

## APPLICATION OF LIGHTING SOURCE MODIFIED WITH AGINS2 QUANTUM DOTS FOR GROWING NOSTOC COMMUNE IN A PHOTOBIOREACTOR

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The work is dedicated to the study of the light source influence that has been modified with AgInS<sub>2</sub> quantum dots on the *Nostoc commune* culture in the photobioreactor. A model of the three-section laboratory photobioreactor has been created. It was investigated that the modification of the light source doesn't lead to the changes in the physical parameters in the photobioreactor. As a result, the light source modified with QD Ag:In = 1:20 resulted in a 1.5x increase in the biomass of *N. commune*. The amount of pigments were also changed: the maximum content of chlorophyll *a* (8.1 mg/g) was established under the conditions 3 of using a light source modified with QD Ag:In = 1:7. The increase in the amount of carotenoids was noticed regardless of the amount of indium in the modifying film. We recommend to use a light source modified with AgInS<sub>2</sub> QDs to obtain *N. commune* biomass enriched with carotenoids.

**Keywords:** *N. commune*, quantum dots, AgInS<sub>2</sub>, biomass, productivity, photobioreactor

**Introduction.** *Nostoc commune* is a colonial cyanobacterium of the genus *Nostoc* that forms slimy colonies on the soil and is often found in freshwater systems. This species is not included in the list of objects that can cause infection or be toxic to humans and animals. *Nostoc commune* has a number of valuable properties (Guiry and Guiry, 2020). The use of *Nostoc commune* biomass is becoming increasingly relevant in Ukraine. Increasingly, the biomass of this cyanobacterium can be used to produce biofuels, food additives, animal feed or other products. It is undemanding to nutrients and can be used, including for wastewater treatment. Cultivation can be carried out in various conditions, including artificial reservoirs, ponds and even water tanks. However, there are a number of problems that hinder the development of *N. commune* cultivation. One of them is the low biomass yield. To obtain significant amounts of biomass, it is necessary to use large areas or volumes of water. To solve this problem, new methods must be developed to increase biomass yield. One promising direction is the use of photobioreactors - devices that use artificial light sources to grow cultures of phototrophic microorganisms (Fernández et al., 1998). Since microalgae require light for photosynthesis, the goal for successful industrial operations is to optimize the wavelength, intensity and duration of LED lighting required for specific microalgae species.

Light is one of the most important factors influencing the growth rate of photosynthetic organisms. The effect of light energy varies for

different processes and species. Both excessive light and lack of light negatively affect biomass production. In a photobioreactor, the conversion of available light depends on the concentration of biomass, which in turn relies on the optimal distribution of radiation energy so that all cells receive the appropriate amount of energy. It is assumed that the optimal value of the photon flux density for algae is in the range of 100–600  $\mu\text{mol}\times\text{m}^{-2}\times\text{s}^{-1}$  and is not specific to a particular species. Excessive light also acts as a brake, causing the so-called photoinhibition phenomenon, i.e. a situation where a photosynthetic microorganism is exposed to more radiant energy than it is able to process. The situation usually occurs in the early stages, immediately after the start of cultivation (Anand Rajendran et al., 2013; Brzychczyk et al., 2020). The growth of phototrophic microorganisms depends, among other things, on the wavelength of the incident light. Most species of microalgae use the visible light spectrum or photosynthetically active radiation (400-700 nm) to produce organic compounds through photosynthesis. The use of different strategies for its control, such as coloring the medium inside the photobioreactors with dyes or applying different coatings on the panels, will allow achieving the desired results in terms of the qualitative and quantitative composition of microalgae.

In artificial light cultures, LEDs are most often used to illuminate photobioreactors due to their narrow wavelength range and the ability to easily select the color of light. Studies show that the

growth of algae is significantly influenced by the red light spectrum, which promotes their faster reproduction and growth. On the other hand, the blue light spectrum causes cell growth. Research on LED lighting for microalgae cultivation has highlighted the advantage of LEDs over direct sunlight, especially in laboratory photobioreactors. Laboratory solutions for photobioreactors of various designs, fully automated, performing the tasks of breeding, research and optimization of the reproduction processes of photoautotrophic microorganisms, despite the fact that they meet the tasks set for them, exhibit a number of shortcomings, including those related to light distribution.

