The creation of the photobioreactor for the effective chlorella growth and study of the light spectral composition influence on its biomass

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Abstract. Chlorella is a green eukaryotic microalgae (*Chlorella vulgaris*). The microscopic cell is spherical, 2–10 μm in diameter. This microalgae is one of the most important and promising for biomass production. Chlorella contains the pool of biologically active substances: about 50% protein (including essential amino acids); a complex of essential unsaturated fatty acids (including Omega-3); vitamins A, B1, B2, B3, B5, B6, E; as well as macro- and micronutrients important for health. This creates the prerequisites for its commercial production for use in medicine, cosmetology and veterinary medicine. Scientists have found that chlorella has an antioxidant, anti-inflammatory, antimicrobial and even wound healing effect due to the presence of this pool of biologically active compounds. Chlorella is cultivated in ponds or bioreactors with specified parameters that create favorable conditions for the growth of chlorella biomass. Each set of conditions creates the prerequisites for changing the growth rate and output of individual products. We studied the influence of the photometric characteristics of the light source on the optical density and, accordingly, the efficiency of growing chlorella. The results of optical density measurements allowed us to note that the largest increase in biomass is observed when using warm white LED lighting (T=2700K).

1 Introduction

Chlorella is a green eukaryotic microalgae (*Chlorella vulgaris*). The microscopic cell is spherical, 2–10 μm in diameter. This microalgae is one of the most important and promising for biomass production [1].

Chlorella contains a pool of biologically active substances: about 50% protein (including essential amino acids); a complex of essential unsaturated fatty acids (including Omega-3); vitamins (A, B1, B2, B3, B5, B6, E) and macro- and microelements. This creates the prerequisites for its commercial production for use in medicine, cosmetology and veterinary medicine. In addition, the complex of substances, in particular, antioxidants, provitamins, vitamins and other substances are often considered as a single pool of substances and are referred to as chlorella growth factor [2,3].

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Food production actively uses chlorella in the form of suspensions, powders and tablets, both as additives and as independent products.

Scientists have found that chlorella has an antioxidant, anti-inflammatory, antimicrobial and even wound healing effect due to the presence of this pool of biologically active compounds [4].

There are studies concerning the radioprotective properties of microalgae under radioactive irradiation of model organisms. It has been shown that the inclusion of chlorella in the diets of rats contributes to an increase in the biochemical parameters of the blood serum of radioactively irradiated animals [5].

In agriculture, the main direction is the use of chlorella biomass or components as biostimulants for plants [6], as well as for feeding farm animals. Even at a commercially acceptable level of chlorella-based supplementation for feeding poultry, pigs and other farm animals, chlorella biomass can effectively work as a feed additive or replacement for expensive feed ingredients [7,8]. An important prerequisite for the large-scale use of algae biomass as a feed additive is the continued reduction in the cost of technological processes.

In the field of ecobiotechnology, chlorella is used for bioremediation of the environment.

For the production of biofuel from chlorella, it is necessary to achieve a certain composition, since the content and qualitative composition of lipids are the most important quality parameters when creating this type of fuel [9,10]. In a study by Mallick et al. (2012) scientists achieved an increase in the lipid pool by 9% (up to 55% dry weight) [11].

Closed life support systems designed to support the life of astronauts, including long-distance flights, are based on bioreactors that produce microalgae that filter waste, which then reused. Experiments to create such systems have been repeatedly carried out by domestic and foreign scientists with varying degrees of success [12,13,14].

Chlorella cultivation usually takes place in special facilities, bioreactors or artificial reservoirs. Each set of conditions creates the prerequisites for changing the growth rate and the yield of individual products, for example, as mentioned above, lipids.

There are data on the effect of different spectra during the day on the growth of chlorella culture. For example, the growth activity of chlorella was correlated using different spectral composition of light at certain intervals of the day: the blue spectrum showed the best effect in the morning, the white one in the afternoon, and the red one in the evening. Chlorella has been found to have growth phases, and at certain times of the day, chlorella needs certain radiation [15,16,17]. The use of these data can reduce costs in the industrial production of microalgae and improve energy efficiency.

