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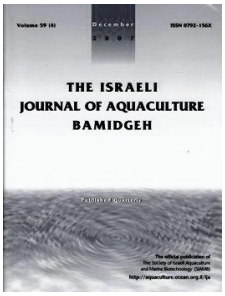

Use of Red and Blue Light-Emitting Diodes (LED) and Fluorescent Lamps to Grow Microalgae in a Photobioreactor

Caner Koc[1]*, Gary A. Anderson[2], Anil Kommareddy[3]

[1] *Department of Agricultural Machinery, Faculty of Agriculture, Ankara University, 06110 Diskapi, Ankara, Turkey*

[2] *Department of Agricultural and Biosystems Engineering, South Dakota State University, 57006 Brookings, SD, USA*

[3] *GIS Center of Excellence, South Dakota State University, 57006 Brookings, SD, USA*

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Key words: microalgae, photobioreactor, *Chlorella kessleri*, LED lamps, biomass

Abstract

High oil content microalgae are a source for biofuel production. They can be produced in open ponds or closed photobioreactors. To efficiently grow such microalgae, illumination for photosynthesis, CO_2 consumption, and the pH and nutrient content of the growth medium must be monitored and precisely controlled. In a closed photobioreactor, illumination is the most critical parameter because it is the most expensive factor of algae production and must be operated 24 h per day. In this research, *Chlorella kessleri* (UTEX 398) microalgae were grown in photobioreactors. All parameters were identical, except the source and intensity of the illumination. The light sources included red light-emitting diodes (LED), blue LED, and fluorescent lights. Growth of the microalgae was observed for seven days and the effects of the three illumination sources on cell count, cell weight, and cell size were determined. In the first experiment, in which the current of all three light sources was the same, red LED produced the highest number of cells with the highest weight while blue LED light produced the largest cells. In the second experiment, in which the light intensity was the same for all three light sources, the highest weight was again achieved with the red LED. Thus, we suggest that most advantageous production system may be to use a red light initially to produce the desired cell concentration, then switch to a blue light to increase cell size.

* Corresponding author. Tel: +90-3125961125, fax: +90-3123183888, e-mail: ckoc@ankara.edu.tr

Introduction

Biomass produced from high oil content microalgae is a plausible source of lipids for biofuel production. High oil content microalgae can be produced in open ponds or closed photobioreactors. To efficiently grow high oil content microalgae, illumination, pH, CO_2, and the nutrient content of the medium must be monitored and controlled. Control of illumination is the most critical parameter because illumination is the most expensive factor of algae production in a closed photobioreactor that operates 24 h per day. Blue light tends to yield the highest oil production on a g oil/g algae basis for *Chorellea* sp. and *Saccharomyces* (Shu et al., 2012). When exposed to blue light, algae fix more carbon on a cell per day basis, but also have a high respiration rate (Rivkin, 1989). Red light generates more cells (Lee and Palsson, 1996; Chen et al., 2009; Shu et al., 2012) Algae are less efficient at converting photons from blue light to biomass while algae cells grown in fluorescent light have twice the cell volume has those grown in red (Chen et al., 2009). Light wavelength may influence cell composition relative to protein, polysaccharides, and lipids (Rivkin, 1989). *Spirulina platensis* and *Nannochloropsis oculata* produced more cells when grown in fluorescent light than in red or natural light but had the same biomass (Chen and Lee, 2012). Although research has not yet delineated its impact, the wavelength of light affects algae cell size, reproduction, and composition, which determine the value of the algae as a biofuel. For the same biomass, smaller cells result in more cells per volume (cell concentration). The depth of light penetration is a function of cell concentration, i.e., a biomass with smaller cells will have less light penetration, affecting the design of the photobioreactor (Lee, 1999).