A number of solutions implement radiant energy artificially, so the light structures are located both inside and outside the photobioreactors. The use of LEDs allows for greater flexibility in the design of a system that is limited in the elements exposed to the sun as a light source (Fernandes et al., 2010). Another significant advantage of using LEDs is that they can be easily programmed to produce the desired power, and they are also very efficient in converting electricity into light. In addition, we can power the LEDs using various renewable energy sources such as solar, wind, hydro, tidal, geothermal and nuclear, creating large-scale algae cultivation systems capable of using a wide range of renewable energy options (Park and Dinh, 2019; Izadpanah et al., 2018; Slegers et al., 2013).

Therefore, the need for artificial lighting that will promote biomass growth and also correspond to the absorption spectrum of pigments for photosynthetic organisms is growing. The solution to this problem may be the use of quantum dots. These are nanoparticles of elements that produce light of different spectra depending on the size of the particles themselves (Miao and Liu, 2015; Božin et al., 2013).

The effect of coatings based on nanosized AgInS<sub>2</sub> particles on cyanobacterial cells, as photosynthetic microorganisms, is interesting (Huong et al., 2022; Cichy et al., 2017). Therefore, the topic of using light modified by QDs is new and the effect on living systems is not fully studied.

Therefore, the aim of the work was to determine the effect of a light source modified by AgInS<sub>2</sub> quantum dots for growing *Nostoc commune* in a photobioreactor.

**Materials and methods.** The material for our study was a culture of the cyanobacterium *Nostoc commune*. The algae have the appearance of a filamentous organism that forms colonies of various shapes (Guiry and Guiry, 2020). The culture was deposited in the Depository of the D.K. Zabolotny

