

**ИЗУЧЕНИЕ ХИМИЧЕСКОГО СОСТАВА И СВОЙСТВ БИОМАССЫ ИЗ  
МИКРОВОДОРОСЛЕЙ *CHLORELLA SOROKINIANA* ПРИ  
РАЗЛИЧНЫХ ВИДАХ ФИЗИЧЕСКИХ ВОЗДЕЙСТВИЙ**

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*В работе представлены данные, касающиеся актуального вопроса переработки микроводорослей *Chlorella sorokiniana*, богатство витаминного и аминокислотного состава которой обеспечивает ее питательную (вдвое больше по сравнению с соевым белком) и биологическую ценность. Приведены сведения об использовании хлореллы в качестве обогащающей добавки в пищевые продукты для создания современной системы жизнеобеспечения пилотируемых космических кораблей. На основании анализа литературных данных по производству астаксантина рекомендовано учитывать установленный положительный эффект светового воздействия на микроводоросли, а варьированием состава питательной среды целенаправленно получать сухую массу микроводорослей с фиксированным содержанием липидов, белков и крахмала. Изучено влияние различных физических факторов на специфику протекания биохимических процессов в клетке и вариативность состава сухой биомассы при культивировании *Chlorella sorokiniana*. Представлены экспериментальные результаты применения с указанной целью красного лазера (выходная мощность 1,6 мВт, длина волны 0,63 мкм), воздействия дневного света (интенсивность 2800 Лк), инфракрасного (мощность 250 Вт, интенсивность 14100 Лк) и ультрафиолетового (длина волны 280-315 нм) облучения образцов микроводоросли хлореллы. Наиболее значимые показатели концентрации белковых соединений (46,0 и 48,4% от сухой биомассы) характеризуют биомассу, полученную при воздействии дневного света и инфракрасного излучения; во втором случае дополнительно дана общая оценка жирнокислотного состава образца, представленного в основном ненасыщенными кислотами (77 мас.%). Повышенное содержание хлорофиллов, *a* - (19,3 мг/г) и *b* - (7,1 мг/г), и общих каротиноидов (2,8 мг/г) отличает биомассу на основе *C. sorokiniana*, выращенную при дневном свете. Выявлено, что ультрафиолетовое воздействие на образцы микроводоросли благоприятствует накоплению в сухом продукте сахаридов (до 430 мг/г). Полученные авторские данные позволяют подобрать подходящие условия для направленного проведения процесса культивирования *Chlorella sorokiniana* в различном оптическом диапазоне и получать биомассу с заданным составом и свойствами.*

**Ключевые слова:** микроводоросли *Chlorella sorokiniana*, физические воздействия, культивирование, биомасса, белки, углеводы, хлорофиллы

## STUDY OF CHEMICAL COMPOSITION AND PROPERTIES OF BIOMASS OF *CHLORELLA SOROKINIANA* UNDER INFLUENCE OF DIFFERENT PHYSICAL FACTORS

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*The paper presents data on the current issue of the processing of microalgae *Chlorella sorokiniana*, the richness of the vitamin and amino acid composition of which ensures its nutritional (twice as much as soy protein) and biological value. This article also provides information on the utilization of *chlorella* as an enrichment supplement to develop a modern Life Support System for manned spacecraft. Based on the data collected from various bibliographical studies, it is recommended to take into account the established positive effect of light exposure on the microalgae for the production of astaxanthin, as well as, to vary the composition of the nutrient medium in order to obtain a dry microalgae biomass with a fixed lipid, protein and starch content. The influence of various physical factors on the specificity of biochemical processes in the cell and the variation in the composition of dry biomass in the cultivation of *Chlorella sorokiniana* was studied. Experimental results of the application of a red laser (output power 1.6 mW, wavelength 0.63 μm), the exposure to daylight (intensity 2800 Lux), infrared (power 250 W, intensity 14100 Lux) and ultraviolet (wavelength 280-315 nm) radiations of the microalgal samples are presented. The most significant concentrations of protein compounds (46.0% and 48.4% of dry biomass) were obtained after the exposure to daylight and to infrared radiation. In the last case, the fatty acid composition of the sample was represented mainly by unsaturated acids (77% by weight). The biomass of *C. sorokiniana* cultivated under the influence of daylight showed an increased content of chlorophylls a (19.3 mg/g), chlorophyll b (7.1 mg/g) and of total carotenoids (2.8 mg/g). Under the action of ultraviolet light, the quantity of saccharides increased (up to 430 mg/g). The data obtained enables to select optimal cultivation conditions of *Chlorella sorokiniana* in order to obtain a biomass with a given composition and properties.*

