Research Article

Sustainable cultivation of microalgae by an insulated glazed glass plate photobioreactor

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Abbreviations: PBR, photobioreactor; **IGP**, insulated glazed glass photobioreactor; **CLP**, cool light photobioreactor; **HLP**, hot light photobioreactor; **IGU**, insulating glazing unit; **PAR**, photosynthetic active radiation; **UV**, ultraviolet; **IR**, infrared; **Chl-*a*** , Chlorophyll *a*; **PSII**, photosystem II; $\mathbf{F_V/F_M}$, maximum quantum yield; **OJIP**, chlorophyll *a* fluorescence induction curve

Abstract

Microalgae growth in closed photobioreactors is greatly inhibited by elevated temperatures caused mainly by the infra-red portion of light. Current passive evaporative cooling systems for temperature control in outdoor photobioreactors are neither economical nor sustainable. Here we built a novel flat plate photobioreactor with its illumination surface customized with insulated glazing units (IGP). The IGP design enabled transmission of more than 50% of visible light while blocking 90% of ultraviolet and infrared radiations. The growth and productivity of *Nannochloropsis* sp. (MUR 267) in the IGP was compared against conventional flat plate photobioreactors subjected to the full spectrum (HLP) and also externally modified spectrum (CLP) of halogen lights. High temperature (up to 42^{o}C) resulted in no growth in the HLP. Biomass productivities of *Nannochloropsis* sp. grown in the CLP was significantly higher than the IGP due to higher light transmission and lower temperature profiles recorded in the CLP. Lipid content of *Nannochloropsis* was highest in the CLP (60.23%) while protein was highest in the IGP (42.43%). All photosynthesis parameters were negatively affected in the HLP. The IGP's ability to remove infrared (heat) makes this newly developed photobioreactor a promising and sustainable cultivation system for mass algal production especially for high value products.

1 Introduction

The continuous increase in world population and expansion of the global economy places an ever-growing demand on the natural resources of our planet. The continued development and improvement of efficient and sustainable energy and food production systems is critical for supporting this requirement. Despite extensive effort in moving towards more sustainable primary industries, no single renewable energy or an alternative food stock is sufficient to reliably alleviate the demand for more traditional resources to any significant degree [1]. Therefore, there is a great need for a combination of various energy and food production systems which can efficiently exploit the different range of conditions experienced around the globe [2, 3]. This would be both the safest and most productive option for future energy and food production [3].

Microalgae biomass cultivation is a promising method for the sustainable production of food, feed, fuel and also high value bioactive products. The cultivation of microalgae has limited competition with food crops over the use of agricultural land and the need of freshwater [4, 5]. This is a key advantage when compared to all other biofuel crops and has led to microalgae biomass being considered a viable raw

material source for bioenergy production [6]. Irrespective of these advantages, due to low biomass productivity and high capital and operating expenses, no economically viable large scale microalgae to biofuel production plant is currently operational world-wide. Presently, microalgae cultivation facilities employ systems such as open ponds and closed photobioreactors for the mass production of algae biomass.

Open ponds are inexpensive and are easy to operate but have major shortfalls. They are susceptible to contamination, and experience extreme conditions (high temperature, pH and salinity) since culture parameters are not easily controlled, making them favourable only for the cultivation of a few robust microalgae strains [7]. On the other hand, closed microalgae systems, such as photobioreactors (PBRs) are currently sought after, as they offer better regulation of optimum growth conditions when compared to open ponds and significantly reduce the risk of culture contamination [8]. Flat-plate PBRs have been widely suggested for the outdoor cultivation of microalgae, since they incorporate a large illumination surface area to volume ratio [9].

