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THE INFLUENCE OF LIGHT INTENSITY ON THE FORMATION OF PIGMENT COMPLEX IN CHLORELLA VULGARIS CELLS

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Abstract. Light is one the most important factors affecting growth and development of microorganisms with high photosynthesis performance such as Chlorella vulgaris. Due to its simple life cycle Chlorella used as model organism to study, the photosynthesis mechanisms and the cell metabolism control. The aim of presented research was to determine the effect of different light intensity on Chlorella vulgaris growth rate, the amount of chlorophyll a and b, composition and formation of pigment complex. In the basis of results, it has been found that when the light intensity increases to 500-µmol m^{-2} s^{-1}, the absorption spectrum peak of chlorophyll a shifted to a short wave for 4-5 nm. Research showed that there are 5 forms of chlorophyll a and 2 forms of chlorophyll b aggregates at the pigment complex of Chlorella vulgaris.

Keywords: *Chlorella vulgaris, light intensity, chlorophyll, pigment complex, microalagae*.

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1. Introduction

Microalgae are photosynthetic eukaryotic microorganisms, with similar organelles to land plants such as chloroplasts and nucleus, yield more efficiently biomass. The reason is their higher performance in photosynthesis by utilizing sunlight, CO_2, water leading to their extremely higher growth rates (Panahi *et al*., 2019). Chlorella, a unicellular green alga, has been widely used in aquaculture, food and biotechnology industries (Dantas *et al*., 2015). Chlorella has a simple life cycle and metabolism similar to the metabolic pathways of higher plants. It is also used as a model organism to study the mechanisms of photosynthesis, carbon dioxide and the metabolism control.

Light is the main factor affecting the growth and life cycle of photosynthesic microorganisms. Intensity is a quantitative characteristic of light. For determination of the intensity of photosynthetically active light, an estimate of the number of photons is widely used, expressing them in micromoles (µmol m^{-2} s^{-1}) per unit area per second (Maltsev *et al*., 2021; Metsoviti *et al*., 2020). Microalgae growth is proportional to light intensity until reaching a saturation point at which the photosynthetic activity of microalgae achieves their maximum value (Qin Zhou *et al*., 2015). When it falls below this optimal value, microalgae growth will be limited. Conversely, if the light intensity values exceed the optimal value, photosynthetic apparatus will be damaged, which causes photoinhibition (Gonzalez *et al*., 2019). It is known from various publications that the

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light-absorbing complex in the photosynthetic apparatus of Chlorella vulgaris is formed from chlorophyll a and b pigments, as in other algae and higher plants (Hasanov & French, 1973). Chlorophyll a is the main pigment that captures light quantums and converts light energy into electron motion energy, while chlorophyll b is an additional pigment that absorbs light with a shorter wavelength and transmits it to chlorophyll a.

In recent years there has been a lot of interest in effect of light intensity on the biophysical characterization of the photosynthetic apparatus and cell metabolism of Chlorella vulgaris. Knowledge of light condition effect on Chlorella vulgaris cell has great importance for determination optimal growth conditions, evaluation pigment complex formation, purposeful accumulation of various bioproducts, such as starch, lipids, antioxidants.

Several studies have suggested that the cells cultivated at high light intensity (>500 μmolfoton m^{-2} s^{-1}), have damages on photosynthetic apparatus, that causes photoinhibition (Maltsev *et al*., 2021; Gonzalez *et al*., 2019). High light intensity (>400 μmolfoton m^{-2} s^{-1}) activates so-called "molecular sunglasses" mechanism by increasing the synthesis of xanthophyll molecules which induces color change of microalgae from green to yellow (Grudzinski *et al*., 2016).

The aim of present research is determining the optimal lighting conditions for the cultivation of chlorella vulgaris, studying the effect of different light intensity on the cell growth and formation of a light-harvesting complex of photosystem.

2. Material and methods

Microalgae cultivation

Chlorella vulgaris cells were cultivated in PSI-MC1000 photobioreactor in 8 tubes for 168 hours at a temperature 30°C, light intensity of 60, 100, 500, 1000 μmol m^{-2} s^{-1}. The culture was grown in Tamiya medium (Tamiya *et al*., 1953; Alyabyev *et al*., 2007). OD(680) of culture was measured by a photobioreactor every 10 minutes during the cultivation period.

