Graphical Abstract

Pigment-Targeted Light Wavelength and Intensity Promotes Efficient Photoautotrophic Growth of Cyanobacteria

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Abstract

A consensus is lacking whether monochromatic rather than broad-spectrum illumination is more efficient for photosynthetic microbe production platforms. Light wavelength and intensity were tuned to pigment composition for growth of the Cyanobacterium *Synechocystis* PCC 6803. Phycocyanin (PC)-targeting LEDs (620 nm) provided more than 6 times the peak efficiency of white LEDs, with peak efficiency growth rates of 0.063 h^{-1} at $81 \mu\text{E m}^{-2} \text{ s}^{-1}$ and 0.039 h^{-1} at $126 \mu\text{E m}^{-2} \text{ s}^{-1}$ for red and white LEDs, respectively. Chlorophyll *a* (Chl *a*)-targeting LEDs (680- and 440-nm) performed poorly. Indeed, 10 times greater mass abundance was observed for PC than Chl *a*. PC levels did not change while Chl *a* levels decreased when *Synechocystis* transitioned from white light at $50 \mu\text{E m}^{-2} \text{ s}^{-1}$ to $250 \mu\text{E m}^{-2} \text{ s}^{-1}$ with 620 nm, 680 nm, or white LEDs. This work demonstrates that light wavelengths and intensity need to be optimized for each strain.

Keywords: Cyanobacteria, Photosynthetic efficiency, Light-emitting diode, Photo-inhibition

1. Introduction

Photosynthetic microbes, such as microalgae and Cyanobacteria, could become a promising production platform for renewable fuels and chemicals that are derived from CO_2 .

However, the barriers to entry for products from microbial photosynthesis – for example, efficient light delivery to support high biomass productivity (Erickson et al., 2011) - have been difficult to overcome. Currently, direct solar illumination is most often used for the growth of microalgae and Cyanobacteria in pilot or semi-industrial scale photobioreactors.

Full industrial employment has been slow due to considerable efficiency limitations of direct solar illumination. Importantly, only a segment of light wavelengths in solar illumination, which has been referred to as photosynthetically active radiation (PAR, 400-700 nm), is usable for photosynthesis (Zhu et al., 2008), and much of the solar energy is wasted or generates heat that may need to be removed (Béchet et al., 2010). In addition, four parameters must be simultaneously met for the practical application of direct solar illumination: 1) location in a geographic area with high annual solar illumination; 2) a temperate year-round climate; 3) photobioreactors with a high surface area exposure to solar radiation, resulting in considerable land areas; and 4) close proximity to industrial CO₂ sources (Blanken et al., 2013; Erickson et al., 2011).

As an alternative, controllable photobioreactor illumination has been proposed to create a more efficient photosynthetic system that is not dependent on broad-spectrum sunlight or solar cycles (Erickson et al., 2011). This can be accomplished by de-coupling sunlight collection from delivery to the microalgae/Cyanobacteria (Erickson et al., 2011) *via* the utilization of: 1) sunlight collectors and filters; or 2) photovoltaics and artificial lighting such as LEDs. Controlled illumination can eliminate the need for geographically sunny/warm regions and large land areas by permitting compact reactors with internal illumination or light guides, and which can be kept in temperature-regulated indoor areas (Jain et al., 2015).

Before implementation, illumination parameters, such as light intensity, light wavelengths, and light-dark cycling (Vejrazka et al., 2013), would need to be optimized to reduce artificial illumination energy cost, improve photosynthetic efficiency, and minimize reactor overheating. Narrow-band monochromatic illumination has been examined for improved

photosynthetic biomass growth in comparison to broad-spectrum light. Some work has shown increased microbe growth with red LEDs (Wang et al., 2007; Wyman and Fay, 1986; Zhao et al., 2013) while others show greater growth with blue LEDs (Atta et al., 2013; Das et al., 2011; Jeong et al., 2013). Monochromatic wavelength illumination can drive differential responses in photosynthetic microbes (Bennett and Bogorad, 1973; Moon et al., 2010). Work by Koc et al. and Shu et al. has shown faster doubling time of the microalga *Chlorella* with red LEDs and greater lipid accumulation per cell (Shu et al., 2012) or larger cell size (Koc et al., 2013) with blue LEDs. However, current literature is generally conflicting and does not point to optimal monochromatic illumination characteristics for photosynthetic growth or production (Zhu et al., 2008). Some of the discrepancies in the results of previous studies, which have examined narrow-band monochromatic illumination, may be influenced by mismatches between the photosynthetic pigment composition of the target microbe and the supplied illumination. While broad-spectrum illumination will almost certainly supply some photons of wavelengths suitable for growth, monochromatic illumination (*e.g. via* narrow-band LEDs) may partially or completely miss the absorption ranges of photosynthetic pigments if not selected appropriately.

