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ВПЛИВ ДОВЖИНИ ХВИЛІ СВІТЛОВОЇ ЕНЕРГІЇ НА КУЛЬТИВУВАННЯ *CHLORELLA VULGARIS*

Показано, що використання світлодіодів в комбінації різних довжин хвиль та інтенсивності освітлення впливає як на приріст біомаси мікроводоростей *Chlorella vulgaris*, так і на зміну їх метаболізму в бік синтезу певних речовин. Найбільший приріст біомаси характерний для співвідношення світлодіодів червоного, синього, зеленого спектрів 1:1:1. При освітленні комбінацією світлодіодів з перевагою червоного кольору у клітинах *Chlorella vulgaris* підвищується вміст хлорофілу *a* удвічі. Використання кольорових світлодіодів збільшує розміри клітин.

Ключові слова: мікроводорості, комбінація світлодіодів, біомаса, довжина хвилі світла, *Chlorella vulgaris*.

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INFLUENCE OF LIGHT ENERGY WAVELENGTH ON CULTIVATION OF *CHLORELLA VULGARIS*

It is shown that use of LEDs in various wavelengths combinations and intensity of lighting affects both growth of algae *Chlorella vulgaris* biomass and changing of their metabolism towards the synthesis of certain substances. The largest growth of biomass is typical for LEDs ratio of red:blue: green spectra as 1:1:1. While illuminating by a combination of LED with a predominance of red light in *Chlorella vulgaris* cells are doubled a content of chlorophyll *a*. The use of colored LEDs increases the size of the cells.

Key words: microalgae, LEDs, LED combinations, biomass, light wavelength, *Chlorella vulgaris*.

Introduction. Considering the gradual decrease of world resources of fossil energy stocks and increase of anthropogenic impact on the environment, alternative fuels getting technologies become more and more attractive, especially biofuels. For Ukraine and other developed countries, microalgae can be considered as a potential and stable source of biofuel [1]. Modern technologies of microalgae cultivation in closed photobioreactors allow to maintain rational parameters providing the mass-exchanging processes, an environment temperature, a nutrient level, etc. [2].

Literature review and problem statement. A microalgae biomass buildup depends on the light energy income to the cell, as on its supply modes, a wave-length of absorption spectrum and its intensity affect not only on the biomass buildup, but also on the biosynthesis of certain substances such as triacylglycerol, raw material for biodiesel fuel material getting [3, 4]. To implement the cultivation technology industrially, it is necessary to provide steady light on microalgae culture, especially in winter when the sunlight is not enough, or in the period of darkness.

Using standard fluorescent lamps, electricity expenses and lamps caducity lead to increasing in the costs of the process and, consequently, in the costs of the final product. Moreover, the fluorescent light has a constant direct relation of different wave-lengths [5, 6]. At the same time various types of algae have a different set and pigments relation in photosynthetic apparatus (chloroplasts) and, accordingly, fundamentally absorb radiation with a different wave-length spectrum [7]. So, *Chlorella vulgaris* absorbs light in a range of blue and red radiation [8].

The use of light by means of LEDs (LED – Light Emitting Diodes) allows ~~you~~ to choose wave-lengths corresponding to the desired range for culture and leads to energy costs reduction. The work authors [9] determined that LED radiation with the capacity of 1000 W for 24 hours in the wave-length range from 400 to 640 nm can completely satisfy the needs of microalgae.

The use of LEDs poses a problem of selection wave-lengths ratio because each LED type radiates only in a narrow range. Such wave-lengths limitation leads to the necessity of establishing a LEDs combination to satisfy the requirements in light energy of microalgae culture. This article is determined to show different wavelength LED rational combination ratios

