

ILLUMINATION INFLUENCE ON *Chlorella sorokiniana* BIOMASS SYNTHESIS

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The aim of the work was to estimate the influence of illumination on the rate of *Chlorella sorokiniana* alga biomass synthesis; kinetic dependencies of the synthesis and unit rate of biomass growth at different illumination conditions. Verification of adequacy of kinetic dependencies has been implemented. The kinetic equations and values of the unit rate of biomass growth derived in studied illumination modes made it possible to calculate the time needed for the synthesis of the set amount of biomass and related growth medium consumption required for a set of cultivation conditions.

Key words: *Chlorella sorokiniana*, biomass growth, cultivation conditions, kinetic regularities.

Technologies employing vegetation biomass as a source of energy and valuable food components are currently considered as biotechnologies being implemented on a large scale. The search for productive types of biomass puts forward phototrophic organisms as a perspective source of energy and raw material for extracting phytocomponents [1–3]. When developing the processes of extracting the valuable phytocomponents from the biomass of phototrophic organisms it is important not only to consider the possibility of obtaining diverse target products from the biomass but also to organize a safe production with a minimal strain on environment [4–8].

These requirements are fairly fulfilled in the case of *Chlorella* alga biomass, which finds its vast scope of application as the source of biofuel of the third generation and raw material for obtaining valuable food components, including lipids and carotenoids.

In this regard, an urgent task is to select optimal biomass cultivation conditions that would not demand substantial energy-related and financial expenditures [9–13].

This work is focused on the studying of kinetic regularities of synthesis of alga *Chlorella sorokiniana* biomass in different

illumination modes and deriving kinetic dependencies that characterize the influence of spectral structure of luminous flux on the biomass accumulation speed.

Materials and Methods

The initial concentration of *Chlorella sorokiniana* alga cells in suspension amounted to 4.2 mln cells per ml; temperature of the mixture is (23 ± 1) °C; intensity of mixture aeration is 1.5 l per min (liters per minute); mixing mode is periodic (15 min once per day); mixing speed is 500 rps (revolutions per second).

Illumination conditions for the biomass suspension are the following:

- Mode 1. Fluorescent lamp, illumination intensity is 2200–2800 lx, $T = 400$ K, daylight (later referred as DFL);
- Mode 2. Infrared incandescent lamp, spectral area of the luminous flux — 3.5–5.0 μm , illumination is 14100 lx (later referred as IR);
- Mode 3. UV-radiation with a mercury gas-discharge lamp with a spectral area of the luminous flux of 320–400 nm during 3 hours on the first day of cultivation (later

referred as UV-A) and further illumination with a fluorescent lamp, illumination intensity 2200–2800 lx, $T = 400\text{ K}$, daylight (later as UV+DFL).

Dimensional parameters of the photobioreactor (Fig. 1) are the following: height — 380 mm, diameter — 50 mm, volume — 500 ml.

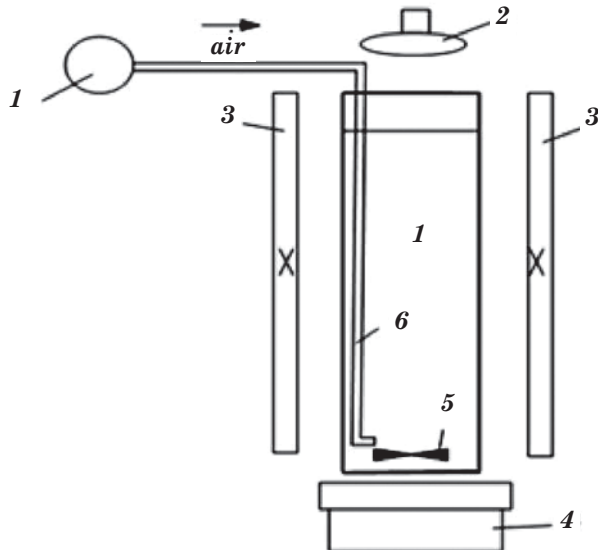


Fig. 1. Laboratory photobioreactor for *Chloralla sorokiniana* microalga cultivation:

1 — pump-aerator; 2 — source of radiation (IR or UV); 3 — daylight lamp; 4 — magnetic agitator; 5 — anchor of magnetic agitator; 6 — aeration tube

The concentration of the cell suspension of microalgae in the culture medium was determined using a calibration graph of dependence of the optical density of the suspension on cell concentration. The optical density of the suspension was determined at a wavelength of 750 nm. Culture medium was used as a reference solution. Sampling for spectrophotometry was carried out after mixing the suspension. The measurements were carried out in the range of optical density of 0.2–0.6, which corresponds to the linearity of the Beer-Lambert law. The cells were counted in the hemocytometer in 5 large squares [14] in the absence of signs of cell flocculation. Dilution of the suspension was carried out at its high concentration. Dilution was taken into account when calculating the concentration.

Concentration of alga cells in the suspension was identified by measuring the optical density using a spectrophotometer with 750 nm wavelength and with a subsequent

conversion to the quantity of cells in 1 ml of suspension in the hemocytometer. The values of optical density of the series of solutions were used for calibration graph construction.

Cultivation was implemented with the use of tap-water based growth medium containing a range of macro- and microelements shown in the table 1 below [15].

Regression analysis was used for mathematical processing of experimental data. Graphic dependences are presented after statistical processing using the least squares method implemented in Microsoft Excel. The degree of reliability of the experimental results is confirmed by their threefold repetition and reproducibility of data.

Results and Discussion

The derived calibration dependency is, as follows: $y = 26.729x - 0.591$ ($R^2 = 0.9883$). It was used for identification of *C. sorokiniana* alga cells quantity at the wavelength $\lambda = 750\text{ nm}$.

Fig. 2 illustrates dynamics of *C. sorokiniana* cells' biomass synthesis in accordance with illumination conditions.

Analysis of experimental dependency of alga cells' biomass concentration on duration of the process demonstrated compliance between the curve manner and Verhulst logistic equation (1) [16]:

$$\frac{dX}{dt} = \mu X \left(1 - \frac{X}{K}\right), \quad (1)$$

where x is concentration of cells' biomass, mln cells/ml at the current moment; K is population capacity, mln cells/ml; μ is unit speed of growth, days⁻¹.

Equation (2) is used to identify the unit speed of growth:

$$\ln X = \ln X_0 + \mu t, \quad (2)$$

where X_0 and X are concentrations of cells' biomass, mln cells/ml at the initial and current moment, correspondingly.

It was established that the speed of alga biomass accumulation reaches its maximum at the concentration of cells of 21 mln cells/ml with a further decrease. Fig. 3 illustrates the relation between cell growth speed and their concentration.

Exponential phase of growth on the graph with vertical $\ln X$ and horizontal t axes represents a line with a tangent of inclination angle equal to the value of μ (Fig. 4).

Equation (3) is likewise appropriate to be used for calculation of the unit speed of growth:

Table 1. The composition of the culture medium for microalgae for a laboratory reactor volume of 10 liters

Culture medium A				
Substance	Molecular weight, g/mol	Concentration in stock solution, mg/l	Concentration in culture medium, µg/l	Amount of substance per reactor, ml
ZnSO ₄ ·7H ₂ O	287.53	0.1	100	10.0
CuSO ₄ ·5H ₂ O	249.66	0.1	10	1.0
CoSO ₄ ·7H ₂ O	281.06	0.1	100	10.0
MnCl ₂ ·4H ₂ O	197.91	0.1	500	50.0
H ₃ BO ₃ ·WF	61.83	0.1	50	5.0
Na ₂ MoO ₄ ·2H ₂ O	241.96	0.1	100	1.0
Culture medium B				
Substance	Molecular weight, g/mol	Concentration in stock solution, mg/l	Concentration in culture medium, µg/l	Amount of substance per reactor, ml
FeCl ₃ ·6H ₂ O	270.21	1.0	4,000	40.0
Na ₂ EDTA·2H ₂ O	372.24	1.0	6,000	60.0
Culture medium C				
Substance	Molecular weight, g/mol	Concentration in culture medium, mg/l	Amount of substance per reactor, g	
KNO ₃	101.1	1,000	3,03	
KH ₂ PO ₄	136.07	100	0,32	
MgSO ₄ ·7H ₂ O	246.48	240	2,4	