The objective of the work presented here was to characterize biomass growth and photosynthetic efficiency for monochromatic wavelength illumination applied to *Synechocystis* PCC 6803, which is a Cyanobacterium that relies primarily on Chlorophyll *a* (Chl *a*), phycocyanin (PC), and allophycocyanin (APC) for photosynthesis (Melnicki et al., 2013). *Synechocystis* PCC 6803 is a widely-used model microbe and has been characterized for lipid production for biofuels (Cuellar-Bermudez et al., 2015) and transformed for

increased generation of fuel products, including ethylene (Ungerer et al., 2012), isoprene (Lindberg et al., 2010), and hydrogen (Cournac et al., 2004).

Chl *a* is considered the primary photosynthetic pigment in Cyanobacteria and green algae because it directly utilizes photon energy to initiate electron transfer at the reaction centres of photosystems I and II to perform photosynthesis. Chl *a* has two primary absorption peaks – a red peak at 680 nm and a blue peak at 440 nm (Nobel, 2009a). Photosynthetic microbes also utilize accessory pigments, which can absorb photon energy and eventually transfer it to a nearby Chl *a* reaction centre. Phycobilisomes (as well as other pigments) act as accessory pigments for Chl *a* (Nobel, 2009a). PC and APC, which are phycobiliproteins present in *Synechocystis*, form a complex together and have absorption peaks at 620 nm and 650 nm, respectively. Photon energy absorbed by accessory pigments is transferred to nearby pigment molecules and eventually photosystem reaction centres *via* highly efficient resonance energy transfer processes (in the case of *Synechocystis* from PC to APC to Chl *a*) (Glazer, 1985). While excess photon energy is lost (mostly as heat) during down-conversion (Nobel, 2009b), the presence of accessory pigments broadens the photosynthetic absorption spectrum. In addition, microbes may present a high number of accessory pigments for each reaction centre (*e.g.* 50-1000 accessory pigment molecules per reaction centre) to improve photosynthetic reaction rates in low light conditions (Glazer, 1985; Nobel, 2009b). Photosynthetic pigment composition can adapt over time to light wavelength and intensity (Bennett and Bogorad, 1973; Mishra et al., 2012). Limitations to the whole-cell photosynthetic rate is dependent on the relative abundance of chlorophylls and accessory pigments presented. Other accessory pigments not considered here, such as carotenoids and xanthophylls, can fill in photon absorption gaps in the solar spectrum and

help provide a photo-protective role (Nobel, 2009b). As a result, the preferred pigment(s) to target with monochromatic illumination is not necessarily straightforward and is dependent on the strain, culture conditions, and illumination history.

It was hypothesized that photosynthetic efficiency could be improved by matching light wavelength and intensity to the host of pigments in the target microbe. *Synechocystis* growth rate and photo-inhibition under monochromatic illumination were examined at several specific wavelengths targeting PC, APC, and Chl *a*. A long-term culture was performed to examine pigment composition and adaptation over time to monochromatic illumination. This work provides a guide for optimizing illumination conditions for

Synechocystis on the basis of photobiology as well as a framework for elucidating optimal illumination conditions for other photosynthetic microbes.

2. Materials and Methods

2.1 Strain and inoculum culture conditions

Synechocystis PCC 6803 (wild type) was a gift from Brad Postier (Phycal LLC, Cleveland, OH) and cultured in BG-11 medium (C3061, Sigma Aldrich, St. Louis, MO), which was supplemented with TES buffer at 4.6 g/L (T5691, Sigma Aldrich) and 100 mM NaHCO₃ at pH 7.5-8. Cultures were mixed on a magnetic stir plate at room temperature and continuously illuminated with a CFL lamp at 50 $\mu\text{E m}^{-2} \text{s}^{-1}$.

