proceedings

MDPI

Proceedings

Influence of Color Temperature of White LED Diodes and Illumination Intensity on the Content of Photosynthetic Pigments in *Chlorella vulgaris* Algae Cells †

Paweł Kondzior [1,*], Damian Tyniecki [2] and Andrzej Butarewicz [1]

[1] Department of Chemistry, Biology and Biotechnology, Bialystok University of Technology, 15-351 Bialystok, Poland
[2] Department of Electrical Power Engineering, Bialystok University of Technology, 15-351 Bialystok, Poland
* Correspondence: p.kondzior@doktoranci.pb.edu.pl; Tel.: +48-85-746-96-59
† Presented at Innovations-Sustainability-Modernity-Openness Conference (ISMO'19), Bialystok, Poland, 22–23 May 2019.

Published: 18 July 2019

Abstract: The purpose of this paper is to determine the influence of color temperature of Light Emitting Diode (LED) diodes and illumination intensity on the content of photosynthetic pigments of chlorophyll a, chlorophyll b and carotenoids in *Chlorella vulgaris* algae cells. Choosing the right color temperature and intensity of illumination can favorably affect the growth of algae. In particular, it can contribute to the efficiency of the photosynthesis process and the amount of produced biomass from *Chlorella vulgaris* algae. In the spectrophotometric studies, the highest content of chlorophyll a, chlorophyll b and carotenoids was found in cultures illuminated with very cold white light (8500 K) with an intensity of 500 µmol/m²s. The highest measured content of chlorophyll a (Chl a) pigments was 48.29 mg/L, Chl b pigment was 23.25 mg/L and carotenoids pigment was 12.65 mg/L; the smallest content of pigments for Chl a (11.48 mg/L), Chl b (4.69 mg/L) and carotenoids (3.03 mg/L) was found in the sample illuminated with warm white light (3200 K) with an intensity of 50 µmol/m²s. The highest amount of dry organic matter amounting to 2.0 g/L was found in a sample illuminated with warm white light (3200 K) with an intensity of 250 µmol/m²s, then 1.91 g dry organic mass (DOM)/L for very cold white light with an intensity of 250 µmol/m²s, and 1.48 g DOM/L for very cold white light with an intensity of 50 µmol/m²s. The obtained results show that a higher content of photosynthetic pigments does not directly affect the increase of the amount of dry organic matter.

Keywords: photosynthetic pigments; algae; *Chlorella vulgaris*; LED diodes; illumination

1. Introduction

The purpose of this paper is to determine the influence of the color temperature of Light Emitting Diode (LED) diodes and illumination intensity on the content of photosynthetic pigments of chlorophyll a, chlorophyll b and carotenoids in *Chlorella vulgaris* algae cells. The photosynthetic pigments transform the energy of light into the energy of chemical bonds, from which algae synthesize organic compounds (saccharides) in the process of photosynthesis from simple inorganic compounds. Choosing the right color temperature and intensity of illumination can favorably affect the growth of algae. In particular, it can contribute to the efficiency of the photosynthesis process and the amount of produced biomass from *Chlorella vulgaris* algae.

2. Material and Methods

Chlorella vulgaris algae from the University of Bialystok were used in the study. BG-11 Medium for Blue Green Algae ATCC Medium 616 was used to grow algae. The volume of culture medium was 500 cm^3, the incubation temperature was 25 °C, and continuous lighting was provided. LED panels based on 84 diodes (Surface Mounted Device (SMD) 5630) were used for lighting. The influence of warm white (3200 K), cold white (6500 K) and very cold white (8500 K) was studied. Each color of lighting was tested at three intensity levels: 500 μmol/m^2s, 250 μmol/m^2s and 50 μmol/m^2s. The intensity of illumination was measured with the Delta OHM HD 2102.1 photoradiometer with the LP 471 Photosynthetically Active Radiation (PAR) sensor. The color temperature measurement was measured using the GL SPECTIS 1.0 Touch GLX10 tf spectrometer. The pigment content was measured spectrophotometrically with a Hach Lange DR 5000 on day 2, day 4, day 6, day 9 and day 15 of incubation. For this purpose, a 5 cm^3 culture was taken as a test sample. The test sample was centrifuged at 4500 Revolutions Per Minute (RPM) for 10 minutes using the MPW-352R centrifuge. The supernatant was decanted and 5 cm^3 of 90% methanol was added to the centrifuged mass of the algae. Then, the test-tubes were closed with a bacteriological stopper and then mixed and placed in a steam bath set at 60 °C for 10 min. After this, the sample was centrifuged again for 10 min at 4500 RPM and the resulting supernatant was subjected to spectrophotometric analysis at wavelengths of 470 nm, 652 nm and 665 nm. The pigment content was calculated from the formulas published in Xiong et al. [1]:

