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The Influence of Artificial Lighting Systems on the Cultivation of Algae: The Example of *Chlorella vulgaris*

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Abstract: Microalgae are a practical source of biological compounds for biodiesel production. This study examined the influence of three different light-emitting diode (LED) systems on the biomass production of green algae *Chlorella vulgaris* BA0002a. The cultivation was carried out in a photobioreactor illuminated from the bottom with a single side light jacket (PBR I), in a photobioreactor illuminated from the bottom with a double side light jacket (PBR II) and in a photobioreactor illuminated only from the top (PBR III). Research has shown that the intensification of algae cell production and growth depends on the light distribution and exposure time of a single cell to radiation. In the experiment, the highest growth of algae cells was obtained in the photobioreactor with double jacket and lower light panel. The lowest cell growth was observed in the photobioreactor illuminated only from above. For cultures raised in the PBR I and PBR II photobioreactors, increased oxygen production was observed, which was directly related to the increased production of biomass, which in turn was dependent on the increased amount of radiant energy.

Keywords: cylindrical LED light coat; tracking lighting; photosynthetic microorganisms; photobioreactor; algae

1. Introduction

Biomass (energy willow, forest waste) or waste from agri-food processing (straw or fruit marc) is currently a cheap and renewable energy source [1–3]. Specially cultivated algae represent one of the promising sources of such energy [4,5]. Biomass derived from algae can be used to produce energy as a raw material for combustion or co-incineration with other waste fuels or through the production of biofuels [6–14]. Biofuels derived from algae can take the form of pyrolytic solid fuels [15], flammable gases (hydrogen and methane) [16] or liquid hydrocarbons and biodiesel [17,18]. Since photosynthetic organisms consume carbon dioxide in photosynthesis, algae cultivation is also a desirable side effect of carbon sequestration [19,20].

Algae are a diverse group of photosynthetic organisms that play an important role in the biosphere. They are characterized by a high growth rate potential compared to typical energy crops [21]. They are increasingly used in agriculture, environmental protection, medicine and energy. There are many known systems for the reproduction of algae, which often have different yields.

The high productivity of good-quality algae and their optimal growth rate depends on a number of factors [22]. The most important factors are the distribution and availability of light with the appropriate wavelength [23] and intensity and the temperature and geometry of the vessels [24].

In addition, in the breeding of algae, the parameters of the production process, such as nutrients supplied during reproduction or the concentration of CO_2, are important.

The dynamics of aeration is another key parameter for improving the growth of microalgae cells. Aeration promotes pH regulation, aids mixing, and allows the elimination of nutrient concentration gradients. It promotes the even exposure of all cells to light radiation and helps to release the oxygen produced to reduce its toxic effect on microalgae. Improper balance of individual elements of the breeding environment may result in the inhibition of growth and reproduction [25,26].

Algae are undoubtedly a potential source of renewable energy and a raw material for the production of biofuels, but unfortunately, their production is very energy-intensive [16,26]. Additional challenges in the conventional method of algae production are their collection and separation [17].

As mentioned above, light energy significantly determines the growth rate of photosynthetic organisms, and its influence is different for different processes and species production [26–28]. Both excessive and insufficient lighting have a negative impact on the amount of biomass [29–32]. When cultivating algae in photobioreactors, the conversion of the available light depends on the degree of biomass concentration and thus on the optimal distribution of radiation energy, so that all cells receive the right amount of it. According to various authors, the optimum photon flux density value for algae is in the range of 50–200 $\mu mol \cdot m^{-}2 \cdot s^{-}1$ [15,33,34] and is characteristic not only for a given species but even for a given varietas.

When choosing a photobioreactor for the production of algae, care must be taken that all algae, regardless of their distance from the light source, can receive the same amount of radiation, which will ensure a constant growth rate. Attention should also be paid to the effect called shading [12]. With the growth of algae, i.e., with the density of cells, the light intensity in the reactor is suppressed, forcing an increase in the intensity of lighting in order to maintain the growth rate. Unfortunately, such a procedure may have the opposite effect, causing photoinhibition, i.e., a situation in which the photosynthetic microorganism is exposed to a greater amount of radiant energy than it needs to develop. It leads to damage of the second-order photositemator [35–37].

For cultures using artificial lighting, light-emitting diodes (LEDs) are most often used to illuminate photobioreactors. LEDs emit radiation with narrow wavelength ranges and offer the possibility of easily selecting the light color. Research suggests that algal growth is greatly influenced by the red light spectrum, which promotes faster multiplication and growth. On the other hand, the blue light spectrum causes cell growth [38].

