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

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The paper presents data on the actual issue ofthe usage of the microalgae Chlorella sorokiniana in the biotechnological field. Its vitamin and amino acid contents, which arehigher than in soy, enriches its nutritional value.This article also provides information on the utilization of chlorella as an enrichment supplement to develop a modern Life Support Systemfor 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 ofa 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 microalgalsamples are presented. The most significant concentrationsof protein compounds (46.0% and 48.4% of dry biomass) were obtained after the exposure to daylightand 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 daylightshowed 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 sorokinianain order to obtain a biomass with a given composition and properties.

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

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 keto-carotenoid 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 [6-8]. The relationship between biomass yield and urea content in wastewater [9] used for microalgae cultivation has been studied. It has also been shown thatamount of proteins and carbohydrates in biomass increases in a nutrient medium low in sulfur and nitrogen [10]. Moreover, it has been established experimentallythat physical factors (PF), including light, applied in different wave ranges, exert a significant influence on the cultivation rate and the composition of the biomass ofduckweed and Eichhornia [11-15].

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 highcontent of valuable nutrients.

EXPERIMENTAL PART

The cultivation of the microalgae *Chlorella sorokiniana* took place in a photobioreactor, which is a cylindrical glass vessel (Figure 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 minutes of stirring with an intensity of 300-500 min^{-1}, then 120 minin rest mode. A compressor model AP-001 (intensity 1.5 l/min) was used for aeration.

The preparation of the culture medium containing the necessary set of macro- and microelements is described in the articles [16, 17]. Algal population growth was monitored by measuring the optical density values using a UNICO 1208 spectrophotometer at a750 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 208V with a nominal output power of 1.6 mW and a wavelength of 0.63μm. In previous studies, it was shown that scattering a laser beam up to 5 cmdiameter accelerates the growth of biomass [16, 17]. 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^2, 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 OUFD-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.

After being exposed to the different physical factors, the *Chlorella sorokiniana* biomass was concentrated by centrifugation at 6000 rpm for 10 minutes. 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:

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

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

$$Cx{+}c = (1000\ A_{470} - 2.13\ Ch\ a - 97.63\ Ch\ b)/209, \quad (3)$$

where:

Ch a and *Chb* – concentrations of chlorophyll a and chlorophyll b, respectively, μg / ml;

C x + c – concentration of carotenoids, μg/ml;

A_{470}, A_{649}, A_{664} – optical densities at wavelengths of 470, 649 and 664 nm.

The mass indices of the lipids isolated from microalgae were extracted by the Soxlet 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 Table 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 changein 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, Table 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 biotechnologicalprocess 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 Figure 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.

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 thecarbohydrates content in *C. sorokiniana* biomass obtained after different types of PF are presented in Table 2. According to the results of the analysis given in Table. 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 (Table 2, sample 1), a tendency to increase the overall concentration of carbohydrates is also observed (from 33 to 39%, or 180.3 mg/ g).

In turn, the analysis of the data (presented in Table 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 (Table 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 toUV and IR radiation (comparable values: 28.4 and 27.6 mg/g, see Table 3), which indicates an active flow of photosynthesis in the cells.

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 (≈ 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 anincreased 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 carbohydratesin 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.

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STUDY OF THE CHEMICAL COMPOSITION AND PROPERTIES OF THE BIOMASS OF *CHLORELLA SOROKINIANA* UNDER THE INFLUENCE OF DIFFERENT PHYSICAL ACTIONS

Table 1.

The nitrogen-containing compounds and proteincontents in *C. sorokiniana* biomass under different cultivation conditions

№ of sample	Type of PF in cultivation of *C. sorokiniana*	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

**without Physical Factors (PF)*

Table 2.

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

№ of sample	Type of PF in cultivation of *C. sorokiniana*	Total content		Monosaccharides, mg/g	Oligo-, poly-saccharides, 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

Table 3.