2.2 Flask reactor and LED array setup

133 Stock cultures were prepared for batch flask inoculation by diluting to 25-50% in fresh BG-
134 11 medium 24 h prior to inoculation to maintain exponential growth. Cells were inoculated
135 for batch culture into sterile 25 cm² untreated polystyrene tissue cell culture flasks (431463,
136 Corning Inc., Corning, NY) in fresh BG-11 with TES buffer and NaHCO₃ at an OD_{750 nm} of
137 0.05. A culture volume of 10 mL was used, providing a short light path length of
138 approximately 4 mm. Exponential growth condition were maintained by culturing at low
139 density, short light path length, and high abundance of carbon and nutrients. Therefore,
140 light wavelength and intensity were the primary effectors of growth rate. Flask caps were
141 pierced with sterile 27G needles (BD305120, Beckton Dickinson, Franklin Lakes, NJ) for
142 sampling and sealed with epoxy (1324007, Loctite, Düsseldorf, Germany) to prevent gas
143 exchange. Light delivery was confined to the top of the flasks by black masking on the
144 sides of the culture flasks. The incident-light surface of the flask was covered with neutral
145 density filter sheets (B&H Photo Video, New York City, NY) to attenuate light across
146 roughly two orders of magnitude ranging from 1.3 to 100% transmission. Flask reactors
147 were illuminated with high power LEDs (3W, 700mA) on a 16:8 h light:dark cycle (**Fig. 1**).
148 Colored LEDs were obtained from Ledfedy.com (Shenzhen, China): 620-630 nm; 660 nm;
149 680-690 nm; 440-450 nm; and 460-470 nm. Cool white LEDs were obtained from
150 Ledsupply.com (Randolph, VT) (**Fig. 1a**). LEDs heat dissipation was provided by heat
151 sinks and cooling fans to maintain ambient sample temperature (19-25°C) during LED
152 operation. Incident light was measured directly at the culture surface (on the culture side of
153 the polystyrene flask) with a PAR meter sensitive to 400-700 nm (model 2319, LI-COR,
154 Lincoln, NE). Chambers that held LED arrays, cooling fans, and cyanobacterial cultures
155 were constructed of a black acrylic sheet to block background light (**Fig. 1b**), and mounted

on orbital shaker plates (EW-51820-40, Cole Parmer, Vernon Hills, IL) at 70 rpm to provide mixing.

2.3 Flask reactor culture conditions under LED illumination

To generate data for photosynthesis-irradiance curve model fitting, *Synechocystis* batch cultures were maintained in flask reactors under LED treatment. Cultures were maintained in BG-11 (same composition as stock cultures) for three days and 7-10 illumination intensities were examined for each monochromatic wavelength with four biological replicates, as well as a dark control. The full range of light intensities was covered from low intensity with no photosynthetic growth to high intensity with photoinhibition ($0 - 960 \mu\text{E m}^{-2} \text{ s}^{-1}$) when applicable. Growth was measured daily by measuring the OD at the beginning of the 16-h light cycle.

Long-term pigment composition testing was performed for 14 days with 620 nm, 680 nm, and white LEDs each at a moderate intensity of $250 \mu\text{E m}^{-2} \text{ s}^{-1}$. The experimental setup was similar to the previous test except that, starting on Day 6, cultures were maintained semi-batch by replacing 20% of the culture volume daily with fresh BG-11 containing 100 mM NaHCO_3 . Growth measurements were performed daily by measuring the OD. Headspace CO_2 was measured by an infrared gas analyser (model 6250 LI-COR). Pigment composition was measured at Day 14 by extracting PC and Chl *a* and measuring content by spectrophotometry using known extinction coefficients. For comparison, pigment extraction was also performed on the stock culture used to inoculate the long-term semi-batch cultures on Day 0.

2.4 Photosynthetic pigment extraction and measurement

Samples were prepared for extraction and spectroscopy measurement of PC and Chl *a* by centrifuging cultures. The supernatant was removed and replaced with either 0.1 M phosphate-buffered saline (for PC) or 90% ethanol (for Chl *a*). Samples were vortexed to resuspend cells in the extraction solvent. PC samples were sonicated on ice for 3 min at 15 W (Sonifier 150, Branson Ultrasonics, Danbury, CT). Chl *a* samples were allowed to sit in the dark for 5 min for extraction. Samples were centrifuged again and the supernatant was used for spectroscopy measurement of PC at 620 nm and Chl *a* at 665 nm. Extinction coefficients were measured from a standard (P2172, Sigma Aldrich) for PC (0.9971 mL * mg⁻¹ * cm⁻¹) or derived from literature for Chl *a* (84 mL * mg⁻¹ * cm⁻¹) (Namsaraev, 2009).