The most important factor in the design of high density and successful photobioreactors is the lighting (Barta et al., 1990; Richmond, 2004) because most of the energy used in a photobioreactor is for lighting. Photobioreactors must be economical, durable, and reliable, and have an efficient light source, for widespread commercial use. When choosing a light source, the spectral characteristics of the light flux and the wavelength range are important. Different kinds of algae may require different nutrients in the growth medium, different photon flux density, and different light wavelengths. Dense algae cultures tend to reflect, refract, and absorb light in a manner that prevents the light from traveling through the entire depth of the photobioreactor, making it difficult to maintain a dense culture. The structure of microalgae and the way that microalgae use light is complicated. Sometimes algae cells are unable to use all the photons absorbed for photosynthesis in the photosynthetic active radiation (PAR) range because the light intensity (photon flux density; photons/m^2/s) is too great. This situation can cause photo-saturation, photo-inhibition, or photo-oxidation which reduce photosynthesis, possibly injuring cells, and may result from cells being too close to the light source. If the light intensity is too low because of shading, then photosynthesis will be slower, reducing biomass production. Excessive or inadequate illumination may cause problems through the depth of the culture medium. High intensity light will not resolve the problem of shading in a dense culture and may lead to excessively high temperatures that can inhibit algae growth and production and damage the cell (Park and Lee, 2000).

LED light sources are suitable for use in photobioreactors. Algae use light in the PAR range for photosynthesis making PAR an important factor that affects the microalgae production and must be considered in the design of LED lighting systems for photobioreactors. High luminous efficiency, low energy consumption, and long life span are the major advantages of LED lights compared to other light sources. LED have relatively narrow wavelength bands allowing them to produce specific wavelengths (color) that can provide significant benefits such as energy efficiency. LED lights can reduce stress from excessive illumination on living organisms (algae) and, compared to other lights, are easily obtained. Their small structure, low energy consumption, and ease of installation make LED more environmentally friendly than other sources. In addition, LED lights do not produce as much heat as other types of lights, preventing overheating of the growth medium surrounding the microalgae cells (Koc et al., 2009).

The objectives of this research were to determine the effects of LED (blue and red) and fluorescent light sources on cell count, cell weight, and cell size of *Chorella kessleri* (UTEX 398) microalgae grown in closed photobioreactors.

Materials and Methods

Photobioreactor. Photobioreactors were made from Plexiglas. Clear plastic tubes (Corning Inc., Corning, NY, USA) were connected to a porous tube diffuser placed at the bottom of the photobioreactors to inject air into the growth medium. The injected air mixed the microalgae and growth medium. The air that passed through the tubes was a mixture of air from an air compressor and commercially-purchased gas that was 95% CO_2 and 5% nitrogen. The carbon dioxide and air flow were measured by flow meters (Cole Palmer Inc.).

Illumination. Panels (270 x 270 mm) with 59 blue and 384 red LED lights were obtained from Daktronics (Brookings, SD). Two experiments were conducted. In the first, each LED color was supplied by a constant current of 2.6 mA and constant voltage of 6.5 V for the blue LED and 11.0 V for the red. Thus, the actual power inputs were 16.9 W for the blue LED and 28.6 W for the red. The third illumination source was a fluorescent light powered by a 110 V line source. A three-channel fiber optic spectrometer (Avantes Inc., Broomfield, CO, USA) was used to measure the wavelengths and intensities of the light sources. The peak wavelengths were 467 nm for the blue LED and 659 nm for the red. The panel light intensity, measured 1 cm from the light source, was 557 $\mu W/cm^2/nm$ for the blue LED, 272 $\mu W/cm^2/nm$ for the red LED, and 163 $\mu W/cm^2/nm$ for the fluorescent light. The intensity was 3019 lux for the blue LED, 1389 lux for the red LED, and 2936 lux for the fluorescent lights. In the second experiment, all three illumination sources had an intensity of approximately 6030 lux, obtained by adjusting the distance between the photobioreactor and the light source. In the second experiment, power inputs were 18 W for the blue LED and 6.042 W for the red LED. Power for the fluorescent light was maintained at the same level as in the first experiment.

