Enhancement of biomass production in *Scenedesmus bijuga* high-density culture using weakly absorbed green light

Erico R. Mattos^a, Manjinder Singh^a, Miguel L. Cabrera^b, Keshav C. Das^{a*}

Biorefining and Carbon Cycling Program,

^aDepartment of Biological and Agricultural Engineering

^bDepartment of Crop and Soil Sciences

The University of Georgia, Athens,

GA 30602, USA

* Corresponding author. Tel.: +1 706 542 8842

Fax: +1 706 542 8806

Postal Address: Driftmier Engineering Center, University of Georgia, Athens, Georgia 30602

E-mail address: kdas@engr.uga.edu

Abstract

To increase microalgae biomass production and support high density cultures in photobioreactors artificial illumination systems have been designed to increase photosynthetic activity. Supplemental lighting systems are commonly composed by a combination of chlorophyll (*a + b*) strongly absorbed wavelengths, while weakly absorbed wavelengths are not present. At this work we compared the photosynthetic activity and biomass production induced by chlorophyll (*a + b*) strongly versus weakly absorbed wavelengths in *Scenedesmus bijuga* microalgae cultures at different biomass densities. Photosynthetic activity and biomass production induced by 4 different wavelengths using LEDs (blue - peak at $\lambda 470$ nm; green - peak at $\lambda 530$ nm; red - peak at $\lambda 655$ nm; and white-4100 K) were measured and analyzed on high-density cultures of *Scenedesmus bijuga*. As culture density increased the chlorophyll (*a + b*) weakly absorbed green light penetrated deeper into the samples inducing higher oxygen evolution at culture concentration of 1.45 g L^{-1} compared to the chlorophyll (*a + b*) strongly absorbed red light. High-density culture (2.19 g L^{-1}) cultivated under green light showed higher biomass production rate ($30 \text{ mg L}^{-1} \text{ d}^{-1}$) with a 8.43% biomass growth in a 6-day period compared to the same quantum flux of red light that induced 4.35% biomass growth on the same period. The integration of green LEDs into photobioreactors lighting apparatus could improve the existing systems composed predominantly by red and blue LEDs increasing biomass productivity of high-density cultures at latter stages of microalgae cultivation.

KEYWORDS: *Scenedesmus*, green, light, biomass, photosynthesis, photobioreactor

1. Introduction

Microalgae cultivation has been used since 1960 for water treatment and high-value compounds commercialization [1]. Most recently, it has aroused great interest as a feedstock for biofuels production and biodegradable products [2-4]. However, the high cost of harvesting and de-watering due its intrinsic low biomass concentration is still a major drawback to scale up [5]. To increase microalgae cultures' cell density, different kinds of closed photobioreactors using artificial illumination systems have been developed, capable of achieving higher biomass concentrations under controlled environmental conditions [6-9]. Artificial illumination systems can increase the volumetric productivity and high-density cultures can only be achieved when the cells receive appropriate levels of light energy along the entire growth cycle [8]. Exposure of cells to excessive light at early culture stages leads to photodamage and photoinhibition that can cause a decline in photosynthetic capacity and biomass growth, whereas low levels of light energy at later stages become a growth-limiting factor. Providing optimal light supply throughout the different developmental stages over the entire culture cycle can significantly increase biomass production [8,10]. To optimize light supply, illumination systems have been designed to match microalgae absorbance spectrum based on its main pigments – chlorophyll *a* and *b*. These systems are normally composed of chlorophyll's strongly absorbed wavelengths at blue ($\lambda 400 - \lambda 500$ nm) and red ($\lambda 600 - \lambda 700$ nm) spectrum and do not include the chlorophyll's weakly absorbed wavelengths at green and