The same as any other outdoor cultivation system, flat-plate PBRs are subjected to two critical environmental factors regulating algal growth: sunlight and temperature [8]. Considering that sunlight is the main source of illumination for any outdoor microalgae cultivation system, biomass productivity is observed to be directly dependent on the climatic conditions of a particular locality or region. Excessive light can lead to photo-oxidation and photoinhibition, bringing forward a significant decline in the growth and productivity of microalgae grown in outdoor PBRs while insufficient supply of light can be of growth-limiting [10]. Variations in seasonal temperature coupled with extreme daily temperature fluctuations also yield significant changes in microalgae growth, photosynthesis efficiency and biomass productivity when grown outdoor [11]. In tropical regions, microalgae grown in outdoor PBRs with no temperature control mechanisms can be subjected to temperatures as high as 60^{o}C [12]. Such temperatures are well above the tolerance thresholds of most commercially grown microalgae species [11-14]. Among the various metabolic processes in plants and algae, photosynthesis is reported to be the most heat sensitive reaction [15]. As in any other chemical reaction, it is observed that the rate of microalgae photosynthesis increases with temperature until optimum temperature is reached [16]. However, above optimum temperature, the rate of photosynthesis is seen to decline drastically due to the denaturation of vital enzymes and the inactivation of the PS II of the photosynthetic machinery [17, 18]. Evaporative cooling systems using freshwater are currently the most effective method for

controlling overheating in PBRs. However, the lack of freshwater availability in most regions with high solar irradiation makes such a process non-sustainable and economically unfeasible.

In this study, we designed and developed a novel glass plate photobioreactor, which allows more than 50% of the photosynthetic active radiation (PAR) to pass through while blocking and capturing over 90% of ultraviolet (UV) and infrared (IR) spectra. Infrared wavelengths in particular are the main reason for the temperature increase in PBRs which can negatively affect microalgal growth and photosynthesis [11, 19]. Ultraviolet has also been reported to damage algal cells resulting in their mutation or death [20] . Through our customized glass plate photobioreactor design, these captured spectra can be further converted to electricity through the integration of external photovoltaics. The electricity generated can be used for plant operation, supply extra artificial lighting, or to provide a heating option for the cultures at night [21]. In here, we also investigated the effect of heat stress on the photosynthesis response of *Nannochloropsis* sp. in our customized PBR compared to the conventional systems.

2. Materials and methods

2.1. Organism and culture medium

The marine Eustigmatophyceae, *Nannochloropsis* sp. (MUR 267) used in this study was obtained from the Murdoch University Algae culture collection. *Nannochloropsis* sp. was grown in F/2 medium as formulated by Guillard [22] made from natural sea water (collected from the coastal waters off Hillary's Beach, Perth, Western Australia). The seawater was charcoal filtered and autoclaved prior to the addition of sterile nutrients. The total culture volume of microalgae in each plate reactor was standardized to 5 litres and the initial cellular concentration of each reactor was adjusted to 2.2×10^7 cells mL^{-1}.

2.2 IGP design

The developed photobioreactor (IGP) was constructed using two different types of insulated glazing units (IGUs), namely standard low emissivity units (low-e) and customized low-e IGUs. Three of the four sides of the photobioreactor were built using standard low-e IGUs. The fourth light-exposed side of the reactor was constructed using a customised low-e IGU unit known as Tropiglas which employed an internal OptiWhite clear glass panel in conjunction with an external glass panel coated with a 7-layer metal-dielectric thin film [23]. This thin film enabled more than 50% of the visible light to pass through,

while blocking more than 90% of the UV and IR radiations (Figure 1). The air gaps of the IGU units also provided additional thermal insulation, making the visible light the dominant radiation illuminating the algal culture. The IGP was directly exposed to the full spectrum of an Arlec 500W portable halogen lamp. Halogen lamps were used as the source of illumination due to their continuous light emission spectrum similar to sunlight, especially their peaks in the IR region.

2.3 Experimental Setup

To validate the performance of the customized photobioreactor (IGP), we compared the growth and productivity of *Nannochloropsis* sp. with two other standard flat plate glass PBR designs. The first of these consisted of a standard photobioreactor positioned behind an external heat filter (CLP) which eliminated most of the incoming IR (positive control). The external heat filter compromised of a transparent glass vessel with cool water being continuously bubbled in it. The second standard PBR was exposed to the direct illumination of the halogen lamp (HLP) and represented the negative control. The base of each reactor was designed as a V-shape for better mixing of the microalgae culture and to prevent sedimentation of cells. The light: dark cycle was 12 h: 12 h similar to sunlight. Each reactor was mixed via uniform bubbling of sterile air from the bottom of each reactor panel, providing a liquid flow rate velocity of 1.2 cm s^{-1} [24, 25]. Underwater temperature data loggers (TINYTAG AQUATIC 2) were used for the continuous recording of culture temperature in each reactor. The irradiance and spectra composition of light was measured both at the outer center surface and also inside of each PBR using a StellarNet Spectrometer, Model BLACK-Comet CXR-SR-50 (Figure 2). The incident photon-flux density of PAR on the surface center point of each reactor was standardized to 285 µmol photons m^{-2} s^{-1} by varying the distance of the reactors from the light source (Figure 2). This was in accordance to make sure all reactors received the same input of light energy at its surface level.