Light (or radiance) is the main source of energy for algae to produce food by photosynthesis. Light propagates as both wave and discrete packets called photons. Each photon has a discrete energy known as quanta, computed as: $E = h \times v$ (Ryer, 1997) and $E = h \times c/\lambda$ (Kommarredy and Anderson, 2003), where E = energy of a photon (J), h = Planks constant (6.626×10^{-34} J/s), v = frequency of light (Hz), c = speed of light (299,792,458 m/s), and λ = wavelength of light (m). The energy of a photon was calculated as 4.25×10^{-19} J for the blue LED, 3.014×10^{-19} J for the red LED, and 3.97×10^{-19} J for the fluorescent light, based on an average wavelength of 500-nm.

An average PAR of 26 $mol/m^2/day$ is required for plant photosynthesis in a greenhouse environment or culture in growth chambers (Barta et al., 1990). This average provides 300 $\mu mol/m^2/s$ of instantaneous radiation. In the first experiment (constant-current), PAR values were 217.17 $\mu mol/m^2/s$ for the blue LED, 123.16 $\mu mol/m^2/s$ for the red LED, and 71.115 $\mu mol/m^2/s$ for the fluorescent light. In the second experiment (constant illumination intensity 6030 lux), PAR values were 257.06 $\mu mol/m^2/s$ for the blue LED, 472.05 $\mu mol/m^2/s$ for the red LED, and 38.896 $\mu mol/m^2/s$ for the fluorescent light.

Penetration depth. Microalgae are very efficient at absorbing light energy. As the depth of a photobioreactor increases, high intensity light intended to increase the depth of light penetration can cause micro damage to algae by overheating or photo-oxidation (Lee, 1999). Therefore, the light sources were positioned about 1 cm from the photobioreactor.

Culture medium. Chlorella kessleri microalgae (UTEX 398) were grown in an N-8 culture growth medium obtained from the Culture Collection of Algae at the University of Texas, Austin, TX, USA. The medium consisted of 30 ml $NaNO_3$ (Fisher BP360-500), 30 ml $CaCl_2 \bullet 2H_2O$ (Sigma C-3881), 30 ml $MgSO_4 \bullet 7H_2O$ (Sigma 230 391), 30 ml K_2HPO_4 (Sigma P 3786), 30 ml KH_2PO_4 (Sigma P 0662), and 30 ml NaCl (Fisher S271-500), mixed in 2820 ml reverse osmosis/deionization (RO/DI) water. To avoid bacterial contamination, the water was treated by an RO/DI device that uses ultraviolet light to

destroy bacteria (Thermo Fisher Scientific Inc.). The initial cell concentrations for the first experiment were 1.25 x 10^6 cells/ml for the blue LED, 1.35 x 10^6 cells/ml for the red LED, and 1.24 x 10^6 cells/ml for the fluorescent lights. Cell concentrations for the second experiment were 1.0 x 10^5 cell/ml for all illumination sources.

Data collection. Temperature, pH, oxidation reduction potential (ORP), and gaseous CO_2 were monitored by sensors (Cole-Parmer Instrument Co. Vernon Hills, IL, USA) powered by a constant 13 V power supply. A program was developed in the G-programming language using Labview 8.5 (National Instruments) to collect and store the data.

Mixing. Mixing is important to obtain high cell density cultivation of microalgae in a photobioreactor. With effective mixing, algae cells in the photobioreactor come into contact with nutrients in the growth medium and metabolites produced by the algae (such as oxygen) are removed. Each cell benefits from light as circulation moves the cells back and forth from lit to shaded areas in the photobioreactor. Mixing was accomplished by air supplied by a 5.5 HP compressor (Coleman Power, USA) through the porous membrane tubes (Penn-Plax) placed at the bottom of the photobioreactors; bubbles rose and escaped from the top. The size and frequency of the air bubbles were established by the openings in the porous membrane and the air flow rate was measured with a flow meter (Cole Palmer Inc.).