2 Methods

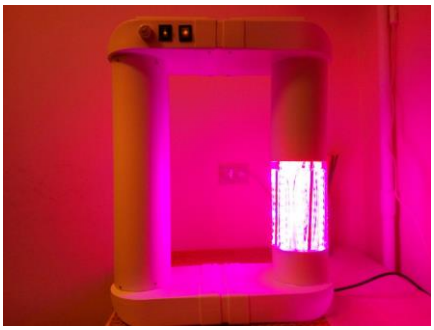
The objects of the study were two strains of chlorella: 1 - with a thin cell wall (*Chlorella vulgaris* VKPM Al-24); 2 - with a thick cell wall (*Chlorella vulgaris* Beijer), provided by Algotek LLC and the Department of Hydrobiology of Moscow State University, respectively.

Prototypes of the photobioreactor for efficient cultivation of chlorella microalgae were developed within the framework of the «435nm» project together with engineers from the «Your Sector of Space» community. Experimental stands and prototypes of the photobioreactor were designed to formulate and test the hypotheses. During the research, we created four photobioreactor prototypes, which were designated as 200, 401, 402 and 402.3.

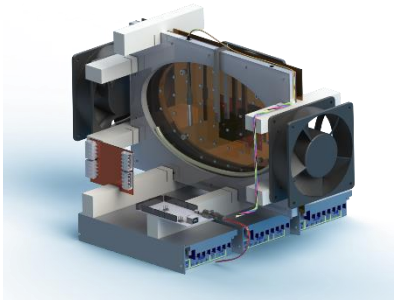
Each bioreactor had the following blocks: body; frame; medium circulation system; medium supply system; acidity regulation system; system for measuring the optical density of the medium; system for ensuring the temperature regime of the environment; gas supply

system; inlet gas composition control system; outlet gas composition measurement system; system for cleaning the cavity of the photobioreactor; lighting system; control system; power unit.

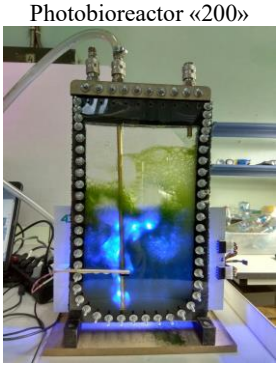
Photobioreactors differed in the materials from which the case was made: plexiglass; fluoroplast; aluminum alloys; stainless steel (Fig. 1).



Photobioreactor «200»



Photobioreactor «401»



Photobioreactor «402»

Photobioreactor «403»

Fig. 1. External view of photobioreactors.

The chlorella culture was cultivated on a modified Tamiya nutrient medium, at a temperature of 24±10C and around the clock illumination. We determined the growth dynamics of chlorella using optical density sensors built into the phytobioreactors.

In addition to creating photobioreactor prototypes, we studied the effect of different light sources on chlorella biometrics under different growing conditions. Chlorella was grown in 1000 ml flasks in opaque grow boxes (Urban Grower 60x60x200 cm (Gorshkoff, Russia)), in which different lighting modes were set (ratio of red (R) and far red light (FR)). Illumination options: 1 - R/FR = 1, PPFD = 142 (±10) µmol/m2s (R=FR); 2 - R/FR = 2, PPFD = 142 (±10) µmol/m2s (R>FR); 3 - R/FR = 1/2, PPFD = 142 (±10) µmol/m2s (FR>R). The control variant was grown in a light room with white fluorescent lamps - FL (OSRAM AG brand, manufactured in Germany) with an intensity of 150 µmol/m2s, and the culture was also grown in the dark. In all variants, the culture was grown for 5 days.

The optical density of the chlorella culture was determined in dynamics on days 1, 3, and 5 on a Cary-50 spectrophotometer, Varian, USA.