orange ($\lambda 500 - \lambda 600$ nm) spectrum [9]. Chlorophyll ($a + b$) strongly absorbed wavelengths can indeed stimulate higher photosynthetic activity in low-density microalgae cultures [11, 12] and have been shown to induce high biomass production [7]. However, in high cell density cultures, light penetration is limited [13] and these strongly absorbed wavelengths are mostly absorbed by the layers of cells close to the illuminated surface resulting in a large fraction of its energy dissipated as heat by non-photochemical quenching mechanisms, whereas cells at the center of the sample may not be exposed to enough light energy. On the other hand, chlorophyll ($a + b$) weakly absorbed wavelengths which have a high scattering coefficient and a low absorbance coefficient [14] can penetrate deeper into high-density cultures and can be more homogeneously distributed among the cells as well as within the chloroplasts of individual cells. Along with deep penetration, the diffusive nature of the algal culture and the changes in spectral reflectance associated with algal densities indicates an increase on chlorophyll ($a + b$) weakly absorbed wavelengths pathway inside the culture compared to strongly absorbed wavelengths as culture depth increases [15], thus increasing the opportunity for light to encounter chloroplasts. This phenomenon is described by Terashima et al. [16] as the “detour effect” and it can possibly increase the absorbance of weakly absorbed wavelengths in microalgae cultures. In intact cells present in microalgae culture, pigments are concentrated into chloroplasts, which decrease the opportunity for light to encounter pigments. This is referred to as the ‘package effect’ (also known as sieve effect) and results in a reduced light absorption at the absorption maxima of the pigments, while

there is little reduction in absorption at those wavelengths that are weakly absorbed by the pigments [16,17]. Package effect is a function of cell density, size and pigment content and its magnitude becomes more severe as cultures cell density increases [18].

Recent studies with higher plants show that the chlorophyll ($a + b$) strongly absorbed wavelengths get completely absorbed by the initial cell layers whereas green light can penetrate further into the leaves [19] and drive carbon fixation more efficiently than red and blue light in cells located deeper in the leaves [20]. In high-density microalgae cultures, the increased number of cells have the same effect as the multiple cell layers present in higher plant leaves and light penetration becomes limiting as the culture gets denser [13]. Providing high cell densities microalgae cultures with supplemental chlorophyll ($a + b$) weakly absorbed wavelengths may allow for deeper light penetration and homogeneous light distribution in high-density algae culture, promoting extra biomass growth. In the present study we compared the effect of different wavelengths (red – peak at $\lambda 655$ nm; blue – peak at $\lambda 470$ nm; green – peak at $\lambda 530$ nm) as well as a full spectrum neutral white light (4100 K) on dissolved oxygen evolution and biomass production of the microalga *Scenedesmus bijuga* at high biomass concentrations.

2. Materials & Methods

2.1 Algal Culture

Scenedesmus bijuga (UTEX 2980) was previously isolated from waste water [21] and maintained in modified BG-11 medium (Na_2CO_3 , 20 mg L^{-1} ; NaNO_3 , 1.07 g L^{-1} ; K_2HPO_4 , 6.1 mg L^{-1} ; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 7.5 mg L^{-1} ; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 36 mg L^{-1} ; citric acid $\cdot \text{H}_2\text{O}$, 6 mg L^{-1} ;

ammonium ferric citrate, 6 mg L⁻¹; Na₂MGEDTA, 1 mg L⁻¹; H₃BO₃, 2.86 mg L⁻¹; MnCl₂·4H₂O, 1.81 mg L⁻¹; ZnSO₄·7H₂O, 0.222 mg L⁻¹; Na₂MoO₄·2H₂O, 0.391 mg L⁻¹; CuSO₄·5H₂O, 0.079 mg L⁻¹; and CoCl₂·6H₂O, 0.05 mg L⁻¹). The pH of culture medium was adjusted to 7.0 +/- 0.2 with hydrochloric acid, 3.65 g L⁻¹ before inoculation, and the algae were maintained in a temperature-controlled, illuminated growth chamber at 25 +/- 1°C and 250 µmol m⁻² s⁻¹ light intensity provided 12 hours a day by cool-white, fluorescent (6500K) T-8 bulbs.

2.2 Chlorophyll extraction and light absorbance

For chlorophyll extraction and light absorbance measurements, 10 cm³ of homogenized algal cells suspension was collected and centrifuged (3,075 RCF, 10 min). The supernatant was removed and re-suspended with the same amount of 95% methanol solution. The new solution was placed on hot water (60°C) for 30 min. After further centrifugation the supernatant was collected and analyzed. Absorbance spectrum from 300-700 nm was measured using a Varian Cary 50 UV/Visible spectrophotometer (Varian, Inc, Santa Clara, CA, USA).