Cell analysis. Cells were analyzed by microscope and hemocytometer. Samples (10 ml) were taken from the photobioreactor and coated with 1 ml isotonic diluents (Fisher Scientific) to make the microalgae cells visible. Later, three 1-ml samples were mixed and counted. The number of cells was counted under a microscope (Electron Microscopy Sciences). Pictures were taken with a digital camera mounted on the microscope and transferred to a computer with Infinity 2 software. Measurements were repeated every 24 h. Three samples were taken each time and the average cell concentration was recorded. The number of cells in an area of 0.01 mm^2 on the hemacytometer was multiplied by 10^4 to calculate the total number of cells per 1 ml.

Filter paper with pores of 10 µm was used to measure biomass. The filter paper was weighed on a precision balance, then a 10-ml sample of the algae solution was placed on it. The filter paper was drained, placed in a vacuum oven (Cole-Parmer), and dried for 5 h at 75°C. The filter paper with the dried algae biomass was weighed a second time. The difference between the initial weight of the filter paper and the weight of the paper with the algae was considered the weight of the microalgae. Doubling time is an important parameter for estimating the microalgae biomass produced in a given time period. In this experiment, doubling time was calculated as $N(t) = C2^{t/d}$, where N(t) = the number of objects at time t, d = doubling period (time it takes for object to double in number), c = initial number of objects, and t = time (Richmond, 2004).

Statistical analyses. The microalgae were grown using two treatment methods (constant current, constant light intensity) and three illumination sources in the Department of Agricultural and Biosystems Engineering of South Dakota State University. ANOVA was applied to determine the effects of treatment method and illumination source on the average cell count, weight gain, and biomass. Statistical analyses were made using SPSS 11.0 (SPSS Inc., Chicago, IL, USA). When differences between groups were found to be significant by ANOVA, then Tukey HSD and T2 post-hoc tests were applied (Ozdamar, 1999). The effects of pH, ORP, temperature, and CO_2 on cell count and microalgae mass were determined using covariance analysis (ANCOVA; Frigon and Laurencelle, 1993).

Results

For both experiments, pH was kept at 6.2-7.0 (Fig. 1); when the growth medium became more basic, CO_2 was added to the photobioreactor. Only atmospheric carbon dioxide was used as the carbon dioxide source. The highest CO_2 concentration in the exhaust gas of the photobioreactor was over 1900 ppm on the fifth day of the first experiment. The highest cell concentration was 22.5 x 10^6 cells/ml, obtained in the first experiment with the red LED. Similarly, the highest weight was achieved with the red LED in the second

experiment. During the first experiment (constant current), biomass doubled by 97 h for the blue LED, 41 h for the red LED, and 49 h for the fluorescent lights. In the second experiment (constant intensity), biomass doubled by 99 h for the blue LED, 96 h for the red LED, and 76 h for the fluorescent lights.