2.4 Cell density and growth measurements

Cell count was measured daily using a Neubauer haemocytometer to determine cellular concentration. *Nannochloropsis* sp. in all reactors were grown in both batch and semi continuous modes. However, all measurement regarding the growth and productivity of the microalgae were only performed in the semi continuous mode [26]. Cultures were acclimated to each reactor conditions for at least 18 days prior to any measurements. The specific growth rate, µ, and ash free dry weight (AFDW) of *Nannochloropsis* sp. in each reactor was measured following the methods of Moheimani et al. [27]. Chlorophyll *a*

concentration was spectrophotometrically determined based on the methods of Jeffrey and Humphrey using 90% ice cold acetone [27].

2.5 Protein, carbohydrate and lipid determination

The relative percentage of protein, carbohydrate and lipid over organic biomass (AFDW) of microalgae in each reactor was determined in triplicate sets during each harvest. Samples were concentrated on Whatman GF-C filters and stored at -5℃ till further analysis. Total carbohydrate, lipid and protein content were determined following the methods of Moheimani et al. [27].

2.6 Photosynthesis measurements

The photosynthetic performance of cultures was investigated via the polyphasic chlorophyll *a* fluorescence induction (OJIP) curve using an AquaPen-C portable fluorometer (Photon Systems Instruments, Czech Republic). Measurement were made at pre-dawn (Hour 0) and also at pre-dusk (Hour 12) to study the response of the microalgae to the increase in temperature during the light illumination period and also the recovery potential of photosynthesis after the lights were switched off. On each sampling occasion, 20ml culture was collected from each PBR and incubated for 20 minutes in the dark according to the temperature of the corresponding PBR. After this period of dark-adaptation, a minimum of three 3mL aliquouts were used to perform pseudo-replicate measurements of Chl-*a* fluorescence parameters for each sample. The AquaPen-C was set to maximum saturation pulse intensity of 3000 µ mol photon m^{-2} s^{-1} red light (625nm) for 2 s and the Chl-*a* fluorescence OJIP curves were recorded using the supplied *FluorPen* software (Photon Systems Instruments). The O(K)JIP features of the fluorescence induction curve were defined following Strasser et al. [28] however F_O was defined at $F_{30\mu s}$ and, due to dark adaptation, $F_P=F_M$ where the peak occurs between 0.15 – 1.0 s. Analysis of the double-normalised variable fluorescence $[(F_t-F_O)/(F_M-F_O)]$ was performed to allow empirical extraction of all Chl-*a* fluorescence phenomenological information from the OJIP curve [29]. The estimated maximum quantum yield of PSII photochemistry (F_V/F_M) is regularly used as an indicator of plant stress [30, 31] and was employed here along with the related but less commonly used parameter F_V/F_O, which reflects the relative number of PSII reaction centres capable of evolving oxygen, to provide measures of photosynthetic capacity [32]. The shape of the Chl-*a* fluorescence induction curve in the range of $F_{0.1ms}$ – $F_{0.3ms}$ is recognised as being responsive to the impact of heat and we utilised the parameter W_K =

$(F_{0.25ms}-F_{0.03ms})/(F_{2ms}-F_{0.03ms}) = (F_K-F_O)/(F_J-F_O)$ as employed by Brestic et al. [33] and ΔW_{OJ}, following Oukarroum et al. [34], to investigate impacts to this region of the fluorescence transient.

