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DYNAMICS OF CHLOROPHYLL A AS AN INDICATOR OF LASER IRRADIATION OF ALGAE AND CYANOBACTERIA

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The paper evaluates the possibility of using the indicator of the amount of chlorophyll a as an indicator of the light supply of green algae and cyanobacteria cultures. The study was conducted on the culture of Acutodesmus dimorphus (green algae) and Nostoc commune (cyanobacteria). They studied the effect of lasers that generate light waves of 405, 532, and 650 nm for different durations on the accumulation of chlorophyll a. It was noted that the amount of chlorophyll a in the biomass of the studied crops varies depending on the wavelength of light and the duration of irradiation. As the duration of irradiation increases, the content of chlorophyll a in the biomass of the studied objects decreases.

Ключові слова: A. dimorphus, N. commune, chlorophyll a, laser irradiation

Introduction. Microalgae are a collective group of aquatic organisms that combine prokaryotic and eukaryotic divisions capable of light-dependent CO2 fixation (Paper et al., 2022). This makes microalgae a promising object in the biotechnology of obtaining biomass enriched with target metabolites. Algae grow quickly, do not require complex multicomponent environments, and the technology for separating their biomass is not complicated (Lopez-Hernandez et al., 2022; Sancher-Bayo et al., 2020). The only limitation is the need to illuminate the cultivation containers, observing certain photoperiods. The presence or absence of light can significantly affect the growth of biomass and the synthesis of important biomolecules of microalgae (Klimek-Szczykutowicz et al., 2022; Helamieh et al., 2021).

In temperate climate zones, closed cultivation systems with artificial lighting are used for successful industrial cultivation of microalgae. For this, it is necessary to use light sources with optimal wavelength and light intensity (Kendirlioglu and Cetin, 2017; Vialet-Chabrand et al., 2017). The duration of illumination, as well as the method of supplying light rays, will directly affect the provision of insolation of algocultures. Light waves of different lengths have individual effects not only on the proliferation of microalgae cells, but also on the accumulation of metabolites (Paik et al., 2019; Fukuda, 2013).

One of the promising and newest methods of correction of microalgae biotechnology is the use of laser irradiation. A laser is a device for generating or amplifying a monochromatic beam of light. Depending on the nature of the biological agent, the laser can cause a photostimulating or photoinhibiting effect on the cell (Haonan et al., 2017). In each specific case, the effect of lasers on a cell of microscopic algae will depend on many factors: the wavelength of the lasers, the duration and frequency of irradiation, and the individual sensitivity of the organism. It is obvious that there will be differences in the reaction of prokaryotic and eukaryotic representatives of microalgae.

Considering the fact that most representatives of algae are photoautotrophs, chlorophyll *a* was chosen as a test molecule for studying the effect of laser irradiation (Bianchi and Canuel, 2011).

Chlorophylls are the color pigments of all photosynthetic organisms. These molecules are photoreceptors. In algae, 6 types of chlorophyll have been identified, but the main one of them, which is found in the cells of all divisions of algae, is chlorophyll *a*. Other types of chlorophylls are found as auxiliary in various algae, this feature is also used in taxonomy. Cyanobacterial cells contain only chlorophyll a (Da Silva and Lombardi, 2020).

The work is devoted to the study of changes in the amount of chlorophyll *a* depending on the effect of laser irradiation of different wavelengths on cultures of green algae and cyanobacteria.

Materials and methods. The material in this work was: culture of green algae Acutodesmus dimorphus (Turpin) Tsarenko and cyanobacteria Nostoc commune (IMV K-19). Cultivation was carried out in Falcone tubes with a volume of 50 ml on a nutrient medium with a minimum mineral composition (Table 1.) under the conditions of a 16-hour photoperiod, under the illumination of an LED lamp and a temperature of 26 ± 2˚C.

Table 1.

The composition of the nutrient medium with a minimum content of mineral

dry residue	**200-500 mg/l**
fluorides	**2 mg/l**
Na^+	**20 mg/l**
K^+	**20 mg/l**
Ca^{2+}	**100 mg/l**
Mg^{2+}	**50 mg/l**

Algae and cyanobacteria were irradiated with 5 mW lasers that generate light waves of different lengths: 405 nm, 532 nm, and 650 nm. The duration of irradiation was 5, 10 or 15 minutes. After a single exposure, the algae continued to grow for 14 days in a climate chamber under the above conditions.