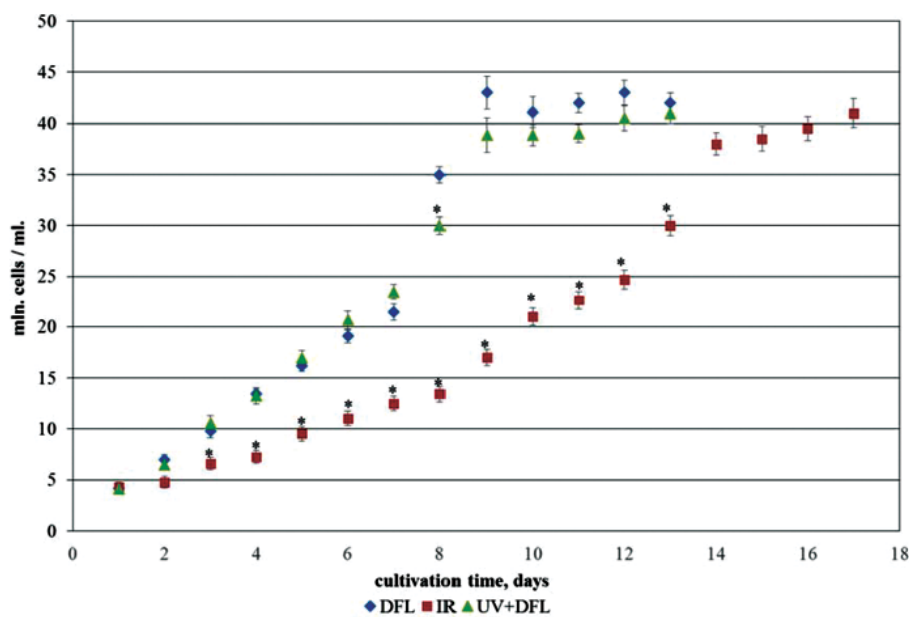


Fig. 2. Dynamics of *C. sorokiniana* cells' biomass synthesis in different illumination modes: **P* < 0.05 compared with the concentration of cells in the variant with the DFL lighting mode on the same day of cultivation