Spectrophotometric analysis

Samples for spectrophotometric studies were obtained from 4 tubes with cell culture which were grown at different light intensities**.** 3 ml suspension was taken from each tube and centrifuged at 2800 rcf for 5 minutes. Methanol was added as solvent to precipitat and kept for 5 minutes to extract chlorophyll molecules, then extract was centrifuged at 2800 rcf mode for 5 minutes to obtain a transparent suspension.

Chlorophyll a, b, and Chla/Chlb ratio determination

The determination of the amount of chlorophyll a and B and the ratio of xla/Xlb was carried out by the spectrophotomectic method. The light absorption of each sample was measured at wavelengths of 350-750 nm on a JENWAY 7315 spectrophotometer. Absorption value of the samples at 652 nm and 665 nm wavelengths was substituted to the following formulas and the amount of chlorophyll a and b was calculated (Porra *et al*., 1989):

$$Chl_a = 16.29 \times A^{665.2} - 8.54 \times A^{652}$$

$$Chl_b = 30.66 \times A^{652} - 13.58 \times A^{665.2}$$

$$Chls_{a+b} = 22.12 \times A^{652} + 2.71 \times A^{665.2}$$

Fluorescence spectroscopy of FSII and light-harvesting complex

Samples with chlorophyll concentration of 15 µg/ml were used to measure the fluorescence of FSII and light-harvesting complex. Excitation and emission spectrum were recorded on a CARY ECLIPSE VARIAN spectrofluorimeter with a cross section of both monochromators of 5 nm, a scan speed of 600 nm/min, a resolution of 1 nm, and capture data point time of 100 ms.

3. Results and discussion

The growth dynamics of Chlorella vulgaris cells cultivated under constant nutrient and temperature conditions at 4 different light intensities (60, 100, 500, 1000 µmol m^{-2} s^{-1}) completely corresponds to the theoretical conditions of the growth process of unicellular organisms (Figure 1). The growth curve is characterized by a lag phase, an exponential phase and a stationary phase.

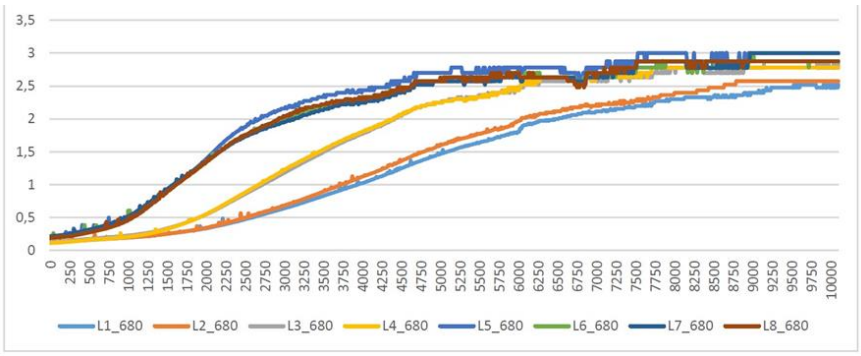


Figure 1. Growth dynamics of Chlorella culture in PSI MC-1000 photobioreactor

Analysing growth dinamic curve, it can be concluded that the optimal light intensity for the adaptation of cells to medium, exponential division and transition to the stationary phase is between 100 to 500 µmol m^{-2} s^{-1}.

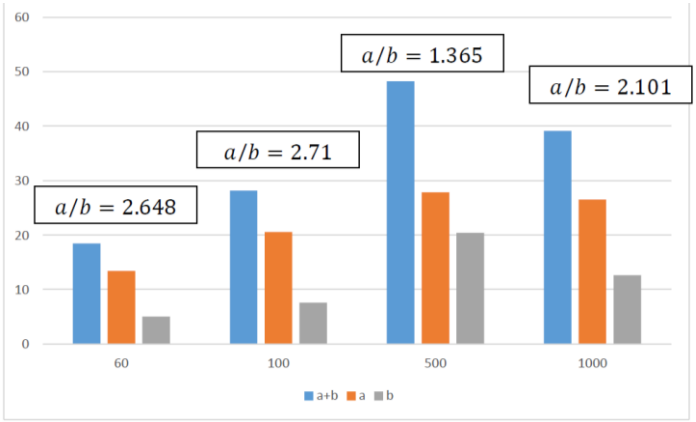


Figure 2. Values of chlorophyll a and b, a+b, a/b depending on different light intensity

The amount of chlorophyll a and b, summary and ratio of Chla/Chlb in the suspension obtained from Chlorella vulgaris cells cultured at different light intensity, were studied (Figure 2). The total content of chlorophyll a and chlorophyll b was

determined to be µmol, 28 µg/ml, 48 µg/ml and 39 µg/ml in 100, 500 and 1000 µmol m^{-2} s^{-1}, respectively.