**Key words:** microalgae *Chlorella sorokiniana*, physical factors, cultivation, biomass, proteins, carbohydrates, chlorophylls

### Для цитирования:

Смятская Ю.А., Кузнецова Т.А., Политаева Н.А., Амира Туми, Атаманюк И.В., Разговоров П.Б. Изучение химического состава и свойств биомассы из микроводорослей *Chlorella sorokiniana* при различных видах физических воздействий. *Изв. вузов. Химия и хим. технология*. 2019. Т. 62. Вып. 2. С. 72–78

### For citation:

Smyatskaya Yu.A., Kuznetsova T.A., Politaeva N.A., Amira Toumi, Atamanyuk I.V., Razgovorov P.B. Study of chemical composition and properties of biomass of *Chlorella sorokiniana* under influence of different physical factors. *Izv. Vyssh. Uchebn. Zaved. Khim. Khim. Tekhnol.* 2019. V. 62. N 2. P. 72–78

## INTRODUCTION

According to the "Strategy for the development of the chemical and petrochemical complex up to 2030" and to the "Comprehensive Program for Development of Biotechnology in the Russian Federation

through 2020", the key problems of the chemical and biotechnological industries in the Russian Federation are the high costs of the raw materials and the lack of assortment. One of the possible solutions to such problems is to create new economically effective and environmentally friendly chemical industries based on the

use of microalgae. This kind of vegetal raw material has a number of advantages over others, consisting in a high area yield throughout the year with significantly large amounts of raw materials.

In this case, big plans are laid out for the microalga *Chlorella* (*C.*). Its rich vitamin and protein contents allows it to be used effectively as a feed additive for cattle and domestic animals. However, the *Chlorella* protein contains all essential amino acids, and its nutritional value exceeds twice that for soy protein. Since *Chlorella* contains more than 50% protein (in comparison, the indicator for wheat is about 12%) and is not inferior in value to meat, in many countries it is actively introduced into the human food diet [1]. In Japan, *Chlorella* is added to bread, confectionery and ice cream. Russian scientists associate the technological process of making dry *Chlorella* algae with the production of combined dairy products. Questions concerning the chemistry of *Chlorella* and the prospects for using its unique composition in creating a life support system for manned spacecraft are considered [2].

It is possible to obtain biologically valuable substances from *Chlorella* such as astaxanthin (a ketocarotenoid pigment, found in aquatic animals). In the technology of obtaining astaxanthin, it is characteristic that both freshwater (*C. sorokiniana*) and marine (*Tetraselmis sp.*) microalgae can be processed [3]. In this case, the effect of light and the presence of inorganic carbon and nitrate compounds in a system suitable for cultivation allows to effectively control the process of producing astaxanthin of improved quality [3]. In [4], it is proposed to use waste water of pig farms (rich in carbohydrates) as a nutrient medium for the technological processing of the algae *C. Vulgaris*. The realization of such an idea will allow solving several problems simultaneously: water purification, cultivation of microalgae and obtaining valuable components.

Studies on three species of the strain *C. Sorokiniana* in the treatment of wastewaters taken from cattle-breeding farms have revealed [5] that varying the composition of the nutrient medium leads to different protein, lipid and starch content in the dry biomass of microalgae. Similarly, the addition of nitrogen in nitrate and ammonium forms can change the biomass composition [6-9]. The relationship between biomass yield and urea content in wastewater [10] used for microalgae cultivation has been studied. It has also been shown that amount of proteins and carbohydrates in biomass increases in a nutrient medium low in sulfur and nitrogen [11]. Moreover, it has been established exper-

imentally that physical factors (PF), including light, applied in different wave ranges, exert a significant influence on the cultivation rate and the composition of the biomass of duckweed and *Eichhornia* [12-18].

In this regard, the purpose of this study is to examine the effect of various types of PF on the process of obtaining a *C. sorokiniana* biomass with a high content of valuable nutrients.

#### EXPERIMENTAL PART

The cultivation of the microalgae *Chlorella sorokiniana* took place in a photobioreactor, which is a cylindrical glass vessel (Fig. 1) with a height of 380 mm and a diameter of 50 mm (500 ml volume). The process was carried out in the "day-night" function in batch mode: first 15 min of stirring with an intensity of 300-500 min<sup>-1</sup>, then 120 min in rest mode. A compressor model AP-001 (intensity 1.5 l/min) was used for aeration.