The influence of light energy varies in different processes and for different species. Knowledge of radiation energy density distribution in photobioreactor (PBR) and energy absorption by algae biomass is essential for PBR analysis, modeling and design.

The aim of the study was to investigate the influence of three different artificial lighting systems on the cultivation of *Chlorella vulgaris (C. vulgaris*) algae.

2. Materials, Methods and Methodology

2.1. Characterization of Biological Material Chlorella vulgaris

The studies used the *C. vulgaris* type BA0002a inoculum from the Culture Collection of Baltic Algae (CCBA) in the Institute of Oceanography at the University of Gdańsk. These algae belong to the group of green algae; they inhabit a brackish water environment and are one of the most widespread algae species in the Baltic Sea.

2.2. Characteristics of the Test Stand

The research stand consisted of three identical cylindrical glass photobioreactors, designated as PBR I, PBR II and PBR III, with an active capacity of 1.79 dm^3 (Figure 1). Each of the photobioreactors was equipped with an artificial lighting system based on RGB LEDs (LED RGBW).

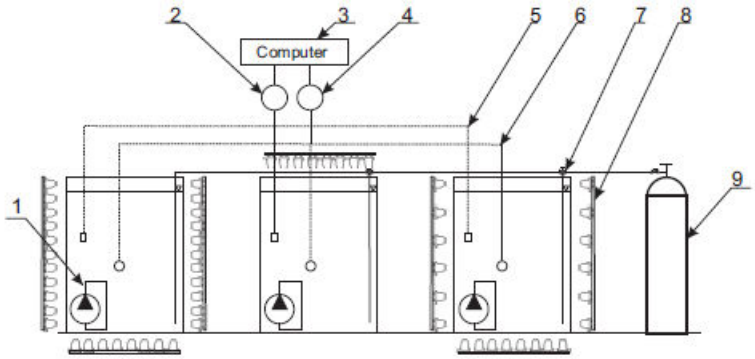

Figure 1. Scheme of the test bench: (1) circulation pump, (2) temperature meter, (3) computer, (4) photosynthetically active radiation (PAR) meter, (5) thermocouple, (6) PAR probe, (7) dosing regulator CO2, (8) lighting system, (9) CO2 feeding system. Source: own work.

The PBR I reactor was equipped with LED directional lighting, in the form of a bottom panel that illuminated the conducted culture through the bottom of the glass reactor and a single, cylindrical RGB LED. The bottom panel had 18 W of power and contained 30 points of light with a luminous flux of 1100 lm and a color temperature of 3000 K (warm white color), while the cylindrical mantle consisted of 150 light points with a total power of 36 W.

The PBR II reactor was equipped with LED directional lighting which came from the bottom of the reactor and had 18 W of power. The bottom panel contained 30 lighting points with a luminous flux of 1100 lm and a color temperature of 3000 K (warm white color) and a double, cylindrical LED light jacket. The double light jacket consisted of 150 RGB LED light spots with 36 W of power and 150 lighting points with 18 W of power and a 950 lm luminous flux with a color temperature of 3100 K (warm white).

The PBR III reactor was equipped with directional lighting on the upper surface (seminar), with a power of 13.5 W, a flux of 1200 lm and a color temperature of 6500 K. In all the reactors, the cultures were illuminated in the 14/10 system.

The geometric dimensions of the cylindrical light sheaths of the PBR I and PBR II reactors were the same: diameter 24.5 cm ± 1.0 mm, height 19.5 cm ± 1.0 mm.

In addition, each reactor was equipped with the same mixing systems (circulating pumps with an average capacity of 80.0 $L \cdot h^{-1}$), temperature and pH measurement meters, probes for measuring photosynthetically active radiation (PAR) (Quantum MQ-100) and CO_2 feeding systems. Figure 2 presents a cross-section of an example photobioreactor (PBR I).

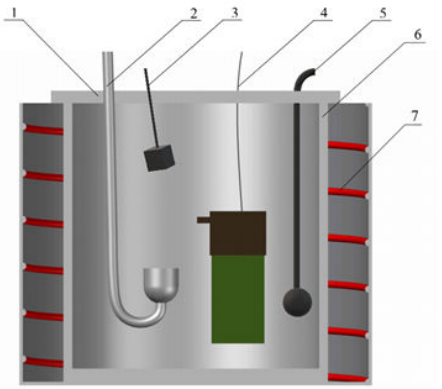

Figure 2. Technical illustration through a cross-section of an example PBR: (1) reactor cover, (2) diffuser feeding system, (3) temperature sensor PT100, (4) circulating pump Eden 128, (5) PAR probe, (6) glass reactor, (7) cylindrical LED light jacket. Source: own study.