Chlorophylls were extracted with a mixture of chloroform and ethanol in a 2:1 ratio. Determination was carried out by the spectrophotocolorimetric method (CaryWin UV 60). The amount of chlorophyll *a* was calculated according to typical formulas (Da Silva and Lombardi, 2020).

Statistical processing of the obtained results was carried out using Microsoft Excel software. Differences in the results discussed in the work are probable at the level of significance $p \leq 0.05$ according to the Student's test.

Results and discussion. The main photosynthetic pigment in plants and algae is chlorophyll *a*, other available pigments perform an auxiliary function (Bianchi and Canuel, 2011). Different pigments are able to absorb light waves of only certain lengths. It is known that the light absorption spectrum of chlorophyll a lies in two peaks, namely: 430 and 662 nm. But the spectrum of action of photosynthesis lies within 400-700 nm. Therefore, it is expected that lasers that generate light waves in the range of 400-700 nm will affect both photosynthetic pigments and photosynthesis in general. Not only the region of the visible spectrum, but also the time of irradiation will affect the synthesis of chlorophyll a.

After analyzing the obtained data, it was found that with five minutes of exposure, no significant difference between different lengths of light waves was found. The amount of chlorophyll *a* after five minutes of irradiation. Short-term laser irradiation can cause the effect of photobiostimulation. But by increasing the irradiation time, we also increase the amount of light absorbed by microalgae cells. Ultimately, too much light can cause a photoinhibition effect.

After analyzing the data, it was found that the amount of chlorophyll *a* significantly decreased during a ten-minute irradiation with a laser that generates light waves with a length of 650 nm (table 2). This is because chlorophyll *a* absorbs less light in the typical red spectrum (640 nm) than in blue-violet. Therefore, an excessive amount of red light expectedly led to the inhibition of the synthesis of this pigment. A significant increase in exposure time, compared to short-term exposure, also led to a decrease in the amount of chlorophyll *a*.

Table 2.

The amount of chlorophyll a after a single irradiation of A. dimorphus and N. commune culture (mg/g) (M±m, n=4)

t, m	**Irradiation conditions**			
	control	**405 нм**	**532 нм**	**650 нм**
	A. dimorphus			
5	16,7±0,81	25,7±1,05	30,1±1,22	28,4±1,31
10		24,8±0,89	25,7±0,93	18,8±0,77
15		13,2±0,67	21,9±0,81	21,2±1,01
	N. commune			
5	3,8±0,16	4,6±0,13	4,9±0,16	4,2±0,11
10		4,1±0,10	4,1±0,13	3,8±0,09
15		2,9±0,08	3,6±0,13	3,1±0,12

The amount of chlorophyll a after 15 minutes of irradiation decreased compared to 10 and 5 minutes. And with 15-minute irradiation with a 405 nm laser, photoinhibition of the synthesis of this pigment occurred for both studied cultures.

Laser irradiation is characterized by various parameters such as: wavelength of light, duration of irradiation, frequency of irradiation, laser power, etc. Each of these parameters has an individual effect on the accumulation and synthesis of primary and secondary metabolites. Low-intensity laser irradiation (5 min) led to an increase in the amount of chlorophyll a in both studied cultures. Increasing the duration of irradiation to 10 or 15 minutes led to a decrease in the amount of chlorophyll *a*.

Therefore, the amount of chlorophyll *a* can be used as a criterion for evaluating the effect of laser irradiation on cultures of green algae and cyanobacteria.

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ДИНАМІКА ХЛОРОФІЛУ *А* ЯК ІНДИКАТОРА ЛАЗЕРНОГО ОПРОМІНЕННЯ ВОДОРОСТЕЙ ТА ЦІАНОБАКТЕРІЙ

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У роботі оцінено можливість використання показника кількості хлорофілу а як індикатора забезпеченості світлом культур зелених водоростей та ціанобактерій. Дослідження проводилося на культурі Acutodesmus dimorphus (зелені водорості) та Nostoc commune (ціанобактерії). Вивчали вплив лазерів, які генерують світлові хвилі довжиною 405, 532 і 650 нм за різної тривалості на накопичення хлорофілу а. Відмічено, що кількість хлорофілу а у біомасі досліджуваних культур змінюється залежно від довжини світлової хвилі та тривалості опромінення. Із зростанням тривалості опромінення вміст хлорофілу а у біомасі досліджуваних обєктів зменшується.

Ключові слова: A. dimorphus, N. commune, хлорофіл а, лазерне опромінення

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