To characterize the growth of chlorella under different lighting conditions, two indicators were used: growth index (I) and specific growth rate (µ), which were calculated by the formulas:

$$I = \frac{X_{\max} - X_0}{X_0}, \tag{1}$$

$$\mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1}, \quad (2)$$

where X_{max} and X_0 are the maximum and initial values of optical density, units, X_2 and X_1 are the value of optical density (mm) at time t2 and t1, days, respectively.

The studies were carried out in 3 biological and 5 analytical replicates. Averages of all data were calculated using Microsoft Excel 2013 (Microsoft Corporation, USA). Analysis of variance (ANOVA) was performed using Statistica version 10.0 and means were compared using Fisher's least significant difference (LSD) test at a $p \leq 0.05$ significance level.

3 Results

Conducted laboratory experiments aimed at studying the effect of the spectral composition of light on the growth of two strains of chlorella culture allowed us to identify some patterns: 1 - the largest increase in biomass is observed when using white fluorescent lamps (T = 2700K); 2 - in the case of using FR>R or FR=R, their inhibitory effect on the growth of the studied strains of chlorella was observed. In addition, when determining the optical density of cultures, similar results were obtained, which indicate the same perception of the studied strains of chlorella to the effect of different spectral composition of light.

It was experimentally established that during the cultivation of thick-walled chlorella (*Chlorella vulgaris* Beijer), the greatest increase was observed when it was grown under conditions of illumination with white fluorescent lamps and the smallest - when using R=FR or FR>R (Table 1).

Table 1. The results of measurements of optical density, growth index (I) and specific growth rate (μ) of a suspension of chlorella with a thick cell wall using various light sources.

Lighting type	At a wavelength of 440 nm					At a wavelength of 690 nm				
	D_0	D	D-D_0	I	μ	D_0	D	D-D_0	I	μ
FL. T=2700K control	0.515	2.811	2.296	4.45	0.42	0.458	2.492	2.029	4.43	0.42
R=FR	0.527	1.634	1.107	2.10	0.28	0.472	1.510	1.039	2.20	0.29
R>FR	0.523	1.898	1.375	2.62	0.32	0.464	1.666	1.202	2.59	0.32
FR>R	0.530	1.654	1.124	2.12	0.28	0.474	1.467	0.994	2.10	0.28
darkness	0.526	0.463	-0.063	-0.12	-0.03	0.472	0.413	-0.059	-0.13	-0.03

Figure 2 shows the growth dynamics of thick-walled chlorella depending on the optical density and duration of cultivation.

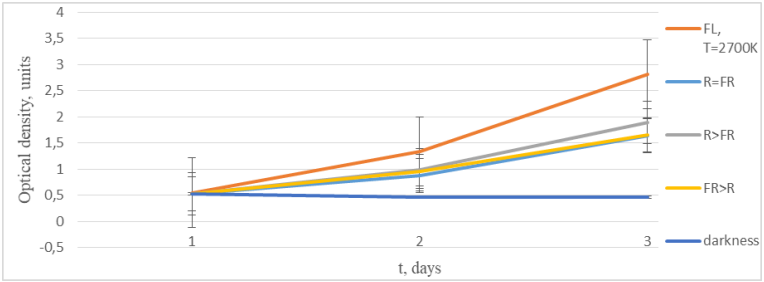


Fig. 2. The dependence of the optical density of the suspension of *Chlorella vulgaris* Beijer on the duration of cultivation at λ=440 nm.

When analyzing the absorption spectrum, it should be noted that it has a continuous character. The maximum absorption of light quanta is observed at different wavelengths in two peaks. It was experimentally established that the first maximum is located in the red region (from 660 to 690 nm), the second - in the blue region (430 to 450 nm). The minimum absorption is observed in the green region of light (500 to 610 nm). The data obtained are consistent with the results of other authors, indicating that it is in these regions of the world that the efficiency of photosynthesis is the highest (Fig. 3).