3 Results

3.1 Light and temperature profile in each reactor

All three PBR setups were prepared and run in parallel to investigate both the effect of the customized IGUs on the developed PBR temperature profile and also to compare the growth and productivity of *Nannochloropsis* sp. between all three PBRs. The difference in light distribution inside all three PBRs is presented in Figure 2 illustrating that the HLP transmitted the highest proportion of infra-red spectra (750-1100nm) when compared to the CLP and IGP. The diurnal variation in temperature of *Nannochloropsis* sp. cultures in each PBR during the study is presented in Figure 3, illustrating that the temperature in all reactors was continuously increasing during the light illumination period. The highest temperature in all reactors was recorded at the final hour of illumination (12^{th} hour) after which temperature began to drop once the halogen lights were switched off and the dark cycle began (Figure 3).

3.2 Specific growth rate and biomass productivity

The growth of *Nannochloropsis* sp. in all three PBRs is also presented in Figure 3 where the cultures were initially operated in batch mode. Cultures were later operated semi-continuously when they reached a predetermined late logarithmic growth phase (90% of the maximum cell concentration) in which analytical measurements were performed (Figure 3). The specific growth rate (μ) of *Nannochloropsis* sp. was found to be significantly higher in the CLP when compared to the others (Table 1) (One Way Repeated Measures ANOVA $P < 0.05$). Culture in the IGP reached temperatures as high as 35.5 °C and exhibited a decrease in its μ when compared to that of the CLP, which recorded a maximum temperature of around 30.6 °C (Table 1). Elevated temperatures of up to 42.4 °C recorded in the HLP was found to be lethal and was responsible for the immediate decline in the growth of the algae which eventually led to the death of most cells. This consequently led to negative growth rates in the HLP and prevented the culture from being maintained under the semi continuous mode. For this reason, no further analyses were carried out using the HLP culture. In this study the highest biomass productivity for *Nannochloropsis* sp. was found to be in the CLP which recorded a value of 90.5 ± 8.16 mg L^{-1} d^{-1}. As

seen in Table 1, the biomass productivity in the CLP was significantly higher than the customized IGP (52.7 ± 5.70 mg L^{-1} d^{-1}). In terms of the amount of PAR transmitted into the reactors, *Nannochloropsis* grown in the CLP resulted in a productivity of 0.218 ± 0.02 [mg L^{-1} d^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$] which was 1.35 times higher than the productivity of this alga when grown in the IGP (Table 1).

3.3 Biomass composition

In this study, no significant difference was observed between chlorophyll *a* content for *Nannochloropsis* sp. grown in both the CLP and IGP (Table 1). Nevertheless, total lipid content over biomass was found to be significantly higher in the CLP when compared to that of the IGP while no significant difference was observed in the total carbohydrate content in both the PBRs (Table 1). In terms of total protein content, the microalgae grown in the IGP recorded a significantly higher value than that of the CLP (Table 1).

3.4 Chlorophyll *a* fluorescence analysis

Just before light exposure on day 1, each of the reported photosynthetic parameters derived from Chl *a* fluorescence data were reasonably uniform between the reactor types (Figure 4). By the end of 12 hour light illumination on the first day of the experiment, the estimated maximum quantum yield of PSII photochemistry (F_V/F_M) was severely reduced in the HLP reactor as a result of the treatment conditions. Fluorescence yield had decreased substantially in this reactor, with F_O and F_M only 30 % and 12 % of their original values, respectively (Figure 4). This reflects both the observed decline in cell density as well as PSII inhibition in the HLP reactor. F_V/F_M remains very low throughout the remaining measurement period in the HLP reactor but recovery is apparent on day 4. The IGP reactor also exhibited a decline in F_V/F_M relative to the 'control' CLP reactor during the day but this was marginal and, by the end of the 12 h dark period, the values were again similar. The parameter F_v/F_o represents the proportion of PSII reaction centres that are capable of evolving oxygen [32, 35] and was presented here to show that it follows the same pattern as the more commonly used F_V/F_M parameter but may be a more useful parameter as it exacerbates the differences between treatments. It was interesting to note that, in the longer term, F_v/F_M became significantly higher in the IGP when compared to the CLP (Figure 3) (One Way Repeated Measures ANOVA $P < 0.05$). While the W_K parameter, previously used to assess sensitivity to heat [33], and was observed to increase in the HLP reactor (and very marginally in the IGP reactor) the parameter was not as responsive to the heat treatment as the other parameters (Figure 4). While many have found that heat stress induces a K-peak in the fluorescence induction curve (0.2 – 0.3

ms time range), our measured ΔW_{OJ} (Figure 5) show that F_K actually tended to be a smaller fraction of F_J in treatment relative to control conditions. Very low variable fluorescence in the HLP reactor resulted in noisy ΔW_{OJ} data however there was a trend toward positive values (Figure 5).

