanin (C-PC) in the supernatant

was measured spectrophotometrically at 615 nm and 652 nm and calculated based on Eq. (5–9). The C-PC purity was calculated as the ratio of the absorbances at 615 nm and 280 nm.

$$C - phycocyanin\ concentration\ (g/L) = (OD_{615} - 0.474\ x\ OD_{652})/5.34 \qquad (5–9)$$

The total protein content of cell biomass was determined using standard methods (Moheimani et al., 2013).

5.3.7 Measurement of photosynthesis

The effective quantum yield (F_q'/F_m'), an important indicator of the operating efficiency of photosystem II (PS II) photosynthetic competence, was measured, based on the pulse modulation principle, using a Microscopy-Pulse Amplitude Modulation (MC-PAM) fluorometer (Heinz Walz GmbH, Effeltrich, Germany). This fluorometer comprises a Zeiss AxioScope.A1 epifluorescence microscope (equipped with a modulated light emitting diode source and a photomultiplier to detect chlorophyll fluorescence), a PAM control unit and a desktop monitor equipped with the WinControl software package for instrument operation and fluorescence analysis (Kim et al., 2006). The MC-PAM was also fitted with a pinhole (0.2 mm) micro quantum sensor for the measurement of quantum flux density of the blue excitation light. Uniform PAM settings were ensured throughout the experimental duration. Thus, the measuring light frequency, saturating light intensity, saturating light width, and photomultiplier gain were set at 3, 10, 0.10, and 5, respectively. The MC-PAM uses a blue light-emitting diode lamp at 470 nm peak wavelength for measuring light, saturating, and actinic illuminations (Dijkman and Kromkamp, 2006). Chlorophyll *a* fluorescence was measured on a small portion of *A. platensis* cells by focusing the objective lens (200x) on an active circular diameter on a standard glass slide (7.6 x 2.6 cm) with a coverslip. A reasonable volume (200 µL) of the culture was used to ensure that the coverslip floated on the cells and did not apply pressure against the glass slide. Rapid light curves (RLCs) of photosynthetic

electron transport rates were obtained for samples immediately withdrawn from the culture systems at 10 s illumination time intervals, for actinic light irradiances from 126 to 1718 µmol photons m^{-2} s^{-1}. The RLCs were monitored diurnally with measurements before sunrise until after sunset. Fluorescence measurements on each replicate took only 45 s on each slide, after which a new slide was made. Three pseudo-replicates of samples from each treatment, with measurements lasting no longer than two minutes for treatments, were ensured for each sampling time. Correction of background fluorescence was also ensured by the measurement of fluorescence signals on the blank portion of the glass slide. The photosynthetic index, F_q'/F_m', was determined from the RLCs (Genty et al., 1989). The PSII maximum electron transport rates ($rETR_{max}$) was estimated from a waiting-in-line equation of electron transport rates (ETR) vs. irradiance (E) fitted using a non-linear least-squares model (Eq. (5–10)) (Ritchie and Bunthawin, 2010)

$$ETR = [(ETR_{max} . E)/E_{opt}]. e^{1-E/E_{opt}} \quad (5–10)$$

5.3.8 Statistical analysis

Statistical data analysis was carried out using repeated measures one-way analysis of variance (RM-ANOVA) to determine significant differences among treatments. All data were subjected to normality via Shapiro-Wilk and homoscedasticity tests. A threshold of $p < 0.05$ was deemed significant, and the post-hoc Holm-Sidak, multiple comparison test, was used to separate the means. All statistics were done using SigmaPlot v14.0, and the values reported were mean and standard error, mean ± std error.

5.4. Results and discussion

5.4.1 Climatic and culture conditions during outdoor cultivation of *A. platensis*

This study was carried out between 28 April and 19 June 2019 during the austral winter season at Murdoch University, Perth, Western Australia (-32°03'59.5" S, 115°50'6.3" E). Solar radiation and ambient air temperature profiles showed a time-dependent variation over the duration of the culturing event (Fig. 5–2). The solar radiation was mostly stable at 700 W m^{-2} from late April to late May. Strong fluctuation and reduction in solar intensity were observed in June due to an increase in cloud cover (Fig. 5–2A). Reduction in solar radiation corresponded to the time of maximum rainfall (07 – 15 June) during *Arthrospira* culture (Fig. 5–2B) but did not correspond to the lowest air temperature (2.1 ˚C on 19 May) (Fig. 5–2C).

The various culture systems responded in the direction of ambient temperature on 19 May (Fig. 5–(2D – G)), a function of the ambient temperature; however, no loss of culture viability was observed even though *A. platensis* is cold-intolerant (Borowitzka, 2018a). In this study, thermally-insulated IGP (not heated throughout the cultivation period) and heated (PEC–heated at night only, IRF–heated at night only, ORP–heated continuously) culture systems had minimum temperatures that were respectively 80 % and 69 – 75 % higher than the minimum air temperature. For 98 % of the cultivation days, the minimum culture temperatures in the PEC (control), IRF, IGP photobioreactors and ORP did not fall below 14 ˚C, 14 ˚C, 17 ˚C, and 15 ˚C, respectively. On the other hand, maximum culture temperatures ranged between 27.7 ˚C in ORP and 39.9 ˚C in the PEC photobioreactor. On average, culture temperatures were statistically different (One-way ANOVA, F = 2603, P = < 0.001) among cultivation systems, ranging between 17.9±0.03 ˚C in the raceway pond and 22.3±0.05 ˚C in

the control (Table 5–1). Despite the nocturnal heating cycles, the control photobioreactor displayed greater variability in culture temperature than the other cultivation systems (Fig. 5–2G). This variability is most likely the result of the photobioreactor design not having the insulative double glazing of the IGP nor the infrared blocking and reflecting capacity of the IRF. The control PEC design is the conventional closed flat plate photobioreactor that typically exhibits rapid increases and decreases in culture temperature in response to ambient conditions (de Jesus et al., 2018; Nwoba et al., 2020c). The ORP and the IGP photobioreactor showed the least variation in day and night temperatures. The IRF, which has the lowest heat application (50 W), was intermediate in culture temperature variations, demonstrating that the low-e film has both spectral selection and thermal insulation properties (Nwoba et al., 2020b; 2020c; Nwoba et al., 2019b). The culture temperature in the control is also the most extreme, with daytime temperatures routinely above 36 ˚C. The temperature optima for *A. platensis* is 35 – 38 ˚C, whereas the lowest temperature for growth is 15 – 20 ˚C (Borowitzka, 2018a; Richmond et al., 1993). The double-glazing of the IGP photobioreactor with an embedded low-emissivity film in the illumination surfaces filters incoming sunlight by reflecting heat-inducing infrared radiation from the system and transmitting photosynthetically-beneficial visible photons to the culture. As a result, the system warms up slowly during the day and minimises heat dissipation at night, thereby reducing diel temperature fluctuations and ensuring that the culture is in the optimal biomass productivity temperature range for longer. Periods outside the optimal temperature range are shorter, meaning the culture is less likely to be temperature stressed, thus improving survivability.

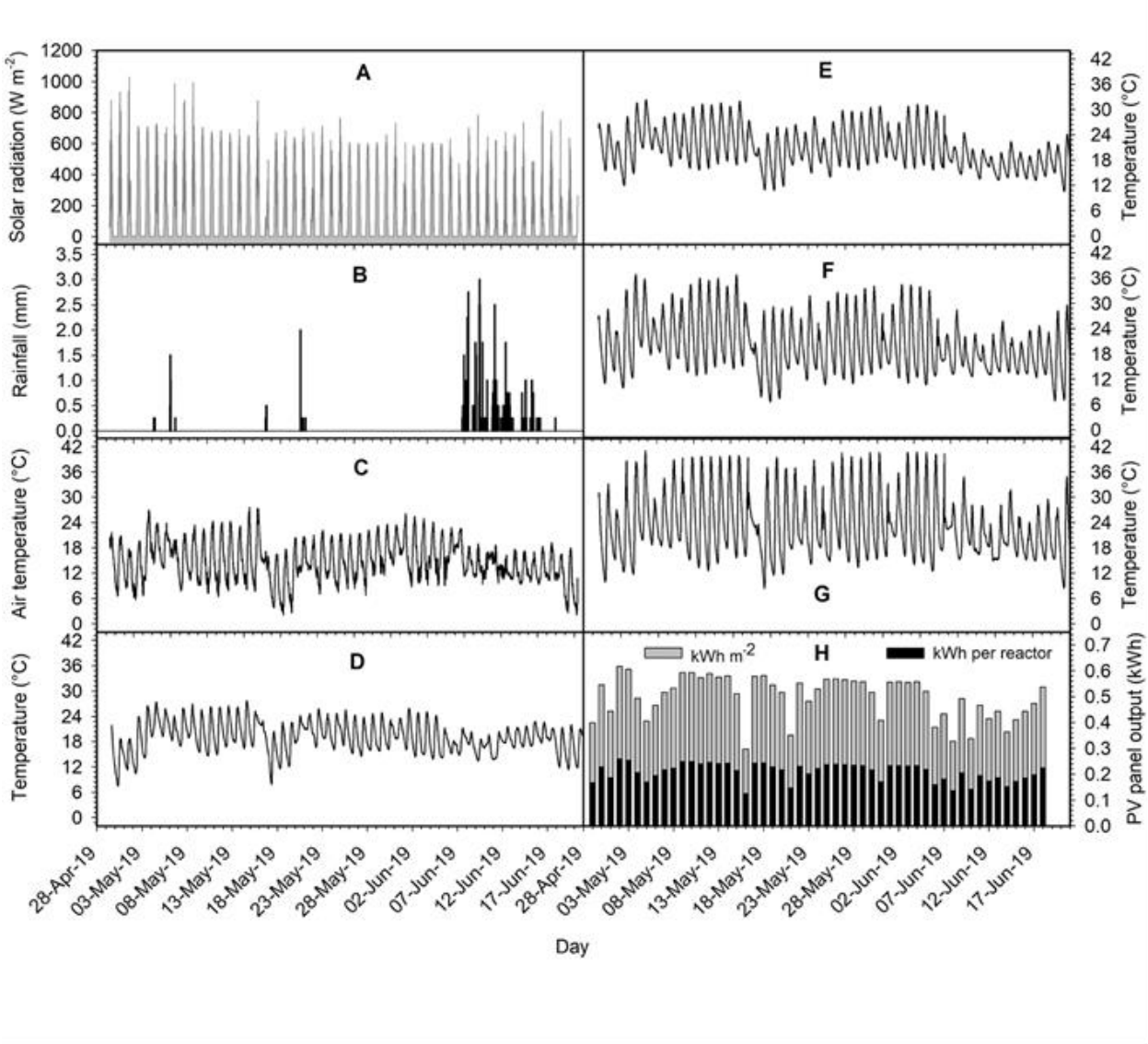

Fig. 5–2. Daily climatic and culture conditions. A) 10-min solar radiation, B) 10-min rainfall, C) 10-min air temperature, D) 5-min temperature in open raceway pond, E) 5-min temperature in thermally-insulated glazed photovoltaic reactor, F) 5-min temperature in infrared reflecting film photobioreactor, G) 5-min temperature in a passive evaporatively cooled photobioreactor, H) Photovoltaic panel output. D was under a 24-hour continuous heating condition; F and G culture systems were under 13-hour heating conditions starting from 19:00.

5.4.2 Biomass production of *A. platensis* in culture systems

Given the differences in the stability of the culture temperature in the photobioreactors, it might be expected that biomass production would be different in each of the cultivation systems. This was tested by analyzing the growth performance of *A. platensis* MUR 126 when operating in semi-continuous mode (Figs. 5–3, 5–4). The highest biomass yield (1.6 g L^{-1}) was obtained in the control photobioreactor while the lowest (0.9 g L^{-1}) was found in the continuously heated raceway pond (Fig. 5–(3C, D)). The instability in the biomass trend observed in the raceway pond (Fig. 5–3D) was due to exposure to unfavorable weather events such as rainfall, which effectively diluted the culture (Fig. 5–2B). The specific growth rate in the control PEC photobioreactor was statistically significantly higher (One-way RM ANOVA, $F_{3,15} = 11.2$, $P = 0.001$) than the IGP and IRF, which were statistically higher than the ORP (Table 5–1). *Arthrospira* productivity in thermally-insulated IGP photobioreactor was 32 % less than control and 67 % higher than the continuously heated ORP (Fig. 5–4, Table 5–1). The difference in productivities could be explained by the behavior of each system in response to climatic conditions during the day (Fig. 5–2), with thermally-insulated IGP showing a considerable reduction in solar energy input due to its spectral selection (Nwoba et al., 2020c; Nwoba et al., 2019b).

In winter, extended times of low temperature and solar radiation, as well as high rainfall, are common and, therefore, strongly affect microalgal growth performance under outdoor conditions. For instance, the metabolic activity of *Arthrospira* sp. LEB-18 was shown to be reduced at temperatures below 17 ˚C, with a consequential reduction in its growth (de Jesus et al., 2018). Similarly, García-López et al. (2020) reported that temperatures below 15 ˚C reduced the areal biomass density of *A. maxima* LJGR1 under outdoor conditions.