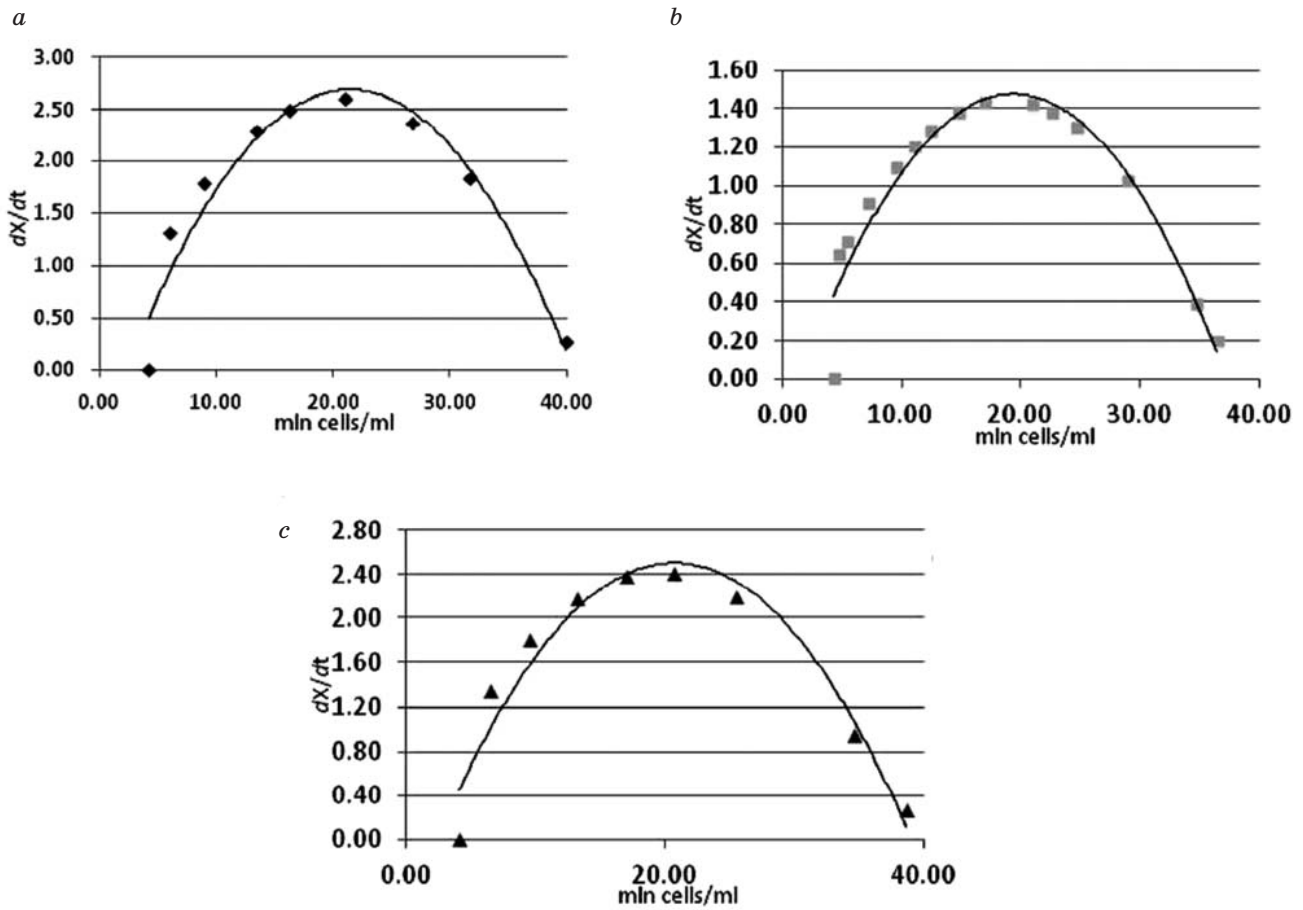


Fig. 3. Dependency of growth speed of alga *C. sorokiniana* cells on their concentration (equation 1):
 a — DFL; b — IR; c — UV+DFL

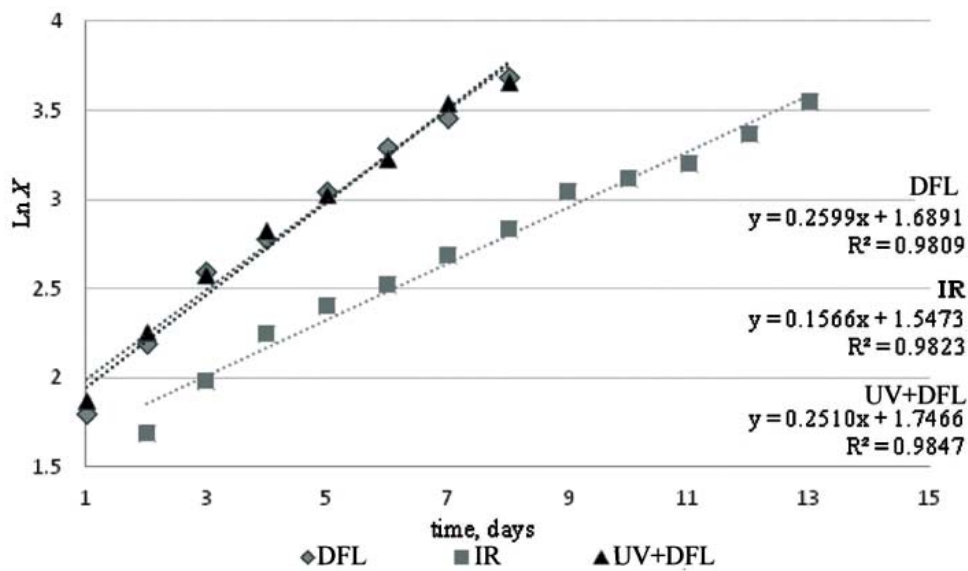


Fig. 4. Exponential phase of growth of *C. sorokiniana* alga cells under various illumination condition modes in semi-log coordinates:
 the points represent experimental data; lines – calculated kinetic model

$$\mu = \frac{\ln(X_2/X_1)}{t_2-t_1} \quad (3)$$

With X_1 and X_2 as concentrations of cells' biomass, mln cells/ml at time t_1 and t_2 , correspondingly.