Breeding of *C. vulgaris* BA0002a microalgae was carried out in cylindrical glass reactors with module L·day^{-1} equal to 0.985 and capacity 1072.0·10^{-3} m^3, including working capacity 1.79·10^{-3} m^3. The glass cylinders were sterilized before the start of cultivation in an autoclave at 120 °C for 20 min. The studies were carried out in a 14/10 batch culture system (day/night) of *C. vulgaris* BA0002a inoculated on a medium of the general composition (Table 1).

Table 1. Composition of culture medium.

Component	Concentration, mg·dm^{-3}
total nitrogen concentration	184.5
nitrate nitrogen	84.0
ammonium nitrogen	52.0
urea nitrogen	49.0
total concentration of phosphorus (soluble in neutral ammonium citrate and in water)	49.2
total potassium concentration (water-soluble)	192.0
water-soluble magnesium	3.0
water-soluble sulfur	2.5
boron	0.025
copper EDTA	0.010
iron EDTA	0.070
manganese	0.040
molybdenum	0.004
zinc EDTA	0.025
pH	6.4
salinity	5.84 g NaCl dm^{-3}
oxygen content	6.93
oxygen content including salinity	7.77

Determination of the basic components of the medium was carried out by means of tube colorimetric tests using a single-beam NANOCOLOR PF-12 PLUS photometer, with autocalibration and self-control functions in the spectral range of 340–860 nm. The main methods of determination are summarized in Table 2. The NANOCOLOR VARIO C2 M thermostat for chemical mineralization was also used. Wherever the range of the method did not allow for direct determination, the sample was diluted and the result recalculated. Other determinations were also carried out according to the reference methods enclosed in accordance with the device data.

Table 2. List of measurement methods.

Designation, $mg \cdot dm^{-3}$	Description of the Method	Ref:
total nitrogen N_{og} scope: 5–220 wavelength: 385 nm	mineralization in a thermostat with compensation of interfering substances and photometric determination with 2.6-dimethylphenol in a mixture of sulfuric and phosphoric acid	985083
nitrate nitrogen N-NO_3 scope: 4–140 wavelength: 365 nm	a color reaction with 2.6-dimethylphenol in a mixture of sulfuric acid and phosphoric acid	91865
ammonium nitrogen N-NH_4 scope: 30–160 wavelength 585 nm	determination of blue indophenol dye on the basis of reactions with hypochlorite and salicylate at pH 12.6 in the presence of sodium nitroprusside	985006
phosphorus P 50 scope: 10.0–50.0 wavelength: 436 nm	photometric determination as molybdenum blue after acid hydrolysis and oxidation at 100–120 °C	985079
O_2 oxygen range: 0.5–12.0 wavelength: 445 nm	determination of dissolved oxygen content in water by Winkler method, color reaction with free iodine	985082
potassium K 50 scope: 2.0–50.0 wavelength: 690 nm	determination of the turbidity caused by the formation of potassium tetraphenyl borate	985045
iron Fe range 0.01–2.00 wavelength: 470 nm	color reaction with 1.10-phenanthroline	91836
copper Cu^{2+} range 0.01–2.00 wavelength: 585 nm	color reaction with cuprizone (oxalic acid bis(cyclohexylidene hydrazide))	
sulfur S range: 0.1–3.0 wavelength: 660 nm	color reaction with N,N-dimethyl-1,4-phenylenediamine	91888
zinc Zn^{2+} range: 0.20–6.0 wavelength: 490 nm	determination of the concentration of zinc ions in aqueous samples by complexing the contained metal ions with cyanide, followed by selective release of zinc ions by addition of chloral hydrate, by reaction with 4-(2-pyridylazo) resorcin (PAR) to form an orange complex whose color intensity is proportional to that of zinc ions	985042
manganese Mn range: 0.10–3.00 wavelength: 436 nm	photometric determination of manganese content by TMB method according to Serrat	918126

During the tests, the following measurements were recorded: temperature (T), using PT100 resistance sensors, and pH, conductivity, oxygen content and salinity, using a multifunctional CX-401 meter. In addition, the concentration of carbon dioxide (CO_2) was measured by colorimetric tests. The optical density was determined using a DEN-1B densitometer with a wavelength of l = 565 ± 15 nm and a measurement range of 0.00 to 15.00 McF. Biomass increment was assessed by the direct method of counting the cells and measuring their size under the Motic RED 233 optical microscope using the Thoma chamber, three times a day at regular intervals. The results (in the graphs) are given as the arithmetic mean of these measurements.