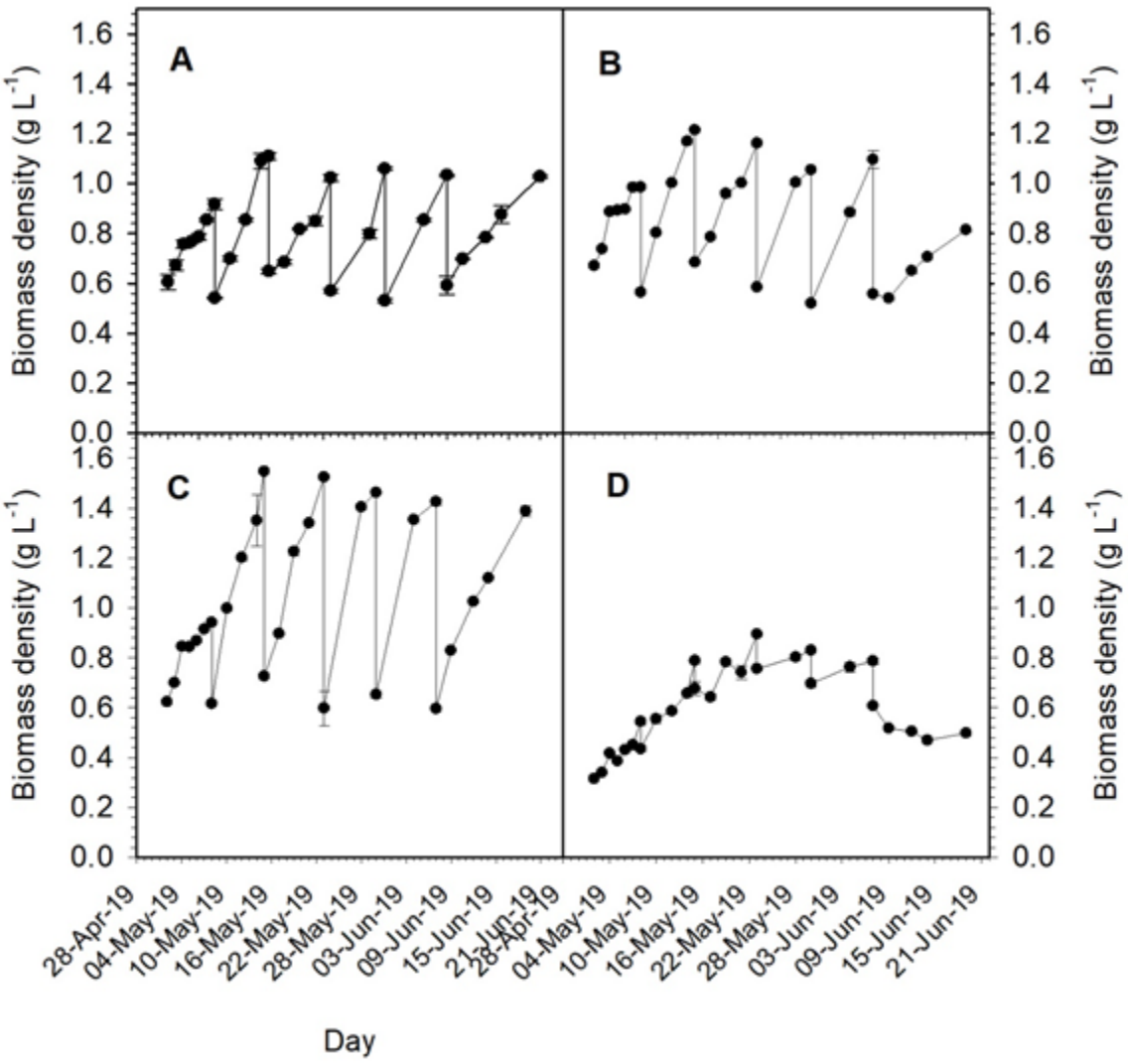

Fig. 5–3. Biomass density of *Arthrospira platensis* in cultivation systems during austral winter. A) thermally-insulated glazed photovoltaic IGP), B) infrared reflecting film (IRF), C) passive evaporative cooling (PEC) flat-plate photobioreactors, D) open raceway pond (ORP).

Therefore, large-scale production of *Arthrospira* requires long periods of sunshine and high temperature for optimal growth. This high-temperature requirement shows that the year-round production of this alga in many areas of the world is not feasible (Vonshak, 2014; Vonshak et al., 1982). For example, Earthrise Farms (California, USA) grows certain *Spirulina* sp. for seven months in a year due to low temperatures in winter. However, Hainan DIC

Microalgae (Hainan, China) operates for nearly 12 months over a year due to warmer tropical conditions (Borowitzka, 2018a). In the current study, attempts to cultivate *A. platensis* in conventional raceway pond and flat plate photobioreactors during this time of the year without heating the culture were unsuccessful (data not shown). This was solely due to cold weather during this time of the year. Although the productivity in the IGP with thermal insulation was lower than that in the PEC (under a cycle of cooling and heating), the thermal control measures required for the PEC are energy-intensive, expensive, and unsustainable (see Béchet et al., 2010; Nwoba et al., 2020b; 2020c). Interestingly, results here suggest that the average areal biomass productivity of the IGP photobioreactor (Fig. 5–4) is similar to typical productivity values (10.0 – 15.0 g m^{-2} d^{-1}) achievable in the summer operation of an ORP (Borowitzka, 2018a; García-López et al., 2020; Nwoba et al., 2019b). Considering that *Arthrospira* cannot be grown in ORP in the austral winter without a heating system (which introduces complications for cultivation at large-scale), and that previous work (Nwoba et al., 2020b; 2020c) has indicated that the same reactor can be used to cultivate in the summer with no additional cooling required, the IGP bioreactor could conceivably be used to cultivate *A. platensis* over a whole year. Hence, the thermally-insulated IGP photobioreactor is a viable and useful technology for reliable microalgae production.

5.4.3 Photochemical efficiency of *Arthrospira* cultures

The F_q'/F_m' ratio, a standardized parameter that provides a scientific evaluation of the instantaneous operating efficiency of photosystem II primary photochemistry (conversion of sunlight energy to biomass), was assessed diurnally (Fig. 5–5). This ratio trended with the same pattern for all systems; lowest in the morning, increased to a steady maximum value as the solar energy, ambient and culture temperatures increased (Fig. 5–5A) followed by a fallback, to levels observed at dawn as temperatures and solar energy diminished toward the

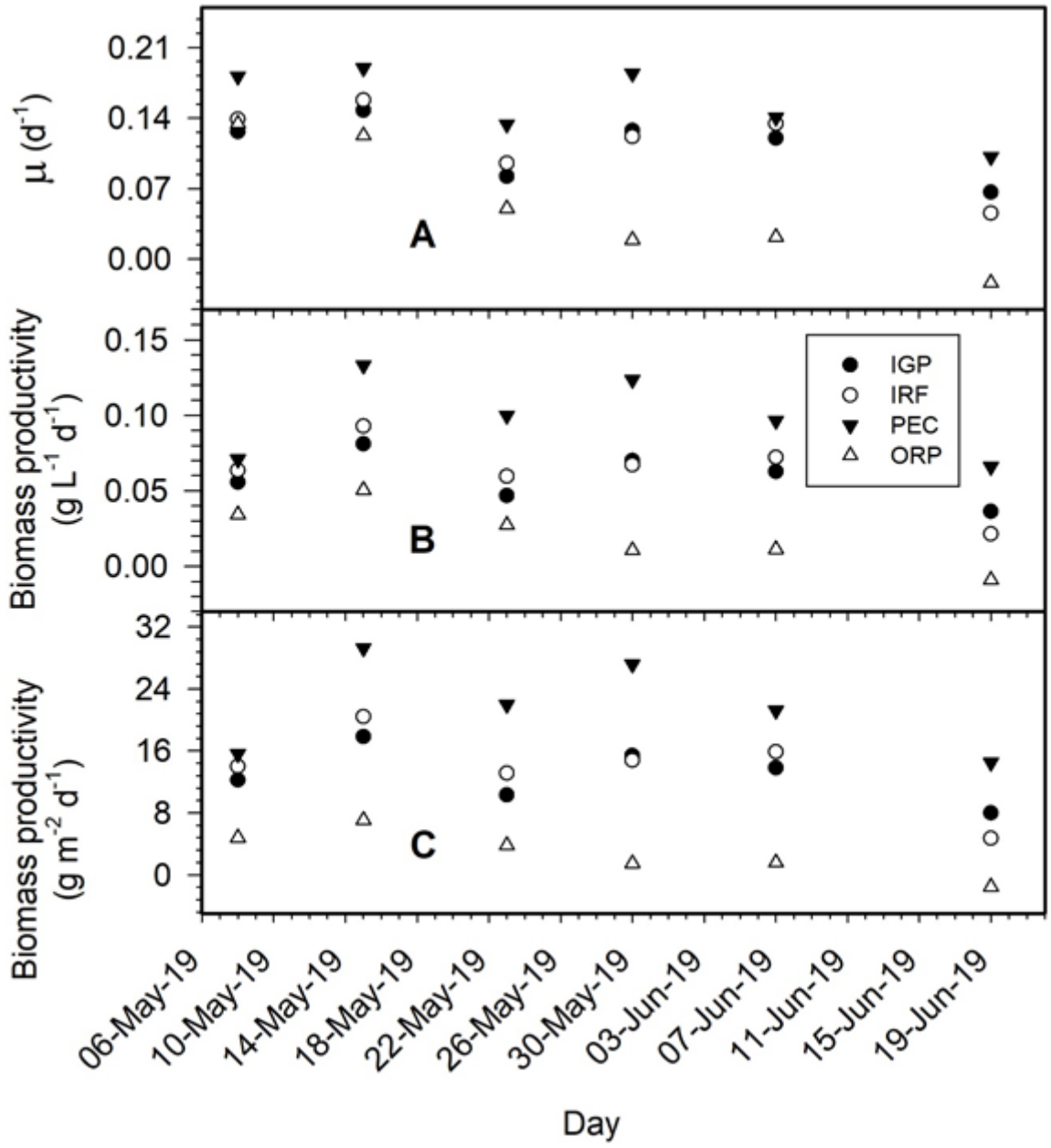

Fig. 5–4. Specific growth rate, volumetric, and ground areal biomass productivities of *A. platensis* in culture systems during austral winter.

end of the day.

The low F_q'/F_m' values at dawn synchronized with the time of minimum culture temperatures (Fig. 5–(5A, C)). Even though there was some diurnal variation, this was very limited and the F_q'/F_m' values were essentially constant for each treatment. In normal summer conditions, a drop in the effective quantum yield would suggest that mechanisms

for photoprotection or to avoid photodamage had been instigated by the alga (Borowitzka, 2018b). However, during this winter period, the irradiance and culture temperatures were considerably less than those that would tend to induce photoinhibition. There was some variation in the F_q'/F_m' between treatments with the ORP and IRF having the lowest value and the IGP the highest. However, none of these ratios for effective quantum yield were at levels that suggested stress in the cultures. Overall, the F_q'/F_m' values obtained here were comparable to the previous report for a healthy *Arthrospira* culture (Nwoba et al., 2019b; Zeng et al., 2012), and more than the 0.32 reported for outdoor cultured *Spirulina platensis* M2 under low temperature (25 ˚C and high O_2 concentration) by 24 – 35 % (Torzillo et al., 1998).

The maximum electron transport rates ($rETR_{max}$) showed no changes for the photobioreactors (Fig. 5–5B), probably as a result of the similar configuration of the cultivation systems, which allowed for maximum capture of solar radiation. In contrast, the $rETR_{max}$ value for ORP increased by 70 % from 20 µmolelectrons m^{-2} s^{-1} two hours after sunrise to 42 µmolelectrons m^{-2} s^{-1} and remained steady until sunset (Fig. 5–5B). The difference in this value between photobioreactors and ORP could be due to differences in reactor geometry (Fig. 5–1), with the former having a large surface area to volume ratio compared to the latter. Less variation in $rETR_{max}$ value is attributed to a balance between the electron transport processes and carbon reduction in *A. platensis* cell (Nwoba et al., 2019b). The relatively low value at the morning hour shows minimal light saturation and electron transport rates leading to the downregulation of enzyme activities (Szabó et al., 2014). However, values increased in response to increasing solar energy and culture temperatures.

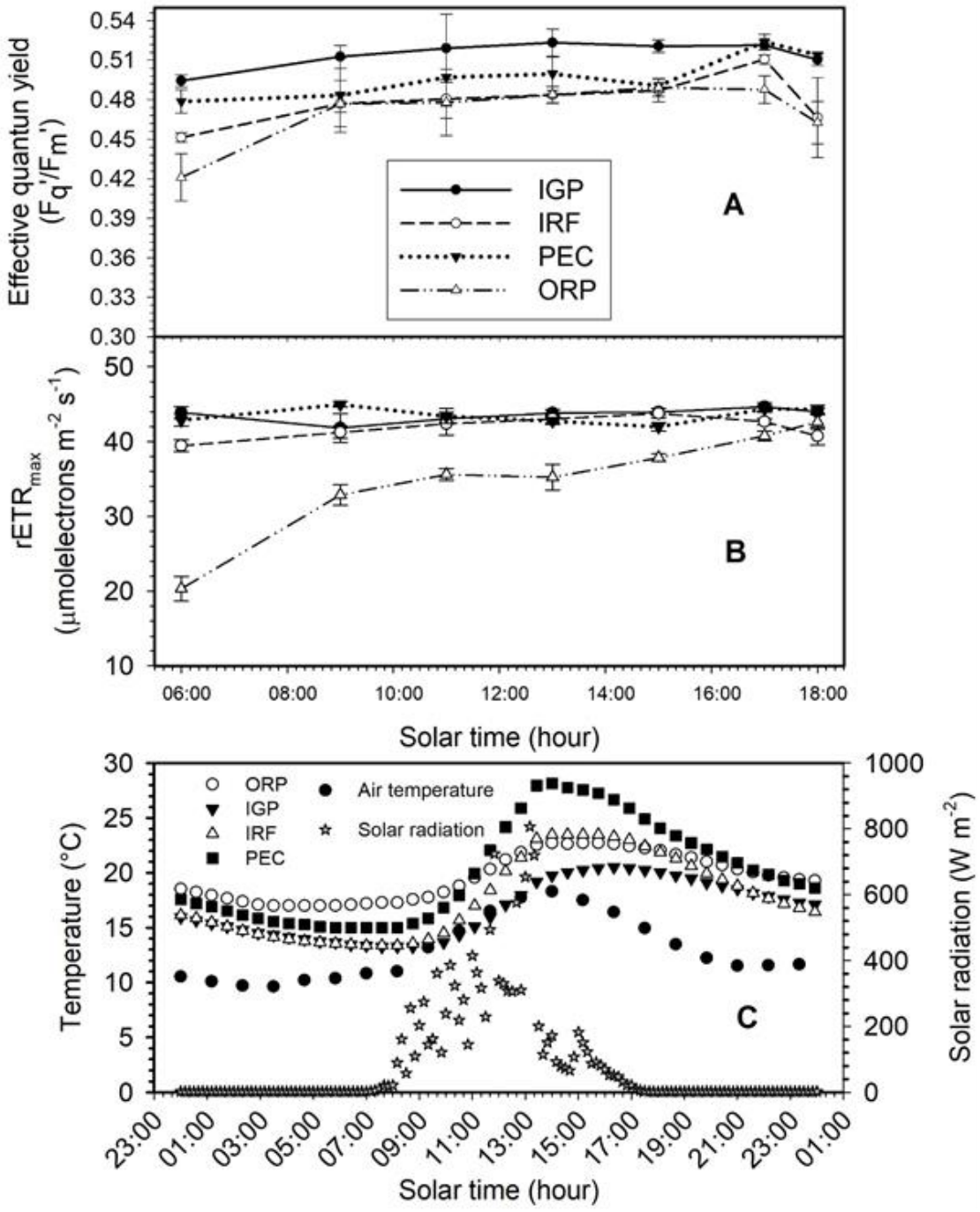

Fig. 5–5. Photosynthetic performance (effective quantum yield and maximum electron transport rate) of *A. platensis* cultivated in heated and non-heated systems during austral winter. Error bars indicate standard error, n = 3.