Table 2 shows kinetic equations characterizing the speed of *C. sorokiniana* alga cells' biomass synthesis and unit speed of growth.

Thus, the maximum value of unit speed of growth of cells' biomass is reached at DFL illumination mode.

Table 2. Kinetic equations of *C. sorokiniana* alga cells' biomass synthesis

Cultivation conditions	Verhulst model and unit speed of growth, μ , days ⁻¹
DFL, illumination intensity is 2200-2800 lx, T = 400 K (daylight)	$\frac{dx}{dt} = 0.26x \left(1 - \frac{x}{41}\right)$ $\mu = 0.26 \pm 0.01$
IR, spectral area of luminous flux is 3.5–5.0 μm illumination intensity is 14100 lx	$\frac{dx}{dt} = 0.15x \left(1 - \frac{x}{38}\right)$ $\mu = 0.15 \pm 0.01$
UV-radiation during 3 hours of the first day of cultivation using a mercury gas-discharge lamp with a spectral area of luminous flux 320–400 nm. DFL, illumination intensity 2200–2800 lx, T = 400 K (daylight)	$\frac{dx}{dt} = 0.25x \left(1 - \frac{x}{39}\right)$ $\mu = 0.25 \pm 0.01$

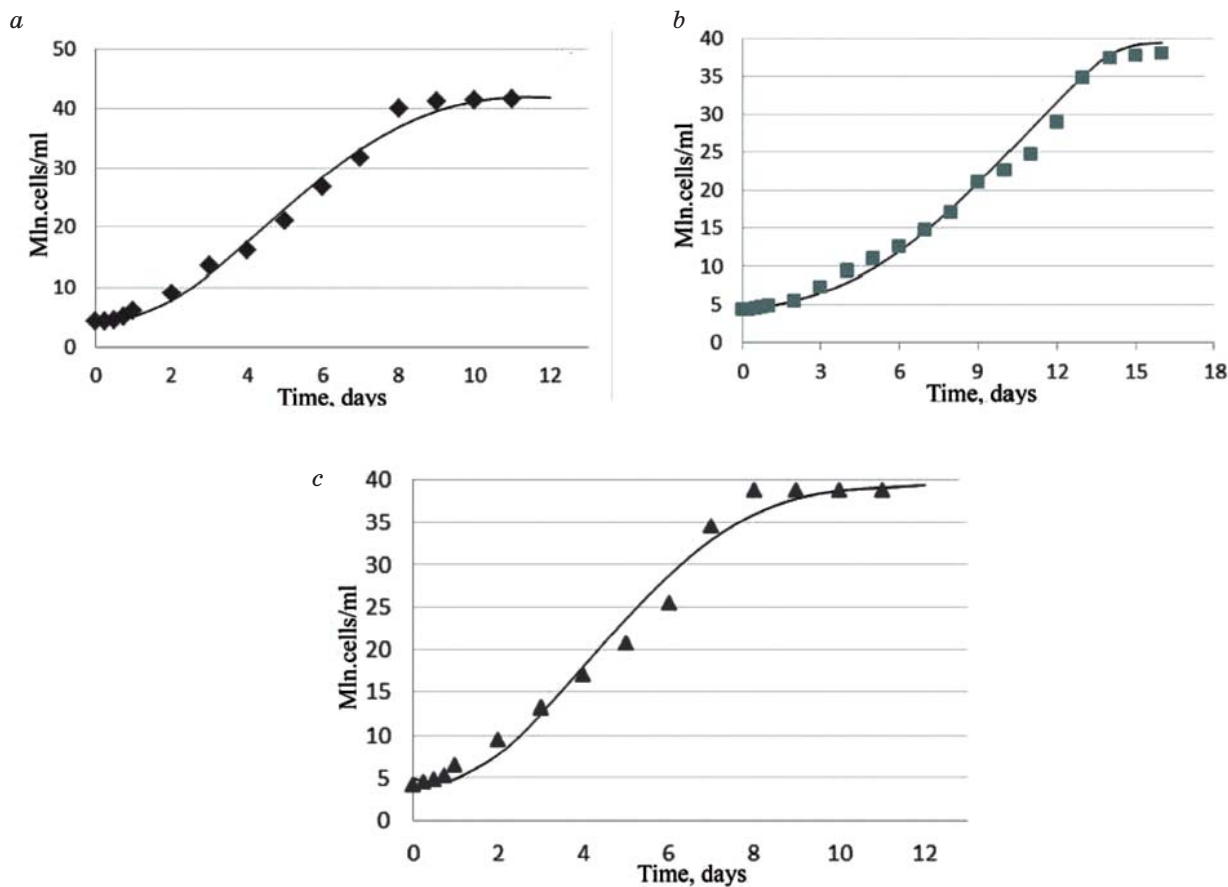


Fig. 5. Kinetic dependencies of *C. sorokiniana* alga cells' biomass synthesis in various illumination modes: a — DFL; b — IR; c — UV+DFL

Fig. 5 comprises results of adequacy verification for derived kinetic dependencies. The results of modelling of synthesis kinetics for *Chlorella* alga biomass complies with data obtained by the authors [17].

This way, the following observation has been established: the peak K population capacity in the set temperature conditions (23 ± 1) °C amounts to 41 mln cells/ml during DFL illumination mode, 2200–2800 lx, (daylight) which is 1.7 times bigger than during IR illumination mode with radiation intensity of 14100 lux. It was identified that a short-time radiation during the initial stage of alga cells' biomass synthesis in a spectral diapason of 320–400 nm does not results in a significant increase of the synthesis speed.