2.3 Oxygen Evolution Experiment

2.3.1 Light system

An LED panel was built above an aluminum heat sink containing one Luxeon® III Star LED: green-λ530 nm (min λ520 nm/max λ550 nm), two Luxeon® K2 Emitter LED: blue-λ470 nm (min λ460 nm/max λ490 nm) and neutral white (4100 K), and a Luxeon® Rebel Star LED: red-λ655 nm (min λ650 nm/max λ670nm). Each LED was used to illuminate the microalgae samples through a 6.35 mm (¼ inch) diameter fiber-optic light guide

1 positioned 1 mm away from the cuvette surface. A light condenser was attached to the
2 flat end of the fiber optic cable to the LED to collect and direct the LED output through
3 the light guide. The LEDs were powered by an Agilent power supply (Agilent Technologies,
4 Santa Clara, CA, USA). Radiometric light intensity measurements were performed at the
5 tip of the fiber optic light guide using a quantum sensor (LI-COR, Quantum Sensor LI-190,
6 and Datalogger, LI-1400 Lincoln, NE, USA).

7 2.3.2 Culture cultivation

8 A 100-cm³ sample of *S. bijuga* in its exponential phase of growth was inoculated in
9 900 cm³ BG-11 medium and cultivated for 12 days in a controlled-environment growth
10 chamber under 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity (T-8 bulbs), at 25°C temperature, and a
11 12-h light/12-h dark regime. Ambient air enriched to 5 % volume fraction of CO₂ was
12 supplied 12-h per day following the light regime. The set up was the same as described in
13 Mattos et al. [22].

14 2.3.3 Dissolved oxygen measurements

15 Measurements of dissolved oxygen were conducted for four days. Samples were
16 collected at the exponential growth phase (8th to 11th d) and its cell suspension adjusted
17 to the lower biomass concentrations (0.7 and 0.8 g L⁻¹) by dilution with fresh BG-11
18 medium and to the higher biomass concentrations (1.0 and 1.45 g L⁻¹) through
19 centrifugation at room temperature (3,075 RCF, 10 min). After adjustment, samples were
20 kept in the dark to bring the dissolved oxygen concentration down. Aliquots of 5 cm³ of *S.*
21 *bijuga* were illuminated by the four different LEDs at 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$. For each different

light color, dissolved oxygen evolution was measured for 15 minutes using a Clarke type dissolved oxygen micro electrode (LAZAR , DO-166MT-1, USA) attached to the top of a 5-cm³ cuvette in a closed system isolated from external illumination. The cuvette was placed in a horizontal position so that the light path was 6 cm. For all measurements, agitation was provided by a micro stir bar. To establish dark respiration, dissolved oxygen consumption was measured for 15 minutes in control samples under dark conditions. To minimize the differences in cultures between experiments, samples at the same biomass concentration were analyzed on the same day. Results for dissolved oxygen evolution and consumption were reported in units of mg L⁻¹ h⁻¹. All measurements were conducted in triplicates.

2.3.4 Light transmittance

To establish the effect of culture density on light transmittance of the different LED light sources (red, blue, green, neutral white) through *S. bijuga* culture, samples at different biomass concentrations (0.7, 1.0 and 1.45 g L⁻¹) were placed in cuvettes with different path lengths (1, 5, 10, and 20 mm) and light transmittance was measured using the apparatus described in section 2.3.1 Lighting system. Light intensity passing through the samples was measure in units of $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the cuvette surface across the illuminated surface. Light intensity was set at 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the tip of the fiber optic cable.