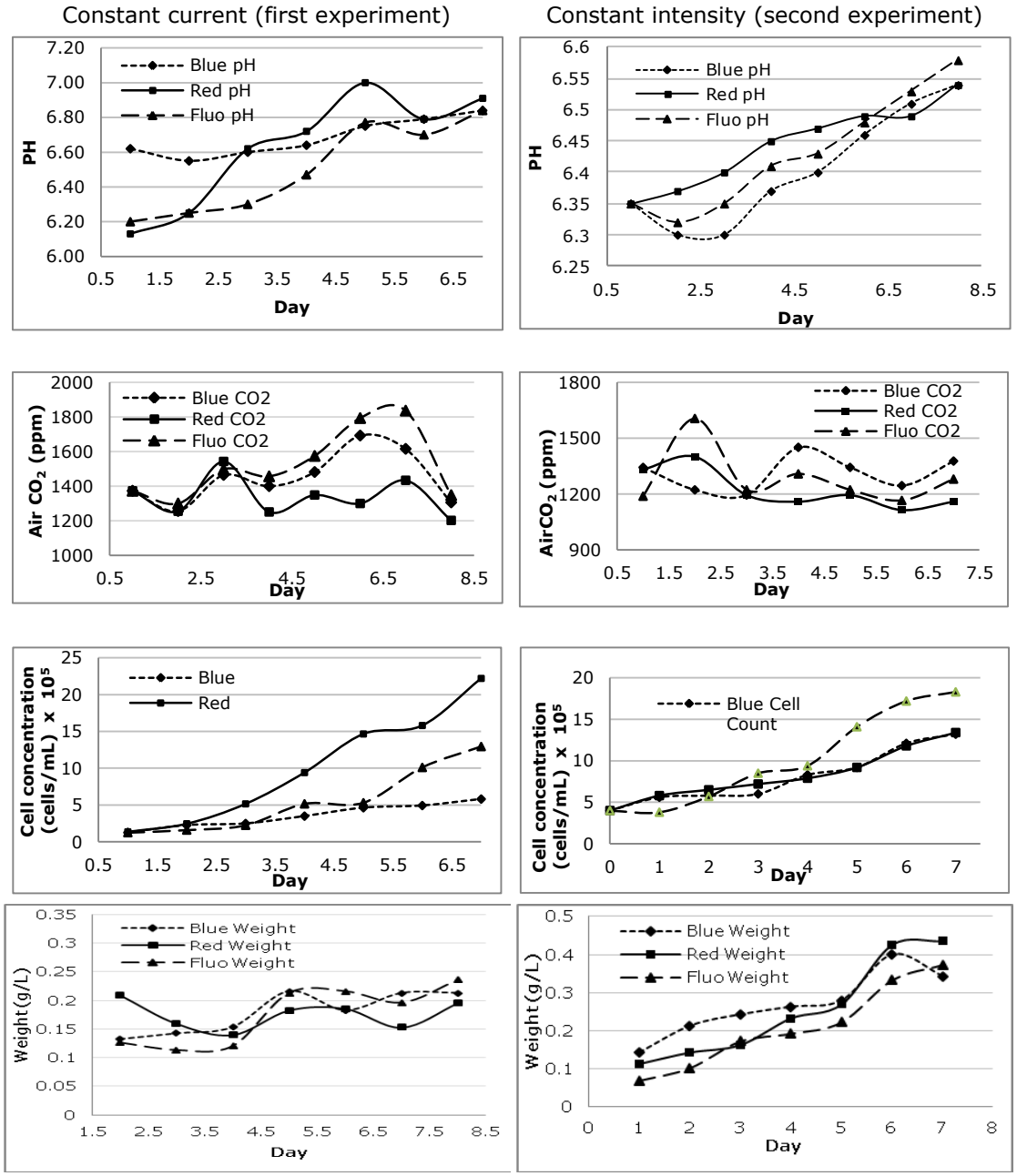

Fig. 1. pH, CO_2, cell concentration, and cell weight of the microalgae *Chlorella kasseri* grown under constant-current light sources and constant light intensity.

The size of the microalgae was determined with ImageJ software (Wayne Rasband National Institutes of Health, USA). All algae grown under all lights maintained a spherical shape. Cells grown in the blue LED were larger than those grown in the red or fluorescent lights and reached a maximum diameter of 25 µm. The average diameter of the microalgae grown in the blue LED was 16 µm, most were larger than those grown under the other two lights, and they were more circular. The microalgae grown under the red LED reached a maximum diameter of 20 µm. The average of the microalgae grown under red LED was 12 µm, they were an average of 40% smaller than the microalgae

grown under the blue LED, and were not as circular. The microalgae grown under the fluorescent light reached a maximum cell diameter of 25 μm, like those grown under the blue LED, but their average diameter was 12 μm, similar to those grown under the red LED. The size and shape of the microalgae were much more varied when grown under the fluorescent light than under the blue or red LED, however, a large cell size does not necessarily mean more algae biomass. The red LED produced more biomass even though the average cell was smaller than the blue LED, producing a higher cell concentration.