In each bioreactor, 2 mL of *C. vulgaris BA0002a* inoculum was inoculated in an amount of 1.79×10^{-3}·m^3. Each inoculum contained on average 2.7×10^6 cells·mL^{-1}.

3. Results

Measurements of the cultivation reaction with the CX-401 multimeter showed that the pH of the crop was 6.5 ± 0.2. It maintained the same level for all lighting systems. The reason for the lower pH could be the excessive distribution of carbon dioxide using a gas diffuser. The pH distribution over time is shown in Figure 3.

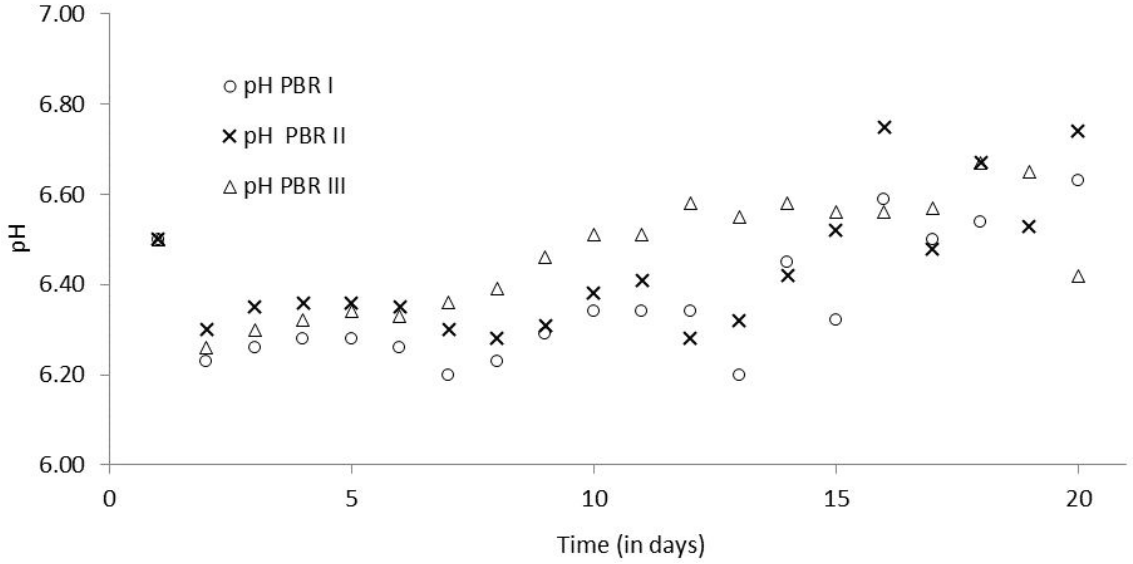

Figure 3. Distribution of acidity over time, depending on the type of lighting system. Source: own study.

The graph in Figure 4 contains the distribution of oxygen content over time. The concentration of oxygen, except between days 11 to 14, for all lighting systems was maintained in the range of 5 to 15 mg·dm^{-3}. Between days 11 and 14, oxygen concentration was observed to rapidly increase by over 100% for both PBR I and PBR II, which correlated fully with the maximum increase in the number of cells for these photobioreactors at that time. Increased oxygen production was observed for PBR I and PBR II photobioreactors.