2.4 Biomass Production Experiment

2.4.1 LED table

1 An LED panel containing 12 LEDs was built above an aluminum heat sink (30 x
2 30cm). The LEDs were dispersed in 4 rows of 3 LEDs connected in series. Each row was
3 composed by the same color LEDs: Luxeon® III Star green- λ 530 nm (min λ 520 nm/max
4 λ 550 nm); Luxeon® III Star blue- λ 470 nm (min λ 460 nm/max λ 490 nm); Luxeon® K2
5 Emitter LEDs neutral white (4100 K); and Luxeon® Rebel Star LED red- λ 655 nm (min
6 λ 650 nm/max λ 670 nm). Each LED row was controlled by potentiometers that enable
7 regulation of light intensity. Each potentiometer was connected to a Luxdrive™ 1000mA
8 Buckpucks 3021-D-E-1000 that were all connected to a single power transformer (12V).
9 O-rings were mounted at the LED table to keep the erlenmeyer flasks 1 cm above from
10 the LEDs and radiometric light intensity measurements were performed at the flasks'
11 illuminated surface using a quantum sensor (LI-COR, Quantum Sensor LI-190, and
12 Datalogger, LI-1400 Lincoln, NE, USA).

13 2.4.2 Culture cultivation

14 A 300-cm³ sample of *S. bijuga* in its exponential phase of growth was inoculated in
15 2,700 cm³ nutrient enriched BG-11 and cultivated for 28 days in a 4-L erlenmeyer flask in
16 a controlled-environment growth chamber under 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity (T-8
17 bulbs), at 25 °C temperature, and a 12-h light/12-h dark regime. To ensure satisfactory
18 conditions, samples were collected every 2 days and the concentrations of the two main
19 nutrients necessary for algae growth, nitrogen and phosphorous, were periodically
20 monitored and adjusted to its original concentration (176 and 10.8 mg L⁻¹, respectively)
21 when the level of one of them dropped under 10% of its original concentration.

Micronutrients had their concentration adjusted following the stoichiometrically calculated nitrogen adjustment. Ambient air enriched to 5 % volume fraction of CO₂ was supplied 12-h per day following the light regime. To avoid algal sedimentation, the culture flask was positioned in an inclined position as described in Mattos et al. [22].

2.4.3 Biomass Production

On the 28th day, 2.0 L of sample collected at biomass concentration of 1.8 g L⁻¹ was concentrated by centrifugation at room temperature (3,075 RCF, 10 min) to a higher biomass concentration of 2.19 g L⁻¹ and samples were transferred to the LED plate to grow above the different LED lighting sources for 6 additional days. Dissolved oxygen evolution was measured in the culture before it was transferred to the LED plate. Aliquots of 100-cm³ samples were placed in 125-cm³ erlenmeyer flasks that were placed above the LEDs. The flasks were coated black to avoid external illumination and ensure that the light provided by the LED was the sole light source. Light intensity was adjusted to 500 μmol m⁻² s⁻¹. To avoid algae sedimentation the LED plate was placed on an orbital shaker at 1.67 Hz.

2.5 Biomass and pH

Culture biomass concentration was determined gravimetrically as described by Chinnasamy et al. [21] and pH measurements were taken using an accumet® portable AP62 pH/mv Meter (Fisher Scientific, USA).

2.6 Nitrogen and Phosphorous

Culture media nitrogen content was measured as nitrate using chromotropic acid method using HACH Test'N tube™ kits (HACH, USA) and results were expressed as $\text{mg L}^{-1} \text{NO}_3^- \text{-N}$. Culture media phosphorous content was measured as reactive phosphorous (orthophosphate) using the molybdovanadote method using HACH Test'N tube™ kits (HACH, USA) and results were expressed as $\text{mg L}^{-1} \text{PO}_4^{3-}$.

2.7 Experimental Design

The flaks containing microalgae high biomass density culture were randomly assigned to the spots on the LED table. Treatments were carried in triplicates and final biomass production for each treatment was reported as the final average and standard deviation obtained from the 3 replicates.