5.4.4 C-phycocyanin, chlorophyll a, carotenoid and protein contents

C-phycocyanin (C-PC) content and its purity trended similarly in all cultivation systems, with values in photobioreactors usually higher than that in the raceway pond (Fig. 5–(6A, B)). A significant variation (RM ANOVA, F = 67.88, p = <0.001 for C-PC content, F = 27.07, p = < 0.001 for C-PC purity) existed for C-PC content and purity between the photobioreactors and the raceway pond, with a 44 % higher content in the former compared to the latter (Table 5–1). However, pairwise comparison procedures revealed no significant differences in C-PC content and purity (Holm-Sidak, p > 0.05) among the photobioreactors (Table 5–1). In terms of C-PC productivity, significantly higher productivities (RM ANOVA, F = 39.92, P < 0.001) were found in the photobioreactors (15.08 – 16.30 mg g^{-1} d^{-1}) than in the raceway pond (8.78 mg g^{-1} d^{-1}). The C-PC content in photobioreactors (12 % organic biomass) achieved in this study is similar to previous reports (7 – 17 % dry weight) (García-López et al., 2020; Ho et al., 2018; Nwoba et al., 2019b; Wood et al., 2015). The purity (i.e. contamination with other protein products) of the C-PC in crude phycocyanin extracts was found to be ~ 39 % higher in the photobioreactors than the product from raceway ponds (Pearson correlation, R = 0.91, P = 0.08), possibly due to reduced biological contamination of the cultures in the photobioreactors. A maximum average C-PC purity of 1.28±0.06 was found for the PEC but this was not significantly different from that of the thermally-insulated photobioreactor. High C-PC purity in crude extracts is advantageous because of the potential to lower the cost of downstream purification. Phycocyanin purity absorbance ratios corresponding to 0.7, 3.9, and 4.0 are regarded as food, reagent, and analytical grades, respectively (Eriksen, 2008; Wood et al., 2015). Results here suggest that crude phycocyanin of C-PC purity level higher than those for food grade can be produced from all culture systems, especially in thermally-insulated photobioreactor during austral winter

Chlorophyll *a* in the ORP, IRF, and PEC were relatively constant over time. Chlorophyll content in the IGP was steady up to 22 May but then dropped by 30 % and remained steady for the rest of the study. This was a reflection of the changes in solar radiation for the experiment (Fig. 5–6D). There were differences in the absolute amount between treatments, but these were not statistically significant. All cultivation systems showed no variation in total carotenoid contents at each measurement period (Fig. 5–6E). On average, chlorophyll *a* and total carotenoid contents showed no significant variation among treatments (RM ANOVA, $p > 0.05$). Although pigment concentrations in photobioreactors and raceway pond were similar (except for C-PC content), a considerable biomass difference was observed. This scenario has been reported in *Spirulina* cultures subjected to low temperatures (Torzillo et al., 1998).

Regarding the biochemical constituents of *Arthrospira* biomass, it is a common knowledge that protein is the principal component and can reach 50 – 70 % dry weight under optimal growth conditions (García-López et al., 2020). In this study, the organic biomass normalized total protein contents (479.2±26.8 – 534.9±18.1 mg g^{-1}) showed no significant variation across treatments (Table 5–1) and are similar to the previous report on *A. maxima* LJGR1 (García-López et al., 2020).

Therefore, the technological innovation in thermally-insulated IGP photobioreactors related to minimizing thermal energy dissipation at night and keeping daylight temperature below the upper critical limit for most microalgae was effective. Critical growth parameters such as chlorophyll *a*, carotenoids, C-phycocyanin, and protein productions were unaffected by this innovation, thus representing an attractive approach.

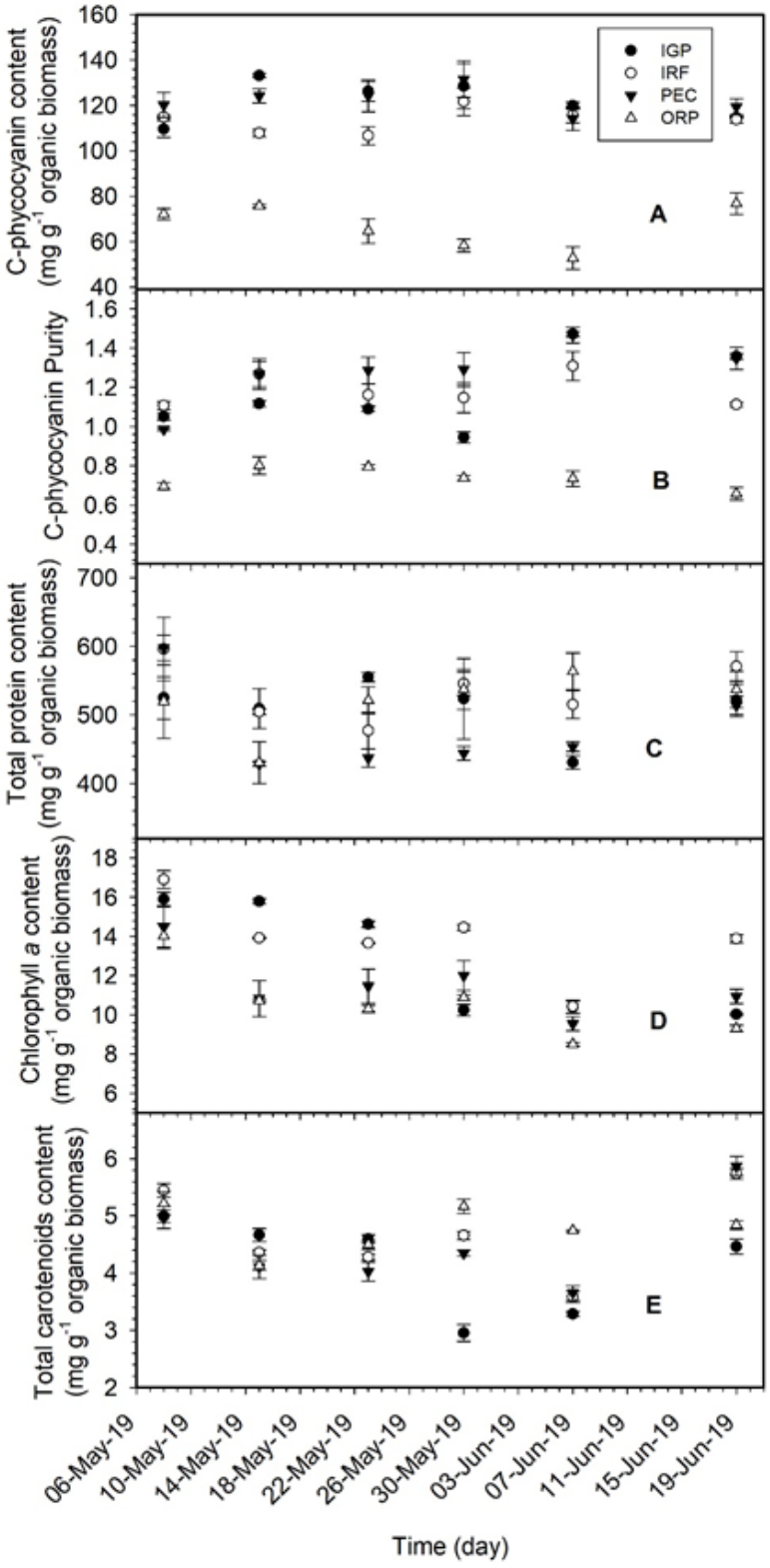

Fig. 5–6. Biopigments and total protein accumulation kinetics of *A. platensis* under different thermal control measures during austral winter. Error bars indicate standard error, n = 6.

5.4.5 Hydrodynamics of the photobioreactor

Hydrodynamic characteristics of photobioreactors influence light energy availability to microalgal cells, thereby affecting the efficiency of light conversion in such systems. The airflow rate suitable for the culture operation without cellular rupture of the trichomes and settling of cells was 24 L min^{-1} (0.17 vvm). At this airflow, the mixing time was measured to be 112.8±2.29 s and superficial gas velocity (U_G) and Reynolds number (Re) of 0.0034 m s^{-1} and 1174, respectively, were calculated. Hernández-Melchor et al. (2017) have characterized the hydrodynamics of a 150 L flat plate photobioreactor under air-water biphasic system for the production of microbial consortium. They reported mixing time of 103.8 s, U_G of 0.0068 m s^{-1}, and Re of 1176. In contrast, Sierra et al. (2008) have investigated a 250 L flat plate photobioreactor in a biphasic system for the production of microalgae. These authors reported mixing time and U_G at 51.9 s and 0.0076 m s^{-1}, respectively. Although the Reynolds number calculated here is similar to that reported in previous studies, the differences in the values of mixing time and U_G are due to the use of different volumes, airflow rates, and reactor heights. When *Nannochloropsis* sp. MUR 267 was grown in these reactors at the same culture volume (140 L), optimal mixing time, and U_G were 106.3 s and 0.0039 m s^{-1}, respectively (Nwoba et al., 2020c), clearly indicating that these parameters also may be species-specific. Values of 0.03 and 16.8 W m^{-3} were obtained for gas holdup and volumetric power (P/V), respectively, for the cultivation systems, which are lower than the 0.046 and 33.6 W m^{-3} figures, which have previously been reported for these parameters in a similar reactor (Hernández-Melchor et al., 2017). The volumetric expansion of gas in photobioreactors guides the characterization of transport phenomena in such systems, and the low value found here shows a short residence time of the gas in the culture systems. The small P/V values indicate yields on energy savings, though strongly dependent on volume,

can also vary with species. For instance, a P/V of 12.4 W m^{-3} was reported for the culture of *Spirulina* in a flat-panel photobioreactor with a working volume of 50 L (Reyna-Velarde et al., 2010). In contrast, 33.6 W m^{-3} was reported when a microbial consortium was grown in the same system (Hernández-Melchor et al., 2017). In the present study, experimental results suggest that these photobioreactors (with IGP inclusive) are suitable for *Arthrospira* production with the capability to effectively distribute nutrients in a short time, reduce heat and gas build-up, transit cells rapidly between photic and non-photic zones, and lower power consumption.

5.4.6 Operational energy input, biomass energy, and photovoltaic energy outputs

With a typical biomass elemental constituents of $CH_{1.78}O_{0.36}N_{0.12}$ (21.22 g mol^{-1}) and its enthalpy of combustion at 547.8 kJ mol^{-1} (152.17 Wh mol^{-1}) (Reyna-Velarde et al., 2010), the energy produced by biomass achieved in the thermally-insulated IGP with a productivity of 0.07 g L^{-1} d^{-1} (i.e., 2.92 g m^{-3} h^{-1}) was 20.94 W m^{-3}. The volumetric power input (due to mixing only) to each of the plate photobioreactors at a superficial gas velocity of 0.0034 m s^{-1} was 16.8 W m^{-3}. Therefore, a net energy output of 4.14 W m^{-3} was obtained using the IGP photobioreactor. This energy output was four times less than the 16.04 W m^{-3} achieved in the PEC reactor with a productivity of 0.11 g L^{-1} d^{-1}. Considering that the PEC system required heating at night and cooling on specific days during austral winter (Table 5–1), it was not energy-efficient for the culture of *A. platensis* in the winter season. In contrast, IGP photobioreactor generated additional energy from the integrated photovoltaic panels irrespective of seasons. Based on basic energy calculations of the PV panel at the experimental facility (See Nwoba et al., 202b; 2020c for details), the daily average energy

output of the PV panel during this experiment was simulated to be 0.29±0.007 kWh m^{-2} (0.21± 0.005 kWh $reactor^{-1}$) (Fig. 5–1H). Comparing this energy to the daily mixing energy need of 0.38 kWh m^{-3} (0.053 kWh $reactor^{-1}$) for the operation of blower sparging the culture, the IGP system uses dramatically less energy efficient for the production of *Arthrospira* biomass at 0.17 vvm. Therefore, PV output from the IGP photobioreactor during winter far exceeded the energy required for its operation by 75%, corroborating our previous finding (Nwoba et al., 2020 b; 2020c).

Some energy external to the photobioreactor is required to run normal operations, e.g., mixing, manage gas flow, etc., and there may be conditions were some external temperature control (e.g., cooling, heating) is still necessary. Incorporating an integrated photovoltaic panel into the photobioreactor design supplies local electricity for grid-independent operation. This is especially appealing in remote areas (often well suited to microalgal culture), neutralizing the compelling economic costs of energy required for cultivation. Tredici et al. (2016) reported the cost of *Tetraselmis suecica* biomass production in a Green Wall Panel flat plate photobioreactor as US$12.4 kg^{-1} and indicated that this cost could be significantly reduced if the reactors are integrated with photovoltaic elements. Moreover, thermal insulation of photobioreactors based on illumination spectral filtering have been shown to diminish external cooling and heating for temperature regulation while improving culture reliability (Nwoba et al., 2020b; 2020c). The combination of passive temperature control and photovoltaic energy generation forms a crucial design criterion for conjoint production of the desired chemical product, e.g., high-value pigment (phycocyanin), and electrical energy for operational needs from a single photobioreactor. Though the current solution is technologically attractive, its economics can be questionable given the investment cost and efficiency of PV technology.

Table 5–1. Average culture temperatures, biomass productivities, biopigments, and total protein contents of *A. platensis* under different thermal control measures during austral winter. Different superscript letters indicate significant differences in rows, Holm-Sidak test (RM ANOVA, P < 0.05), n = 6.

Parameter	IGP	IRF	PEC	ORP
Thermal control mechanism	Low-e film + double glazing	Low-e film only + heating	Freshwater cooling + heating	Heating
Heater rating (W)	None	50	300	305
Average temperature (˚C)	20.98 ± 0.03^{a}	19.63 ± 0.04^{c}	22.27 ± 0.05^{b}	17.66 ± 0.03^{d}
μ day^{-1}	0.13 ± 0.01^{b}	0.13 ± 0.01^{b}	0.17 ± 0.01^{a}	0.07±0.02c
Volumetric biomass productivity (g L^{-1} d^{-1})	0.07 ± 0.01^{b}	0.07 ± 0.01^{b}	0.11 ± 0.01^{a}	0.04 ± 0.01^{c}
Ground areal productivity (g m^{-2} d^{-1})	14.82 ± 1.19^{b}	15.63 ± 1.28^{b}	23.06 ± 2.49^{a}	5.23 ± 0.96^{c}
Chlorophyll *a* content (mg g^{-1} biomass)	12.83 ± 1.18^{a}	13.88 ± 0.84^{a}	11.55 ± 0.68^{a}	10.63 ± 0.78^{b}
Total carotenoids content (mg g^{-1} biomass	4.16 ± 0.34^{a}	4.68 ± 0.33^{a}	4.50 ± 0.33^{a}	4.77 ± 0.17^{a}
C-phycocyanin content (mg g^{-1} biomass)	122.06 ± 3.64^{a}	113.46 ± 2.25^{a}	122.37 ± 2.39^{a}	66.76 ± 4.01^{b}
C-phycocyanin productivity (mg g^{-1} d^{-1})	16.30 ± 1.43^{a}	15.08 ± 1.16^{a}	16.30 ± 1.31^{a}	8.78 ± 0.69^{b}
Total protein content (mg g^{-1} biomass)	510.92 ± 17.18^{a}	534.93 ± 18.06^{a}	479.19 ± 26.77^{a}	518.26 ± 18.68^{a}
Crude CPC purity	1.17 ± 0.08^{a}	1.18 ± 0.03^{a}	1.28 ± 0.06^{a}	0.74 ± 0.02^{b}

For example, it could be argued that a standalone PV system that incorporates higher efficiency panels would be a better option. Such a scenario could increase solar energy conversion to electricity and increase the optimum light harvesting area of the flat plate photobioreactor. Additional costs regarding cabling, junction losses and reduction in modularity of the overall installation would need to be factored in. The authors acknowledge that the actual impact and viability of new technologies such as the IGP photobioreactor need to be rigorously assessed based on energy, life-cycle and techno-economic analyses. Currently, effort is underway on detailed techno-economic and life-cycle analyses for an IGP based facility.

