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The influence of PAR irradiance on yield growth of *Chlorella* microalgae

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Abstract. The experiment indicated the influence of different PAR irradiance conditions on biomass growth of *Chlorella vulgaris* Beijer stock culture. The absorption spectrum of suspension was evaluated with spectrophotometer SF-256 UVI LOMO Photonika. In this work, effects of LED lamps with various radiant powers on yield gain were compared by analyzing the relation between cells concentration increase and time. It was concluded, that light is the most important factor influencing growth and cells development, as well as nutrient solution, temperature and carbon dioxide provision.

1. Introduction

Over the last decade, microalgae had been introduced to livestock animals' diet along with premix, vitamins and biologically active additives. *Chlorella* is a green microscopic algae species, widely regarded for its active biomass production [1]. It contains high-grade proteins, fats, carbohydrates and vitamins and is included in the category of "superfoods". Among all plants, *Chlorella* is at the top position in terms of many factors. For example, 40-60% of proteins, 30-35% of carbohydrates, 5-10% of lipids and up to 10% of mineral substances are included in the biomass of *Chlorella* [2].

Cell fission speed, which determines the survival of microalgae population, is mostly dependent on accessibility of energy sources and nutritional substances. As for all phototrophic organisms, light supply plays vital part throughout vegetation period of microalgae, while its properties, such as intensity and spectral composition, are of great importance [3, 4].

2. Materials and methods

Duration of the experiment – 54 hours.

Initial optical density of three samples: from 0.16 to 0.19 RU.

Table 1. Initial data.

	<i>Installed power (W)</i>	<i>Ee(PAR) (W/m²)</i>	<i>PPFD(PAR) (μmol/s/m²)</i>	<i>PAR irradiance (nm)</i>
Sample 1	4	10.3	48.6	
Sample 2	10.5	49.1	227.4	400...700
Sample 3	15	67.9	321	



Temperature (T , $^{\circ}\text{C}$): during the entire experiment temperature was maintained at optimal range $36.5 - 37.5^{\circ}\text{C}$.

The experiments were carried out to observe the influence of irradiating source power on *Chlorella* microalgae cells concentration growth. Method was developed for the current labor facility, which was used for conducting the experiment in cultivating microalgae *Chlorella*.

Measurements of optical density spectrum were performed throughout the entire experiment period once every 3 hours 3 times a day with spectrophotometer SF-256 UVI. By analyzing concentration-time ration, we aimed to evaluate, how radiation, provided by LED lamps with diverse output, affected yield gain of the culture.

The schematic diagram of experimental facility is illustrated in figure 1. The setup consists of a parallelepiped-shaped case (2) and cover plate (1) made of Plexiglas [5].

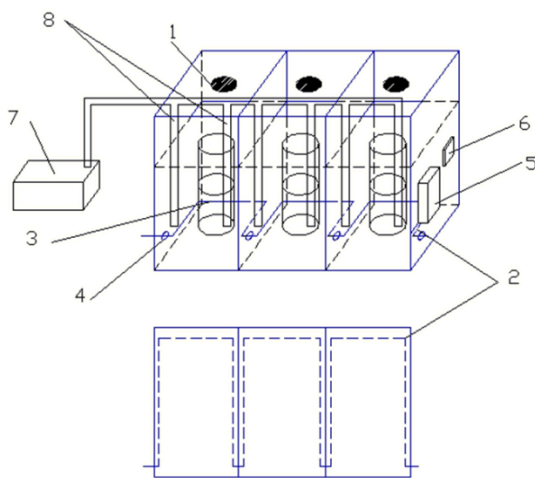


Figure 1. Scheme of experimental setup, (a) top view, 1 – cover plate; 2 – case; 3 – irradiating source; 4 – heating element; 5 – measuring containers; 6 – ports for heating element; 7 – thermostatic regulator; 8 – deferential breaker; 9 – pipes of aerator; 10 – aerator; 11 – ports between units.

300 ml measuring containers (5) are installed at the center of each unit of photobioreactor for the direct cultivation of *Chlorella*. Containers are filled with 250 ml suspension, contained 50% Tamiya medium and *Chlorella* stock culture.