2.5 Photosynthesis-Irradiance curve model fitting

Optical density data (OD_{750 nm}) was used to calculate the specific growth rate, μ (Day 3 minus Day 0), and this data was fitted to two photosynthesis-irradiance curve models. In these models, photosynthesis rate is a function of light intensity. In this work the specific growth rate (μ) was used as a proxy for photosynthesis rate, since cultures were limited to exponential growth conditions. The Platt model (Eq. 1) is a widely-accepted empirical model that accounts for a decline in growth rate due to photo-inhibition at high intensities (Macedo et al., 1998; Platt et al., 1980):

$$P = P_{max} (1 - \exp(-\alpha I / P_{max})) (\exp(-\beta I / P_{max})) \quad (1)$$

where

P = specific growth rate, μ (h⁻¹) at photon flux (I)

P_{max} = the maximum growth rate possible (in the absence of photo-inhibition for the Platt model)

202 α = the slope of the linearly increasing region of the curve at low intensity

203 β = the slope of the declining region of the curve at high intensity ($\beta = 0$ if there is no
204 photo-inhibition)

205 In addition to the Platt model, a rational model was used, which also accounts for photo-
206 inhibition. The Eilers/Peeters model (**Eq. 2**) has the same fitting parameters as the Platt
207 model ($P_{\max}$, α , β) with the exception that the $P_{\max}$ value represents the true maximum
208 photosynthetic rate and accounts for photoinhibition (Eilers and Peeters, 1988; Macedo et
209 al., 1998). The parameters a, b, and c are functions of $P_{\max}$, α , and β .

$$210 \quad P = 1 / (aI^2 + bI + c) \quad (2)$$

$$211 \quad a = 1 / \alpha I_{opt}^2 \quad (3)$$

$$212 \quad b = 1 / P_{\max} - 2 / \alpha I_{opt} \quad (4)$$

$$213 \quad c = 1 / \alpha \quad (5)$$

$$214 \quad I_{opt} = \sqrt{c / a} \quad (6)$$

215 where

216 I = photon flux ($\mu\text{E m}^{-2} \text{s}^{-1}$)

217 I_{opt} = optimal photon flux ($\mu\text{E m}^{-2} \text{s}^{-1}$)

218 The α and β values were estimated from the data and the best-fitting $P_{\max}$ value was
219 iteratively determined. Fitting of data to the models was performed for each light
220 wavelength separately by non-linear least squares regression.

221 From the best-fit Eilers/Peeters models, nominal biomass yield on light energy ($\text{g L}^{-1} \text{J}^{-1}$)
222 was calculated as the amount of biomass produced per unit of illumination energy and
223 normalized against the peak productivity of the broad-spectrum white LEDs. This provides
224 a calculation of growth efficiency as a fraction of the maximum efficiency under control

(white, broad-spectrum) LEDs. The total incident illumination energy was calculated from the peak output wavelength of each LED (or the weighted average output for the white LED), the total illumination time, and the illuminated culture surface area (25 cm²). The incident illumination energy calculated from the flux of photons at the culture surface did not account for LED efficiency or light losses, and other energy expenditures (such as mixing energy) were not considered. As a result, this data is meant to convey only biological parameters of growth energy efficiency.

3. Results and Discussion

3.1 Model fitting allows prediction of peak growth rates and optimum intensities for each wavelength

The Eilers/Peeters photosynthesis-irradiance curve model generally provided a similar quality of fit as the Platt model (by R² value) but a better estimate of P_{max} (maximum growth rate in h⁻¹) in comparison to the experimental data (**Fig. 2**) – results will primarily be discussed using the Eilers/Peeters model (**Table 1**). The highest specific growth rate was predicted from the models for the PC- and APC-targeting, 620-nm and 660-nm LEDs (0.071 and 0.070 h⁻¹, respectively; **Fig. 3**). The Chl *a*-targeting, 680-nm and 440-nm LEDs resulted in a lower peak growth rate (0.047 and 0.034 h⁻¹, respectively) compared to the 620-nm and 660-nm LEDs and also compared to the white LEDs (0.063 h⁻¹). The lowest peak growth rate was observed for the 460-nm LEDs (0.030 h⁻¹) due to the absence of a photosynthetic pigment absorption peak at this wavelength. Photo-inhibition was not observed with 680-nm and white LEDs for the light intensity range examined (**Fig. 3**). The absence of photo-inhibition in 680-nm LEDs is likely due to their low brightness, which