4. Discussion

4.1 Growth and biomass productivity

This is the first study investigating the potential use of insulating glazing units (IGU) in a customized PBR in terms of eliminating harmful wavelengths such as IR and UV. Our results clearly indicated that using IGUs for the construction of plate photobioreactors will allow for the production of microalgae independent of the need of freshwater for evaporative cooling. The difference in growth and productivity of *Nannochloropsis* sp. in all three PBRs was found to be mainly due to the variation in temperature and light distribution between reactors. The extremely low Fv/F_M values and negative growth in the HLP highlights the importance of managing culture temperature within PBRs.

Of the two remaining PBRs mitigating heat delivery to the culture, the standard CLP method was identified to be more successful. The IGP was found to a) transmit 14% less PAR and b) have at least 4.9^{o}C higher temperature when compared to the CLP. Increase in light irradiance has been reported to bring forward an increase in biomass productivity of most microalgae, as higher light irradiance is seen to increase the reproduction of microalgae through its impact on photosynthesis until a saturation point is reached, after which photoinhibition is normally observed [36, 37]. Most microalgae increase their exponential growth rates as a response to increase in temperature until their optimum growth temperature is reached. Any further elevation of temperature is accompanied by rapid decrease in growth rates and productivity due to loss of structural integrity caused by heat stress [38]. Heat stress affects the growth of microalgae through the alteration of vital enzyme activities (denaturation, inactivation) or the modification of integral proteins which are part of the photosynthetic apparatus [16, 39]. Relatively poorer productivity in the IGP compared to CLP was most likely due to a combined outcome of both the lower transmission of PAR into the reactor and elevated culture temperatures, with higher temperatures being the primary factor. The higher temperature profile experienced by the IGP was likely due to the accumulated heat from the remaining 10% of infra-red (IR) radiation transmitted and also the conductive heating of the IGU coating itself through the absorption of the un-transmitted portions of IR. For the Eustigmatophyte, *Nannochloropsis* sp., the optimum growth temperature has been outlined to be between 24–27°C, and values exceeding this range have been seen to negatively affect growth and the photosynthetic ability of this alga [40, 41].

4.2 Biomass composition

The biochemical composition and content of microalgae such as protein, lipid, carbohydrate and pigments have been reported to vary according to different growth conditions. Light (quality and quantity) and also temperature have been observed to alter the overall biochemical composition of most microalgae [42, 43]. Chemical energy stored in carbon based compounds is derived from light harvested by microalgae while temperature is seen to manipulate the biochemical composition of algae through its effects on both cell composition and enzyme reactions [44, 45]. Previous studies have reported that increasing growth temperature results in lowering the overall lipid content of microalgae [46, 47]. This was in accordance to the culture grown in the CLP which had higher lipid content than the IGP. It is believed that most organism including microalgae manipulate their lipid composition to preserve their membrane fluidity at various temperatures [48]. In general, increase in growth temperature has been observed to bring forward a significant decline in protein concentration while increasing the amount of carbohydrates and lipid which contradicts the results of this study [49, 50]. The protein content of *Nannochloropsis sp.* grown in the IGP was observed to be significantly higher than that of the CLP. Nonetheless, this outcome is seen to be species specific as other studies have also shown contradicting outcomes, such as higher protein content at inflated temperatures in microalgae [51, 52]. Thus, it is safe to say that these changes are generally species specific and may differ when subjected to different growth conditions [53].