To ensure temperature conditions, the case of photobioreactor (1) is flooded with water. Water temperature is maintained by heating element (4) and stabilized by digital thermostatic regulator (7) with remote sensor, attached to one of the measuring containers (5). Current system is introduced due to optimal *Chlorella vulgaris* Beijer cultivating temperature range of $36.5-37.5^{\circ}\text{C}$.

Inner units are connected with ports (11), located in the separation walls 2 cm above the bottom. The case is filled with water at the same level as measuring containers (5).

Aerator (10) is designed to accomplish two functions:

1. air distribution into each container to provide *Chlorella* cultivation;
2. air agitation; air mixture is supplied by pipes with diffusive-end pipes.

Differential breaker (8) usage assures fail-safe functioning of the facility.

Test samples of suspension from each container (5) were registered every 3 hours, using spectrophotometer SF-256. We investigated optical density of *Chlorella* suspension for yield gain occurrence and compared temperatures by thermometers.

The results we obtained were processed in Microsoft Excel, the average was calculated. Afterwards, readings were used to receive the optical density-time relation (figure 2).

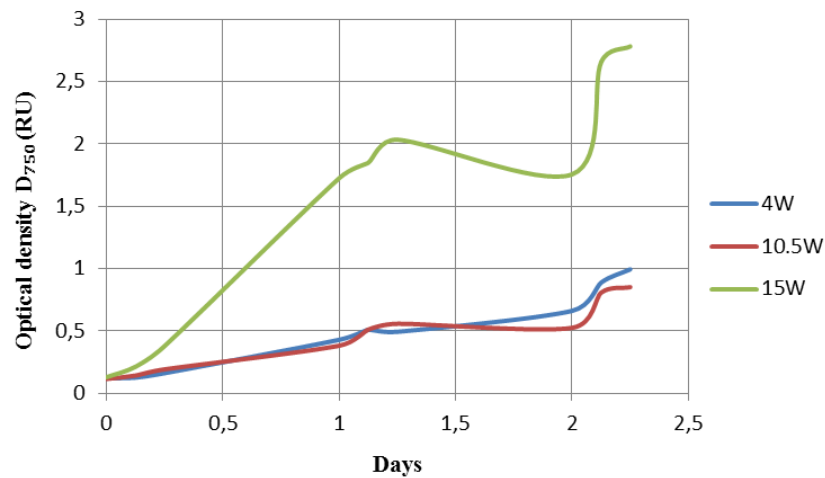


Figure 2. Diurnal variation of optical density at 750 nm D_{750} for 3 radiation sources of different power (container 1 – 4W; container 2 – 10.5W; container 3 – 15W).

The degradation of optical density on days 1 and 2 was caused by continual light emission and stress factor absence. Furthermore, some cells died out partly and precipitated. In the day time light sources and aerator were deenergized within sampling, what lead to stress initiation and ongoing growth of Chlorella.

To reduce the measuring error while defining optical density, containers were held in flask clamps and placed as close as possible to the photoelectric detector. The detector is designed for translucent medium measurement by standard. In turbid medium light dispersion appears.

Data processing is not needed to show optical density related to wavelength (figure 3) [6].