3. Results & Discussion

3.1 Light absorption, transmittance and penetration

Light absorbance by the extracted chlorophyll content of *S. bijuga* samples showed a pronounced increase at the blue ($\lambda 470 \text{ nm}$) and red ($\lambda 655 \text{ nm}$) spectrum with a minor increase at the green ($\lambda 530 \text{ nm}$) spectrum as the culture biomass concentration increased (Fig 1). Attenuation of red and blue light penetration in green microalgae is mostly due to light absorption by chlorophyll pigments, which have high absorption coefficients in these wavebands, whereas green light attenuation is caused by light scattering [14]. The light transmittance results (Tab 1) demonstrated that the chlorophyll ($a + b$) weakly absorbed green light has highest transmittance values compared to the other light sources at all biomass concentrations. The general trend of highest

1 transmittance of green light followed by white, red and blue was consistent at the
2 different measured path lengths. As culture density and path length increase, red and
3 blue wavelengths become less available to the culture than green light. The difference on
4 the amount of green light available to *S. bijuga* cells measured at a culture density of 0.7 g
5 L⁻¹ at 1mm path length which was 12.8, 33.3, and 48.6% higher than white, red and blue
6 light constantly rises as the path length increases. At a distance of 20mm from the
7 illuminated surface the amount of green light measured (7.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was twice the
8 amount of white light (3.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$) whereas less than 1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of red and blue
9 light could be detected. At the highest culture density of 1.45 g L⁻¹ green light was 40.4
10 and 75.2% more available to *S. bijuga* cells at 1mm path length compared to red and blue
11 light and a 100% more available to cells at 10mm path length compared to white light.
12 Provided at the same initial light intensity green light was able to better penetrate the
13 high-density *S. bijuga* samples illuminating more cells than red and blue light deep into
14 the culture reducing the non-illuminated areas. The differential increase in light
15 absorption at different wavelengths as the culture biomass concentration increases is
16 reflected in the light penetration pictures (Fig 2). The decrease in light penetration is
17 more noticeable in samples illuminated with red and blue compared to the samples
18 illuminated with green and white light. In agreement with the light absorption results
19 observed in this study (Fig 1), Yun and Park [13] reported that light attenuation in *C.*
20 *vulgaris* samples at increasing cell concentrations was higher for the strongly absorbed

λ450 and λ650 nm light than for the weakly absorbed λ550 nm light, which could penetrate further into the algal suspension.

3.2 Oxygen Consumption

Total dissolved oxygen consumption measured on control samples increased as culture density increased. The higher amount of cells present at high biomass concentrations consumed more oxygen compared to samples with lower biomass concentrations going from 1.52 mg L⁻¹ h⁻¹ at the lowest biomass concentration (0.7 g L⁻¹) to 4.23 mg L⁻¹ h⁻¹ at the highest biomass concentration (1.45 g L⁻¹). When the dissolved oxygen consumption is expressed as a function of dry biomass in units of g kg⁻¹ h⁻¹, the oxygen consumption by kilogram of microalgae increases from cell density 0.7 to 1.0 g L⁻¹ then decreases at the cell density 1.45 g L⁻¹ (Fig 3). At the highest cell density (1.45 g L⁻¹) the increased amount of cells competing for the same amount of oxygen diluted on the sample results in less oxygen consumption by individual cells compared to the samples at cell density of 1.0 g L⁻¹.

3.3 Oxygen Evolution

At the four biomass concentrations selected, the full spectrum white LED induced the highest dissolved oxygen evolution whereas blue light induced always the lowest (Fig 4). For the 3 lower biomass concentrations, the chlorophyll (*a + b*) strongly absorbed blue light had the poorest light penetration as it gets completely absorbed by the cells closer to the illuminated surface resulting in the lowest oxygen evolution rates compared to the other light colors. At the highest biomass concentration blue light penetration was

1 severely limited and the total amount of oxygen produced by the photosynthetic process
2 was not enough to compensate the amount of oxygen consumed by the respiration
3 process resulting in oxygen consumption instead of net oxygen evolution (Fig 4). The
4 overall decrease in net oxygen evolution by all light colors at the highest biomass
5 concentration (1.45 g L^{-1}) was attributed to the high oxygen consumption due respiration.