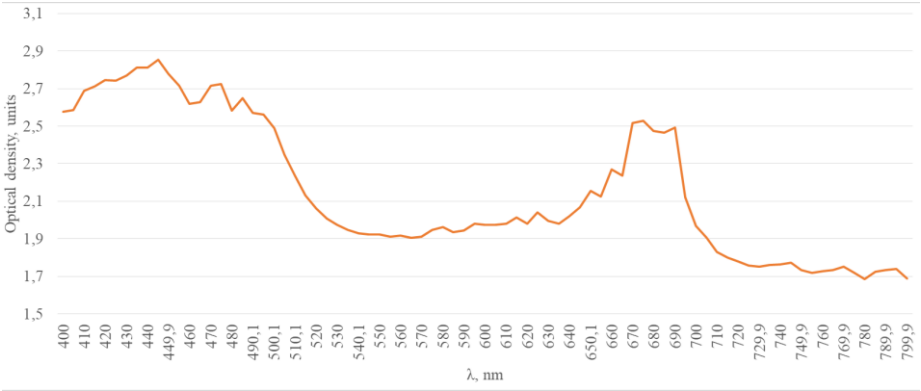


Fig. 3. Dependence of the optical density of the suspension of *Chlorella vulgaris* Beijer cultivated under LED illumination, T=2700K on the fifth day in the wavelength range from 400 to 800 nm.

Studies conducted with a strain of chlorella with a thin wall (Chlorella vulgaris VKPM Al-24) showed similar results with a strain of chlorella with a thick wall. The greatest increase was observed when illuminated with white fluorescent lamps, and the minimum increase was observed when using FR>R (Table 2).

Figure 4 shows the growth dynamics of thin-walled chlorella, depending on the optical density and duration of cultivation.

The absorption spectrum of thin-walled chlorella is similar to that described above and has two maxima: the first is located in the blue region (from 445 to 500 nm), the second is in the red region (from 670 to 690 nm) (Fig. 5).

Table 2. The results of measurements of optical density, growth index (I) and specific growth rate (μ) of a suspension of chlorella with a thin cell wall when using different light sources.

Lighting type	**At a wavelength of 440 nm**					**At a wavelength of 690 nm**				
	D_0	**D**	**D-D_0**	**I**	**μ**	**D_0**	**D**	**D-D_0**	**I**	**μ**
FL. T=2700K control	0.317	1.742	1.425	4.50	0.43	0.269	1.605	1.336	4.97	0.45
R=FR	0.384	0.895	0.511	1.33	0.21	0.333	0.881	0.549	1.65	0.24
R>FR	0.442	1.195	0.753	1.70	0.25	0.391	1.068	0.678	1.73	0.25
FR>R	0.377	0.725	0.349	0.93	0.16	0.323	0.709	0.387	1.20	0.20
darkness	0.368	0.596	0.229	0.62	0.12	0.334	0.492	0.158	0.47	0.10

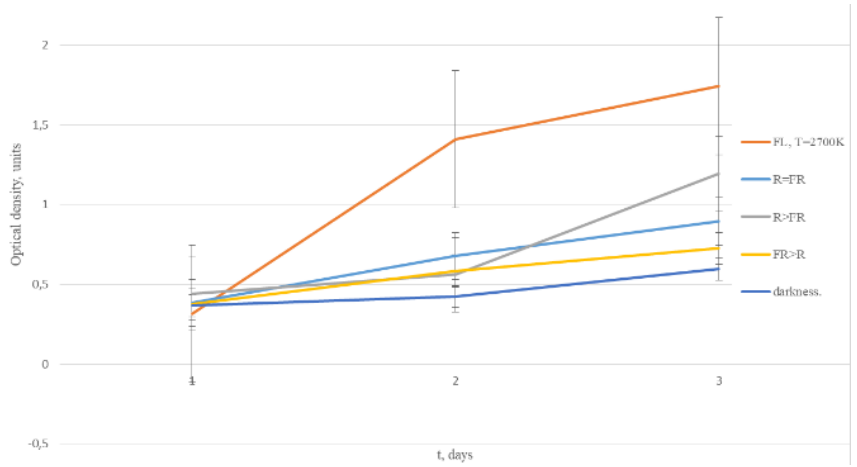


Fig. 4. Dependence of the optical density of a suspension of *Chlorella Vulgaris* VKPM Al-24 on the duration of cultivation at λ=440 nm.