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

№ 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

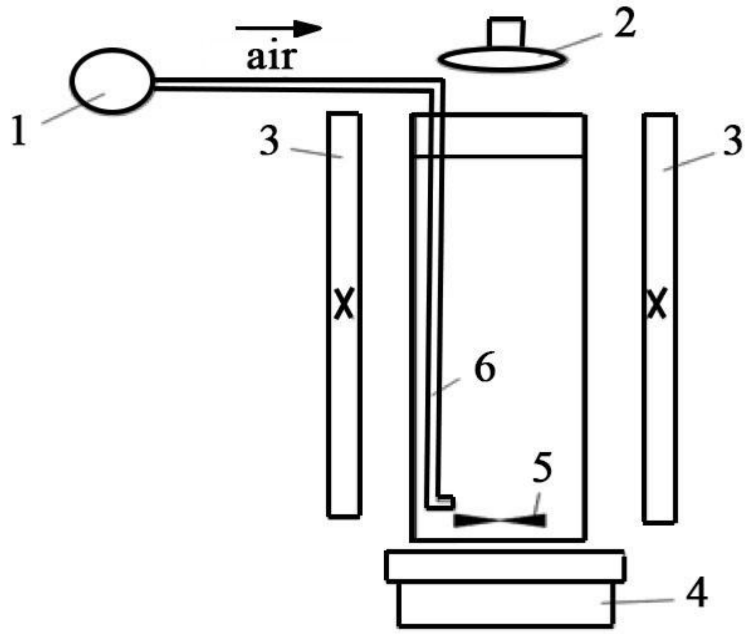

Fig.1. Schema of the photoreactor used for the cultivation of *Chlorella sorokiniana*: 1 – pump-aerator; 2 – source of radiation (laser, IR, UV); 3 – phosphorescent lamps; 4 – magnetic stirrer; 5 – anchor agitator; 6 – air supply tube

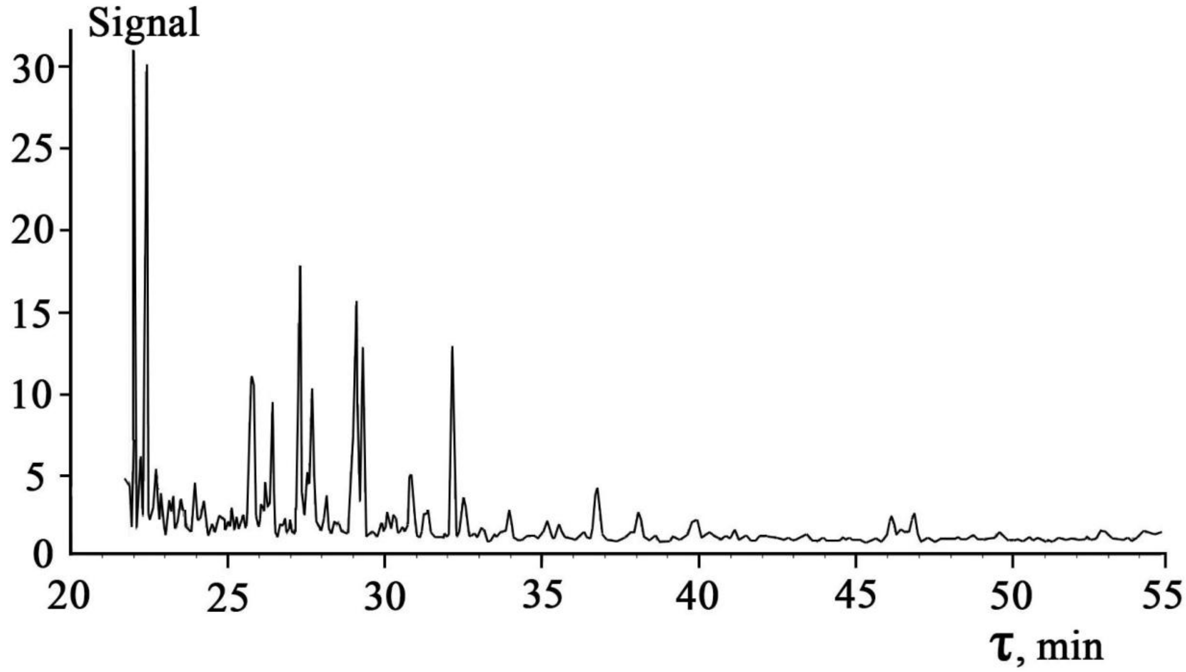

Fig. 2. Chromatogram of a lipid sample obtained from the microalgae *C. Sorokiniana* exposed to infrared light

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