5.5 Conclusions

Arthrospira was successfully cultivated in a 140 L thermally-insulated photovoltaic photobioreactor under outdoor climatic conditions of austral winter with biomass productivity and corresponding c-phycocyanin content that are respectively, 67% and 45% higher than those achieved in a classical raceway that was heated continuously. This study demonstrated that the technological innovation in a thermally-insulated photobioreactor could be used to intensify the reliability of outdoor *Arthrospira'*s cultivation in photobioreactors during austral winter seasons with suboptimal climatic conditions without the requirements for extraneous cooling and heating systems. This design presents a less expensive and more energy-efficient pathway to large-scale microalgal culture. However, optimization of the novel photobioreactor is crucial for the cultivation of a wider spectrum of microalgae.

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Link to the next chapter

Thermal insulation of a photobioreactor that is based on illumination spectral filtering has been shown to diminish external cooling and heating for temperature regulation while improving culture reliability. The combination of passive temperature control and photobioreactor energy generation provides a notable pathway for conjoint production of desired microalgal bioproducts (e.g., eicosapentaenoic acid, c-phycocyanin) and electrical energy for operational needs in a single photobioreactor. This is especially appealing in remote areas (often well suited to large-scale microalgal culture and lack access to grid electricity and freshwater), neutralizing the compelling economic costs of energy required for cultivation. Whereas on the surface, this technology appears attractive, the actual energy balance of the system needs to be rigorously assessed. In this chapter, the net energy ratio (NER) of this novel photobioreactor was evaluated. The result of the energy analysis of this novel photobioreactor is compared to a photobioreactor under a passive evaporative cooling.

Chapter 6

Energetic performance of co-producing biomass and electricity in photovoltaic photobioreactors

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Energy efficiency analysis of outdoor standalone photovoltaic-powered photobioreactors coproducing lipid-rich algal biomass and electricity

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HIGHLIGHTS

- 1-ha microalgal plant based on novel photobioreactor design has NER of 3.0.
- Integrated PV module produces energy excess to requirements for plant operation.
- NER for novel photobioreactor comparable to agricultural biofuel crops.
- Spectral selection and PV technologies result in standalone operation.
- Novel photobioreactor offers energy and water sustainable production of microalgae.

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ABSTRACT

The need for thermal regulation in microalgal photobioreactors is a significant impediment to their large-scale adoption. The energy costs associated with thermal regulation alone can easily result in a negative energy balance. Self-sustaining photovoltaic powered photobioreactors that do not require cooling systems provide an opportunity to maximize biomass productivity, generate local electricity, reduce thermal regulation requirements, and significantly improve the energy balance of the system. Net energy analysis of a spectrally-selective, insulated-glazed photovoltaic photobioreactor (IGP) with an integrated capability for renewable electricity generation used to cultivate *Nannochloropsis* sp. without freshwater-based cooling resulted in a net energy ratio of 2.96, a figure comparable to agricultural bio-oil crops such as *Jatropha* and soybean. Experimental data from pilot-scale operation of this novel photobioreactor producing *Nannochloropsis* biomass under outdoor conditions was extrapolated to a 1-ha IGP installation. Annual biomass productivity reached 66.0-tons dry weight ha^{-1}, equivalent to overall energy output of 1696.2 GJ ha^{-1}. The integrated semi-transparent photovoltaic panels generated an additional 1126.8 GJ ha^{-1} yr^{-1} (313.0 MWh ha^{-1} yr^{-1}). Energy demands from plant building materials, machinery, fertilizers, plant operations, and biomass harvesting constituted total energy input with a combined value of 707.3 GJ ha^{-1} yr^{-1}. Comparison with a conventional photobioreactor requiring passive evaporative cooling showed novel photobioreactor had a 73% greater net energy ratio. *Nannochloropsis* cultivation in IGP system ensured co-production of lipid and protein of 34.7 and 25.7-tons ha^{-1} yr^{-1}, respectively. These results suggest that this novel photobioreactor could be a viable and sustainable biomass production technology for mass microalgal cultivation.

1. Introduction

Microalgae represent an efficient solar-driven biotechnology resource for the environmentally sustainable production of biofuels, food, feed, cosmetics, fine chemicals, fertilizers, and biopharmaceuticals. The basis of the promise of microalgae as a biorenewable

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David A. Parlevliet	Conceptualization, Formal analysis, Resources, Methodology, Validation, Supervision, Writing – review and editing.
Damian W. Laird	Conceptualization, Resources, Supervision, Writing – review and editing.
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Julien Louveau	Formal analysis, Validation, Writing – review and editing.
Jeremy Pruvost	Formal analysis, Resources, Methodology, Validation, Writing – review and editing.
Navid R. Moheimani	Conceptualization, Resources, Methodology, Supervision, Writing – review and editing.

Principal supervisor confirmation

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

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6.1 Abstract

The need for thermal regulation in microalgal photobioreactors is a significant impediment to their large-scale adoption. The energy costs associated with thermal regulation alone can easily result in a negative energy balance. Self-sustaining photovoltaic powered photobioreactors that do not require cooling systems provide an opportunity to maximize biomass productivity, generate local electricity, reduce thermal regulation requirements, and significantly improve the energy balance of the system. Net energy analysis of a spectrally-selective, insulated-glazed photovoltaic photobioreactor (IGP) with an integrated capability for renewable electricity generation used to cultivate *Nannochloropsis* sp. without freshwater-based cooling resulted in a net energy ratio of 2.96, a figure comparable to agricultural bio-oil crops such as *Jatropha* and soybean. Experimental data from pilot-scale operation of this novel photobioreactor producing *Nannochloropsis* biomass under outdoor conditions was extrapolated to a 1-ha IGP installation. Annual biomass productivity reached 66.0-tons dry weight ha^{-1}, equivalent to overall energy output of 1,696.2 GJ ha^{-1}. The integrated semi-transparent photovoltaic panels generated an additional 1,126.8 GJ ha^{-1} yr^{-1} (313.0 MWh ha^{-1} yr^{-1}). Energy demands from plant building materials, machinery, fertilizers, plant operations, and biomass harvesting constituted total energy input with a combined value of 707.3 GJ ha^{-1} yr^{-1}. Comparison with a conventional photobioreactor requiring passive evaporative cooling showed novel photobioreactor had a 73 % greater net energy ratio. *Nannochloropsis* cultivation in IGP system ensured co-production of lipid and protein of 34.7 and 25.7-tons ha^{-1} yr^{-1}, respectively. These results suggest that this novel photobioreactor could be a viable and sustainable biomass production technology for mass microalgal cultivation.

6.2 Introduction

Microalgae represent an efficient solar-driven biotechnology resource for the environmentally sustainable production of biofuels, food, feed, cosmetics, fine chemicals, fertilizers, and biopharmaceuticals. The basis of the promise of microalgae as a biorenewable resource, include: (a) high lipid yields (up to 60 m^3 ha^{-1} vs. 2 m^3 ha^{-1} for *Jatropha*, or 0.2 m^3 ha^{-1} for corn [1]); (b) high conversion of solar energy to product (theoretical maximum values of 10 % for microalgae vs. 6 % for C_4 plants [2]); (c) rapid reproduction cycles allowing for semi-continuous or continuous harvesting; (d) potential for CO_2 sequestration (1 kg biomass is equivalent to 1.8 kg CO_2, [3]); (e) no requirement for high-value agricultural land, reducing competition with food-based crops; and (f) flexible inputs for culture systems including sea-/industrial, domestic and agricultural waste-water, flue gas, thus avoiding freshwater dependence [4, 5] and valorize waste streams [6, 7, 8, 9].

This combination of features has led many to view microalgal production as a panacea to the environmentally sustainable production of many bio-based products, rather than current efforts using agricultural plant-based systems. However, microalgal culture requires far greater energy input than the production of traditional terrestrial crops. For example, in their study of the environmental impact of oil production in Italy, Jez et al. [10] reported that oil production from microalgae still has greater negative environmental impacts compared to traditional crops (e.g., sunflower and rapeseed) due to excessive energy demand and input material consumption. The cultivation of microalgae, in addition to harvesting of biomass, was by far, the biggest contributor (60.9 %) to the electrical energy needs and environmental impact [10]. The authors did note that the environmental impact of algal production could be reduced considerably by the use of renewable, specifically solar energy to provide the

electricity to drive cultivation. Recently, Morales et al. [11] have indicated that there is a balance to be achieved between environmental impacts and energy when integrating photovoltaic panels with microalgal cultivation. Thus, to effectively exploit microalgae as a renewable bioresource, the energy efficiency of cultivation systems need to be considerably improved for commercial-scale production.

The two conventional systems for the commercial production of microalgae are open pond systems and closed photobioreactors [12]. Open ponds (e.g., classical raceways) are attractive commercially due to their low capital investment costs and are considered the cheapest technology for mass microalgal production [13, 14]. However, low biomass productivity and the inability to sustain year-round production due to a high rate of culture contamination are significant limitations associated with open ponds [1, 13, 15]. Closed photobioreactors can offer optimal biophysiological conditions that lead to higher biomass productivity with a lower tendency for contamination. Unfortunately, photobioreactors are prone to overheating under outdoor conditions, which results in lower productivity and high cell mortality. As such, temperature and thermoregulation of cultures in photobioreactors is a well-recognized problem in solar microalgal farming [16]. This can also contribute to high environmental and energy costs in those temperate areas of the world where the solar resource is ideal for microalgal culture, e.g., western USA, Israel, north-western Australia.

Under outdoor conditions, >50 % of the solar radiation hitting the photobioreactor surface is within the infrared region (i.e., wavelengths above 700 nm) and directly contributes to overheating the culture [17]. Consequently, up to 95 % of collected solar spectral energy is transformed to heat by the culture [18]. Microalgae have optimal temperature windows, in which maximum bioproductivity is achieved. In summer (especially in the tropics), supra-optimal (high) temperatures that are lethal to microalgae are easily reached in closed

photobioreactors necessitating the use of cooling systems. In contrast, sub-optimal (low) temperatures occur in temperate regions, especially during winter, and these can lead to deterioration in growth and loss of productivity, making it necessary to heat cultures [19]. Year-round productivity in photobioreactors can then really only be achieved by cooling and heating photobioreactors and this is enough to lead to a negative energy balance of the system, even before considering other inputs such as materials, mechanical operations, and required nutrients. Therefore, effective, temperature control of algal solar photobioreactors is a serious challenge to the overarching goal of cost-effective, environmentally sustainable, low-energy consuming microalgal production.

The problem of photobioreactor temperature control lends itself to novel approaches for the design of photobioreactors that are self-cooling (and require no heating in winter) and integrate photovoltaic electricity generation. These could then be optimized for maximal biomass productivity over the year to significantly decrease energy demand and address the negative net energy balance of algal photobioreactors. To this end, Moheimani and Parlevliet [20] proposed a microalgae production plant utilizing semi-transparent, spectrally-selective photovoltaic (PV) filters positioned above the culture facilities. This system could transmit a specific light spectral range to the culture while capturing and redirecting the remaining wavelengths to the PV cells for electrical energy generation. This idea paved the way for the design and development of an energy-harvesting spectrally-selective insulated glazed photovoltaic (IGP) photobioreactor [21, 22]. The IGP photobioreactor has a transparent (thin-film, CdTe) PV panel (40 % transmission) and a low-emissivity (low-e) film [21]. The PV panel is glued to the upper part of the reactor to generate electrical power for production operations, removing the requirement for grid electricity. The low-e film is embedded in the illumination surface and selectively allows >70 % of photosynthetically-

beneficial wavelengths from sunlight to reach the microalgae culture, while simultaneously reflecting > 90 % of ultraviolet and infrared radiation [21]. Filtering out the non-photosynthetic wavelengths (e.g., above 700 nm), should keep the temperature in the photobioreactor below the upper critical limit without the need for freshwater-related cooling during the day [22]. In the same vein, the large temperature drops at night typical of conventional photobioreactors can also be mitigated by the insulated panels, ensuring a culture temperature above the lower critical limit for most microalgae species. Although on the surface this solution sounds attractive, the actual energy balance of the technology needs to be rigorously assessed.

The net energy ratio (NER) is a standardized parameter used to evaluate the energetic productivity of a system [23] and represents a quantitative and scientific evaluation of the ratio between total energy production and primary non-renewable (fossil) energy requirements in the production process during a technology's life cycle [24, 25]. An NER ≥ 1 corresponds to the energy output exceeding the energy input, and such a system is obviously desirable [25, 26, 27]. Assessment of process sustainability for algal production systems (especially for biofuels) has been carried out mainly on systems based upon open ponds [23, 28, 29, 30, 31, 32]. The energy balance of closed photobioreactors, and particularly flat panel reactors, have been subjected to far less scrutiny. The reported range of the calculated NER for these types of systems varies widely. For example, Jorquera et al. [23] used a GaBi program to produce values of 4.5 and 1.7 for production of biomass and oil, respectively, from *Nannochloropsis* sp grown in a flat panel photobioreactor, while another research focussed on *Scenedesmus obliquus* reported values between 0.39 and 7.81 when cultured at mid-temperate latitudes [33]. In the most comprehensive treatment of photobioreactor energy efficiency, Tredici et al. [34] recently reported an NER of 0.6 for biomass production in an

industrial-scale Green Wall Panel photobioreactor system culturing *Tetraselmis suecica* in Italy and 1.7 for a similar silicon-based PV-integrated system located in Africa. The data from the limited number of studies on photobioreactors to date seem to indicate that overall NER is due to both photobioreactor design, the species being cultured and the location of the facility. In fact, Morales et al. [11] have indicated that there is a compromise that needs to be made between optimizing energy efficiency and environmental impact when assessing commercial microalgal production using photobioreactors.

Only a few studies have investigated the supply of energy to the system using PV, but none of those have explored the actual integration of PV panels into photobioreactors themselves. Combining spectral filtering technology and PV electricity generation into an individual photobioreactor module should mean that more modules can be placed per hectare as well as reducing heating and cooling costs to maintain the microalgal cultures at temperatures for maximum biomass productivity.

This study aims to evaluate the NER of a pilot-scale flat panel photobioreactor that incorporates self-cooling and integrated photovoltaic energy generation for cultivation of *Nannochloropsis* sp.; a microalga often touted as a potential biofuel feedstock. The result of the energy analysis of this novel photobioreactor is compared to a photobioreactor utilizing a passive evaporative cooling (PEC) using the same system boundaries. The strength of this analysis is the use of experimental biomass productivity and power efficiency data obtained from the operation of both types of photobioreactor. However, the authors emphasize that the validity of the conclusions is only applicable within the defined boundary limits and use of the IGP photobioreactor system.