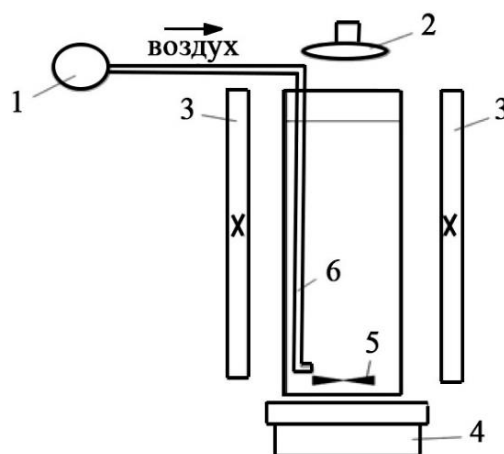


Fig. 1. Scheme of the photoreactor used for the cultivation of *Chlorella sorokiniana*: 1 – pump-aerator; 2 – source of radiation (laser, IR, UV); 3 – phosphorescent lamps; 4 – magnetic stirrer; 5 – anchor agitator; 6 – air supply tube

Рис. 1. Схема фотобиореактора для *Chlorella sorokiniana*: 1 – насос-аэратор; 2 – источник излучения (лазер, ИК-, УФ-); 3 – лампы дневного света; 4 – магнитная мешалка; 5 – якорь мешалки; 6 – трубка для подачи воздуха

The preparation of the culture medium containing the necessary set of macro- and microelements is described in the articles [19, 20]. Algal population growth was monitored by measuring the optical density values using a UNICO 1208 spectrophotometer at a 750 nm wavelength; and further, by a recalculation in a Goryaev chamber by the enumeration of cells present in 1 ml of suspension.

In order to study the effects of different physical factors on the growth and the chemical composition of microalgal cells, various optical ranges were used: a fluorescent lamp (FL) with an intensity of 2800 Lux separately and in combination with a laser radiation

(LR), ultraviolet (UV) and infrared (IR) radiation. The LR was produced by a red laser LGN 208 V with a nominal output power of 1.6 mW and a wavelength of 0.63 μm. In previous studies, it was shown that scattering a laser beam up to 5 cm diameter accelerates the growth of biomass [17, 18]. In the present study, a telescope was used to create such a beam on the object. The radiation density was maintained at 0.3 W/m<sup>2</sup>, and the illumination was at a 40 Lux intensity, respectively. The distance between the laser and the telescope (angular magnification of 30x) was 2.1 m, and between the telescope and the sample: 0.1 m. The cultivation was carried out at a temperature of 21.0 °C with a drift of ± 1.0 °C.

The source of IR (thermal) radiation was an IRPC lamp operating under the following conditions: voltage 220 V, power 250 W, intensity 14100 Lux. The temperature during the exposure increased to (28.0 ± 2.0) °C. The UV radiation was provided by the OUFД-01 lamp (wavelength: 280-315 nm), which was switched on for 3 hours on the first day of cultivation; and later, the process was carried out using FL at a fixed temperature (21.0 ± 1.0) °C [19, 20].

After being exposed to the different physical factors, the *Chlorella sorokiniana* biomass was concentrated by centrifugation at 6000 rpm for 10 min. After removal of the supernatant, the precipitate was subjected to freeze-drying and the concentration of valuable components in the resulting biomass was determined. In particular, the protein content in the dry biomass of *Chlorella sorokiniana* was determined by the Bradford test in the presence of Coomassie Brilliant Blue G-250 at λ = 595 nm. The calibration curve was constructed using standard albumin solutions (fraction V (volume), concentrations of 0.05; 0.10; 0.25; 0.50 and 1.00 g/l).

Saccharides were determined according to the method of Dubois (1956) at wavelengths λ = 480 nm and 490 nm using calibration curves for alginate and dextran solutions, respectively.

The pigment content in the system was assessed by the adapted Sumanta method (2014) at λ = 470, 649 and 664 nm. The concentrations of chlorophylls *a* and *b* and carotenoids were calculated:

$$Ch\ a = 13.36 A_{664} - 5.19 A_{649}, \quad (1)$$

$$Ch\ b = 27.43 A_{649} - 8.12 A_{664}, \quad (2)$$

$Cx + c = (1000 A_{470} - 2.13 Ch\ a - 97.63 Ch\ b)/209$ , (3) where: *Ch a* and *Ch b* – concentrations of chlorophyll *a* and chlorophyll *b*, respectively, μg / ml; *Cx + c* – concentration of carotenoids, μg/ml; *A*<sub>470</sub>, *A*<sub>649</sub>, *A*<sub>664</sub> – optical densities at wavelengths of 470, 649 and 664 nm.