The effects of treatment method (constant current vs constant light intensity) on weight gain and cell count statistically differed; the effects of color did not (Table 1). The effects of treatment method, illumination source, and their interactions with ORP and CO_2 on weight gain were statistically insignificant while the effect of pH on weight gain and the effects of blue-red light and blue-fluorescent light on cell count were significant.

Table 1. Effects on weight gain and cell count of microalgae grown under different light sources.

	N	*Weight*			*Cell Count*		
		F	*P*		*F*	*P*	
Effects of treatment method (constant current vs constant light intensity) and illumination color							
Treatment				*			**
1	21	7.20	0.011	0.176 ± 0.0082^{a}	20.73	0.0001	947.619 ± 87.298^{A}
2	21			0.244 ± 0.0237^{b}			$6.424.286\pm1.245.958^{B}$
Color				NS			NS
Blue	14			0.224±0.0208			2.240.714±475.872
Fluorescent	14	0.58	0.564	0.192±0.0230	2.57	0.09	3.304.286±1.023.525
Red	14			0.215±0.0267			5.512.857±1.904.684
Effects of treatment method, illumination color, and their interactions							
Treatment (T)				*			**
1	21	6.98	0.0121	0.176 ± 0.0082^{a}	22.80	0.0001	947.619 ± 87.298^{A}
2	21			0.244 ± 0.0237^{b}			$6.424.286\pm1.245.958^{B}$
Color (C)				NS			NS
Blue (B)	14			0.224±0.0208			2.240.714±475.872
Fluorescent (F)	14	0.56	0.5741	0.192±0.0230	2.82	0.0726	3.304.286±1.023.525
Red (R)	14			0.215±0.0267			5.512.857±1.904.684
Treatment X color		0.19	0.8277	NS	8.43	0.0011	**
T_1C_B	7			0.179±0.0136			860.000±116.966
T_1C_R	7			0.175±0.0095			882.857±106.876
T_1C_F	7	0.43	0.6562	0.174±0.0199	2.89	0.0684	1.100.000±212.737
T_2C_B	7			0.269±0.0318			3.621.429±576337
T_2C_R	7			0.255±0.0497			10.142.857±2.926.346
T_2C_F	7			0.209±0.0423			5.508.571±1.695.504
Effects of treatment method, illumination color, and their interactions with pH, ORP, temperature, and CO_2							
Treatment				NS			NS
1	21	0.08	0.7844	0.176±0.0082	1.53	0.2255	947.619±87.298
2	21			0.244±0.0237			6.424.286±1.245.958
Color				NS			**
Blue	14			0.224±0.0208			$2.240.714\pm475.872^{A}$
Fluorescent	14	0.23	0.7958	0.192±0.0230	7.76	0.0018	$3.304.286\pm1.023.525^{B}$
Red	14			0.215±0.0267			$5.512.857\pm1.904.684^{B}$
Treatment X color				NS			**
T_1C_B	7			0.179±0.0136			860.000 ± 116.966^{ABab}
T_1C_R	7			0.175±0.0095			882.857 ± 106.876^{ABac}
T_1C_F	7	0.19	0.8277	0.174±0.0199	8.43	0.0011	$1.100.000\pm212.737^{ABab}$
T_2C_B	7			0.269±0.0318			$3.621.429\pm576.337^{Aa}$
T_2C_R	7			0.255±0.0497			$10.142.857\pm2.926.346^{Bb}$
T_2C_F	7			0.209±0.0423			$5.508.571\pm1.695.504^{ABbc}$
pH	-	31.81	0.0001	**	50.90	0.0001	**
ORP	-	2.39	0.1316	NS	5.37	0.0271	*
Temp	-	1.57	0.2188	NS	7.46	0.0102	*
CO_2	-	0.05	0.8320	NS	0.04	0.8402	NS