6.3 Materials and methods

6.3.1 Functional unit, system boundaries, and source of data

For clarity and easy comparability, this energy balance analysis is carried out following the methodology of Tredici et al. [34], and utilizes similar system boundaries. The functional unit chosen for the current analysis is a 1-ha IGP photobioreactor plant. The choice of a 1-ha plant is not a reflection of the appropriate scale of an algae facility but a manageable size for industrial food or fuel-based applications of algae. It could be argued that a larger plant size would be needed to provide a realistic estimate of energetic efficiency for the production of biocommodities, but larger scales may vary the outcome of the analysis. The experimental data collected from the operation of a single pilot-scale IGP photobioreactor was extrapolated to a 1-ha plant located in Western Australia.

The analysis begins with the cultivation and terminates with the production of a biomass paste containing 70 – 80 % (passing through centrifuge) moisture content. The boundary limits of the pathways for the production of biomass and downstream processing are contained in Fig. 6–1. The analysis was tailored to focus exclusively on the processes required for wet biomass production so as to remove the uncertainty associated with the choice of upstream and downstream processing possibilities. As the IGP unit is a standalone photobioreactor with a self-cooling mechanism, access to a freshwater source for cooling is not considered. Site selection for large-scale microalgal production is largely determined by the topography, climate, weather conditions, land cost and availability, and the engineering of the cultivation systems [35, 36, 37, 38]. Consequently, geospatial factors, such as temperature, solar radiation, water availability, rainfall pattern, and length of season, govern algal productivity.

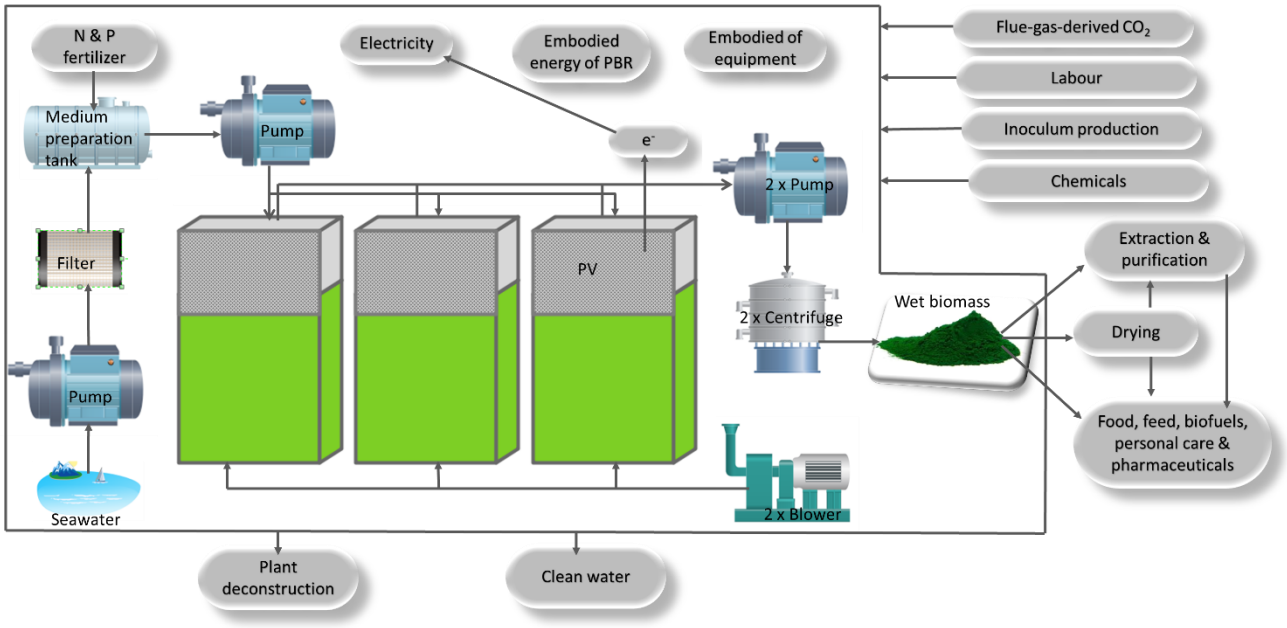

Fig. 6–1. A schematic of microalgal biomass production using a standalone IGP photobioreactor. The solid box shows system boundaries for the energy analysis.

In this context, Karratha (20˚43'56.32" S, 116˚35'57.97" E, elevation 5 m) in Western Australia was chosen as a suitable area for locating the plant as it has previously been identified as a potential location for large-scale cultivation of microalgae [35] and is considered comparable to a number of similar temperate climate cultivation sites around the world. Further, Karratha is close to the sea (Euclidean distance between the plant and seawater intake of 1 – 1.5 km) ensuring availability of seawater for medium preparation, has high solar irradiance (16 – 28 MJ m^{-2} yr^{-1} and 7 – 9 sunlight hours per day), favorable climatic conditions (average temperature, 24 – 35 ˚C) (Fig. 6–2), proximity to industries for flue gas availability, a history as a trial site for large-scale cultivation of microalgae [35], and the climatic conditions are known to support high biomass productivity over the course of a whole year (Emeritus Prof. Michael Borowitzka, pers. comm). Utilizing seawater for microalgae cultivation does not necessarily eliminate the requirement for freshwater, as that will be needed to compensate for evaporation losses and consequential increase in culture

salinity. Energy and life-cycle analyses of microalgal culture typically include freshwater in their parameter set, usually because of the high evaporative losses from open pond systems. However, evaporative losses in photobioreactors are significantly lower; thus, freshwater resources have not been factored into the current study.

Energy output is defined as the summation of the chemical energy stored in the microalgal biomass and the surplus electrical energy produced by the PV modules per hectare per annum. Calculation of the energy input required to operate the plant was based on three main parameters as outlined by Tredici et al. [34], namely: (i) the embodied energy of materials; (ii) the energy of fertilizers, and; (iii) the energy required for operating pumps, centrifuges, and thermal regulation. The energy required for plant dismantling, and that provided by labor are excluded as their contribution to inputs have been shown to be marginal [34].

6.3.2 Sizing and operation of 1-ha IGP photobioreactor plant

The IGP unit used in the experiments is a customized flat panel photobioreactor constructed of insulated glass units (IGUs) with an integrated energy-generating photovoltaic (PV) panel (Fig. 6–3). It is comprised of five 5 mm thick IGUs, each having two glass panels sealed together with an airspace between them to ensure high thermal insulation properties. The solar facing 120 cm × 150 cm (length × height) IGU has a low-emissivity (low-e) thin film deposited on the outer surface. The low-e film is spectrally-selective, allowing > 75 % of visible light to pass through while blocking > 90 % of the ultraviolet and infrared spectral components. This reduces heat loss in winter by reflecting the heat escaping the photobioreactor back into the culture and reduces heat gain during summer via spectral selection/reduction, and avoids freshwater cooling. The rear and bottom IGUs of the photobioreactor do not contain the low-e film, but those on the sides do.

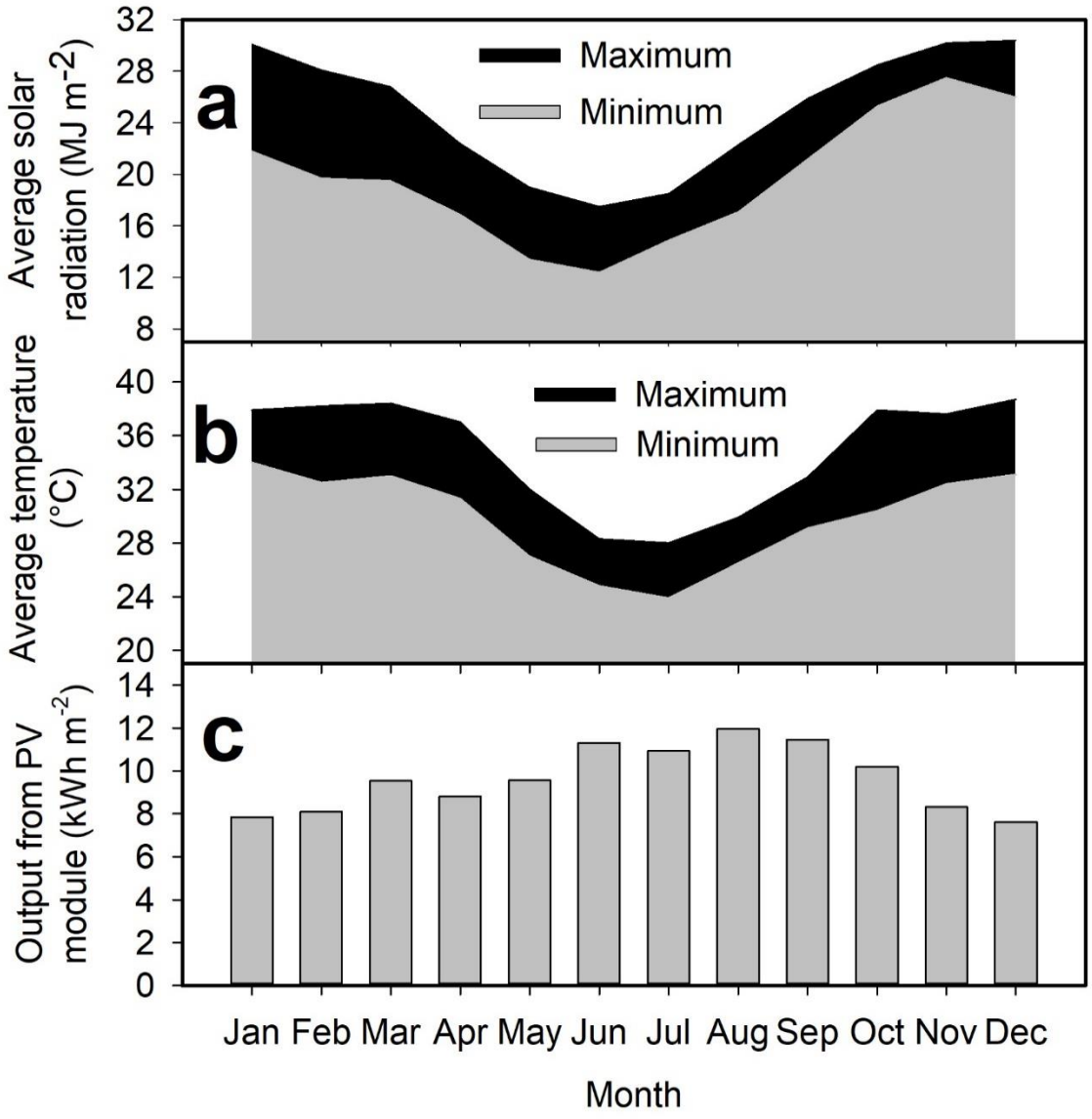

Fig. 6–2. (a) Monthly average daily maximum and minimum solar radiation, (b) air temperature (www.bom.gov.au), and (c) photovoltaic panel output in Karratha from 1990 – 2018.

A 120 cm × 60 cm (length × height) semi-transparent solar glass CdTe PV panel was glued to the upper part of the external surface of the solar facing IGU. This allowed 40 % of the incident sunlight through to the interior of the photobioreactor while converting the remainder to electricity, which is typically stored in a battery and used for providing electrical power for essential photobioreactor functions (e.g., air pump for mixing). The PV panel was

placed 90 cm above the base of the IGP unit, to allow maximum solar harvesting. The plate reactor was inclined at a tilt angle of 32˚, with a north-south orientation to maximize light capture [36]. The photobioreactor has an internal optical path length of 10 cm and an active culture volume of 140 L. The microalgae suspension was mixed continuously by feeding filter-sterilized ambient air from both ends of 1.20 m long ceramic diffusers installed at the base of the photobioreactor. The airflow was provided by a PondOne O2 Plus 8000 air pump (4,200 L h^{-1}) at an aeration rate of 0.21 vvm (volume of air per volume of culture per minute) and airflow pressure was regulated with a flowmeter. This aeration rate corresponds to a superficial gas velocity of 0.0039 m s^{-1}, mixing time of 106.3 ± 3.20 s, and gas-hold of 0.017 ± 0.0002 in the photobioreactor under a biphasic system composed of air and tap water [39, 40]. Culture pH was unregulated, and no CO_2 gas was infused into the cultures.

It was calculated that a 1-ha plant (100 m × 100 m) would comprise a grid of 71 (row) × 40 (column) IGP units based on measurements obtained during the operation of the pilot-scale photobioreactors at the Murdoch University Algae R&D Centre in Perth. This configuration allows a reactor gap of 20 cm on the east-west axis and a gap of 100 cm between rows. Multiple solar angles, ease of access to modules and space for equipment installation were taken into account when arriving at the optimized configuration for a 1-ha installation. The configuration thus contains 2,840 IGP units with a total culture volume of 398 m^3 and a total IGP surface area of 5,112 m^2, equating to a total illuminated surface area for algal culture of 3,646.6 m^2 and total surface area for PV electricity production of 2,044.8 m^2. The ratios of photobioreactor surface area and illuminated surface area to the occupied land surface area are 0.51 and 0.36, respectively.

This analysis considered ancillary equipment following the procedure of Tredici et al. [34] including: blowers (× 2) for culture aeration; centrifugal pumps (× 4) for culture transfer,

circulation of seawater, medium preparation and distribution; and centrifuges (× 2) for biomass separation. The analysis incorporated both the embodied and operational costs of energy. A photobioreactor with the same optical depth, culture volume, hydrodynamic indices, and system boundaries without the photovoltaic and low-e adaptations but requiring a passive evaporative cooling (PEC, Fig. 6–3) system was utilized as a comparator to the IGP module.

6.3.3 Microalgae, culture medium and cultivation conditions

The marine Eustigmatophyte, *Nannochloropsis* sp. MUR 267, isolated from the Swan-Canning Estuary, Western Australia [41] was obtained from the Culture Collection of Algae at Murdoch University (Algae Research and Development Centre), Australia. This alga is a candidate for large-scale production of biofuel, aquaculture feed, and valuable biochemicals (e.g., ω-3 fatty acids) because of its fast growth, tolerance to biotic pollution and high energy conversion efficiency [42, 43]. The *Nannochloropsis* sp. was cultivated using unsterilized (but filtered, 50 µm) natural seawater (Hillary's Beach, Western Australia) enriched with sterilized F/2–Si nutrients [44]. The growth medium was maintained at the ambient salinity of seawater, 33‰ (parts per thousand) NaCl. The *Nannochloropsis* sp. inoculum used for this study was obtained from a non-axenic unialgal culture maintained in the logarithmic growth phase in a 2 m^2 outdoor raceway pond for more than 12 months. The experiment was carried out during the austral spring (from October to November) of 2018.