4.3 Photosynthesis

Photosynthesis is a highly heat sensitive reaction and measurements of Chl-*a* fluorescence have become a popular tool in investigating and characterizing the impact [15, 54]. The F_V/F_M ratio is a simple but important parameter indicating the primary photochemistry of PS II and the physical fitness of the organism [55]. In general, there was a similar pattern in the F_V/F_M ratio during the first four days in both the IGP and CLP as it was highest during pre-dawn followed by subsequent decrease during the light illumination period indicating that cultures began experiencing some stress due to the continuous rise in temperature (Figure 4). Nonetheless, values recovered to be highest again at pre-dawn when temperature decreased, indicating that the photodamage was reversible. The lower F_V/F_M values recorded during the start of the experiment in the IGP when compared to the CLP was due to the initial stress experienced by the microalgae when exposed to the higher temperature profile in IGP. However values recovered to be similar in the IGP and CLP during day 3 indicating *Nannochloropsis* sp. had

adapted to the growth conditions in the IGP (Figure 4). A possible explanation for the higher F_V/F_M values of *Nannochloropsis* in the IGP after day 7 might be related to the lower amount of UV radiation transmitted into the reactor (Figure 3). UV wavelengths have been found to be inimical for the process of photosynthesis due to their high energy content. Excessive UV radiations have been studied to disrupt molecular bonds and DNA of organisms [56]

The significant decline in the F_V/F_M ratio of *Nannochloropsis* cultivated in the HLP throughout the entire experiment is due to heat stress from the excessive temperature profile of the HLP. High temperature is seen to reduce the photosynthetic capacity and photochemical efficiency of photosynthetic organisms [57]. PS II is by far the most liable machinery of microalgae to undergo damage when subjected to elevated stress such as increase in irradiance and temperature [58]. The most common photosynthetic response of microalgae cells subjected to elevated temperatures well above 40^{o}C is the impediment of charge-separating action of PS II and the inactivation of oxygen evolution ability of PS II [38]. This leads to the production of oxygen radicals which can cause damage to cellular biochemical components such as fatty acid peroxidation, within the cell [59, 60]. This may also offer explanation for the lower lipid content of *Nannochloropsis* sp. in the IGP.

Predominantly, under optimum conditions, the fast phase chlorophyll *a* fluorescence kinetics is summarized by a polyphasic pattern of O-J-I-P from the rise in initial fluorescence level, F_O, to the maximum fluorescence level, $F_{M,}$ during a pulse of saturating light [61]. On illumination of samples with a strong saturating light, a fast electron transport process is observed and recorded by the O-J transition or rise within 2 ms. This is subsequently followed the thermal phase known as the J–I–P rise of the fluorescence transient within 1 s. Nonetheless, several previous studies have reported the appearance and dominance of an additional transient step (K step) at 0.3 ms in photosynthetic organisms when subjected to elevated temperatures (44-48 ^{o}C) [18, 19, 62]. The origin of this step has been associated with the damage of the donor side and the inhibition of the water splitting complex of PSII by heat stress [63]. Therefore, we calculated the W_K parameter, to identify any potential changes due to the heat stress as this has been shown to be a good indicator of heat sensitivity and is sensitive to a restricted range of stressors compare to F_V/F_M [33].

The high temperature profile recorded in the HLP is believed to have led to the increase in the W_K values of *Nannochloropsis* sp. from day 2 and there were no signs of recovery throughout the measurement period indicating that the damage to the photosynthetic apparatus was non reversible [3]. Exposure to high temperatures have been observed to bring forward significant damage to the functions of the

oxygen evolving complex (OEC), leading to an inhibition of electron transport between the OEC and PSII [18, 62, 64]. Heat stress is also observed to damage the membrane integrity of cells and brings forward damage to the antennae pigment-protein structures of PS II [65]. The similarity in the W_K values recorded in both the IGP and CLP throughout the experiment except for Day 4 indicates that the microalgae in both these reactors were not subjected to any major heat stress. However, tracking ΔW_{OJ} during the early "photochemical" phase of the OJIP curve clearly revealed that the photochemistry of all reactors was impacted under prevailing conditions. In both the CLP and IGP reactors this was evidenced by negative values near the K point (which we defined here as 0.25 ms) (Figure 5). That is, a trough occurred rather than the commonly reported peak at this point in response to heat treatment. The trough was greater for the IGP treatment, presumably due to the higher temperatures in this reactor, while the impact observed in the CLP reactor is thought to be due to the brighter light environment resulting from culture dilution as it was split to the three treatments at time zero. Oukarroum et al. [34] has previously described a similar response in barley (*Hordeum vulgare* L.) when exposed to moderate stress but a switch to positive values (a K-"peak") under extreme stress conditions. The negative ΔW_{OJ} values exhibited by *Nannochloropsis* sp. in the CLP and IGP reactors reflects an increase in energetic connectivity between PSII reaction centres and may reflect a photoregulatory mechanism to minimise damage to the reaction centres [34]. ΔW_{OJ} data for the HLP reactor was quite noisy due to the severe quenching of the fluorescence signal however there was a definite trend toward positive values. This can reflect damage to the oxygen evolving complex or significant damage to the PSII supercomplexes resulting in monemerization and poor energetic connectivity [66] (Figure 5).