$$Chlorophyll\ \ a\ \ =\ \ 16.82A_{665} - 9.28A_{652}\ \ [\frac{mg}{\mathrm{L}}], \quad (1)$$

$$Chlorophyll\ \ b\ \ =\ \ 36.92A_{652} - 16.54A_{665}\ \ [\frac{mg}{\mathrm{L}}]\ , \quad (2)$$

$$C_{carotenoid}\ \ = \frac{(1000A_{470} - 1.91C_a - 95.15C_b)}{225}\ \ [\frac{mg}{\mathrm{L}}], \quad (3)$$

3. Results and Discussion

3.1. Characteristics of LED Panels

LED panels have been measured in terms of the emitted spectrum. The graphs depicting the emitted spectrum by the LEDs are shown in Figure 1. The spectra emitted by the sun are also shown for comparison. The color temperature of the panels was also tested, and the result was obtained: the measured value of warm white (3200 K) was 3015 K, the measured value of cold white (6500 K) was 6845 K, and the measured value of very cold white (8500 K) was 15,290 K. The results of the color temperature tests show similarity with the data provided by the manufacturer with the exception of very cold white (8500 K), where the discrepancy is high and the value is almost twice as high.

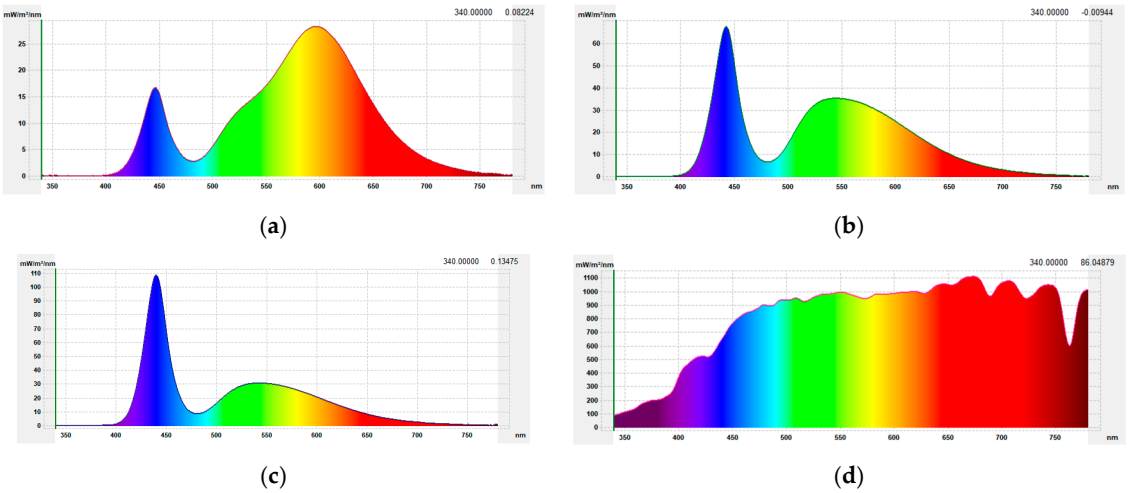

Figure 1. Spectrum emitted by Light Emitting Diode (LED) diodes and the sun: (**a**) warm white (3200 K); (**b**) cold white (6500 K); (**c**) very cold white (8500 K); (**d**) the sun (in the range of 350 nm to 800 nm).

3.2. The Content of Pigments and Cultured Algae Biomass

On the day 15 of incubation, the grown biomass from *Chlorella vulgaris* was analyzed. In the spectrophotometric studies, the highest content of chlorophyll a, chlorophyll b and carotenoids was found in cultures illuminated with very cold white light (8500 K) with an intensity of 500 µmol/m²s. The highest measured content of pigments was 48.29 mg/L for Chl a, 23.25 mg/L for Chl b and 12.65 mg/L for carotenoids. The smallest content of pigments (11.48 mg/L for Chl a, 4.69 mg/L for Chl b, and 3.03 mg/L for carotenoids) was found in the sample illuminated with warm white light (3200 K) with an intensity of 50 µmol/m²s. The remaining results are shown in Table 1. The highest amount of dry organic matter (DOM) amounting to 2.0 g/L was found in a sample illuminated with warm white light (3200 K) with an intensity of 250 µmol/m²s, then 1.91 g DOM/L for very cold white light with an intensity of 250 µmol/m²s, and 1.48 g DOM/L for very cold white light with an intensity of 50 µmol/m²s. The obtained results show that a higher content of photosynthetic pigments does not directly affect an increase in the amount of dry organic matter. When cultivating algae to produce biomass, it is more reasonable to choose the illuminating intensity of 250 µmol/m²s. This will contribute to increasing the amount of biomass (expressed as DOM) for warm white light (3200 K) by 171%, cold white by 147% and very cold white by 205%.