6 A comparative analysis on the effect of red and green light illumination on oxygen
7 evolution rate of various cell densities of algal cultures was conducted based on data
8 presented in Fig 4. At the lowest biomass concentration (0.7 g L^{-1}), oxygen evolution
9 induced by the chlorophyll ($a + b$) strongly absorbed red light was 23% higher than the
10 chlorophyll ($a + b$) weakly absorbed green light. However, as the cell density increased to
11 1.0 g L^{-1} , oxygen evolution was adversely affected in red light illuminated cultures in
12 which an 11% decrease in oxygen evolution rate was observed relative to that in the 0.7 g L^{-1}
13 culture, whereas cultures illuminated by green light showed a 24% increase in oxygen
14 evolution rate (Fig 4). At the highest biomass concentration tested (1.45 g L^{-1}) green light
15 induced 126% more oxygen evolution than red light. This results support the hypothesis
16 that in high-density cultures, the chlorophyll ($a + b$) weakly absorbed green light
17 penetrates deeper into the sample inducing an overall higher oxygen evolution than the
18 chlorophyll ($a + b$) strongly absorbed red light.

19 As previously demonstrated by Lee and Palsson [9], in microalgae cultures, as cell
20 concentration increases the specific photosynthetic activity decreases while the
21 volumetric photosynthetic activity increases as a consequence of higher amount of

photosynthetic pigments present in the culture and limited light penetration [9].

Photosynthetic capacity of *S. bijuga* was calculated as oxygen evolution per biomass in units of $\text{mg g}^{-1} \text{h}^{-1}$ for four different biomass concentrations under the four LED lights at $300 \mu\text{mol m}^{-2} \text{s}^{-1}$. The results indicated that overall photosynthetic capacity of *S. bijuga* decreased as culture density increased. However, the decrease observed in samples illuminated by the green light was less prominent than the decrease observed in samples illuminated by red light, indicating that chlorophyll (*a + b*) weakly absorbed green light sustained higher photosynthetic capacity than red light in microalgae cultures at higher cell densities. Photosynthetic capacity of red light compared to green light was higher in lower density cultures (0.7 and 0.8 g L^{-1}) whereas at the higher density cultures (1.0 and 1.45 g L^{-1}) the chlorophyll (*a + b*) weakly absorbed green light showed higher photosynthetic capacity (not shown).

3.4 Biomass Production Experiment

Before samples were placed on the LED table, oxygen evolution measurements were taken. At $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, the high density samples (2.19 g L^{-1}) showed the highest oxygen evolution rate under green light ($11.07 \text{ mg L}^{-1} \text{h}^{-1}$), followed by white ($10.67 \text{ mg L}^{-1} \text{h}^{-1}$), red ($5.74 \text{ mg L}^{-1} \text{h}^{-1}$) and blue ($1.44 \text{ mg L}^{-1} \text{h}^{-1}$) light (Fig 5).

After 6 days on the LED plate, samples illuminated with chlorophyll (*a + b*) weakly absorbed green light had the highest biomass production (0.18 g L^{-1}) (Fig 6). At the high cell density samples, green light promoted an additional growth of 8.43% of its initial biomass concentration, followed by the white, red and blue light that promoted an

1 additional growth of 6.33, 4.35 and 2.21%, respectively. The growth rate induced by the
2 chlorophyll (*a + b*) weakly absorbed green light ($30 \text{ mg L}^{-1} \text{ d}^{-1}$) was twice that of the
3 chlorophyll (*a + b*) strongly absorbed red light ($15 \text{ mg L}^{-1} \text{ d}^{-1}$).

4 The higher biomass production induced by the green light compared to the other
5 wavelengths confirm the results found in the oxygen evolution experiment supporting the
6 hypothesis that on *S. bijuga* cultures with biomass concentration above 2.19 g L^{-1}
7 chlorophyll (*a + b*) weakly absorbed wavelength can induce overall higher
8 photosynthetically activity than the chlorophyll (*a + b*) strongly absorbed wavelengths
9 under the conditions provided.

10 Previous studies have shown that optimizing photobioreactors light supply might
11 be an efficient means of achieving both a high biomass productivity and a profitable
12 biomass yield on light [8,10,23,24]. Providing supplemental chlorophyll (*a + b*) strongly
13 absorbed wavelengths can increase biomass production of low concentration microalgae
14 samples more efficiently than chlorophyll (*a + b*) weakly absorbed wavelengths [12].
15 However, it was shown that the low light penetration of chlorophyll (*a + b*) strongly
16 absorbed wavelengths become a limiting growth factor in high-density microalgae
17 cultures. In a series of studies Mohsenpour *et al.* [25, 26] demonstrated that green light
18 provided most favorable lighting condition for *C. vulgaris* cultivation compared to red
19 light under static mode cultivation, whereas *C. vulgaris* cultivated in a bubbled
20 photobioreactor with better culture mixing conditions showed higher biomass production

1 under red light compared to green light due the reduced damaging effects of lack of light
2 penetration.