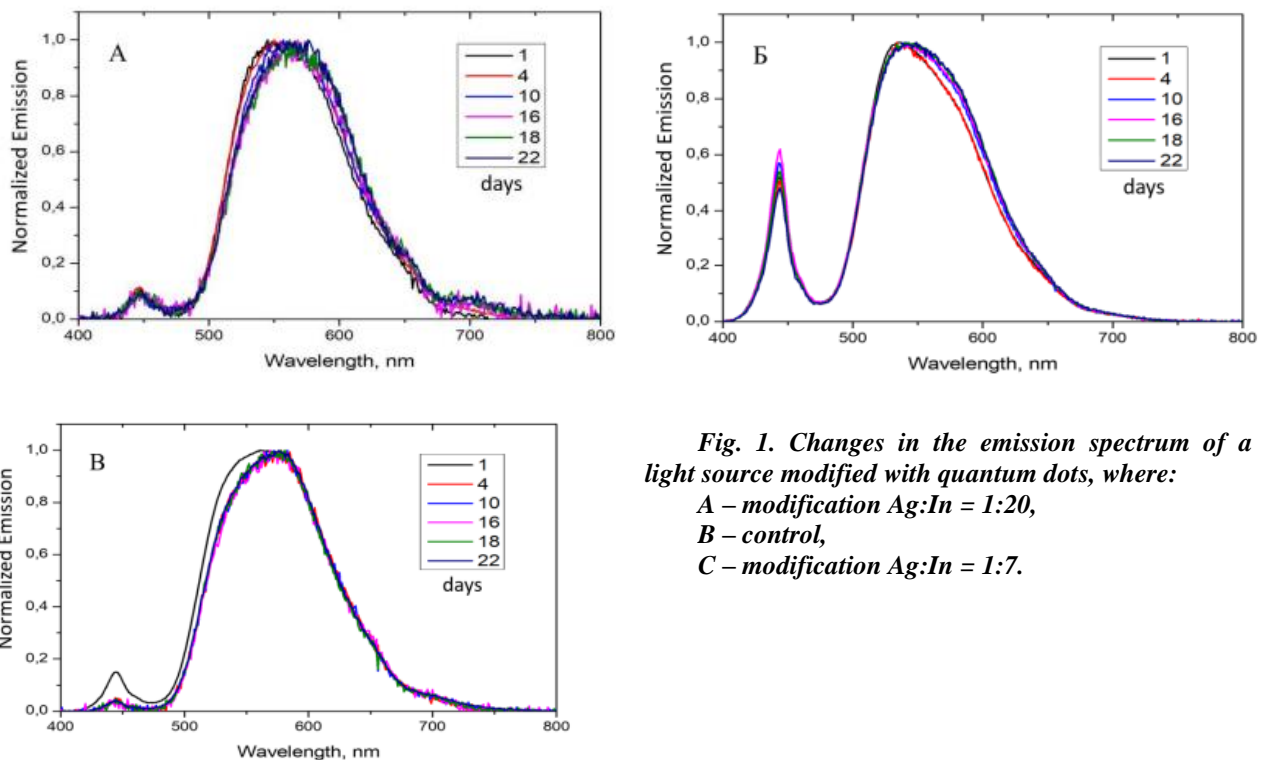
Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine under the registration number *Nostoc* sp. IMV K-19 and was provided for research to the employees of our university under the cooperation agreement. The working culture of *N. commune* was grown on nutrient medium BG-11 and is maintained in sterile conditions with optimal lighting and temperature in the collection of the Department of Biochemistry and Biotechnology of the Yuriy Fedkovych Chernivtsi National University. As part of the work, it was advisable to develop and construct a working laboratory model of a photobioreactor with three tanks for parallel cultivation of the selected object under different lighting conditions. The model we created had three identical cultivation cylinders with a capacity of 2 liters each. Cultivation was carried out under LED lamp illumination: without coating or with applied light-modifying films. Preparation of the lighting source was carried out in three stages. The first stage was to synthesize AgInS<sub>2</sub> (AIS) nanoparticles with sizes of 2 nm and 3.5 nm in a ratio of 1:7 and 1:20, respectively. Quantum dots were synthesized in the laboratory of the Department of Chemistry and Expertise of Food Products of Yuriy Fedkovych Chernivtsi National University, within the framework of the project "Optically active multilayer materials based on semiconductor nanoparticles of type 27 AIVIIIICVI and polymers". The second stage was to prepare PVA solutions. 200 µl of glycerol was added to 50 ml of a 1% solution of polyvinyl alcohol. After that, nanoparticles of the obtained sizes were added to the PVA solutions. The third stage was to pour the solutions into plastic containers measuring 15×8 cm and place them in a drying cabinet for 20 hours at a temperature of 80-84°C to form light-modifying films. The thickness of the formed films (≈50 µm) was measured with the device "HW300PRO". The inoculum was *Nostoc commune* culture. The ratio of inoculum to nutrient medium was 1:10. The duration of cultivation was 22 days at an initial temperature of 24±2°C. Cultivation was carried out under the condition of a 16-hour photoperiod. A nutrient medium with a minimum content of mineral components was used. On the first day, changes in the temperature of the culture medium were recorded every hour. Every third day of cultivation, temperature, pH, and culture density were also measured. Upon completion of cultivation, biomass was separated and the amounts of pigments (chlorophyll a, carotenoids, and phycobilin proteins) were determined. The pH and temperature of the cultivation medium were measured with an ionometer "WaterproofpH-Temp".

Culture densities were determined spectrophotometrically at a wavelength of 750 nm on a CaryWin UV 60 (Agilent, USA). To separate

algae cells from the fugate, the culture of cyanobacteria *Nostoc commune* was centrifuged on Biofuga stratos “Herauses” at 3000 rpm for 15 minutes. After that, the biomass of *N. commune* was disintegrated by ultrasound on an “Ultrasonic Cleaner”, with the addition of an appropriate buffer or solvent. To determine the content of chlorophyll a and carotenoids, pigments were extracted from the hydrated biomass of microalgae *N. commune* with mixtures of chloroform: ethanol (2: 1) or chloroform: acetone (2: 1), respectively. Subsequently, the biomass was centrifuged at 3 thousand rpm. The fugate was used to measure the amount of pigments (Hotos and Antoniadis, 2022; Sanchez et al., 2008). The phycobilin protein complex was obtained from cyanobacterial cells by extraction with 0.2 M phosphate buffer pH 7.0, observing the biomass: extractant ratio (1:3). The amount of phycobilin proteins and the purity of the obtained preparation were determined spectrophotometrically at analytical wavelengths of 280 nm, 562 nm, 620 nm, 652 nm on a CaryWin UV 60 (Agilent, USA) (Chen and Xiong, 2022). The pigment concentration was calculated using standard formulas. All indicators were converted to dry biomass. Statistical processing of the obtained results was carried out according to generally accepted methods, using Microsoft Excel software. All studies were conducted in 4-fold repeatability. Reliability was determined by the Student's t-test. In graphs and tables, the results are presented as the

mean value between replicates and the deviation from the mean value.