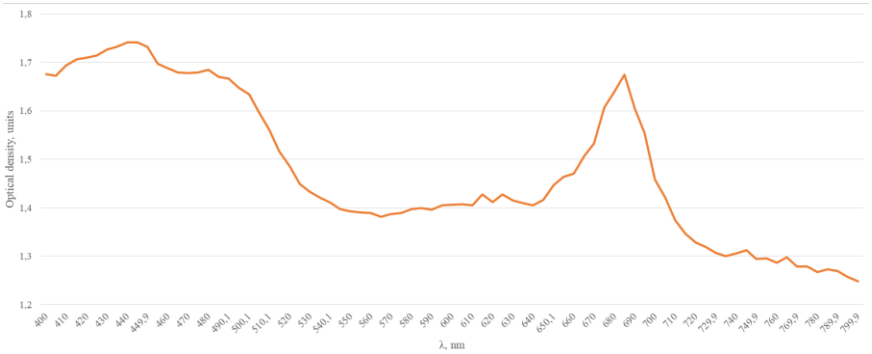


Fig. 5. The dependence of the optical density of a suspension of *Chlorella vulgaris* VKPM Al-24 cultivated under LED illumination, T=2700K, on the fifth day in the wavelength range from 400 to 800 nm.

4 Discussions

Thus, the analysis of modern scientific literature data allowed us to conclude that improving the quality of such a well-known microalgae as chlorella and the production of its biomass, including studies to control the release of individual components, are important tasks of biotechnology. It is widely used in various fields and spheres of human economic activity: from agriculture to ecology and biofuel production. Therefore, it is necessary to constantly improve the technologies for growing chlorella culture in closed systems and to establish optimal modes of its cultivation.

Based on the foregoing, the aim of the work is to study the effect of the spectral composition of light on the growth of chlorella biomass and create a photobioreactor for efficient growth of the culture.

On the basis of the conducted studies, it was found that the studied photobioreactors do not have the final technical solution for the continuous cultivation of chlorella culture. All studied photobioreactors had disadvantages: 1 - difficult access to subsystems, leading to complete dismantling of the system and disconnection of hose connectors; 2 - the presence of stagnant zones due to the incorrect location of the air supply pipes and the circulation

system; 3 - LEDs were overgrown with chlorella, as they were inside the phytobioreactor, which led to a decrease in their efficiency. The identified deficiencies had a negative impact on the growth of chlorella. As a rule, already on the 1st day from the beginning of cultivation, the culture precipitated and stopped growing. After analyzing the identified shortcomings, we designed a new photobioreactor "402.1", under which we observed the active growth of chlorella during long-term cultivation (Fig. 6).

a b

Fig. 6. External view of phytobioreactor "402.1" with chlorella culture: a - the beginning of cultivation, b - the end of cultivation.

5 Conclusion

Thus, chlorella is a relevant object of study due to its wide use in various areas of the national economy, such as agriculture, ecology, biofuel production, and others, as well as the prospects for its use in space biotechnologies. The results of measurements of the optical density of the chlorella strain with a thick and thin cell wall allowed us to note that the largest increase in the biomass of chlorella microalgae is observed when using white fluorescent lighting, and the minimum is observed when using R=FR or FR>R, which contradicts most of the studied sources, where positive influence of the red spectrum (including the far red) on the growth of chlorella biomass have been shown. At the same time, our data confirm the results obtained by other authors, indicating that white light is the optimal illumination for increasing cell biomass.

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