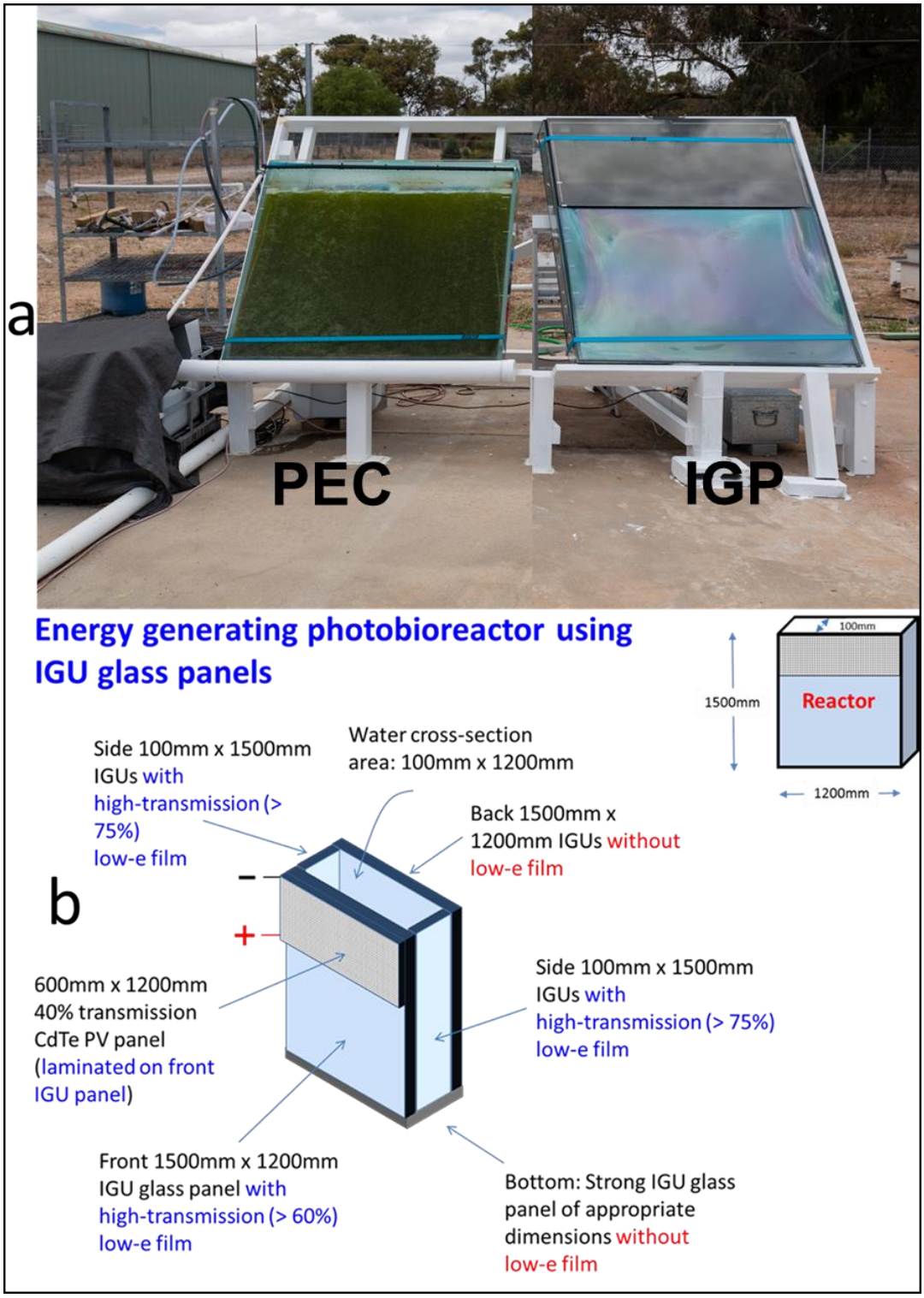

Fig. 6–3. (a) Insulated glazed photovoltaic (IGP) and passive evaporative cooling (PEC) photobioreactors operation at Algae R&D Centre, Murdoch University, Western Australia and (b) schematic showing the construction details of the IGP photobioreactor.

6.3.4 Energy inputs to the 1-ha photobioreactor plants

6.3.4.1 Embodied energy of materials, machinery, and associated equipment

By definition, embodied energy is the "total primary non-renewable energy consumed during the whole lifetime of a product" [45]. The embodied energy data used for this study was based on the information available in the literature [34, 45, 46, 47, 48]. Consideration was given only to the energy consumed during the extraction and processing of raw materials. Energy-related transportation costs and the recovery of materials post lifetime were excluded. The total embodied energy of a manufactured machine consists of the energy content of the materials that form the machine, the energy used for its production, and the maintenance energy [49]. Here, considerations are given to the energy content of the materials and that for machine production only. Lifetime information for machines and plant components are based on manufacturer's specifications and literature data [34]. Lifetime data for assembled machines are 5, 20, and 25 years for pumps, blowers, and centrifugal separators, respectively [34]. Quantity of materials required for the 1-ha plant construction was estimated based on manufacturer's data and pilot-scale operation of single IGP and PEC photobioreactors that were then extrapolated to 1-ha scale.

6.3.4.2 Nitrogen and phosphorus fertilizers input, other nutrients and chemicals

As seawater contains any necessary trace elements required for the growth of microalgae, this analysis considered only nitrogen (N) and phosphorus (P) supplied as sodium nitrate ($NaNO_3$) and sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$), respectively. The fertilizer input (kg ha^{-1} yr^{-1}) into biomass production was obtained from the areal biomass productivity (g m^{-2} d^{-1} or ton ha^{-1} yr^{-1}) based on an average biomass content of

6 and 0.6 % for N and P, respectively [50]. The fertilizer contribution to the total energy input was obtained from their yearly utilization and unit energy cost and expressed in MJ kg^{-1}.

6.3.4.3 Primary energy input for operations

The primary energy used by the electromechanical equipment is related to its total electrical energy production efficiency, which in turn varies with the fuel mix consumed for electricity production. In this analysis, the overall energy production efficiency is assumed to be 58 % [34]. Electrical energy consumed by the equipment such as blowers, pumps, and centrifugal separators (for pumping operation, nutrient preparation, mixing, harvesting) was computed by multiplication of power requirements by working time for each machine.

Operations for medium preparation and culture harvesting comprise transfer of culture from the photobioreactors to the centrifugal separators and renewal of fresh growth medium in the reactors. An energy value of 0.058 kWh m^{-3} is assumed for the specific energy consumption required for culture pumping and medium preparation [34]. A harvesting ratio of 40 % (159 of 398 m^3) and harvesting frequency of three days, based on cell specific growth rate, are used. Given the proximity of the plant to seawater, the water required for medium renewal would be directly pumped from the sea, filtered and transferred to the growth medium preparation tank using a centrifugal pump. Energy for cooling of the IGPs is not considered as previous experimental results have indicated that the elimination of water-related cooling is possible using these IGP photobioreactors [22, 42].

To prevent cells settling, achieve an optimal light/dark regime, and ensure adequate fluid transfer, the culture in the reactor is continuously mixed by way of air bubbling. The power consumption for mixing represents a significant proportion of the total primary energy input [34]. In this analysis, two blowers are considered to provide enough compressed air for mixing in a 1-ha plant. The energy consumed by the blowers is determined from Eq.

(6–1) as described in Chisti [51] and further converted to electrical power by applying an electrical conversion yield of 0.58 [34].

$$P_w/V_l = \rho_l g U_w \quad (6\text{–}\mathbf{1})$$

where P_w = blower power input (W), V_l = unit volume (m^3), ρ_l = density of liquid (kg m^{-3}), g = acceleration due to gravity (9.81 m s^{-2}), and U_w = superficial gas velocity (m s^{-1}).

Considering the proximity of our chosen location to industrial sites in this analysis, CO_2 supply will be sourced from flue gas in order to provide carbon and control the pH of the culture. Therefore, the electrical energy inputs for the supply of CO_2-rich flue gas (12.5 % CO_2) to blowers and inoculum production is assumed to be insignificant [34].

At every semi-continuous harvest (every three days of culture residence time), the culture is passed directly through a centrifuge without pre-concentration. In house experience suggests that the resulting biomass paste has a moisture content between 70 % to 80 %. The specific power consumption of the centrifuge used for culture harvesting is considered to be 1.2 kWh m^{-3} [34].

6.3.5 Energy output

The energy output of the IGP plant was considered as the product of the ground areal biomass productivity (g m^{-2} d^{-1}) and energy content of the biomass (GJ ha^{-1} yr^{-1}) plus the surplus energy produced by the PV panel (GJ ha^{-1} yr^{-1}) for a 1-ha site. The specific enthalpy of nutrient-replete *Nannochloropsis* sp. biomass grown in F/2–Si medium is considered to be 25.7 MJ kg^{-1} [50]. Based on available literature data for the chosen location [35], the annual cultivation period is restricted to the 11 sunniest months of the year (July to May) (Fig. 6–2). Given the experimental annual productivity data collected from microalgal plants previously operating in the chosen location, the average ground areal productivity for this cultivation

period is around 28 – 30 g m^{-2} d^{-1} ([35], Emeritus Prof. Michael Borowitzka, pers. comm.). For this analysis, the average ground areal biomass productivity of the 1-ha IGP plant over a full year was conservatively estimated to be 20 ± 5 g m^{-2} d^{-1}. The data obtained from the basic energy measurement of the PV module was matched with the manufacturer's information sheet and used to simulate monthly average energy output for the chosen location (Fig. 6–2). Based on this calculation, the monthly average energy output from each individual module located at Karratha, Western Australia, is estimated to be 10.02±0.96 kWh. Therefore, the NER for the 1-ha IGP and PEC photobioreactors is calculated based on Eqs. (6–2) and (6–3), respectively.

$$NER = \frac{Overall\ energy\ output\ (energy\ output\ from\ biomass + surplus\ energy\ output\ from\ PV)}{Total\ energy\ input\ (embodied\ energy + energy\ for\ operations + energy\ for\ mixing)} \quad (6\text{–}\mathbf{2})$$

$$NER = \frac{Energy\ output\ from\ biomass}{Embodied\ energy + energy\ for\ operations + energy\ for\ mixing} \quad (6\text{–}\mathbf{3})$$

6.4 Results and discussion

6.4.1 Energy output

The ground areal productivity achieved using the IGP photobioreactor at the Murdoch University Algae R&D Centre in Perth during the austral spring (October – November 2018) was 16 – 23 g m^{-2} d^{-1}, with no CO_2 addition. Productivity could be increased by 70 – 80 % with CO_2 addition to the culture [52]. Notwithstanding this potential increase, a conservative figure of 20 g m^{-2} d^{-1} was chosen for the biomass productivity over the course of a year for this analysis. This level of productivity can be achieved for at least 11 months (330 days), corresponding to 66,000 kg ha^{-1} yr^{-1} (66.0 tons ha^{-1} yr^{-1}) of dry algal biomass and results in energy output of 1,696.2 GJ ha^{-1} yr^{-1} based on a biomass energy content of 0.0257 GJ kg^{-1}. Using an average of five "peak-sun" hours per day and 330 sunny days per year, the

PV panels on the 2,840 units produce a total electrical energy output of 313 MWh ha^{-1} yr^{-1} (67 W m^{-2} per panel), i.e., 1,126.9 GJ ha^{-1} yr^{-1}. Hence, the overall energy output of the 1-ha IGP plant is 2,823.1 GJ ha^{-1} yr^{-1}, with the PV integration contributing 40 % of the total energy generated.

6.4.2 Analysis of energy inputs

6.4.2.1 Embodied energy of photobioreactor, piping, and machinery

The energy embodied in the materials required to build the 1-ha IGP plant is calculated to be 393.8 GJ ha^{-1} yr^{-1} (Table 6–1). Therefore, the total embodied energy of the IGP photobioreactor, piping, fittings and machines adds up to 411.0 GJ ha^{-1} yr^{-1}. The major energy cost comes from the building materials for the IGP reactors, representing approx. 96 % of the annualized energy input. The relative contributions of the energy embodied in fittings, piping, and ancillary equipment is almost negligible. This figure is similar to that reported by Tredici et al. [34], who found that 95 % of the overall embodied energy required was due to the energy cost of the materials used to build a 1-ha Green Wall Panel (GWP) plant made of disposable low-density polyethylene film.

6.4.2.2 Energy consumption for fertilizers and plant operations

Consideration is only given to the nutrients, N and P fertilizer supplied as $NaNO_3$ and $NaH_2PO_4.H_2O$, for the F/2–Si medium [44]. The other major components of the F/2 medium recipe, including trace metals, are derived from seawater. Based on our decades of outdoor growth of *Nannochloropsis* sp., omitting specific trace element additions to seawater-based medium has a negligible effect on biomass productivity [22, 42, 53]. The energy consumption for the production of N and P fertilizers required to grow 66.0 tons ha^{-1} yr^{-1} of *Nannochloropsis* sp. MUR 267 biomass is calculated to be 240.8 GJ ha^{-1} yr^{-1} (Table 6–2).

Table 6–1. Embodied energy of materials for the building of a 1-ha IGP plant

Materials	Embodied energy of material (MJ kg^{-1})	Quantity of materials required (tons ha^{-1})	Lifetime of material (years)	Annual embodied energy required (GJ ha^{-1} yr^{-1})	Percentage contribution to embodied energy cost (%)
Glass (toughened)	23.5[a]	140.3	20[b]	164.9	40.1 (50.0)
Stainless steel for PBR framework and fittings	15.3[b,*]	130.0 (140)	20[b]	99.5 (107.1)	24.2 (32.5)
CdTe PV panel	72[a]	36.9	25[b]	106.3	25.9
PVC pipes and fittings for aeration	52.6[a,*]	2.2 (2.8)	5[b]	23.1 (29.5)	5.6 (8.9)
IGP PBR				**393.8 (301.5)**	**95.8 (91.4)**
PVC for general piping	**52.6[a,*]**	**1.3 (2.8)**	**9[c]**	**7.6 (16.4)**	**1.8 (5.0)**
Centrifuges	56.7[b]	2.0	25[b]	4.5	1.1 (1.4)
Blowers	56.7[b]	1.0	20[b]	2.8	0.7 (0.8)
Pumps	56.7[b]	0.2 (0.4)	5[b]	2.3 (4.5)	0.6 (1.4)
Machines				**9.6 (11.8)**	**2.4 (3.6)**
Total embodied energy				**411.0 (329.7)**	**100**

[a][48]
[b][34]
[c][24]
*Energy content values for recycled materials used in the calculations
Values in parenthesis represent the results for PEC photobioreactor.

Using a culture residence time of three days, nutrient utilization efficiency is known to be 100 % [22]. Therefore, post centrifuge water, that is effectively free of biomass, is clean and nutrient-free and fit for disposal without further treatment.

An airflow rate of 0.21 vvm was maintained in the photobioreactor and found to be suitable for providing mixing throughout the experimental period [22]. The calculated power consumption for blowers was 38 W m^{-3}, which amounts to a yearly electrical energy cost of 430.9 GJ ha^{-1} yr^{-1} (Table 6–3). This cost represents 83 % of the total costs for plant operation and is the major contributor to the primary energy input (Table 6–3). The power consumption for culture harvesting using centrifuges is estimated at 1.2 kWh m^{-3} d^{-1}, equating to electrical energy consumption by the centrifugal separators of 75.5 GJ ha^{-1} yr^{-1}, a value that represents 15 % of the total operational costs and makes this the second-highest contributor to the primary energy input.