The mass indices of the lipids isolated from microalgae were extracted by the Soxhlet method, and

the fatty acid composition of the samples exposed to the IR was determined by gas chromatography on an Agilent 7820AF instrument (GOST 32915-2014).

## RESULTS AND DISCUSSION

As can be seen from the data presented in Tab. 1, the laser and ultraviolet light can have an influence on the formation of additional protein structures in microalgae, but their stability is still not high enough (denaturation and change in their natural properties are possible). In contrast, a high concentration of proteins was recorded in the biomass obtained by exposure to FL and IR irradiation (46.0% and 48.4% of dry biomass respectively, Tab. 1). It should be assumed that the protein content in such systems is associated, first, with the enzymatic activity. Only due to their inclusion in the biotechnological process that high rate of reproduction and metabolism can be achieved (enzymes must be present in the system at a fixed concentration). Moreover, additional information imply that daylight and IR irradiation of the samples also provide a biomass with a high lipid content (77 and 81 mg/g, respectively, which is 45-53% more than in the absence of PF). The chromatographic analysis also established that the fatty acid composition of the biomass obtained by thermal exposure to the infrared range (see Fig. 2) is represented mainly by polyunsaturated acids (77% by weight) with predominance of oleic and linoleic acids. As for the main saturated fatty acids obtained in the studied system, palmitic and stearic acids increased under the conditions of long-wave radiation (IR) from 16 to 23%. Probably, cells of *Chlorella sorokiniana* realize the desire to harden the lipid shell, thus demonstrating a primary protective reaction to the activation of the thermal factor.

**Table 1**  
**The nitrogen-containing compounds and protein contents in *C. sorokiniana* biomass under different cultivation conditions**

**Таблица 1. Содержание азотсодержащих соединений и белков в биомассе на основе *C. sorokiniana* при различных условиях культивирования**

№ of sample	Type of PF in cultivation of <i>C. sorokiniana</i>	Content of total nitrogen, mg/g	Protein content, mg/g dry biomass	Protein content, % of dry biomass
1	LR	61.70	367.12	36.71
2	PL	77.27	459.76	45.98
3	IR	81.30	483.74	48.37
4	UV	59.52	354.14	35.41
5	without PF*	52.98	315.23	31.52

Note: without physical effects

Примечание: \*без физических воздействий

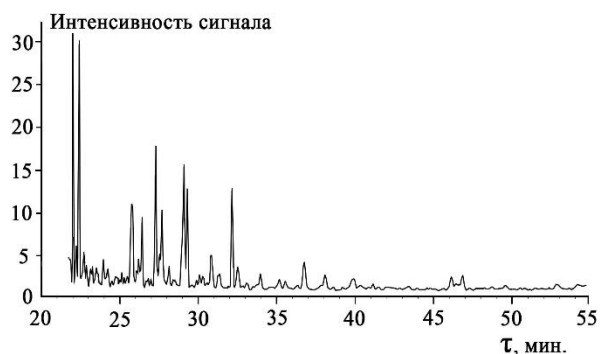


Fig. 2. Chromatogram of a lipid sample obtained from the microalgae *C. Sorokiniana* exposed to infrared light

Рис. 2. Хроматограмма для образца микроводорослей *C. Sorokiniana*, облученной инфракрасным светом

The formation of carbohydrates in the process of photosynthesis is interrelated with parameters such as the light intensity, exposure, temperature, concentration of carbon dioxide, etc. Carbohydrates in the system perform various functions, among which are energetic, storage, and protective functions. In this case, an increase in the concentration of polysaccharides is a sign of an aging cell, and the depletion of the latter indicates a decrease in total carbohydrates. At intensive reproduction, the quantity of sugars can also decrease, as they are involved for the formation of autospores. The data on the carbohydrates content in *C. sorokiniana* biomass obtained after different types of PF are presented in Tab. 2. According to the results of the analysis given in Tab. 2, an increase in the total carbohydrates (43%) characterizes the sample of biomass cultured by UV exposure (sample 4). It is not excluded that the achieved result is a response to ultraviolet radiation. In dry biomass, previously exposed to LR (Tab. 2, sample 1), a tendency to increase the overall concentration of carbohydrates is also observed (from 33 to 39%, or 180.3 mg/g).