An important role in collecting excess energy is played by carotenoids, especially zeaxanthin. The zeaxanthin pigment is synthesized during strong illumination in the xanthophyll cycle, while during a lack of illumination, the pigment passes through the antheraxanthin in the process of epoxidation into violaxanthin. Therefore, the appearance of zeaxanthin can indirectly inform about the saturation of the photosynthesis process, and directly about the excess of light energy [2].

Table 1. Results of testing the content of photosynthetic pigments and the amount of cultured biomass from *Chlorella vulgaris* algae on day 15 of incubation.

Color Temperature	Intensity of Illumination	The Content of Photosynthetic Pigments			Dry Mass	Dry Organic Mass (DOM)	Dry Mineral Mass	Chl a/DOM	Chl b/DOM	Carotenoids /DOM
		Chlorophyll a (Chl a)	Chl b	Carotenoids						
Unit	(μmol/m²s)	(mg/L)	(mg/L)	(mg/L)	(g/L)	(g/L)	(g/L)	(‰)	(‰)	(‰)
Warm white (3200 K)	500	42.92	22.69	11.42	1.84	1.17	0.67	36.68	19.40	9.76
	250	38.69	18.09	10.29	2.63	2.00	0.63	19.35	9.05	5.14
	50	11.48	4.69	3.03	1.87	1.41	0.46	8.14	3.33	2.15
Cold white (6500 K)	500	16.42	7.48	4.99	1.22	0.75	0.47	21.89	9.98	6.65
	250	30.33	14.09	8.43	1.77	1.10	0.67	27.57	12.81	7.66
	50	14.18	6.07	3.78	1.93	1.30	0.63	10.90	4.67	2.91
Very cold white (8500 K)	500	48.29	23.25	12.65	1.43	0.93	0.50	51.92	25.00	13.60
	250	40.46	17.89	10.85	2.41	1.91	0.50	21.18	9.37	5.68
	50	17.31	7.72	4.59	2.04	1.48	0.56	11.69	5.21	3.10

4. Conclusions

The obtained results show that a higher content of photosynthetic pigments does not directly affect an increase of the amount of dry organic matter. The increased amount of photosynthetic pigment in samples illuminated with the intensity of 500 μmol/m²s (except the cold white color of 6500 K) may be caused by the defenses of the *Chlorella vulgaris* algae cells. In order to better understand the processes occurring in the *Chlorella vulgaris* algae cells, future research should extend to determination of content photosynthetic pigments from the xanthophyll cycle.

Author Contributions: P.K. and A.B. conceived and designed the experiments; P.K. performed the biological research and analyzed the data under the supervision of A.B.; D.T. performed the lighting tests and analyzed the data under the supervision of A.B.; P.K., D.T. and A.B. contributed reagents materials and analysis tools; P.K., D.T. they wrote the paper under the supervision and review of A.B.

Acknowledgments: Studies have been carried out in the framework of the own work No. MB/WBiIŚ/5/2017 and funded by the Polish Ministry of Science and Higher Education.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Xiong, J.Q.; Kurade, M.B.; Abou-Shanab, R.A.I.; Ji, M.K.; Choi, J.; Kim, J.O.; Jeon, B.H. Biodegradation of Carbamazepine Using Freshwater Microalgae Chlamydomonas Mexicana and Scenedesmus Obliquus and the Determination of Its Metabolic Fate. *Bioresour. Technol.* **2016**, *205*, 183–190, doi:10.1016/j.biortech.2016.01.038.
2. Gabryś, H.; Kacperska-Lewak, A.; Kopcewicz, J.; Krzymowska, M.; Lewak, S.; Rychter, A.; Starck, Z.; Strzałka, K.; Szymańska, M.; Tretyn, A.; et al. *Plant Physiology (In Polish)*; Polish Scientific Publishers PWN: Warsaw, Poland, 2012.