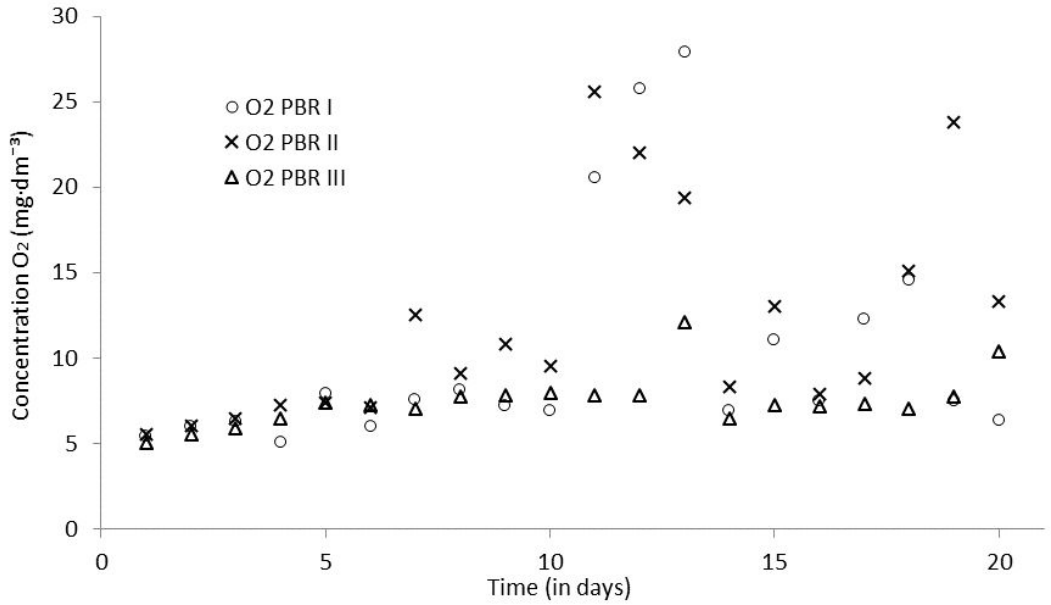

Figure 4. The distribution of oxygen over time, depending on the type of illumination system. Source: own study.

The average temperature of the culture ranged between 25 and 35 °C, although in extreme cases it reached 40 °C. The optimal temperature depends on a given species, although many authors state that for most representatives of this species it is between 20 and 30 °C [19,26,31]. Temperature fluctuations were related to the heat generated by the lighting during its operation in the 14/10 system. The stabilization problem will be eliminated in subsequent tests by removing heat from lighting systems and stabilizing the process temperature. Figure 5 contains a graph of the temperature distribution in the three photobioreactors during cultivation. The highest temperatures were obtained by photobioreactors equipped with cylindrical coats of light and lighting from the bottom panel rather than the photobioreactor illuminated only by light from above.

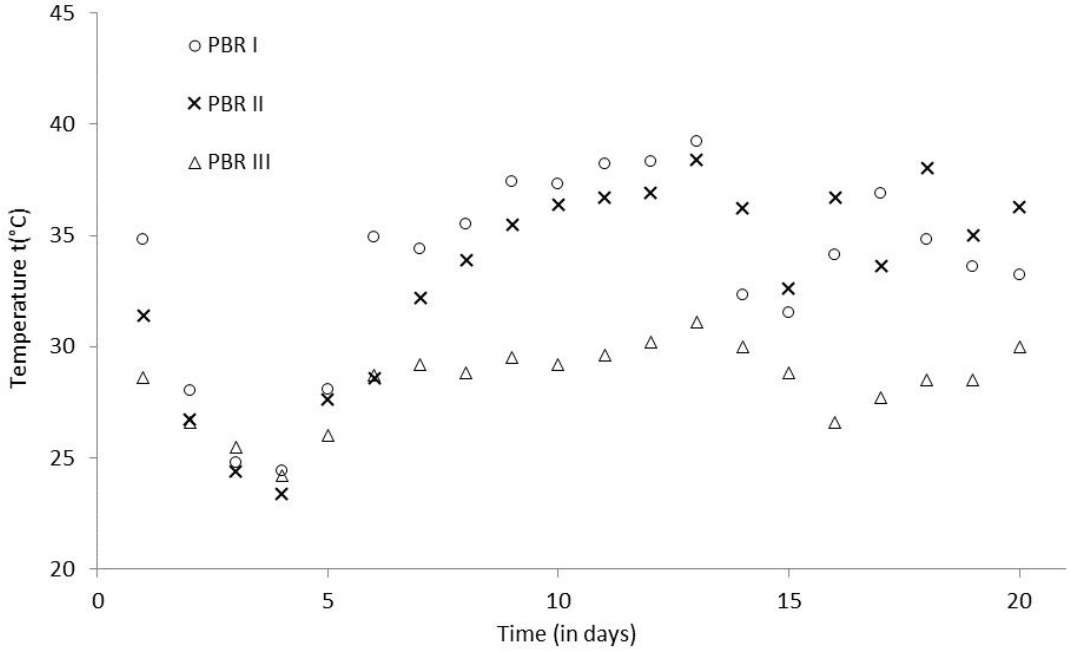

Figure 5. Temperature distribution over time, depending on the type of lighting system. Source: own study.

Optical density measurements were carried out using a densitometer operating in the range of λ = 565 nm ± 15. The absorbance results are shown in Figure 6. From the changes in optical density for different lighting systems, it can be concluded that the largest increase in biomass occurred in the PBR I photobioreactor, which was equipped with a single cylindrical mantle and a lighting panel on the lower surface. The smallest increase was visible for PBR III, which had a single light source on the upper surface. This suggests that, despite the provision of identical conditions of nutrition and mixing, the distribution of available radiant energy translates into the rate of its conversion into biomass and the productivity of the farm.