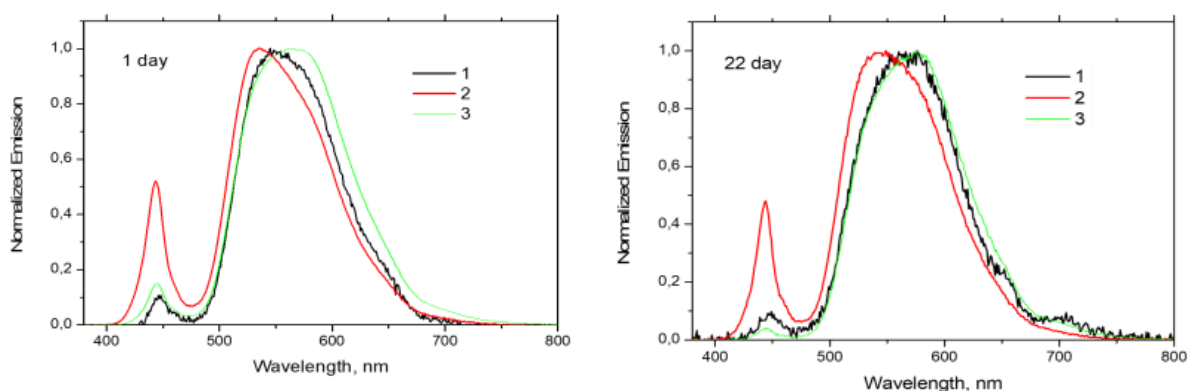
**Results and discussion.** To ensure the lighting conditions, we decided to modify the light sources using quantum dot technology. The purpose of this modification was to bring the spectral region of the emitted light closer to the red peak of the chlorophyll a absorption spectrum. Most often, in scientific practice, solutions of cadmium, zinc, or argentum with the addition of premixes are used to apply QD-based coatings to light sources. When choosing a material for creating modifying films, we were guided by the properties of the elements themselves. When using cadmium as a coating material, we would encounter two problems: a solution of cadmium nanoparticles is toxic to living systems; in addition, when using cadmium sulfate, it is difficult to achieve light emission in the spectrum we need (Chen and Xiong, 2022). Therefore, we chose indium-based nanoparticles as a less toxic element. One of the tasks of our study was to observe the change in the spectrum emitted by the coated sources, as well as the dynamics of the change in the spectrum of the modified films themselves over time. After applying the particles to the lamps, we measured the emission spectrum of the control and modified lamps. The measurements were performed with a Red Tide USB 650 German Ocean Optics in scope mode. The results were presented in the form of graphs.



**Fig. 1.** Changes in the emission spectrum of a light source modified with quantum dots, where:  
A – modification Ag:In = 1:20,  
B – control,  
C – modification Ag:In = 1:7.

Covering light sources with quantum dots allows us to shift the emission spectrum to the required range. In the case of manufacturing films using only Ag<sub>2</sub>S, we would obtain a light source emitting in the infrared spectrum. However, by adding indium particles in different concentrations, it is possible to achieve emission of light sources with different wavelengths (Huong et al. 2022; Cichy et al., 2017).

Therefore, in order to obtain emission in the desired spectral regions, indium was added to silver particles in different concentrations. As a result, we chose the ratio of argentum to indium as 1 : 7 and 1 : 20 as the most successful. By modifying the light source, according to the graphs, we managed to partially approximate the radiation spectrum curve to the solar spectrum.