Total electrical energy consumption for plant operations was calculated to be 581.7 kWh d^{-1} or 517.3 GJ ha^{-1} yr^{-1}. At a 58 % conversion efficiency, this operational energy consumption corresponds to 891.9 GJ ha^{-1} yr^{-1} of primary energy input required.

6.4.3 Energy balance (net energy ratio) of the 1-ha plant

Calculation of the net energy ratio (NER) for any energy generating system entails a judgment on what constitutes the system boundaries. We have chosen to use previously established and validated system boundaries [34] (Fig. 6–1) in order to facilitate meaningful comparison between our results for the novel IGP design and those analyses already present in the open literature. Using these system boundaries, a calculated NER value of 2.96 (Table 6–4) was found for the model 1-ha IGP plant with an annual output of 66.0 tons ha^{-1} yr^{-1} of *Nannochloropsis* sp. biomass.

This value implies that the sum of the photosynthetically-based chemical energy derived from the algal biomass and electrical energy produced by the photovoltaic panels is 66 % higher than the non-renewable fossil fuel input required for the development and operation of the plant. This high NER is possible because the IGP system can be operated at near-maximum productivity without additional thermal regulation and because the PV module provides all of the electrical energy required for plant operation (Table 6–4).

The results show that electrical energy consumption for culturing and harvesting (plant operations) corresponds to 56 % of the primary energy input. The embodied energy of the plant materials and the fertilizers required for the growth of the microalgae represent 25 % and 19 %, respectively, of the overall energy consumption. In the previous energy analysis by Tredici et al. [34] with the same system boundaries and plant size, energy for reactor operation represented the dominant primary energy input (59 % of the total) to the plant. However, the value of the NER obtained in this study is 49 % superior to the 1.7 reported for a hypothetical 1-ha PV-GWP-II integrated system for the production of *Tetraselmis suecica* biomass in Mediterranean African countries, such as Tunisia. The study by Tredici and colleagues assumed that a 25 % coverage of the GWP panels with Si-PV panels (15 % efficiency) was enough to produce all the electrical energy needed for production operations without decreasing the annual biomass productivity. Similarly, Sforza et al. [54] have proposed the use of a photovoltaic-driven photobioreactor and demonstrated that 30 % coverage of the photobioreactor's illuminated surface with a conventional Si-PV panel does not result in a reduction in productivity.

Table 6–2. Energy consumption for N and P fertilizers required to achieve biomass productivity of 66.0 tons ha^{-1} yr^{-1} (66,000 kg ha^{-1} yr^{-1}) of *Nannochloropsis* sp. MUR 267 in a 1-ha IGP plant for 330 days

Nutrient type	Elemental content in biomass (%)	Nutrient source	Energy content of nutrient source (MJ kg^{-1})	Nutrient content in source (%)	Nutrient unit energy cost (MJ kg^{-1})	Daily nutrient usage (kg ha^{-1} d^{-1})	Daily energy cost (MJ ha^{-1} d^{-1})	Yearly energy cost (GJ ha^{-1} yr^{-1})
N	6.0[a]	$NaNO_3$	9.4[b]	16.5	56.9	12.0	683.6	225.6
P	0.6	$NaH_2PO_4.H_2O$	8.6[b]	22.4	38.4	1.2	46.1	15.2
Total							729.7	240.8

[a] [50]
[b] [25]

Table 6–3. Power consumption for operation of the 1-ha IGP plant producing 66.0 tons ha^{-1} yr^{-1} of *Nannochloropsis* sp. biomass for 330 days.

Equipment	Function	Power consumption		Primary energy input (GJ ha^{-1} yr^{-1})	Contribution to operational cost (%)
		kWh d^{-1}	GJ ha^{-1} yr^{-1}		
2 x Centrifuge	Collection of algal biomasses	190.8	75.5	130.2	14.6 (8.8)
4 x Pumps	Seawater pumping for preparation of nutrient medium, medium distribution and culture pumping	27.7	10.9	18.9	2.1 (1.3)
2 x Blowers	Mixing of culture	363.0	430.9	742.9	83.3 (50.7)
1 x Submersible pump (PEC only)	Cooling	280.8	333.3	574.7	39.2
Total		**581.5 (862.3)**	**517.3 (850.6)**	**892.0 (1,466.7)**	**100.0**

Values in parenthesis are percentage contribution for passive evaporative cooling (PEC) option.

In other work, Barbera et al. [55] showed that 50 % coverage with Si-PV modules of the south-oriented roof for an east-west oriented PV-integrated greenhouse with open raceway ponds did not negatively affect biomass productivity. In our previous work with this novel photobioreactor, we have noted that although the integrated PV panel covers 40 % of the total illumination surface, there is no decrease in the overall annual production of biomass [22]. What the current analysis shows is that the integration of the PV panel doesn't reduce achievable biomass productivity and actually produces a surplus energy output of approximately 235 GJ ha^{-1} yr^{-1}, a figure that represents 7 % of total energy output from the IGP plant (Table 6–4). This additional electricity could be used to supply extra illumination and heat at night to increase productivity rates or used for biomass drying and other downstream processing, further improving the economic viability of large scale microalgal culture. Given that a previous report of a comparably sized flat plate photobioreactor producing *Nannochloropsis* sp. biomass determined an NER value of 4.5, the NER for the IGP looks relatively low [23]. However, the high NER value reported in that work is likely a result of a much less conservative estimate of annual biomass productivity (100 tons ha^{-1} *cf.* 66 tons ha^{-1}), the exclusion of significant energy inputs such as nutrients, cooling and harvesting, and a disregard for energy losses due to the conversion of electricity input to primary energy. Those authors also assumed an energy content for the biomass of 30 MJ kg^{-1} (=3,155 GJ yr^{-1}) which is only possible if the biomass has a lipid content at the higher end of expected lipid productivities available in the literature. Given that increase in lipid content of a culture is often associated with a reduction in biomass productivity, an output of 100 tons ha^{-1} yr^{-1} of algal biomass under such conditions appears questionable. We believe that our more conservative lipid and biomass production values, which are derived from validated outdoor

trials using the IGP photobioreactor technology and an analysis that includes realistic energy conversion losses and nutrient inputs provides a more realistic NER for production modelling.

Table 6–4. Net energy ratio for biomass production from *Nannochloropsis* sp. using the 1-ha plant operating for 330 days

Parameter	Unit	IGP	PEC
Ground areal productivity	g m^{-2} d^{-1}	20.0	20.0
Biomass productivity	tons ha^{-1} yr^{-1}	66.0	66.0
Biomass energy content	MJ kg^{-1}	25.7	25.7
Energy output of biomass	GJ ha^{-1} yr^{-1}	1,696.2	1,696.2
Energy output from PV	GJ ha^{-1} yr^{-1}	1,126.8	0
Total energy for operations	GJ ha^{-1} yr^{-1}	892.0	1,466.7**
Excess energy from PV	GJ ha^{-1} yr^{-1}	234.8 (1,126.8 – 892.0)*	0
Energy consumption for fertilizer	GJ ha^{-1} yr^{-1}	240.8	240.8
Energy embodied in materials	GJ ha^{-1} yr^{-1}	411.0	329.7
Total energy output	**GJ ha^{-1} yr^{-1}**	**1931.0 (1,696.2+234.8)**	**1,696.2**
Total energy input	**GJ ha^{-1} yr^{-1}**	**651.8**	**2,037.2**
Net energy ratio	**-**	**2.96**	**0.83**
Net gain	**GJ ha^{-1} yr^{-1}**	**1,279.2**	**-341.0**

*Scenario where the PV is used to offset all the electrical energy for plant operations

**additional submersible pump for pumping cooling water with the exclusion of energy contained in water lost by evaporation.

6.4.4 Comparison of NER of self-cooling PV-PBR (IGP) and freshwater-based passive evaporative cooling (PEC) photobioreactors

The control of temperature in closed photobioreactors is a critical issue in solar microalgal farming and also has an impact on the energy consumption and environmental sustainability credentials of a commercial-scale operation. Passive evaporative cooling systems have commonly been used to maintain an optimum temperature for maximum biomass production [36], but this mechanism of temperature control can be challenging and leads to unfavorable energy balances at large scale. Being able to 'tune down' the energy demand for the thermal regulation of a photobioreactor used in microalgal production could result in significant improvements to the economic and environmental performance of commercial-scale microalgal 'farms'. This scenario was the inspiration for the design of the IGP photobioreactor and the combination of the use of selective filtering of incoming solar radiation to remove infra-red heat-producing wavelengths and recent work has shown that incorporation of infra-red reflecting materials, such as low-e films, on illuminated surfaces can remove up to 90 % of incoming heat-inducing wavelengths without a deleterious impact on cell viability [17, 22, 36]. Use of these technologies has been shown to remove the necessity for extraneous cooling systems in outdoor applications [22].

In order to determine if the IGP based photobioreactor is really an improvement on the use of a cheaper to build flat plate, glass only system, the NER of a flat-panel photobioreactor requiring passive evaporative cooling (PEC) for culture temperature control was determined using the same boundary conditions used for the analysis of the IGP

technology. Results from previous studies on *Nannochloropsis* sp. MUR 267 indicates that maximum bioproductivity was achieved when culture temperature is maintained at < 30 ˚C [43]. Temperature and solar radiation data from our model location of Karratha (Fig. 6–2) indicated that cooling for 5.6 hours per day for 7 – 8 months of the year would be required if using a traditional photobioreactor design. A submersible pump with a power consumption of 0.077 kWh m^{-3} and a lifespan of 5 years [34] was considered adequate for such a cooling operation resulting in a requirement of approximately 1,000 kg m^{-2} yr^{-1} of water for cooling. Due to changes in materials required to construct the photobioreactor itself, the calculated embodied energy of a 1-ha PEC plant actually decreases to 329.7 GJ ha^{-1} yr^{-1} (Table 6–1), but the total operational energy consumption climbs 64 % to 1,466.7 GJ ha^{-1} yr^{-1} (Table 6–4). Using a 58 % energy conversion efficiency, the cooling cost alone reaches 574.7 GJ ha^{-1} yr^{-1}, representing 39.2 % of total operational energy cost (Table 6–3). A similar finding has been reported by Tredici et al. [34]. The overall NER of the 1-ha PEC plant producing 66.0 tons of dry biomass annually is calculated to be 0.83 (Table 6–4). This value is 72 % lower than that calculated for the IGP based plant and well below the minimal threshold considered necessary for the sustainable production of biofuel, food, or feed based on wet biomass production.

6.4.5 Significance of the work

Overheating and high energy input in photobioreactors impede their scalability for microalgae-based biorefinery. Evaporative cooling systems utilizing a freshwater spray on the photobioreactor illuminated surface are not cost-competitive or sustainable due to high energy and water demand, driving the energy balance of closed photobioreactors to a negative value. Recently, the integration of photovoltaic modules into microalgal photobioreactor plant design has been mooted as a way to improve the net energy ratio of

the system by providing locally produced, non-fossil fuel derived energy to generate electrical energy as well as partial shading of photobioreactors to reduce biomass productivity reductions due to photoinhibition. This work has taken that idea to the next step by integrating both spectrally-selective low-e films (to reduce heat gain) and semi-transparent PV panels (to generate electricity) onto the illumination surface of the photobioreactor.

The NER value of 2.96 achieved in this study is at least comparable to, if not better than, that reported for the most efficient photobioreactor based microalgal plants [33]. However, this NER appears insufficient when compared to methods based on converting conventional crops as fuel, feed, or food sources, as the analysis of the energetic efficiency of the plant does not include downstream processing of the wet biomass (see system boundary, Fig. 6–1). Nonetheless, these results do show that the cultivation of microalgae in the IGP photobioreactor is energetically sustainable and can compete favorably with conventional food/biofuel crops considering the market value of the final product or a multi-product biorefinery scenario. For example, one of the most important crops in the world, soybean (*Glycine max*), has average annual grain productivity of 2.6 tons ha^{-1} yr^{-1} (Fig. 6–4). This yield corresponds to energy output and input of 39.2 and 10.6 GJ ha^{-1} yr^{-1}, respectively resulting in a high NER value of 3.7 [56], not that much higher than the 2.96 calculated for the IGP photobioreactor. The primary energy inputs included in the system boundary of soybean grain production were labor, machinery, fertilizers, electricity, herbicides, and transportation. For other microalgal studies with similar plant size and no PV intervention, variable NERs of 0.6 – 7.1 have been reported [33, 34]

The use of microalgal photobioreactor based systems also has the potential to reduce the environmental impact of biofuel and biocommodity production as a result of the

substantially higher concentration of lipids etc. present in microalgae compared to soil-based crops. For example, the lipid content of soybean is 18 % dry weight [57] vs. 50 – 55 % (based on ash-free dry weight) of *Nannochloropsis* MUR 267, [42] which converts to lipid yields of 0.47 tons ha^{-1} yr^{-1} and 33.0 – 36.3 tons ha^{-1} yr^{-1}, respectively. Lipid yield of *Nannochloropsis* sp. in the IGP photobioreactor is 74 times higher than the soybean oil. A similar difference in yield is also seen for non-food bioenergy crops, such as *Jatropha curcas*, where the average dry seed yield and oil content is 5 tons ha^{-1} yr^{-1} and 34.4 %, respectively [58]. That equates to a lipid yield for *Jatropha* crops of 1.72 tons ha^{-1} yr^{-1}, a value that is still 20 times less than that of *Nannochloropsis* sp. Even in a biorefinery scenario, e.g., the conjoint production of lipid and protein, the use of the IGP photobioreactor is comparable to soil-based cropping. The protein content of soybean is 35 % [34] vs. 38 – 40 % for a *Nannochloropsis* sp. MUR 267 maintained at the logarithmic growth phase [41, 42, 53], which translates to a calculated protein yield from nutrient replete *Nannochloropsis* sp. biomass of 25.1 – 26.4 tons ha^{-1} yr^{-1}, compared to a paltry 0.91 tons ha^{-1} yr^{-1} for soybean. These results are not limited to *Nannochloropsis* as Tredici et al. [34] have reported comparable protein yields of *Tetraselmis suecica* (45 % protein content) cultured in a GWP plant as 16 tons ha^{-1} yr^{-1} in Tuscany (Italy) and 30 tons ha^{-1} yr^{-1} in Tunisia (Africa). Overall, *Nannochloropsis* sp. biomass yield in the IGP photobioreactor per unit land area reaches 1,696.2 GJ ha^{-1} yr^{-1}, leading to a net energy balance (gain) of 1,279.2 GJ ha^{-1} yr^{-1} (Table 6–4), which is 45 times higher than that of soybean.

The environmental sustainability credentials of microalgal culture are often touted as being significantly greater than soil-based cropping, particularly as saline microalgal culture can conceivably be achieved in desert and arid zone areas with where cropping is marginal at best. However, Morales et al. [11] have recently reminded us that the actual long term

environmental and financial viability of these facilities is based on a rigorous examination of both environmental, energy, and economic parameters. Thus the actual impact and viability of new technologies need to be assessed not only by energy analysis but the other tools available via life-cycle and rigorous techno-economic analysis. We are currently in the process of undertaking such analyses for an IGP based facility.