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**ВПЛИВ ОСВІТЛЕНOSTI
НА СИНТЕЗ БІОМАСИ
МІКРОВОДОРОСТЕЙ *Chlorella sorokiniana***

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Метою роботи було оцінити вплив освітленості на швидкість синтезу біомаси мікродорості *Chlorella sorokiniana*, кінетичну залежність синтезу і питому швидкість росту біомаси за різних умов освітленості. Встановлено, що найбільше значення питомої швидкості росту клітин біомаси досягається в режимі освітленості 2200–2800 Лк; Т (К) 400 (денне світло). Проведено перевірку адекватності отриманих кінетичних залежностей. Кінетичні рівняння і значення питомої швидкості росту біомаси у вивчених режимах освітленості дадуть змогу розрахувати час, необхідний для синтезу заданої кількості біомаси і пов'язаних з нею витрат живильного середовища в заданих умовах культивування.

Ключові слова: *Chlorella sorokiniana*, зростання біомаси, умови культивування, кінетичні закономірності.

**ВЛИЯНИЕ ОСВЕЩЕННОСТИ
НА СИНТЕЗ БИОМАССЫ
МИКРОВОДОРОСЛЕЙ *Chlorella sorokiniana***

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Целью работы было оценить влияние освещенности на скорость синтеза биомассы микродоросли *Chlorella sorokiniana*, кинетическую зависимость синтеза и удельную скорость роста биомассы в различных условиях освещенности. Установлено, что наибольшее значение удельной скорости роста клеток биомассы достигается в режиме освещенности 2200–2800 Лк; Т(К) 400 (дневной свет). Проведена проверка адекватности полученных кинетических зависимостей. Кинетические уравнения и значения удельной скорости роста биомассы в изученных режимах освещенности позволят рассчитать время, необходимое для синтеза заданного количества биомассы и связанного с ней расхода питательной среды в заданных условиях культивирования.

Ключевые слова: *Chlorella sorokiniana*, рост биомассы, условия культивирования, кинетические закономерности.