4.4 Electricity generation potential

Using the IGP design, a basic energy analysis was made to calculate the amount of electricity that could be potentially generated through the integration of external photovoltaics. Up to 25.2 W m^{-2} of electrical power; can be generated by the un-transmitted solar spectra. This theoretical figure was calculated for a 20cm× 20cm sample design based on the spectral photoresponse of a CIS solar cell with 12% conversion efficiency. This was calculated assuming 1000 W m^{-2} solar flux was the total incoming optical power comprising 500W m^{-2} as the optical power of the visible light, 450W m^{-2} as the optical power of IR, and 50W m^{-2} as the optical power of UV. This electricity produced can be used to run the microalgae cultivation system or redirected to provide extra artificial illumination to improve the productivity of the microalgae. At an efficiency of about 39.8% and 73.9% respectively, around 10W of

blue light (465-485nm) and around 18W of red light (620-630nm) could be produced from the generated electricity using additional LEDs (X Lamp XP-E LEDs).

In practice, the photovoltaics would be attached to the edges of the photobioreactor glass plates and would be internally connected in parallel. Therefore, the electricity produced would be collected from two output wires. When operational, the power output capability of the photobioreactor can be monitored using a maximum power point tracker (MPPT).

4.5 Significance of this study

In an ideal environment, for the maximum production of microalgae biomass, temperature and other confounding variables should be kept as closed to optimal as possible. This would be impossible in a real life outdoor scenario where there is continuous fluctuation in temperature, available sunlight and other climatic conditions. Tropical developing countries are of great interest for the mass cultivation of microalgae as they are subjected to relative long days of sunshine and do not experience extreme conditions such as winter and snowfall. However such regions are subject to high temperature, which is also a critical factor which needs to be addressed in the outdoor cultivation of microalgae. Through this study, we found that the IGP had indeed removed substantial amount of heat as it had a maximum temperature of 7^{o}C lower than that of the HLP. The IGP was also able to sustain the growth of the microalgae *Nannochloropsis* sp. when compared to the HLP, which failed to do so. However, the results also showed that the IGU coating alone was insufficient to control the PBR temperature profile within the optimum range (<27 ^{o}C) for the cultivation of mesophilic microalgae strains such as *Nannochlropsis* sp. and the biomass productivity was still significantly lower compared to the CLP.

In regards of its potential application in an outdoor scenario, the performance of the IGP is expected not to be limited by the variation in transmission and reflection values due to the incident angle of available sunlight. For shallow angle of incidence, there would be fairly minor variations in the intensity of visible light transmitted into the reactor. For larger incidence angle there will be reduced transmission of light into the reactor, however, in an outdoor situation this would correspond to late afternoon light which provides a relatively minor contribution to the total incoming energy. Infrared and UV parts of the spectrum are blocked effectively at all angles of incidence. In terms of energy generation, the average electrical power by the solar cells is almost independent of the orientation of the photobioreactor.

4.6 Concluding remarks

This work has clearly showed that incorporating customized Insulated Glazing Units (IGUs) into a PBR design did in fact manage to significantly reduce the temperature profile and sustain the growth of the microalgae *Nannochloropsis* sp. Through further modifications such as increasing the amount of IGU used and optimizing the reactor design for better heat transfer and light distribution, it will most likely be possible to improve the efficiency of the reactor for the cultivation of various microalgae. Such an innovation would definitely help increase the biomass production of microalgae while at the same time generating electricity from the unused spectra of sunlight. This integrated system would not only significantly help reduce the cost of microalgae cultivation over a long term but also lead to the sustainable production of both electrical and chemical energy from one resource-efficient (sun,light,water) facility.