Another positive factor of LEDs usage is the impact of wave-length radiation on the biosynthesis of certain substances. That means that the LEDs combination usage may increase the biosynthesis of lipid fraction confirmed by work data [10]. The light with the intensity of 1400 lux microalgae *Scenedesmus sp. LX1* was carried out with the following LEDs combinations: white, red, blue, red/blue in the ratio of 3:1. On the 17^{th} day of cultivation the lipid content was correspondingly, %: 43.3; 39.5; 36.3 and 30.1, while during the natural light the lipid content was up to 22.5 %. The biomass growth was higher using white LEDs by 18 % than using only red or blue, and under usage of combination of red and blue the growth hardly varies from the control experiment with sunlight [10]. A set of LEDs with the radiation spectrum in the range of 400 – 800 nm allows to increase biomass output and lipid fractions [11, 12]. Thus, the use of white LEDs with the intensity of 9000 lux increases the rate of growth of *Chlorella vulgaris* biomass by 2.18 times during the cultivation CW mode and lipid content by 2 % compared to the natural light [12]. Most commonly, to increase biomass growth one uses a combination of red and blue radiation [11, 13].

It is also known that increased triacylglycerol biosynthesis occurs during radiation by wave-lengths corresponding to blue range, and polyunsaturated fatty acids corresponding to red [14].

Therefore the problem of selecting LEDs combinations for the cultivation of microalgae is relevant.

Research purpose and objectives. A work purpose is to establish a rational LEDs ratio with different radiation wave-lengths to increase the biomass growth.

To achieve this goal the effect of wavelength and LED ratio on biomass growth, cell size and chlorophyll *a* content was determined.

Materials and methods. The study was conducted by using *Chlorella vulgaris* ACKU 531-06 microalgae, received from the collection of the Taras Shevchenko National University of Kyiv. The microalgae cultivation was conducted in photoreactors with the capacity of 0.15 dm^3. As the nutritional medium it was used standard environment of B. V. Hromov No. 6, where salt concentration was as the follows, g/dm^3: KNO_3 – 1,0; K_2HPO_4 – 0,2; $MgSO_4 \cdot 7H_2O$ – 0,2; $CaCl_2$ – 0,15; $NaHCO_3$ – 0,2; micro-elements were carried based on 1 cm^3/dm^3. The dissolution of micro-elements, g/dm^3: $ZnSO_4 \cdot 7H_2O$ – 0,22; $MnSO_4$ – 1,81; $CuSO_4 \cdot 5H_2O$ – 0,079; $NaBO_2 \cdot 4H_2O$ – 2,63; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ – 1,0; $FeSO_4 \cdot 7H_2O$ – 9,3; $CaCl_2$ – 1,2; Co $(NO_3)_2 \cdot 6H_2O$ – 0,02; EDTA – 10.0. The environment was autoclaved for 1 hour at 1200 °C and the pressure by 250 kPa.

The uterine suspension culture *Chlorella vulgaris*, which was inoculated to photoreactor, made up 20 % of the volume capacity and had an elementary optical density $D_{450} = 0,10 \div 0,11$. Monitoring and the control of purity of *Chlorella vulgaris* culture were conducted by light microscopy using a microscope TM XSP-139TP (Ulab, China). The culture medium was supplied by 100 cm^3 of CO_2 from the balloon (Russia) 1 times per day in order to supply the culture by carbon source. The cultivation temperature was 18 ± 2 °C.

The change in biomass growth was determined by the value of the optical suspension density, measured at a wave-length of 450 *nm*. The measurement of optical density was performed using

spectrophotometer Ulab 102 (China). For research, glass tubes with optical path length l = 1 cm were used. The extraction of chlorophyll was conducted by the method of Richie [15]. Taking into account hydrophobic pigments characteristics, 96 % ethanol was used for extraction.

The microalgae mass was determined with the help of analytical weight ВЛА-200g-М (Ukraine).

Microalgae biomass discharging was carried out by installing a vacuum microfiltration ПВФ-35/2 Б (Russia). The biomass drying was conducted in the baking oven 2В-151 (Russia) at 110 °С.

The *Chlorella vulgaris* cultivation was performed using laboratory facility, which prevents the flow of sunlight. The culture radiation was carried out using combinations of 10 and 20 of LEDs as: 1 – white (colour temperature is 5500 K), 2 – red/blue 3:2, 3 – blue/yellow/red/orange 2:2:5:1, respectively, 4 – blue/red/green 1:1:1. The light measurements were performed with a digital light meter LX101 (China), the light definition spectrum is in the range of 400 – 700 nm. To determine the LEDs power radiation it was used light power detector DT-1307 (China).