Analysis of the light absorption of Chlorella vulgaris extracts showed that the red end of the spectrum contains a complex pigment composition (Figure 3).

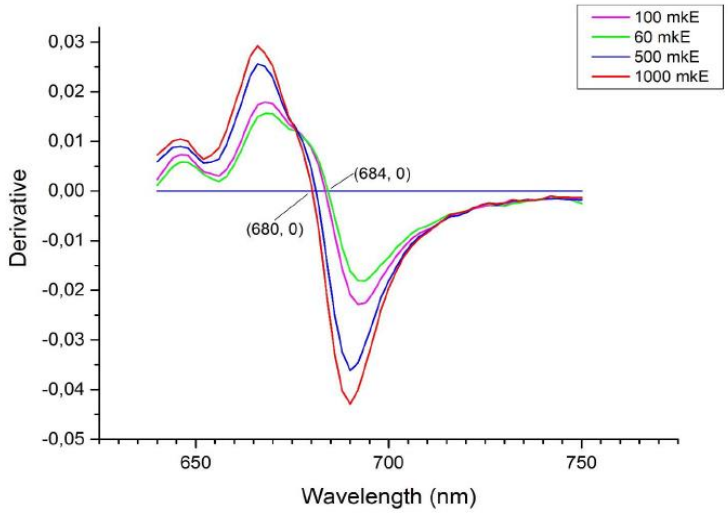
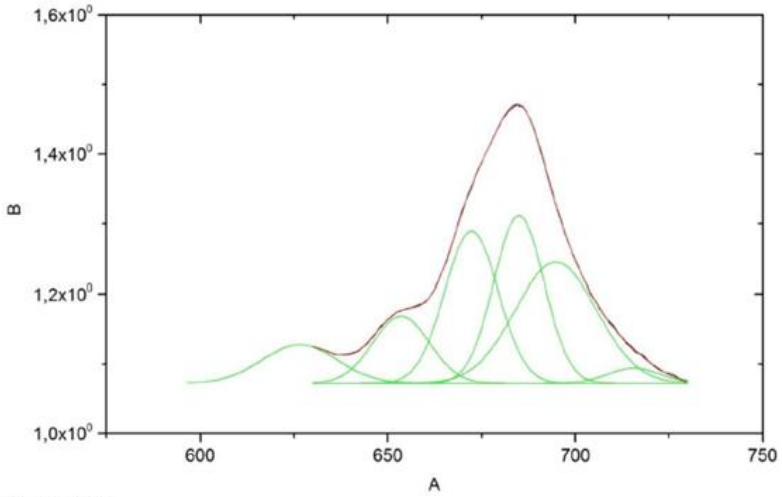


Figure 3. First deriviates of absorbance spectrum of Chlorella vulgaris suspension



Fitting Results

Peak Index	Peak Type	Area Intg	FWHM	Max Height	Center Grvty	Area IntgP
1	Gaussian	4,01899	15,76815	0,23944	684,96561	26,22644
2	Gaussian	0,51012	23,70527	0,05563	626,4828	3,32887
3	Gaussian	1,82592	17,7769	0,09658	653,53701	11,91528
4	Gaussian	3,88626	16,82415	0,217	672,31158	25,36033
5	Gaussian	4,70569	25,61227	0,17271	694,81719	30,70764
6	Gaussian	0,3772	16,58941	0,02182	715,66326	2,46145

Figure 4. Absorbance spectrum analysis of extract from cells cultivated at 60 µmol m^{-2} s^{-1} ligth intensity by Origin Pro 2015