Other options for improving the NER of the integrated photobioreactor system can be explored through optimization of the microalgae cultivation process and optimizing the optical properties of the semi-transparent PV panel and its energy conversion efficiency. Improvement in the PV efficiency should mean higher electricity production for the same PV coverage, and thus, increasing the overall energy output of the plant. Combining the technology with strain selection, e.g., microalgal strains that are resistant to temperature fluctuation and with higher annual biomass productivity, would obviously improve viability. Photobioreactor design parameters can also be investigated in greater detail to better dissipate heat and/or increase thermal inertia to diel variation of culture temperature.

The net energy ratio analysis performed demonstrates that PV integration with microalgae production in photobioreactors is advantageous from an energy efficiency viewpoint. The incorporation of that PV capacity directly into the photobioreactor design only enhances the NER and results in a standalone cultivation system, that provides an excellent energy-efficient technology suitable for the production of high-quality microalgal feedstocks for aquaculture, value-added pigments, pharmaceuticals, nutraceuticals, and bioactive compounds. This photobioreactor provides a reliable experimental platform for studying microalgal performance on a large-scale, especially in remote areas lacking grid electricity and access to freshwater for evaporative cooling.

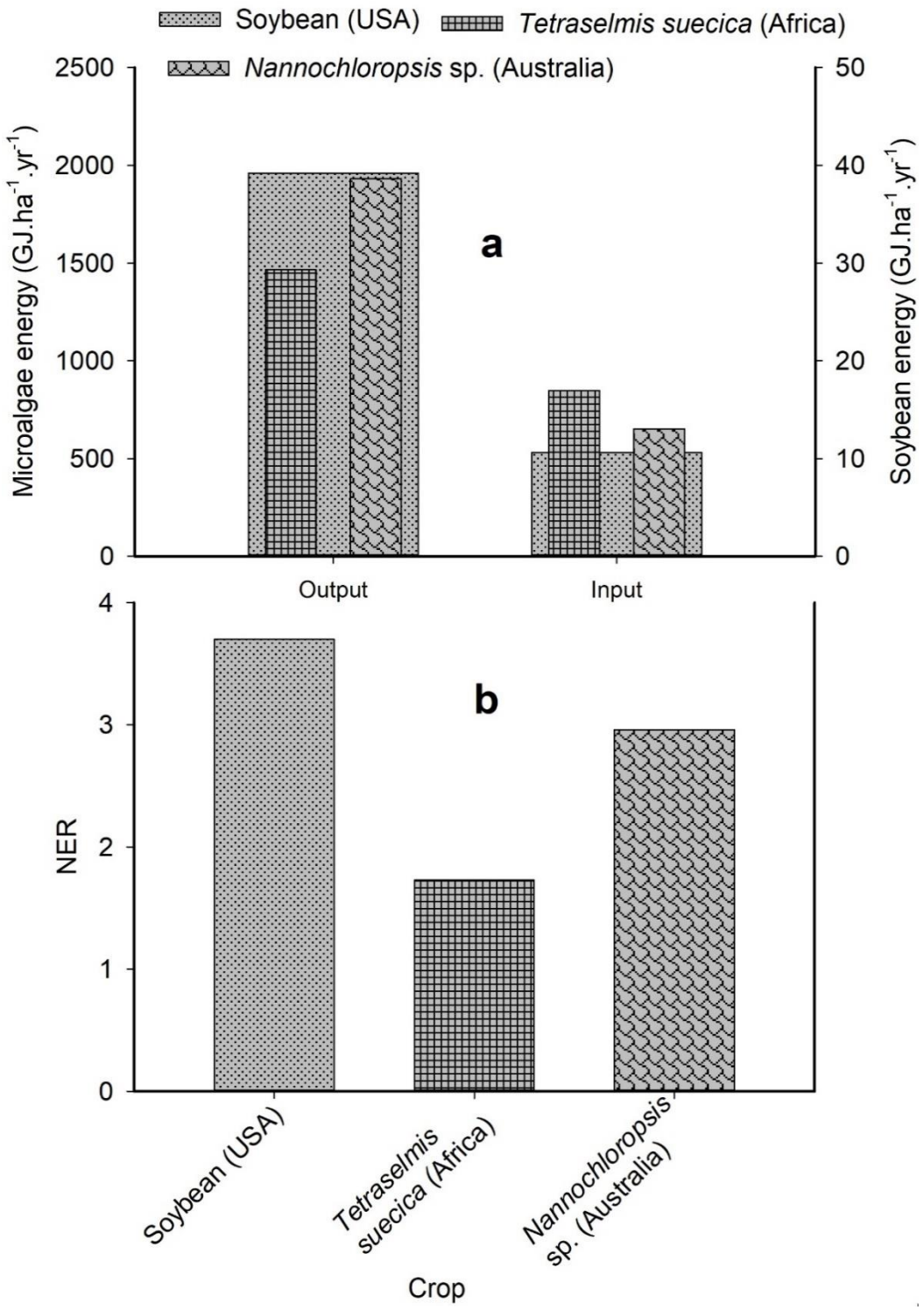

Fig. 6–4. (a) Annual energy output and input and, (b) NER for the production of soybean, *Tetraselmis suecica*, and *Nannochloropsis* sp. MUR 267 biomass in a 1-ha plant. *T. suecica* and *Nannochloropsis* sp. plants are PV-integrated.

6.5 Conclusions

The integration of semi-transparent photovoltaic panels to spectrally-selective insulated glazed photobioreactors offers a trinity of benefits: (a) sourcing local electricity for the plant operation; (b) eliminating freshwater-based cooling of photobioreactors, and (c) a strong reduction in diel temperature fluctuation. These could neutralize the strong external cooling water and electrical energy requirements of microalgal photobioreactors. In this study, the primary energy inputs and outputs for an industrial 1-ha installation of insulated glazed photovoltaic (IGP) self-cooling photobioreactors for the production of *Nannochloropsis* sp. MUR 267 biomass have been investigated. Biomass productivity data for *Nannochloropsis* sp. cultivation in a pilot IGP photobioreactor was scaled to 1-ha and indicated the generation of 66.0 tons ha^{-1} yr^{-1} of biomass and combined energy output (biomass + PV) of 1931 GJ ha^{-1} yr^{-1}. Energy inputs to the plant added up to 652 GJ ha^{-1} yr^{-1}, of which 63 % is contributed by the energy embodied in plant materials and 37 % from fertilizers, culture mixing, and culture harvesting. The analysis indicated that an NER of 3.0 is achievable for the IGP photobioreactor. Comparable analysis for non-PV integrated photobioreactor requiring passive evaporative cooling provided an NER of only 0.8. The calculated NER value for the IGP module is comparable to the best soil-based crops utilized for lipid-based oil production, indicating that this technology could be considered economically viable for mass production of *Nannochloropsis* biomass for various bio-based products. Results show that high net energy gain (1,279 GJ ha^{-1} yr^{-1}) and lipid yield that is at least 25 times higher than dedicated bioenergy crops such as *Jatropha curcas*, can be achieved, demonstrating the high potential of the IGP photobioreactor for the cultivation of microalgae for food, feed, biofuel, personal care, and pharmaceutical applications. The

experimental and calculated results from the standalone IGP photobioreactor suggest that commercially sized installations based on the incorporation of spectral selection and integrated transparent PV should play a significant role in future scenarios for the scaling up of grid-independent production of biomass, bioproducts and renewable electricity from a single algal plant installation.

6.6 References

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Chapter 7
General conclusion

The operation of solar-based microalgal photobioreactors to produce bio-based products requires sufficient cooling and heating technologies to ensure high biomass productivity over the year. Heating and cooling operations are energy-intensive processes and therefore, expensive. Furthermore, conventional evaporative cooling method achieved through freshwater spraying on solar closed photobioreactors for culture temperature control is neither economical nor sustainable. To this end, we have developed a pilot-scale spectrally-selective, insulated-glazed photovoltaic (IGP) flat plate photobioreactor with an infrared reflecting system embedded in the illumination surface for the thermal regulation of outdoor photobioreactors, while coproducing microalgal biomass and electrical energy. The IGP photobioreactor has several benefits over conventional flat plate photobioreactors. The integration of semi-transparent photovoltaic panels results in a sustainable baseload electricity sourcing for the standalone operation of the plant. The spectrally-selective infrared reflective materials on the illumination surfaces limit the input of a substantial quantity of solar-derived heat energy to the culture, thus, diminshing or possibly even eliminating the requirement for external cooling of microalgal photobioreactors with freshwater. The double glazed insulated panels, together with the low-emissivity films, provides a high thermal insulation property contributing to a strong reduction in diel temperature fluctuation.

The proof-of-principle of the IGP photobioreactor is presented in Chapter 2. I demonstrated that coating flat plate photobioreactors with spectrally selective materials could be a viable strategy for managing their internal temperature for sustainable *Nannochloropsis* sp. MUR

267 and *Arthrospira platensis* MUR 126 cultivation. The spectrally-selective insulated photobioreactors supported the growth of *Nannochloropsis* and *Arthrospira*, with productivities and photophysiological values that were similar to those of traditional photobioreactors used as controls. The feasibility experiment paved the way for the design and construction of a pilot-scale IGP photobioreactor under outdoor conditions to test the long-term suitability of proposed systems on the growth and photosynthesis of these microalgal species. Thus, in Chapter 3, I concentrated on the spectral characterisation and biological performance of the IGP photobioreactor under outdoor condition for culturing *Nannochloropsis* sp. in comparison to a standard photobioreactor under passive evaporative cooling and a raceway pond. This study established that the use of IR reflective materials on the illumination surfaces of PBRs to limit the input of heat-inducing wavelengths to the culture can keep the culture temperature within the optimum range with no loss of biomass productivity. The IGP photobioreactor was used to successfully grow the microalga *Nannochloropsis* sp. under outdoor climatic conditions without freshwater-based cooling, with biomass productivity that was like standard plate reactor and significantly higher than the raceway pond. Not having to rely on fresh clean water for cooling has obvious environmental benefits, particularly as many areas suitable for outdoor microalgal production have limited freshwater resources. The standalone IGP generated 72 W m^{-2} of electrical energy, which was approx. three times higher than the mixing energy requirement, while supporting >14% and 71% higher biomass productivity than passive evaporative cooling photobioreactors and raceway pond, respectively. In Chapter 4, I focused on the effect of the temperature control strategy of the IGP on the biochemical constituents and in particular, the fatty acid composition of the *Nannochloropsis* biomass. The biochemical composition of biomass showed a general trend of lipid > protein > carbohydrate, a pattern that is typical of *Nannochloropsis* sp., with no large

variation in each content across treatments. The dominant fatty acid, C16:0 was 24% higher in the photobioreactors than the raceway pond and no other significant shift in major saturated and monounsaturated fatty acid components of this alga were seen among cultivation systems. However, the highest essential fatty acids, eicosapentaenoic acid (EPA, C20:5n-3), 16% and ϒ-linolenic acid (C18:3n-6), 8% of total fatty acid were found in the raceway pond with the lowest average culture temperature and diel temperature variation than photobioreactors. Therefore, constructing photobioreactors with spectrally-selective materials was demonstrated as a viable strategy for managing the internal temperature of photobioreactors, with no significant negative impact on biochemical and fatty acid profiles of microalgae. The reliability of cultures in the developed photobioreactor was investigated by culturing a cold-intolerant microalga, *Arthrospira* during austral winter, and this was the focus of Chapter 5. Interestingly, I found similar average temperatures in heated and thermally-insulated photobioreactors. Consequently, *Arthrospira* was successfully cultivated in the IGP photobioreactor under outdoor climatic conditions of austral winter with biomass productivity and corresponding c-phycocyanin content that were respectively, 67% and 45% higher than those achieved in a classical raceway that was heated continuously. This study demonstrated that the technological innovation in thermally-insulated IGP photobioreactor could be used to intensify the reliability of outdoor microalgae cultivation in photobioreactors during austral winter seasons with suboptimal climatic conditions without the requirements for extraneous cooling and heating systems. In Chapter 6, I evaluated the energy efficiency of an industrial 1-ha installation of the IGP self-cooling photobioreactors for the production of *Nannochloropsis* sp. MUR 267 biomass based on data obtained from the pilot-scale operation of the single photobioreactor. Biomass productivity in 1-ha was 66.0 tons ha^{-1} yr^{-1} of dry biomass and combined energy output (biomass energy + photovoltaic

energy) of 1931 GJ ha^{-1} yr^{-1} was produced. Energy inputs to the plant was 652 GJ ha^{-1} yr^{-1}, of which 63 % was contributed by the energy embodied in plant materials and 37 % from fertilizers, culture mixing, and culture harvesting. The analysis indicated that a net energy ratio of 3.0 is achievable for the IGP photobioreactor. Comparable analysis for a non-photovoltaic integrated photobioreactor requiring freshwater passive evaporative cooling provided an NER of only 0.8. A high net energy gain of 1,279 GJ ha^{-1} yr^{-1} was obtained, demonstrating the high potential of the IGP photobioreactor for the cultivation of microalgae for food, feed, biofuel, personal care, and pharmaceutical applications. The experimental and calculated results from the standalone IGP photobioreactor suggest that commercially sized installations based on the incorporation of spectral selection and integrated transparent photovoltaic panel could play a significant role in future scenarios for the scaling up of grid-independent production of biomass, bioproducts and renewable electricity from a single algal plant installation.

7.1 Future directions

The combination of passive temperature control and photovoltaic energy generation provides a notable pathway for conjoint production of desired microalgal bioproducts and electrical energy for operational needs in a single photobioreactor. This is especially appealing in remote areas (often well suited to large-scale microalgal culture and lack access to grid electricity and freshwater), neutralizing the compelling economic costs of energy required for the cultivation. Previous studies have shown that the actual long term environmental and financial viability of microalgal facilities is based on a rigorous examination of both environmental, energy, and economic parameters. Thus, the actual impact and viability of new technologies such as the insulated-glazed photovoltaic

photobioreactor need to be assessed not only by energy analysis but the other tools available via life-cycle and rigorous techno-economic analysis. Further studies are required in the following areas:

a. Detailed techno-economic and life-cycle analyses of the self-sustainable, standalone insulated glazed photovoltaic flat plate photobioreactor are needed to assess the economic viability and environmental impact of microalgal production using the technology.
b. A wider spectrum of microalgal strains should be cultured in the photobioreactor to understand their physiological and metabolic responses to thermal regulation employed in the system
c. Long-term and continuous culture of microalgae in the novel photobioreactor covering austral winter, spring, autumn and summer need to be conducted to obtain long term reliable biomass productivity and power efficiency data.
d. Scale up to a commercial size must be trialled
e. The novel photobioreactor operations need to be automated based on the growth conditions (high productivity, free CO_2, free/minimum nutrients, minimum mixing).
f. Photobioreactor design parameters can also be investigated in greater detail to better dissipate heat and/or increase thermal inertia to diel variation of culture temperature.
g. Model microalgae growth and biomass productivity of the IGP operating outdoors under local climatic conditions over a whole year of production.