limited testing to $400 \mu\text{E m}^{-2} \text{s}^{-1}$ or less. Under white LEDs, the absence of photo-inhibition may have a variety of causes, including pigment acclimation of the pre-culture (which was illuminated with white light) or the inevitable wavelengths within white light which are not absorbable by pigments and are thus transmitted. As a result, the β value for 680-nm and white LED wavelengths was set to 0 and the model was not extrapolated past the highest light intensity tested. Obviously, at some unknown intensity, photo-inhibition would eventually occur.

These results suggested the presence of differential photo-inhibition under monochromatic light. Growth rates with PC- and APC-targeting, 620-nm and 660-nm LEDs were higher than with white LEDs at low intensities, but lower than with white LEDs at moderate to high light intensities above $475 \mu\text{E m}^{-2} \text{s}^{-1}$ due to photo-inhibition (**Fig. 3**). In other words, if only moderate or high light intensities of these specific light wavelengths had been tested, a lower growth rate would have been observed when compared to the white LEDs. The highest peak growth rate of *Synechocystis* sp., which occurred at low light intensities with PC- and APC-targeting light wavelengths, would, therefore, not have been found. The results of this work are in agreement with trends observed in other studies for which *Synechocystis* PCC 6803 growth in red light was similar to (Singh et al., 2009; Zavřel, et al., 2015) or greater than (Nagasawa et al., 2015) in white light, and much greater than in blue light (Nagasawa et al., 2015; Singh et al., 2009; Zavřel, et al., 2015). Other studies did not show photo-inhibition at high intensities, but this may be due to: 1) long light-path lengths (Zavřel, et al., 2015), and thus self-shading; 2) characterization of photo-inhibition by light-dependent reactions (oxygen evolution rate) (Nagasawa et al., 2015) rather than downstream light-independent steps (biomass growth); and 3) the long duration culture

used in this work (3 days) compared to short-duration exposures (minutes) used for PI curve modelling as in (Nagasawa et al., 2015).

3.2 Derivation of the efficiency (biomass yield from light energy) from model data

The nominal efficiency (*i.e.* nominal biomass yield from light energy when corrected to white LEDs) was calculated as the amount of biomass produced per illumination energy and normalized with the peak productivity of the broad-spectrum white LEDs using the Eilers/Peters photosynthesis-irradiance curve model (**Fig. 4-5**). The PC- and APC-targeting, 620- and 660-nm LEDs showed high nominal peak efficiencies of ~ 5 and $6 \text{ g L}^{-1} \text{ J}^{-1}$, respectively – in other words, they produced up to 5-6 times more biomass per input energy than white LEDs (**Table 1**). The nominal peak efficiency with Chl *a*-targeting, 680-nm LEDs was $1.3 \text{ g L}^{-1} \text{ J}^{-1}$, while the Chl *a*-targeting, 440-nm LEDs and the generic, 460-nm LEDs were 0.37 and $0.25 \text{ g L}^{-1} \text{ J}^{-1}$, respectively (and thus less efficient than white LEDs). While these trends for the peak efficiency were similar to the peak growth rate (**Fig. 3**), it should be noted that the peak efficiency can occur at very low intensities and consequently at impractically low growth rates. For example, the peak efficiency for 680-nm LEDs occurred at $29 \mu\text{E m}^{-2} \text{ s}^{-1}$, but the peak growth rate at this intensity is only 0.018 h^{-1} (**Table 1**).

It was observed that, for the PC- and APC-targeting, 620- and 660-nm LEDs, the efficiency decreased more sharply beyond their peak values than for the other LEDs at increasing illumination (**Fig. 4**). This characteristic will be described as the sensitivity of *Synechocystis* to photo-inhibition under each treatment illumination wavelength. *Synechocystis* was most sensitive to 620-nm LEDs (steepest decline in growth efficiency

from its peak). For these LEDs, the intensity span within $\pm 90\%$ of peak efficiency was only $71 \mu\text{E m}^{-2} \text{s}^{-1}$ (**Fig 5b**). In contrast, this intensity span was $243 \mu\text{E m}^{-2} \text{s}^{-1}$ for white LEDs, showing a much lower sensitivity. These results suggest that, while 620-nm light is very efficient for *Synechocystis*, the illumination intensity must be optimized and then carefully selected to avoid photo-inhibition.