**Fig. 2. Changes in the emission spectrum of different light sources on the 1st and 22nd day of operation**

During the testing of the models, a slight shift in the emission spectrum peaks of the modified light sources was observed without loss of properties. This can be explained by the effect of light on the films, their stabilization, and redox processes leading to photodegradation. In addition to modifying light sources as the main energy source, one should not forget about maintaining the physicochemical parameters of the culture medium, such as temperature, pH, and others, within the optimum values characteristic of the selected producer. In turn, monitoring these physicochemical parameters will allow tracking the stability of the system and, when recording significant deviations from the optimal level, indicating incorrect selection of one of the elements of the cultivation installation, will allow you to quickly react and avoid undesirable results. Among the various factors of the culture medium, temperature is a parameter that directly regulates the growth of algae. The importance of this parameter lies in its direct impact on the activity of enzymes in the cell, the rate of nutrient uptake, carbon dioxide fixation and the growth rate of each type of microorganisms. Biomass growth increases at optimal temperature values, and deviations from the optimal indicators

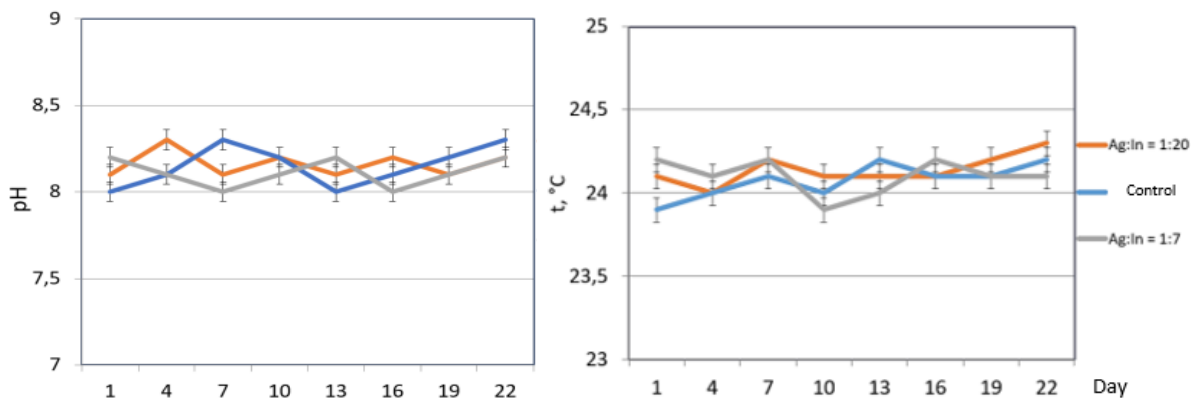
However, these films are not completely stable. The graphs show that after coating the photovoltaic cells with films, the latter stabilize for a certain period of time (about 4-10 days). It is reported that the photostability of semiconductor quantum dots is higher than that of organic dyes of the medium, which are introduced into the culture medium to change the absorption spectrum (Miao and Liu, 2015; Božin et al., 2013). Although the FBI lighting system is not completely stable, the appearance of defects is caused by changes in the diode, and not by changes in the modifying films. During the experiment, no significant loss of properties was detected in the formed films, therefore, the use of such coatings can be recommended as stable.

can be observed in the reduction of biomass growth rates, and in some cases even a change in the shape of algae. The process of photosynthesis is one of the most sensitive to temperature, especially compared to other metabolic cycles. The heat created by temperature stress disrupts the supply and consumption of energy in photosynthesizers (Ahmad et al., 2020). Therefore, when testing our system for cultivating *Nostoc commune*, there was a need to record temperature values. This is necessary in order to be able to assess changes in the physical state of the system and, if necessary, influence it. Therefore, we monitored the temperature value every third day. The obtained data were systematized in the form of a graph, which shows the trend in the temperature of the culture medium (Fig. 3).