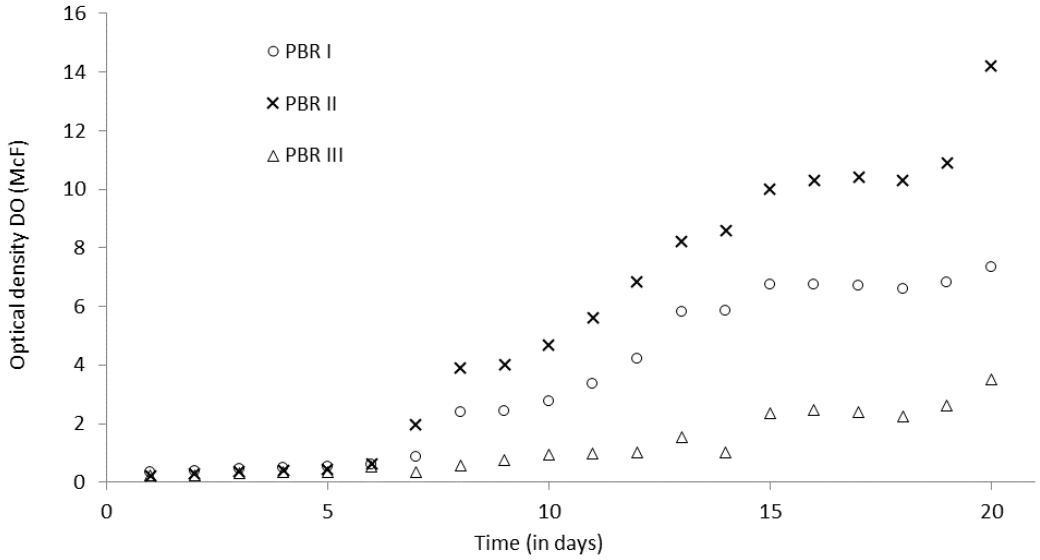

Figure 6. Changes in the optical density of *Chlorella vulgaris* cultures conducted in photobioreactors with various lighting systems. Source: own study.

To determine the correlation between the number of cells and the optical density, the algae cells were counted under the microscope using the Thoma chamber. Next, the correlation between the optical density and the number of cells obtained from the cultures bred in reactors with different lighting systems was determined (Figure 7).

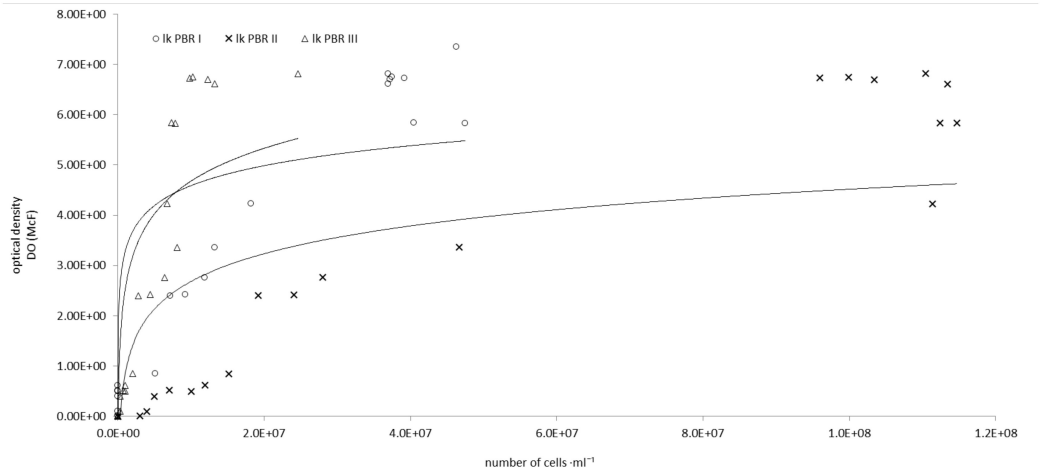

Figure 7. Correlation of the optical density of *C. vulgaris* cultures and number of cells for different lighting systems. Source: own study.

Mathematical models were selected for the plotted characteristics. Table 3 contains the most accurate adjustment of the correlation models of the amount of *C. vulgaris* BA0002a algae cells contained in 1 mL of culture, obtained under different light distribution conditions as a function of optical density. There were similar results from the direct determination of the number of cells in Thoma chambers, although in different conditions they received [32] comparable ranges of optical density.