After combining the Eilers/Peeters modeling results from the peak growth rate and nominal efficiency model curves (**Fig. 3-5a**), the nominal efficiency at the maximum growth rate possible (at P_{max}) was calculated for each treatment illumination wavelength (**Table 1**). These nominal efficiencies at P_{max} 0.070 and 0.071 h^{-1} were highest for the PC- and APC-targeting, 620- and 660-nm LEDs at 4.525 and $3.905 \text{ g L}^{-1} \text{ J}^{-1}$, respectively, compared to 0.063 h^{-1} and $0.490 \text{ g L}^{-1} \text{ J}^{-1}$ for white LEDs (**Table 1**). Since white LEDs did not induce photo-inhibition, the peak growth rate occurred at the inefficient maximum intensity ($960 \mu\text{E m}^{-2} \text{s}^{-1}$). Nonetheless, even when compared at peak efficiencies rather than peak growth rate, the 620-nm and 660-nm LED provided growth at 6.218 and 4.967 times greater efficiency, respectively, than white LEDs. Conversely, the other LEDs showed a nominal efficiency at P_{max} between 1.19 and 0.38 times that of white LEDs. This indicates that optimized monochromatic illumination (targeting PC and APC pigments) provided a faster growth rate for *Synechocystis* than for broad spectrum light, but that peak growth rate also occurred at up to 8-9 times greater energy efficiency than was observed for white LEDs. Theoretical work by Blanken et al. has similarly suggested maximum algal photosynthetic efficiencies as high as 17% with monochromatic red LEDs compared to 4% with direct sunlight (Blanken et al., 2013). The white LEDs used in this study should provide a much higher photosynthetic efficiency than sunlight since their emission is approximately 90%

within PAR compared to 42.5% for sunlight, which may be expected to narrow the performance gap between red and white LEDs. True estimation of photosynthetic efficiency in this work would require accounting for light losses and conversion to heat, which was outside of the scope of this project.

3.3 Long-term pigment composition changes in Synechocystis during monochromatic illumination

Despite the utilization of both phycobiliproteins (PC, APC) and Chl *a*, the results indicated unexpectedly poor performance of the Chl *a*-targeting 680- and 440-nm LEDs. To further investigate, longer-term culture experiments were performed with *Synechocystis* to characterize: 1) the pigment composition of *Synechocystis*; and 2) the pigment concentration changes following treatment with either monochromatic or broad-spectrum LEDs. Under semi-batch culture conditions, PC-targeting, 620-nm and white LEDs obtained similar maximum densities around $OD_{750\text{ nm}} = 4.0$, though 620-nm LEDs produced this result approximately two days earlier (**Fig 6a**). Headspace CO_2 measurements near zero ppm at Day 14 suggested that carbon limitations were the cause for the similar maximum density of cultures exposed to 620-nm and white LEDs during longer-term operating periods. Conversely, Chl *a*-targeting, 680-nm LEDs resulted in a culture with a lower biomass yield and higher headspace CO_2 levels (approximately 100 ppm) at Day 14 (**Fig 6a**). Pigment composition results show a ratio of PC to Chl *a* ($g\ L^{-1}\ OD^{-1}$) greater than 10 for the stock culture (Day 0) and greater than 30 for adapted cultures (Day 14) (**Fig. 6b**). Though Chl *a* has a much greater extinction coefficient than PC (~ 84 compared to $\sim 1\ mL * mg^{-1} * cm^{-1}$), this result may explain the superior performance of PC-targeting, 620-nm

LEDs compared to Chl *a*-targeting, 680-nm LEDs. In addition, these results demonstrate a statistically significant reduction in Chl *a* content for each culture (620-nm, 680-nm, and white LEDs) during the experiment, while no statistically significant decrease was observed in PC content (**Fig. 6b**).