The graph shows that the *N. commune* cyanobacterial culture grew within the optimal temperature range, which is  $24 \pm 2^\circ\text{C}$ . Minor temperature fluctuations can be explained by the influence of the environment on the system. Another important indicator of the state of the medium is pH. First, different microalgae cultures have their own optimal pH range, the maintenance of which will allow for the intensification of growth processes. Second, algae need carbon dioxide for growth. In

turn, the form of dissolved carbon dioxide and the level of its availability in the culture liquid are affected by the acidity index. Also, pH affects the processes of photosynthesis, and deviation from the optimal range can lead to a decrease in the growth of

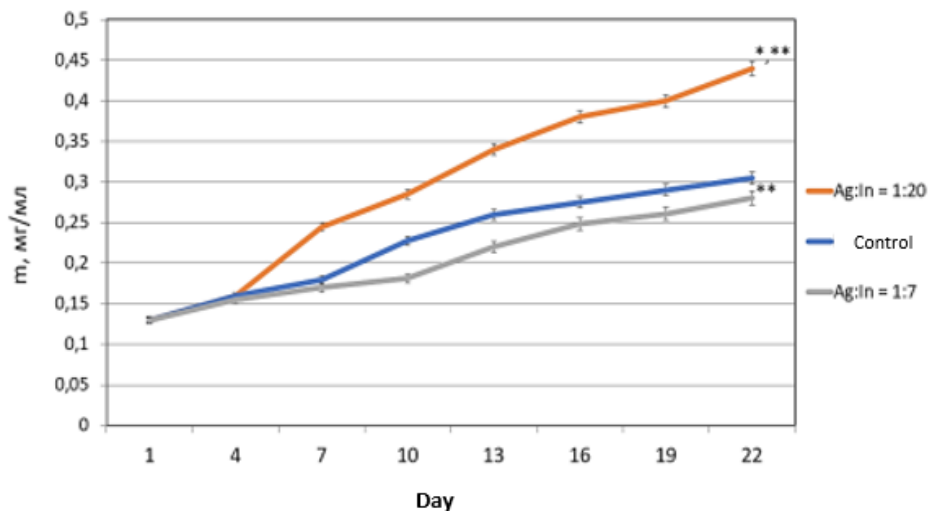
the culture as a whole. The graph shows that the pH fluctuation during the cultivation of *N. commune* cyanobacterial culture was within the optimal range, which is 7.4.



**Fig. 3. Changes in pH and temperature of the *N. commune* cultivation medium under different lighting conditions**

Minor fluctuations in pH can be explained by the influence of metabolites released by the culture into the medium during growth. In addition, the acidity of the medium can be influenced by the composition of the air used for bubbling, the intensity and time of the process itself. In order to assess the effectiveness of modifications to the light sources of the

constructed photobioreactor, we decided to compare the results of cultivating the research object. During the entire cultivation of the cyanobacterium *Nostoc commune*, the optical density of the culture fluid was measured every 3 days. The measurement results are presented in the form of a graph showing the trend of increasing biomass (Fig. 4).



**Fig. 4. Changes in the amount of biomass of *N. commune* under the action of different lighting**

Note: \* - significant difference relative to the control values, \*\* - significant difference between the experimental variants