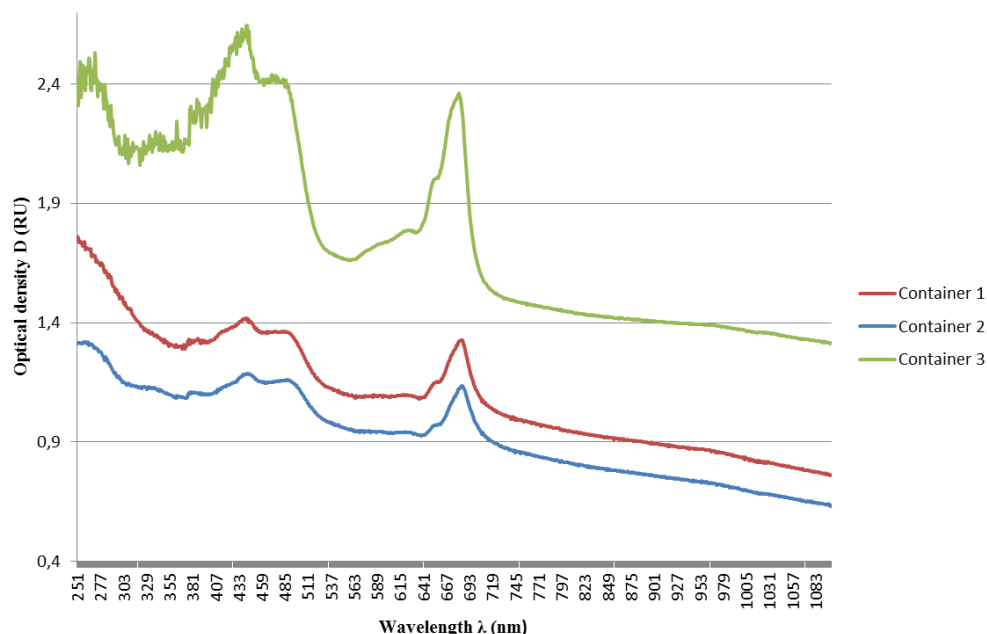


Figure 3. 54 hours variation of optical density for 3 radiation sources of different power (container 1 – 4W; container 2 – 10.5W; container 3 – 15W).

To determine the relation concerning cells concentration increase and time, we calculated number of cells in Gorjaev's chamber. The number of cells was calculated as,

$$n = N_{b.sq.} \cdot 2.5 \cdot 10^6 \quad (1)$$

where n is the number of cells per ml, $N_{b.sq.}$ is the number of cells within Gorjaev's chamber big square.

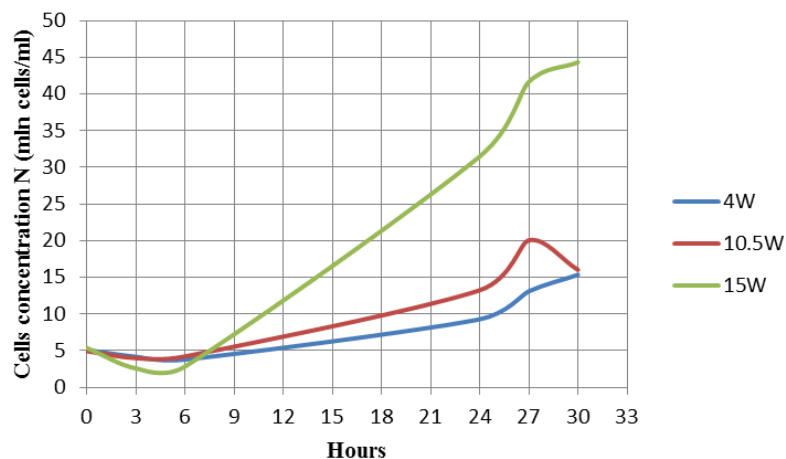


Figure 4. 30 hours variation of cells concentration for 3 radiation sources of different power (container 1 – 4W; container 2 – 10.5W; container 3 – 15W).

3. Results

Based on conducted subsidiary experiments, we proved, that with the increase of light source power level from 4W to 10.5W, without changing the spectrum of the source, optical density value multiplied by 1.5 times. The rise of light source power level from 10.5W to 50W caused 1.4 times increase of optical density. According to the results, it is not necessary for the light source power level to be at maximum to assure optimal level of algae cell growth. Maximum PAR irradiance at a small-size *Chlorella* suspension volume suppresses rapid growth and cell fission.

Three patterns of culture development can be identified in the figure 4: from stock culture seeding until the 6th hour of growth period – exponential growth phase, from the 6th hour until 25th hour of growth period – linear growth phase. Concentration decrease at the stationary phase can be observed in the figure 2. *Chlorella* cells fission occurs 40 times every 24 hours approximately. In accordance with this fact, cells from the second sample had not divided once again after 30 hour cultivation, when they were counted, due to lighting condition difference with the other samples.

4. Conclusion

Light radiation plays essential part in microalgae development, specifically light intensity and spectrum content [7]. According to the experimental data (Figures 1 - 4), highest gain of optical density and concentration was observed during 15 W LED lamp illuminance of suspension, in comparison to LED lamps with less power. This occurred as a result of photosynthesis process of microalgae: the more appropriate the conditions were, the faster cell fission speed and growth appeared.

Based on the experiment, optimized illuminance influences microalgae growth on equal terms with nutritional medium and CO₂ supply. The diagrams of chamber 1 and 2 show, how photosynthesis process in cells is decelerated by insufficient illuminance conditions.

Acknowledgments

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