Cyanobacteria are known to modulate chlorophyll content to adapt to illumination conditions (increasing light sensitivity in low-light conditions and resisting photo-inhibition in high-light conditions) (Kehoe and Gutu, 2006). No adaptation was observed in PC content, which is likely responsible for the high sensitivity to illumination intensity from 620-nm and 660-nm LEDs (**Fig. 5**). Other work shows a decline in both chlorophyll and phycocyanin in *Synechocystis* PCC 6803 when transitioned from low to moderate intensity white light (Pojidaeva et al., 2004) – however, **this study** applied continuous illumination in comparison to the 16:8 h on:off regime used in the present work. Another Cyanobacterium, *Synechococcus* PCC 7002 was shown to decrease phycobilisome content under bright red light, but not chlorophyll (Bernstein et al., 2014), which is the opposite of what was observed in *Synechocystis* in this study. These differences reinforce the necessity of selecting monochromatic spectra and intensity on a per-microbe basis, and that attempts to implement a ‘one size fits all’ illumination regime across multiple microbes may be unreliable. **Future work should be performed to investigate illumination with multiple monochromatic wavelengths targeting more than one pigment, which may yield even greater biomass growth efficiency (Bernstein et al., 2014) while minimizing the sensitivity to illumination of the most prevalent pigments.**

4. Conclusions

When illumination intensity was optimized, *Synechocystis* growth with 620-nm LEDs targeting PC, which is the most abundant pigment by mass, was 6 times as efficient as broad-spectrum light. Conversely, Chl *a*-targeting, 680-nm illumination resulted in slow growth due to the low abundance of this pigment. The observed capacity of *Synechocystis* to adapt Chl *a* content, but not PC content, underlies the importance of balancing wavelength and intensity to maximize growth while minimizing photo-inhibition under PC-targeting monochromatic illumination. This work demonstrates that tight control of illumination must be based on biological information from the available photosynthetic pigments for each microbe of interest.

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Figure Headings

Fig 1 Illumination treatment regime comprising 440 nm, 460 nm, 620 nm, 660 nm, 680 nm, and white LEDs with intensity attenuated from 1.3% to 100% by neutral density filters.

Fig 2 Data and fit for the photosynthesis-irradiance curve model for cultures exposed to: (a) 660-nm LEDs showing photo-inhibition; and (b) white LEDs showing no photo-inhibition.

Data are from both Eilers/Peeters (E/P) and Platt models. Note that a decrease in biomass was observed for control (dark) samples, resulting in a predicted specific growth rate $\mu < 0$ at very low light intensities.

Fig 3 Best-fit Eilers/Peeters photosynthesis-irradiance curve models for experimental data for different LEDs.

Fig 4 Nominal biomass yield from light energy (nominal efficiency) derived from Eilers/Peeters models. All data was normalized against the peak efficiency of white LEDs (control).

Fig 5 Sensitivity of *Synechocystis* to changes in light intensity of each treatment wavelength as derived from Eilers/Peeters photosynthesis-irradiance models. This was measured by the span of intensities within 90% of the peak efficiency for that wavelength.

Fig 6 Adaptation results. Error bars represent standard deviation: (a) Growth of *Synechocystis* in semi-batch culture for 14 days; and (b) changes in PC and Chl *a* content. The concentration of Chl *a* in the stock culture was significantly different than the concentration of Chl *a* after treatment with all LEDs ($p < 0.01$). Stock cultures (white (D0)) were maintained in a stir flask that was illuminated under continuous white fluorescent light at $50 \mu\text{E m}^{-2} \text{s}^{-1}$, and were used to seed 620-nm, 680-nm, and white LED flask cultures.

Tables

Table 1 Eilers/Peeters model data

λ (nm)	R^2	$P_{\max}$ (h ⁻¹)	I at $P_{\max}$ ($\mu\text{E m}^{-2} \text{s}^{-1}$)	NE^{a} at $P_{\max}$ ($\text{g L}^{-1} \text{J}^{-1}$)	μ at NPE^{a} (h ⁻¹)	I at NPE^{b} ($\mu\text{E m}^{-2} \text{s}^{-1}$)	NPE^{b} ($\text{g L}^{-1} \text{J}^{-1}$)
620	0.59	0.070	147	4.525	0.063	81	6.218
660	0.69	0.071	215	3.905	0.066	122	4.967
680	0.90	0.047	400	0.583	0.018	29	1.317
440	0.70	0.034	348	0.297	0.030	147	0.372
460	0.66	0.030	413	0.187	0.025	162	0.249
White	0.90	0.063	960	0.490	0.039	126	1.000

^aNE = Nominal energy efficiency (biomass yield per light energy) normalized against peak efficiency for white LEDs

^bNPE = Nominal peak energy efficiency (biomass yield per light energy) normalized against peak efficiency white LEDs

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6