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Conflict of interest

The authors declare no financial or commercial conflict of interest

6. References

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Figures

Figure 1. Schematic representation of the low-e IGU advanced energy harvesting flat glass panel (Tropiglas) used to build the IGP. The glass panel uses spectrally-selective IGU coatings and luminophores to transmit selected wavebands of the visible spectrum, while portions of UV and IR light is routed through scattering and reflections between the glass surfaces to the edges for collection by additional integrated photovoltaic cells

Figure 2. The relative spectra composition of incident light on the surface and inside of each PBR. (**A**) The full light spectrum (200-1100nm) distribution in all PBRs. (**B**) The PAR spectrum (400-700nm) distribution in all PBRs.

Figure 3. (**A**) The daily average temperature profile of *Nannochloropsis* sp. cultures in all three PBR setups (data are mean and range (n=3) while error bars represent the standard error). (**B**) The growth curve of *Nannochloropsis* sp. cultivated in all PBRs during both batch and semi-continuous mode (***** indicates the start of the semi-continuous mode in the reactors). (**C**) The long term maximum quantum yield values (F_V/F_M) of *Nannochloropsis* sp. cultures cultivated in the PBRs at different time intervals (data are mean and range (n=3) while error bars represent the standard error)

Figure 4. The various Chl- *a* parameters derived from the OJIP curve of *Nannochloropsis* sp. cultivated in the PBRs at (**A**) Hour 0 and also (**B**) Hour 12(data are mean and range (n=3) while error bars represent maximum and minimum values)

Figure 5: The deviation in ΔW_{OJ} plots of *Nannochloropsis* sp. in the different PBRs at (**A**) Hour 0 and (**B**) Hour 12 when compared to the control of Day 1. The control for Hour 12 measurements in the IGP and HLP was based on the values of Day 1 Hour 12 of the CLP. □ represents the deviation in ΔW_{OJ} plots of the IGP and HLP when compared to the Day 1 Hour 12 control of the CLP.

Table 1. Growth parameters, specific growth rates, ash free dry weight biomass productivities, chlorophyll *a* and biochemical content of *Nannochloropsis* sp. (n = 3 ± standard error) cultured semi-continuously inside each reactor.

Parameters	Unit	CLP	IGP	HLP
Temperature control system		Heat Filter	IGU	None
Maximum temperature achieved	^{o}C	30.6 ± 0.53	35.2 ± 0.05	42.4 ± 0.15
IR transmitted into reactor (700-1100nm)	(%)	9.1	10.4	62.1
UV transmitted into reactor (300-400nm)	(%)	59.6	24.8	59.2
PAR transmitted into reactor (400-700nm)	(%)	73.5	59.4	78.2
μ	d^{-1}	0.288 ± 0.01[a]	0.226 ± 0.01[b]	-0.461 ±0.04 [c]
Biomass Productivity	($mg\ L^{-1}\ d^{-1}$)	90.5 ± 8.16 [a]	52.7 ± 5.70 [b]	-
Biomass Productivity Per Number of PAR Photons Transmitted Into Each Reactor	[$mg\ L^{-1}\ d^{-1}$ (μ mol photons $m^{-2}\ s^{-1})^{-1}$]	0.218 ± 0.02[a]	0.161 ± 0.01[b]	-
Chlorophyll *a*	(microgram per ml)	0.70 ± 0.06[a]	0.65 ± 0.03[a]	-
Carbohydrate	% (g/AFDW)	7.98 ± 0.42[a]	7.72 ± 0.24[a]	-
Lipid	% (g/AFDW)	60.23 ± 1.84[a]	49.84 ± 1.98[b]	-
Protein	% (g/AFDW)	31.84 ± 1.07[a]	42.43 ± 0.77[b]	-

Along the rows the same letter above each value indicates no significant differences (One Way Repeated Measures ANOVA $P > 0.05$)