Table 3. Mathematical form of the approximate number of cells per mL read from the densitometer indications for *C. vulgaris* BA0002a, carried out under strictly defined conditions.

Type of Photobioreactor	Model Number of Cells mL^{-1}	R^2
PBR I	y = 0.7971ln(x) − 10.167	0.50
PBR II	y = 0.5731ln(x) − 4.649	0.73
PBR III	y = 0.9429ln(x) − 10.521	0.61

During experiments, the growth of algae biomass was studied over time. Figure 8 shows the increase in the number of algae cells of *C. vulgaris* BA0002a over time with respect to 0.001 dm^3. The maximum daily increase in the number of cells for PBR I (the reactor in which a double light jacket and lighting from the bottom were used), which took place on the 13th day after inoculation of the culture, amounted to 2.93×10^{10} dm^{-3}. For PBR II (with a single cylindrical mantle and lower lighting), the maximum daily increase in the number of cells was 6.48×10^{10} per dm^3 and also took place on the 13th day of breeding. It was 50% higher than the results for PBR I. For PBR III, which was lit from the top, the maximum daily growth only took place on the 20th day after the establishment of the farm and was the lowest relative to the other two photobioreactors 1.13×10^{10} dm^{-3}. The highest productivity of the algae cultures was obtained for integrated lighting in which the double light jacket and the bottom panel (PBR II) were placed.

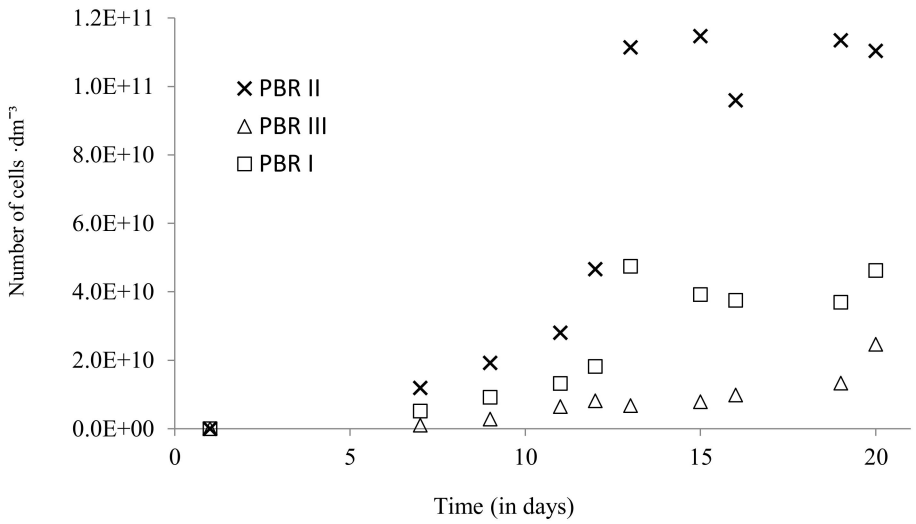

Figure 8. Increase in the number of cells over time, calculated on dm^{-3} culture of *C. vulgaris* BA0002a. Source: own study.

The mean diameter of the obtained cells on the last day of cultivation, measured with a Motic Red 233 optical microscope, was 9.75 µm (Figure 9).

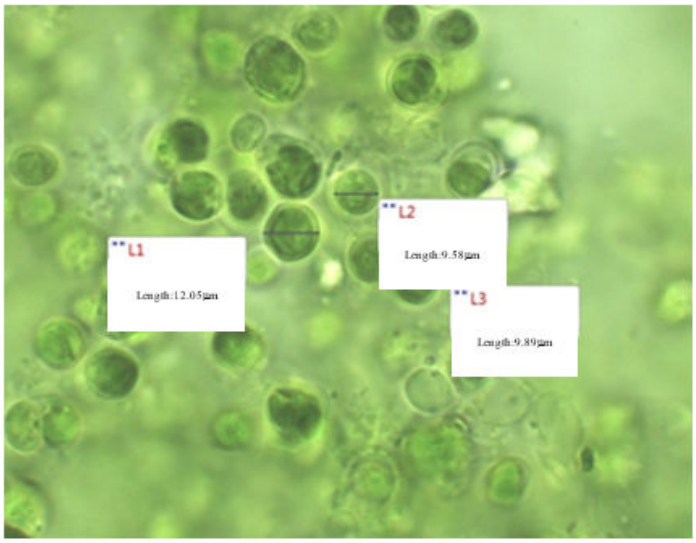

Figure 9. Measurement of the diameter of cultured cells on the 20th day. Source: own study.