3 According to the findings of Pickett and Myers [27] on their study of light-
4 saturation curves of photosynthesis in microalgae cultures no more than one cell thick,
5 the wavelengths tested on this study have no effect on the photosynthetic oxygen
6 production rate or specific biomass growth rate in very dilute cultures where all cells
7 receive above saturating light-intensities (i.e., light attenuation is not a factor). Therefore,
8 under this study's environmental circumstances we attributed the increased biomass
9 productivity of samples illuminated with green versus red light to the deeper green light
10 penetration.

11 **4. Conclusion**

12 As *Scenedesmus bijuga* cell density increased, chlorophyll ($a + b$) weakly absorbed
13 green light (peak at $\lambda 530$ nm) which was able to penetrate deeper into high-density
14 samples became photosynthetically more effective than strongly chlorophyll ($a +$
15 b) absorbed red (peak at $\lambda 655$ nm) and blue (peak at $\lambda 470$ nm) lights. The integration of
16 green LEDs into photobioreactors lighting apparatus could improve the existing systems
17 composed predominantly by red and blue LEDs increasing biomass productivity of high-
18 density cultures at latter stages of microalgae cultivation. Additional experiments should
19 be conducted to define the best balance between strong and weakly absorbed
20 wavelengths provided throughout microalgae culture cultivation to maximize overall
21 production efficiency.

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Figure 1: Light absorbance of extracted chlorophyll content of *Scenedesmus bijuga* cultures at different biomass concentrations.

Figure 2: Light penetration at *Scenedesmus bijuga* samples at different biomass concentrations illuminated by four LED light sources (white-4100 K; red- $\lambda 655$ nm; green- $\lambda 530$ nm; blue- $\lambda 470$ nm) at $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity. Illumination was provided through the left side of the microcuvett.

Figure 3: Dissolved oxygen consumption by *Scenedesmus bijuga* cultures at different biomass concentrations in darkness.

Figure 4: Net dissolved oxygen evolution by *Scenedesmus bijuga* cultures at different biomass concentrations induced by four LED light sources (white-4100 K; red- $\lambda 655$ nm; green- $\lambda 530$ nm; blue- $\lambda 470$ nm) at $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity.

Figure 5: Net dissolved oxygen evolution by *Scenedesmus bijuga* cultures on day 0 at biomass concentration of 2.19 g/L induced by four LED light sources (white-4100 K; red- $\lambda 655$ nm; green- $\lambda 530$ nm; blue- $\lambda 470$ nm) at $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity.

Figure 6: *Scenedesmus bijuga* biomass production induced by four LED light sources (white-4100 K; red- $\lambda 655$ nm; green- $\lambda 530$ nm; blue- $\lambda 470$ nm) during a 6 days cultivation period.

Table 1: Monochromatic (red, blue and green) and full spectrum (neutral white) light transmittance in $\mu\text{mol m}^{-2} \text{s}^{-1}$ measured through *Scenedesmus bijuga* cultures at different biomass concentrations (0.7, 1.0 and 1.45 g L^{-1}) across cuvettes with different path

lengths (1, 5, 10, and 20mm). Light intensity was set at $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the cuvette illuminated surface and light transmittance was measured at the opposite surface.

Table 1

	0.7 g L ⁻¹				1 g L ⁻¹				1.45 g L ⁻¹			
	1mm	5mm	10mm	20mm	1mm	5mm	10mm	20mm	1mm	5mm	10mm	20mm
Green	220	111	66	7.9	215	89	29	3.8	198	61	16	1.3
White	195	84	40	3.8	181	61	15	1.7	162	47	8	0.6
Red	165	61	27	0.64	160	33	4	0.17	141	19	1.3	0.05
Blue	148	38	16	0.18	136	21	1.5	0.05	113	10	0.3	0.02

Figures
Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6