The power of radiation by 10 LEDs consisted: white – 7.5 W, which corresponds to the intensity of radiation by 983 lux; red/blue – 6.2 W, 334 lux; blue/yellow/red/orange – 5.6 W, 435 lux; 9 LEDs blue/red/green – 6.9 W, 476 lux. As control it was used the natural light for 15 hours, which varied widely of 1000 – 9000 lux.

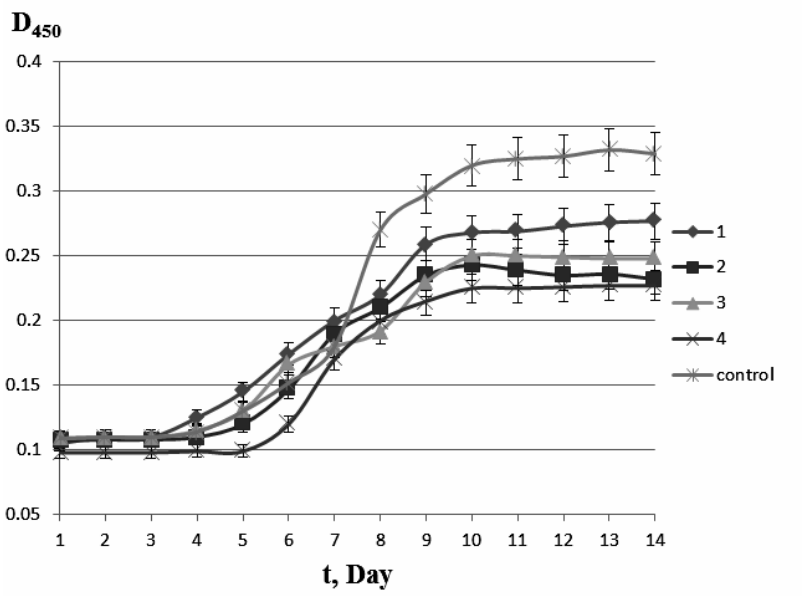
The results of research. The range of radiation spectrum was chosen according to the specific pigment contents of *Chlorella vulgaris* [13]. As chlorophyll *a* has a maximum absorption with wave-length of 450 nm and 675 nm, to provide such ranges indigo LEDs (430 – 450nm) and red LEDs (640–700 nm) were used. Chlorophyll *b* is characterized with the absorption of 475 nm and 625 nm with blue LEDs (450–480 nm) and orange LEDs (615–625 nm) corresponding to it. The chlorophyll a_o contained in photosystem 1 is the primary electron acceptor with maximum absorption of 695 nm, the end of the red spectrum. Next to these main pigments in the cell are carotinoids that have maximum absorption in the range of 341–451 nm and 520–580 nm, corresponding to the LEDs of ultraviolet spectrum of A type 320–395 nm, violet 395–430 nm, indigo 430–450 nm, green 520–555 nm and yellow-green 555–585 nm.

So, theoretically, it is necessary to use all LED radiation spectrum, corresponding to the lengths of pigment absorption, for the culture optimum growth. As there are all wave-lengths in the white light, white LEDs were chosen to compare with different combinations, the combination of red and blue was suggested as the basis with other LEDs with different wave-lengths added to it. They correspond to the absorption of the main pigment of chloroplasts such as chlorophyll *a*. This choice may be explained by the chance to increase both the biomass and lipid growth.