The growth of algae is dependent on many factors [39], including temperature, pH, CO_2 concentration, type and chemical composition of the nutrients and the intensity and color of light. The structure of the photobioreactor also significantly influences the growth of algae biomass [40].

Li et al. [28] and Hu et al. [41] consider that light intensity and color are among the most important factors influencing algae cultivation, including biomass composition and production volume. As stated in [15,30,42–44], light intensity influences the process of photosynthesis and, consequently, the rate of growth.

For the production of ATP and NADPH and the synthesis of particles needed for growth, algae need light of appropriate wavelength and color temperature [45–47]. It would seem that since the light intensity improves the growth rate of the algae, the bioreactors should be illuminated as intensely as possible. However, as reported in [29,33], too high levels of light intensity can cause photoinhibition, which reduces the growth rate of microalgae [48]. Also, cultivation under stress conditions caused by poorly chosen lighting of the bioreactor leads to a decrease in the growth rate and the production of algal biomass [31,32].

Chávez-Fuentes and others [34] used fluorescent lamps (60 W) in the range of 70 to 140 $mE \cdot m^{-2}\ s^{-1}$ for the continuous illumination of the bioreactor. The highest growth of *C. vulgaris* was observed at 140 $mE \cdot m^{-2}\ s^{-1}$ for cold white light and blue light. Khalili et al. [33] studied the influence of different light intensities on the growth of *C. vulgaris.* Of the selected intensities (50, 80 and 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and wavelengths corresponding to red, blue and natural and warm white, 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and warm white proved to be the best for algae cultivation.

George et al. [46], for the example of *Ankistrodesmus falcatus* cultivation, found that low light intensity caused an increase in lipid content; for *Monoraphidium sp.* and *Botryococcus braunia*, they found that the increase in lipids took place at higher light intensities. For *C. vulgaris* cultures, the increase in lipids occurred with blue and white light [49,50]. Slightly different results were obtained by Zhang et al. in a *C. vulgaris* lipid yield study using LED lights [44]. The authors obtained the maximum value of lipids by illuminating the cultures with blue, then red and, lastly, white light. Different results in both of the above-mentioned experiments may be because of the use of lamps with different spectra. The positive influence of the change of blue light intensity from 100 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on the growth of lipids in *C. vulgaris* was also confirmed by studies of Atta et al. [51].

However, the effect of light intensity on protein content is ambiguous. This may be due to different experimental procedures and different test facilities [52,53]. For example, Kendirlioglu and Cetin [54] studied the growth rate and protein amounts of *C. vulgaris* grown at different light wavelengths (blue,

red and white light) and found that the best results were obtained by illuminating the crop with red, then white and, finally, blue light. Asuthkar et al. [55] studied the effect of light wavelengths (blue, white and red LED lamps) on the growth and protein content of *C. pyrenoidosa*. They found that the fastest growth occurred with blue light ($\mu = 0.51$ day^{-1}) and the slowest growth occurred with red light ($\mu = 0.22$ day^{-1}). The protein content was also highest when the culture was lit with blue light.

According to [56], an increase in the closed bioreactor lighting ratio from a range of 420–520 nm to a range of 580–680 nm resulted in an increase in algal biomass productivity. The authors also observed an increase in the specific growth rate. This effect observed in the range of 420–520 nm could be explained by auxiliary pigments (carotenoids) also absorbing photons during photosynthesis and transferring part of the absorbed light to chlorophylls.

4. Conclusions

1. Studies have shown that the intensification of culture and the increase in the content of *C. vulgaris* cells is closely related to the distribution of lighting and the time of exposure of a single cell to radiation.
2. The best outcome was observed with the PBR II photobioreactor, which was equipped with a double light jacket and a lower lighting panel. The least favorable was the reactor with the only light source located above the sample (PBR III).
3. For PBR II, the maximum daily increase in the number of cells was 6.48×10^{10} per dm^3. It also took place on the 13th day of breeding and was 50% higher than in the case of PBR I.
4. Between days 11 and 14, the oxygen concentration was observed to rapidly increase by over 100% for both PBR I and PBR II, which correlated fully with the maximum increase in the number of cells for these photobioreactors at that time.
5. For photobioreactors PBR I and PBR II, an increased production of oxygen was observed, which was directly related to the increased production of biomass and was dependent on a larger amount of radiant energy.

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