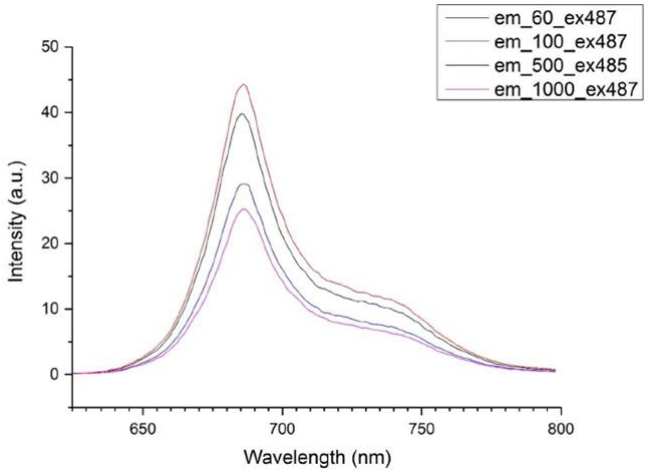


Figure 5. Fluorescence spectrum of Chlorella vulgaris depending on the light intensity during cultivation

The shape of absorbance spectrum indicates the presence two forms of chlorophyll b , 5 forms chlorophyll a, and various carotenoid pigments in the cell. Analysis of spectrum by Origin Pro 2015 software verify this determination. It was found that, at light intensities of 500 and 1000 μmol m^{-2} s^{-1}, the maximum spectrum of chlorophyll a has a wavelength of 680 nm, but at light intensities of 60 and 100 μmol m^{-2} s^{-1}, this maximum has a wavelength of 684 nm. In addition, analysis of extract from cells cultivated at 60 μmol m^{-2} s^{-1} ligth intensity shown that various forms of chlorophyll a are seen at wavelengths of 660 Nm, 669 nm, 677 nm, 683 nm, 691 nm, 704 nm (Figure 4). Also two forms of chlorophyll b are shown at wavelengths of 649 nm and 655 nm.

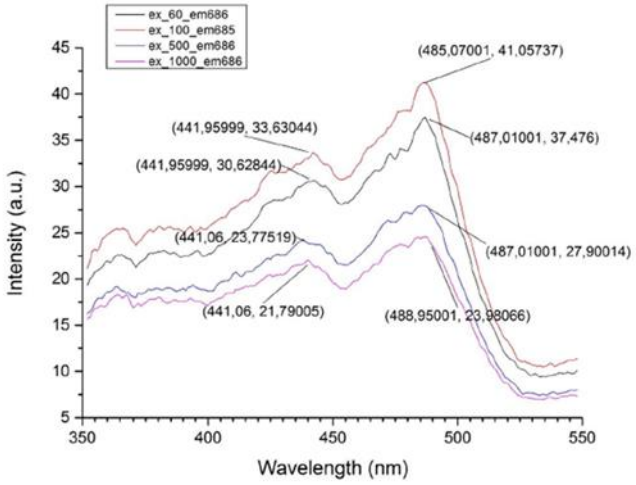


Figure 6. Analysis of fluorescence excitation spectrum of Chlorella vulgaris extracts

Another method for determining the effect of different light intensity on the pigment complex structure is fluorescence. The fluorescence spectrum for analyzing the change in the pigment complex structure of Chlorella vulgaris cell is shown in Figure 5. The structure of the fluorescence spectrum is characterized by two maximum peaks at red end of the spectrum (686 nm and 735 nm). It has been found that when the light intensity increases to 500 μmol m^{-2} s^{-1}, the absorption spectrum peak of chlorophyll a is shifted to a short-wave for 4-5 nm. This indicates the degradation of the form of chlorophyll a molecule in Chlorella vulgaris cells.

The influence of light intensity (60, 100, 500, 1000 μmol m^{-2} s^{-1}) on Chlorella vulgaris cell growth rate was studied. The range of 150-300 μmol m^{-2} s^{-1} of visible light was assumed as the optimal light condition for researced cell culture. Analysis of the light absorbance spectrum, fluorescence and their primary derivative showed that there are 5 forms of chlorophyll a and 2 forms of chlorophyll b aggregates at the pigment complex of Chlorella vulgaris cells, what similar to other algae and land plants. In addition, this analysis showed that chlorophyll b is more resistant to high light conditions compared to chlorophyll a (>500 μmol m^{-2} s^{-1}).

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