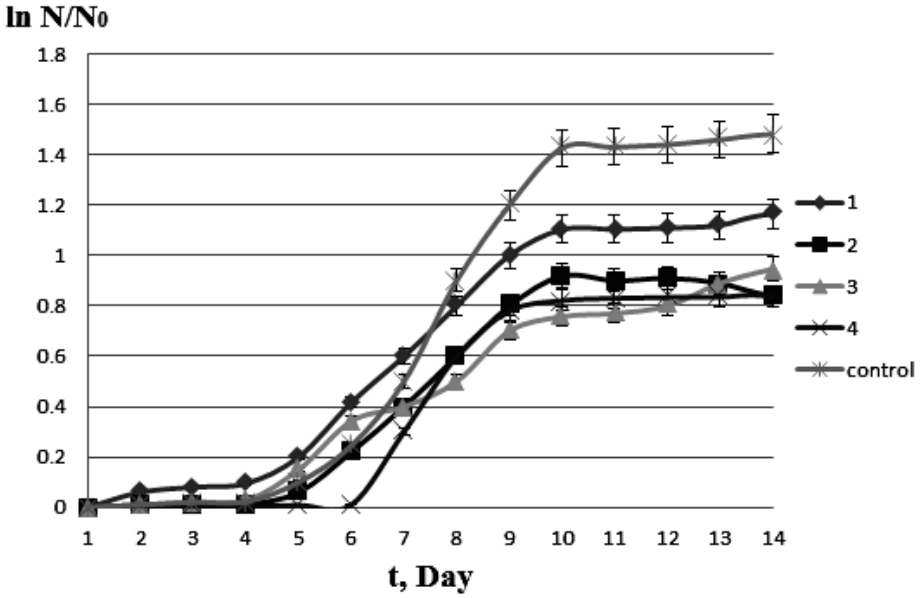
Picture 1 shows the change in optical density during the process of *Chlorella vulgaris* cultivation. Within 6 days, the greatest change in density was observed for the culture with white spectrum (1) and the combination of red/blue/yellow/orange (3). In this period cells adapt to the change of light spectrum and to the reduction of its intensity with the use of LED, temperature changes from 30 to 20 ºС. In the exponential phase the maximum growth was observed with natural lighting.

Picture 2 shows the change in the growth of *Chlorella vulgaris* cell number in the cultivation with the use of different light spectra. The change dynamics corresponds to the change of the suspension optical density.

Under microscopic examination was defined that during the cultivation under the white lighting (1) the cells were yellowish-green with the smallest size of 2.5–2.7 micrometers. Cultivated under the natural lighting cells' size increases up to 2.8–3.1 micrometers. All cells, cultivated with the use of white and natural lighting were easily separated from each other. With the use of the red/blue (2) LEDs combinations and blue/yellow/red/orange (3) LEDs combinations the cells were green and bigger in size, than those cultivated under natural lighting, with their size in the range from 3.7 to 6.2 micrometers, but reduced in number (pic. 2). They formed filamentary and circular colonies, separated only with the use of carbon dioxide. With the use of the blue/red/green (4) LEDs combinations, the cells had the biggest size of 7.5–10 micrometers, which is in average 3–4 times bigger than those cultivated under white lighting. The cells had intense green colour, sometimes dark green. They formed floccular structures that could not be destroyed during mixing.



Picture 1. The change in optical density of cell suspension (D_{450}) during the process of Chlorella vulgaris cultivation under lighting with 10 LEDs with different wave-lengths: 1 – white, 2 – blue/red (2:3). 3 – blue/yellow/red/orange 2:2:5:1, correspondingly, 4 – blue/red/green 1:1:1, control – natural lighting



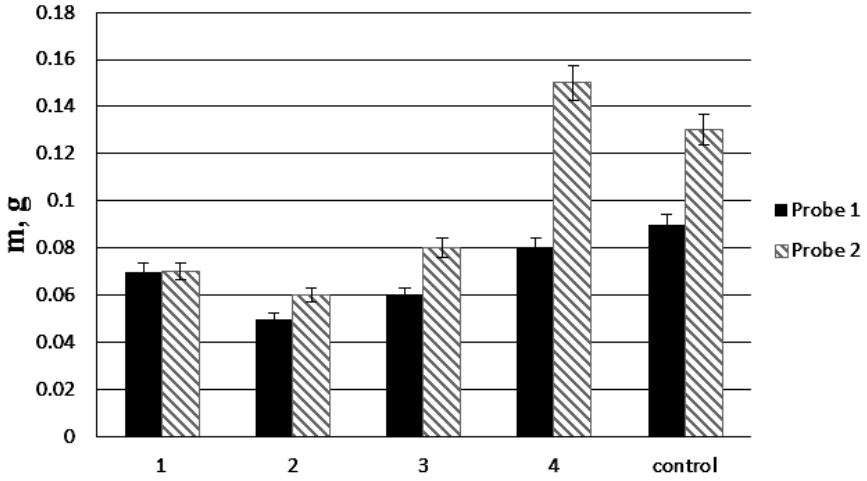
Picture 2. The change dynamics of Chlorella vulgaris cell number (lnN/N_o) during the cultivation under lighting with 10 LEDs with different wave-lengths: 1– white, 2 – blue/red (2:3). 3 – blue/yellow/red/orange 2:2:5:1, correspondingly, 4 – blue/red/green 1:1:1, control – natural lighting