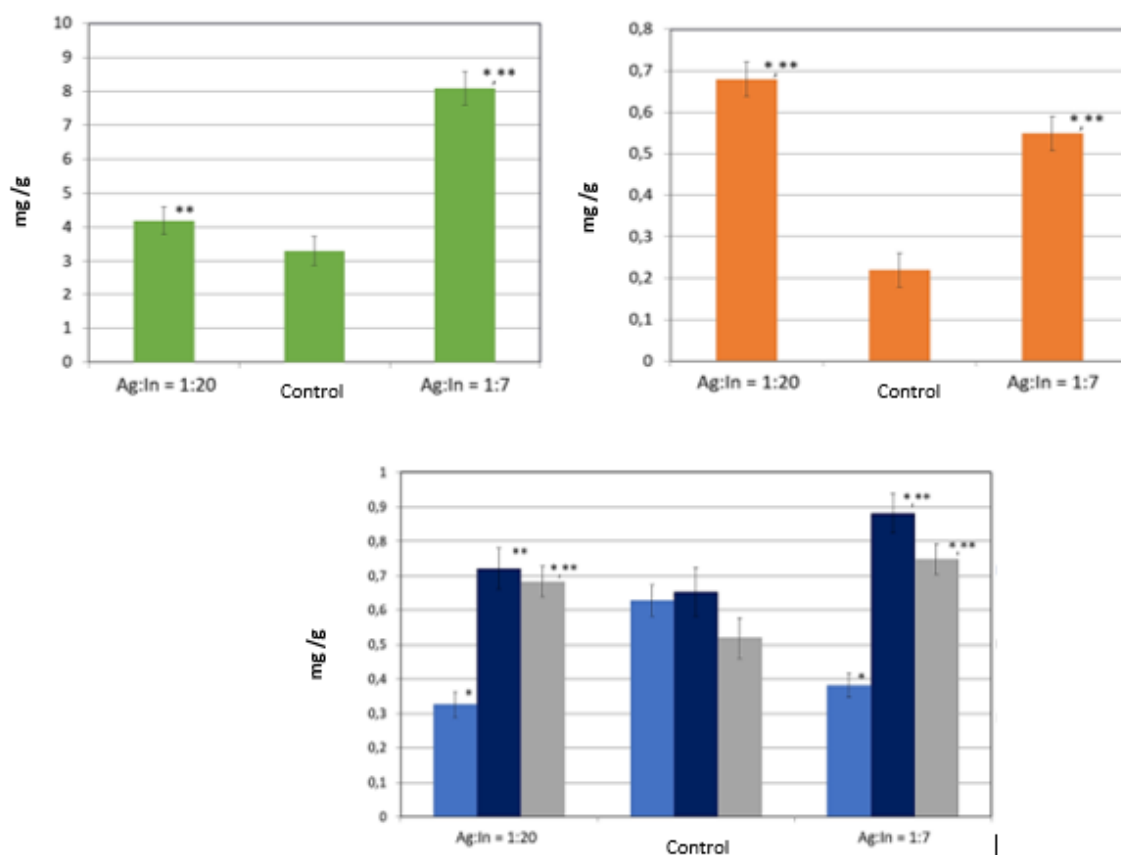
After analyzing the obtained data, it can be stated that the greatest increase in biomass was in tank 1, under the action of 3.5 nm QDs. While in tank 3, with a light source modified with 2 nm quantum dots, a slight inhibition of growth indicators was observed. It can be assumed that such changes in biomass indicators are caused by a shift of the light spectrum into a suboptimal zone.

Therefore, when using light-modifying quantum dots, no deviations from the optimal temperature and pH indicators were observed. Modification of the light source with a 3.5 nm nanoparticle size showed a better effect on the growth indicators of the *Nostoc commune* culture.

Photosynthetic organisms, such as algae, use electromagnetic radiation in the visible spectrum to

stimulate the synthesis of sugar molecules. Special pigments in the chloroplasts of plant cells absorb energy from specific wavelengths of light, triggering a molecular chain reaction known as the light-dependent reactions of photosynthesis. The best wavelengths of visible light for photosynthesis lie within the blue range (425-450 nm) and the red range (600-700 nm). Accordingly, the best light sources for photosynthesis should ideally emit light in the blue and red ranges (Mercado et al., 2004). Wavelengths of light outside the red and blue ranges are not used by most plants and can contribute to the

accumulation of heat in the system. Excessive heat can interfere with photosynthesis and even have a detrimental effect on both the cells of photosynthetic organisms. Therefore, to optimize the emitted light, we modified LED lamps with AgInS<sub>2</sub> quantum dots of different concentrations, in order to approximate the emission spectrum of light sources to the absorption spectrum of target pigments. To track the quantitative changes in chlorophyll a in the biomass of *Nostoc commune*, we extracted them. The results are presented in the form of a diagram (Fig. 5.).



**Fig. 5. The amount of chlorophyll A, carotenoids and phycobiliproteins in the biomass of *N. commune* for 21 days of cultivation under the influence of different lighting**

In comparison with the control values, the concentration of chlorophyll a in tank 1, illuminated by an LED lamp with applied QDs in the ratio Ag:In = 1:20, does not differ significantly. However, a twofold increase in its amount was observed in tank 3. We assume that this result was provoked by a change in the spectrum due to the modified light source with a QD concentration of 1:7. This effect was also manifested in the appearance of the culture. The amount of accumulated carotenoids in the biomass of the *N. commune* culture was also measured (Sanchez et al., 2008). In turn, the amount of carotenoids was significantly higher under the influence of both modifications of the light source.