Table 2

The carbohydrate content obtained from the biomass of the microalgae *C. sorokiniana*, formed under various types of physical influences

Таблица 2. Найденные углеводы в биомассе микроводорослей *C. sorokiniana*, сформированной при различных видах физических воздействий

№ of sample	Type of PF in cultivation of <i>C. sorokiniana</i>	Total content		Monosaccharides, mg/g	Oligo-, polysaccharides, mg/g
		%	mg/g		
1	LR	39.04	390.40	180.25	210.15
2	PL	37.26	372.57	170.30	202.27
3	IR	34.03	340.31	140.22	200.09
4	UV	43.03	430.27	190.16	240.11
5	without PF	33.11	331.14	130.90	200.24

In turn, the analysis of the data (presented in Tab. 2) shows that in sample 3 (obtained by IR exposure), "old cells" prevail; the biomass composition includes, mostly, polysaccharides, and the total amount of monosaccharides in it is 34% by weight. The sample without PF (sample 5) leads, with a high probability, to cell depletion. In this last case, the risk of the influence of extraneous microflora, competing for the consumption of carbohydrates, highly increases. In such a biomass, the concentration of monosaccharides is 8 to 38% lower in comparison with the other samples (Tab. 2), and the populations are characterized by the predominance of "old cells".

It should be assumed that pigments in the studied systems are responsible for the intensity of anabolic processes in chlorella cells, and information on the ratio of chlorophyll concentrations allows one to evaluate the stress conditions in which the microalgae was put through. A relatively high pigment content (29.2 mg/g) was found in the chlorella sample exposed to daylight, as well as in the samples exposed to UV and IR radiation (comparable values: 28.4 and 27.6 mg/g, see Tab. 3), which indicates an active flow of photosynthesis in the cells.

Table 3

Pigment composition of biomass from microalgae *C. sorokiniana* under different cultivation conditions

Таблица 3. Пигментный состав биомассы микроводорослей *C. sorokiniana* при различных условиях культивирования

№ of sample	Type of PF	Total content		Ch. a, mg/g	Ch. b, mh/g	Carotenoids, mg/g	Ch. a/Ch. b
		%	mg/g				
1	LR	2.40	23.98	15.40	6.08	2.50	2.53
2	PL	2.92	29.25	19.32	7.11	2.82	2.72
3	IR	2.76	27.59	18.87	6.45	2.27	2.93
4	UV	2.84	28.36	17.61	7.94	2.81	2.22
5	without PF	2.34	23.40	14.39	6.78	2.23	2.12

In sample 2, in the sampled exposed to FL, intensive population growth was observed. However, it can be clearly seen that laser radiation as well as the absence of any PF negatively affected the synthesis of common chlorophylls (21 mg/g in both cases). In the samples exposed to UV and IR lights, common chlorophylls were detected much more ( $\approx 20\%$ ). The most significant concentrations of chlorophylls a are observed in biomass obtained in daylight (19.3 mg/g). On the contrary, a small amount of chlorophylls a (14.4 mg/g) corresponds to an experiment in which PV was absent altogether, which should be attributed to the stress effect of low illumination on *C. Sorokiniana* during cultivation.

## CONCLUSIONS

In this way, it was found that an increased content of chlorophylls *a* (19.3 mg/g) and in (7.1 mg/g) and total carotenoids (2.8 mg / g) characterizes the biomass based on *C. sorokiniana* grown in daylight conditions. The influence of ultraviolet on these microalgae favor the accumulation of common carbohydrates in the dry product (430 mg/g) while an infrared radiation provides the highest number of protein compounds (484 mg/g) and lipids (81 mg/g).

The data obtained from this study give the possibility to select optimal conditions for the cultivation of *Chlorella sorokiniana* in order to obtain a biomass with a given composition and properties.

*The research was carried out within the framework of the implementation of the Federal Target Program "Research and Development in Priority Areas for the Development of the Russian Science and Technology Complex for 2014-2020" on the theme of the project "Development and Implementation of Innovative Biotechnology for Processing Chlorella sorokiniana microalgae and Lemna minor duckweed" (AGREEMENT No. 14.587. 21.0038, dated July 17, 2017); the unique identifier of the project RFMEFI58717X0038.*

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Поступила в редакцию 26.03.2018

Принята к опубликованию 18.12.2018

Received 26.03.2018

Accepted 18.12.2018