The colour and biosynthetic activity of cells may be explained by the change in chlorophyll *a* and *b* contents. The amount of chlorophyll has no proportional relation to the respiration and photosynthesis, but the contents of chlorophyll always increases with the lack of lighting [16], which is proved by the data received (Table 1). Thus, the content of chlorophyll *a* with the use of natural lighting and white LEDs is equal, but the contents of chlorophyll *b* increases in the second case. The use of LED combinations, where red light prevails (Table 1, position 2, 3), results in the increased amount of chlorophyll *a* in the relation to the control 1.9 and 2.6 times correspondingly. While the use of red/blue/green LEDs (Table 1, position 4) reduces the chlorophyll biosynthesis 4.8 times in the relation to the natural lighting, which changes cell colour into green.

The Content of Chlorophyll *a* and *b* in *Chlorella vulgaris* cells under the lighting with different wave-lengths

LEDs (20 pcs)		chlorophyll *a* mg/g	chlorophyll *b* mg/g	chlorophyll a/*b* interrelation
1	white	10.34±0.31	4.01±0.1	2.58
2	red/blue 3:2	19.34±0.58	3.40±0.1	5.69
3	blue/yellow/red/orange 2:2:5:1	26.79±0.80	6.91±0.2	3.88
4	red/blue/green, 1:1:1 (18 pcs)	9.81±0.29	0.314±0.01	31.2
Control sample (natural lighting)		10.29±0.31	1.51±0.05	6.81

In particular, the size and the form of grouping can explain the differences in biomass growth (picture 3) and the optical density of cell suspension (picture 1). Pic. 3 shows the biomass growth within 14 days of cultivation with different light intensity. The maximum biomass growth is specific to natural lighting with low light intensity (dull days, 10 LEDs), while within the usage of spectra combinations of blue/red/green the growth reduces by 11 %, with white – by 22 %, with blue/yellow/red/orange combination – by 33 % in average, with red/blue – by 45 %.



Picture 3. Biomass yield of Chlorella vulgaris cultivation during 14 days with different lighting of low intensity (10 LEDs, Probe 1) and high intensity (20 LEDs, Probe 2): 1 – white LEDs, 2 – blue/red (2:3), 3 – blue/yellow/red/orange 2:2:5:1, accordingly, 4 – blue/red/green 1:1:1, control – natural lighting

Thus, greater biomass growth and lower optical density, in case when blue/red/green combination is used, may be explained by the bigger size of cells, less cell number and their grouping, as the change in size and quantity influences diffusion and optical density accordingly. The biomass growth, in case where LED system 4 was used, in the relation to other LEDs combinations, may be also explained by the presence of radiation peculiar to the green colour. As green lighting supports CO_2 capturing in the depth of photoreactor and increases the speed of CO_2 transformation into organic substances, which also expedites its consumption and contributes to biomass growth [18]. So, the use of LEDs combinations with equal part of LEDs with radiation corresponding to red, blue and green spectrum wave-lengths were defined as more effective than the use of white lighting.

Discussion of results. The reduction in biomass growth with the use of 10 LEDs compared to natural lighting may be related to lack of light intensity used. As with the use of sun lighting the cell achieved 1.5 – 2 times more energy than with the use of LEDs. It can also explain the increase in cells' size with the use of LEDs combination of different colours, as the photons of different colours have different energy and thus their amount influence the photosynthetic activity. So, a green photon (510 nm) has 20 % more energy than a red one (680 nm) and 15.5 % less than a blue one (470 nm) [19], i.e. the radiation with low wave-lengths of visible light range contributes to the increase of biomass growth.