The resulting difference, illustrated in the diagram, in the amount of accumulated carotenoids can be explained by the creation of stress on the photosystems, and as a consequence an increase in the amount of accumulated carotenoids as photoprotective pigments. In addition, according to the literature, only 2 to 10% of photons are absorbed by pigments, so the reduced number of photons of the blue spectrum is still sufficient for the synthesis of carotenoids. Also, when assessing the pigment composition, in addition to observing the content of carotenoids and chlorophyll a, it was decided to pay attention to the indicators of accumulated phycobilin proteins - colored and water-soluble biliproteins

contained in cyanobacteria. According to their spectral properties, they are divided into three main types: allophycocyanin, phycocyanin and phycoerythrin. These compounds function as the main light-harvesting antennas for absorbing light energy and transferring it to the reaction centers of the photosystem. Photosynthetically active radiation in the spectral range from 400 to 700 nm. is absorbed by phycobilin proteins and converted into chemical energy to support metabolism inside the cell. An increase in the amount of allophycocyanin in the biomass was also observed under the action of modified light with a QD size of 2 nm. As well as a slight accumulation of phycoerythrin in both reservoirs under the action of modified light sources. Therefore, we can assume that quantum dots with a size of 2 nm are able to positively affect the accumulation of allophycocyanin and phycoerythrin. However, a negative effect of such light modifications on the accumulation of phycocyanin was noted. Therefore, we can conclude that modifications with quantum dots of different concentrations of the light source do not equally affect the synthesis of various pigments in the cell. Such results can be explained by a shift in the spectrum of emitted light, which in turn may not coincide with the absorption range of the studied pigments. Thus, a change in the pigment composition is a kind of reflection of the quality and

effect of light on the cells of the selected producer species.

**Conclusions.** Therefore, the modification of the light source does not lead to changes in the physical parameters of the photobioreactor. Daily and periodic fluctuations in temperature and pH remain within optimal values. The use of a light source modified with quantum dots (Ag:In = 1:20) also leads to an increase in the growth activity of *N. commune*. The amount of biomass for 21 days of cultivation is 0.44 mg/ml. The use of a light source modified with quantum dots leads to an increase in the amount of pigments. The maximum content of chlorophyll a (8.1 mg/g) was established for the culture of *N. commune* under lighting conditions using a light source modified with KT Ag:In = 1:7. Modification of the light source leads to an increase in the amounts of carotenoids, allophycocyanin and phycoerythrin and a decrease in the amount of phycocyanin, regardless of the concentration of indium in quantum dots. The light source modified with Ag:In = 1:20 quantum dots allows to increase the biomass yield of cyanobacteria *N. commune* by 1.5 times compared to the control values. The use of such a light source can be recommended for growing valuable biomass of *N. commune* culture enriched with carotenoids.

**Conflict of interest.** The authors declare no conflict of interest.

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## ЗАСТОСУВАННЯ МОДИФІКОВАНИХ КВАНТОВИМИ ТОЧКАМИ AGINS<sub>2</sub> ДЖЕРЕЛ ОСВІТЛЕННЯ ДЛЯ ВИРОЩУВАННЯ NOSTOC COMMUNE У ФОТОБІОРЕАКТОРІ

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*Робота присвячена вивченню впливу джерела світла, модифікованого квантовими точками AgInS<sub>2</sub>, на культуру Nostoc commune в умовах фотобіореактора. Створено модель лабораторного трисекційного фотобіореактора. Досліджено, що модифікація джерела освітлення не призводить до змін фізичних параметрів (температура та рН) роботи фотобіореактора. Наслідком використання джерела освітлення, модифікованого квантовими точками Ag:In = 1:20, стало збільшення кількості біомаси N. commune у 1,5 рази. Також відмічено зміни кількості пігментів: максимальний вміст хлорофілу a (8,1 мг/г) встановлений за умов використання джерела світла, модифікованого КТ Ag:In = 1:7. Збільшення кількості каротиноїдів спостерігалось незалежно від кількості індію в складі модифікуючої плівки. Рекомендовано використання джерела світла, модифікованого квантовими точками AgInS<sub>2</sub>, для отримання біомаси N. commune, збагаченої каротиноїдами.*

*Ключові слова: N. commune, квантові точки, AgInS<sub>2</sub>, біомаса, продуктивність, фотобіореактор*

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