NS = not significant, * = $p<0.05$, ** = $p<0.01$

Discussion

Photosynthesis requires light in the vicinity of the absorption peaks of chlorophyll *a* and *b* (662 nm and 642 nm, respectively), the most important photosynthetic pigments. The red LED peak wavelength was 659 nm, between the chlorophyll *a* and *b* peaks. The blue LED peak wavelength was 467 nm, shorter than the peak chlorophyll *b* absorption wavelength. Chlorophyll *a* has an absorption peak at approximately 430 nm, but it drops to near zero at 655 nm, leaving only a small part of the narrow blue LED spectrum overlapping the chlorophyll spectrum. Therefore, the red LED would be expected to be more effective for photosynthesis and, in fact, the cell mass concentration of the microalgae grown in the red light was highest. This is logical since microalgae cells absorb red light through the green pigment chlorophyll (Matthijs et al. 1996).

The increase in pH indicates that carbon dioxide was consumed by the algae faster than it was supplied, or that the algae were producing a metabolite that tended to be basic. The highest pH was measured in the photobioreactors illuminated with the red LED and fluorescent lights, indicating that the algae in these photobioreactors were consuming more carbon dioxide and growing faster in number of cells, quantity of biomass, and size. The CO_2 concentration in the exhaust air of the first experiment (constant current) was reasonably steady even though the pH increased, which could mean that more CO_2 remained in the growth medium as bicarbonate or carbonate and was not off-gassed. Alternatively, it is possible that the time it took for the bubbles to pass through the photobioreactor was insufficient for the CO_2 to transfer from the bubble to the growth medium, causing the increase in pH. In the constant intensity experiment, exhaust air CO_2 concentration increased towards the end of the trial when the pH increased, supporting the hypothesis that the bubbles were not fully exchanging CO_2 with the growth medium.

Phototropic processes, which control the motion of plant organs in response to light and ensure optimization of biophysical and biochemical reactions, are triggered by light with wavelengths in the range of 400-500 nm. The blue LED had a wavelength of 467 nm. Blue light can lead to larger cells (Lee and Palsson, 1994, 1996; Oldenhof et al., 2006) as a result of delayed cell division (Munzner and Voigt, 1992). The delay can be as much as two hours longer than with red light (Oldenhof et al., 2006). Red light caused mother cells to divide at the smallest size (approximately twice the size or one round of replication) regardless of how many autospores were present (Lee and Palsson, 1996; Oldenhof et al., 2006). The average cell volume of *Chlorella* decreased when mother cells divided into daughter cells under higher light intensity but the total cell volume remained the same (Lee and Palsson, 1994).

A large cell size does not necessarily mean more algae biomass. The constant current red LED produced more biomass (0.44 g/l) that the blue LED, even though the average cell was smaller. The ratio of red to blue photon flux was 2.38 for constant current lighting. The fluorescent light produced more cells than the blue and red LED with constant current. The ratio of red to blue photon flux was 0.47 for the constant intensity lighting. The photon flux ratio for the constant current lighting is the inverse of that for the constant intensity lighting. The difference in photon flux intensity may explain the different results of the lighting systems. The light intensity ratio for the constant current light of red to blue was 0.46, basically the same as the photon flux ratio for the constant intensity lighting. Light measurements in photon fluxes or light intensities (lux) lead to different conclusions.

In conclusion, *Chlorella* is good for producing biodiesel. With oil contents similar to those of seed plants, microalgae have huge potential for biomass productivity (Mata et al., 2010). Further, the fatty acid composition of *C. kessleri* is suitable for biodiesel production (Sydney et al., 2010). The most efficient illumination source in terms of cell size was the blue LED (16 µm avg diameter, 25 µm maximum). Cells grown under blue light were more circular than those grown under other lights. The red LED was the most efficient light source in terms of cell concentration and weight (22.5×10^6 cells/ml and 0.45 g/l). The pH in all photobioreactors generally rose, indicating that carbon dioxide may have been consumed by the algae faster than it was transferred to the growth

medium, or than the algae released a metabolite that raised the pH. The most advantageous production system may be to use a red light initially to produce the desired cell concentration, then switch to a blue light to increase cell size, as concluded by Shu et al. (2012).

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