The enhancement of light energy increases biomass growth in 2 times (pic. 3). The change in biomass growth with the use of different LEDs combinations remains the same as with the use of less light intensity, except of the blue/red/green combination (position 4 at Pic. 3). With these light frequencies used, biomass growth surpasses 15 % the amount of biomass received with natural lighting (sunny days). The same biomass growth with the increase of white LEDs number may be explained with the high power of lighting of a small photoreactor surface, which leads to the decrease of the intensity of the culture development and to the microalgae cell destruction.

So, in order to reduce energy consumption during microalgae biomass cultivation, it is possible to use both natural lighting and LEDs combinations in darkness. To provide maximum biomass growth with the lack of natural lighting, it is reasonable to use the LEDs combinations of three long waves – blue, green and red in equal proportions with the radiation power of 13.8 Watt, and the intensity of 952 lux.

Conclusions. The microalgae *Chlorella vulgaris* biomass growth depends on the radiation wave-lengths and their interrelation. With the lack of LEDs lighting intensity, the maximum biomass growth is observed with the use of natural lighting, containing all wave-lengths of the visible light range. However, the usage of white LEDs, also containing all wave-lengths as the sun light, demonstrates by 11 % less biomass growth compared to the combination of blue/green/red LEDs. The use of LEDs combinations with different wave-length results in the increase of cell size and their aggregation related to those radiated with white or sun light. The usage of LEDs combinations, where red light prevails (blue/red 2:3; blue/yellow/red/orange 2:2:5:1; blue/yellow/red/orange 2:2:5:1), increases chlorophyll biosynthesis in 2 times compared to the natural lighting. The highest biomass growth specific for *Chlorella vulgaris* cultivation is observed under usage of blue/green/red LEDs combination in the ratio of 1:1:1 with the light intensity of 952 lux.

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ВИЗНАЧЕННЯ КОНСТАНТИ ШВИДКОСТІ РОЗКЛАДУ 1-АНТРАХІНОЇЛ ДІАЗОНІЮ

Описано реакцію *N*-азосполучення 9,10-діоксо-9,10-дигідро-1-антрацендіазоній катіону з аліфатичними первинними та вторинними амінами та висвітлено фактори, що впливають на перебіг реакції. Для розуміння поведінки 9,10-діоксо-9,10-дигідро-1-антрацендіазоній катіону в реакції азосполучення визначено константу швидкості розкладу залежно від різних значень pH середовища та присутності аліфатичних амінів. Показано, що у разі збільшення співвідношення амін – 9,10-діоксо-9,10-дигідро-1-антрацендіазоній катіон спостерігається суттєве зростання константи швидкості розкладу.

Ключові слова: константа швидкості розкладу, 9,10-діоксо-9,10-дигідро-1-антрацендіазоній катіон, триазен.

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DEFINITION OF RATE CONSTANT DECOMPOSITION OF 1- ANTHRAQUINOYL DIAZONIUM

The reaction of azo coupling of 9,10-dioxo-9,10-dihydro-1-anthracene diazonium cation with primary and secondary aliphatic amines was described, and the factors that influence on the course of the reaction were reported. The reaction rate constant of azo coupling reaction of 9,10-dioxo-9,10-dihydro-1-anthracene diazonium cation in conditions of different pH values of the medium and in a presence of aliphatic amines was determined. It was shown that the significant growing of decomposition constant observes in conditions of increasing the ratio of amine – 9,10-dioxo-9,10-dihydro-1-anthracene diazonium cation.

Key words: Constant of rate decommposition, 9,10-dioxo-9,10-dihydro-1-anthracenediazony cation, tryazene.

Постановка проблеми. Природні сполуки антрахінону є діючою основою багатьох лікарських засобів рослинного походження. Досягненням стало відкриття антрациклінових антибіотиків – похідних антрахінону, що виявляють високу протипухлинну активність. Антрацикліни мають вуглецевий скелет, в якому ядро антрахінону лінійно анельоване з шестичленним насиченим