**УДК 606**

**OPTIMIZATION OF THE CARBOHYDRATES BIOACCUMULATION PATTERNS IN CHLORELLA SOROKINIANA MICROALGAE**

Toumi A., Smyatskaya J. A., Trukhina E. V.

*Abstracts:*

*Microalgae are great candidates for the food, feed and aquaculture industries. Chlorella is one of the most studied microalgae regarding its potential use for human and animal consumption. This microorganism can cumulate high amounts of valuable components such as lipids, proteins and pigments. The purpose of this study is to select the optimal cultivation conditions for Chlorella sorokiniana in order to obtain a biomass with a higher carbohydrate content.*

*Key words: Chlorella sorokiniana, carbohydrates, cultivation, dietary industry.*

**Introduction:**

Microalgae are photosynthetic microorganisms that can offer a variety of advantages that could be exploited in several important areas [1]. For this reason, scientists and industrials have been more and more interested in these organisms in the last few decades.

It seems important to add that the production of microalgae, although requiring aqueous media, consumes less water than terrestrial crops. Microalgae can also be grown on infertile lands [2], which may significantly reduce their environmental impact.

Chlorella is one of the most studied microalgae. Its high protein and nutrient content make it interesting for the dietary industry, i.e. for the production of food additives (such as natural food stains), dietary supplements, and feed for farming and aquaculture [3].

In addition, under specific cultivation conditions (for example, under stress conditions), Chlorella can increase its carbohydrate, lipid and protein contents [4].

In Malaysia and the Philippines, more than 500 tons of chlorella per year are used for human consumption [5]. Russian scientists have developed a technology for making dry algae of chlorella the production of combined dairy products [6].

Chlorella sorokiniana stands out from the other microalgae species. Its rapid growth rates and high photosynthetic efficiency [7] make it a suitable candidate for the production of dietary supplements and even biofuels. Several studies in progress aim to evaluate the best cultivation conditions and the influence of different physical factors in order to produce a Chlorella biomass with higher nutritional values.

The purpose of the present study is to select the optimal conditions for growing a Chlorella sorokiniana biomass with higher carbohydrate contents.

**Experimental part:**

**Material and methods:**

Microalgae as other autotrophic organisms produce carbohydrates during photosynthesis. The carbohydrate formation will therefore depend heavily on the supplied light intensity but also on the temperature, the carbon dioxide concentrations and the composition of the cultivation medium (micro and macroelements).

Carbohydrates fulfill several important functions in living organisms: it is a source of energy, and a form of storing and protection. An increase in polysaccharide concentration is a sign of cellular aging. On the other hand, a drop in the concentration of these is a sign of cellular exhaustion. During intensive division, the amounts of carbohydrates can also decrease as they are used for the formation of autospores.

For this study, the culture was carried out in a photobioreactor (Figure 1), which is a cylindrical glass vessel of 380 mm height, 50 mm diameter, in a volume of 500 ml. The photobioreactor was set under a "day-night" regime with a periodic agitation: stirring time = 15 minutes, resting time (without stirring) = 120 minutes. The stirring speed was set at 500 revolutions per minute (rpm). The aeration was carried out by a compressor (model AP-001) at a flow rate of 1.5 l / min. The nutrient medium used was prepared in a conventional way and contains all the required macro- and microelements described in previous studies [8], [9], [10].



*Figure 1 - Scheme of the photobioreactor:
1 - pump-aerator, 2 - radiation source (IR, UV) 3 - fluorescent lamps, 4 - magnetic stirrer, 5- anchor of magnetic stirrer, 6 - air supply tube.*

Population growth was monitored by measuring the optical density ​​(using a UNICO 1208 spectrophotometer, at a wavelength of 750 nm) and by further counting on the Goriaev chamber by enumerating the number of cells in 1 ml of the cell suspension. The morphological study of the Chlorella sorokiniana cells was performed by microscopy in intravital preparations with a magnification of 640 times.

Radiation source during Laser Radiation (LR): A red laser was used in LGN 208B in a continuous mode with a nominal output power of 1.6 mW and a wavelength of 0.63 micrometers. The use of a telescope made it possible to obtain a cylindrical light beam of 5 cm diameter on the object. The power density of the radiation at the installation was 0.3 W / m2, lighting 40 Lux. The distance between the laser and the 30x angular magnification telescope was 2.1 m, and the distance between the telescope and the sample was 0.1 m.

After exposure to LR for 15 minutes, a significant increase in the number of cells was observed. The cells were characterized by a large vacuole, a thickened lipid shell, a large accumulation of metabolites, and the cells were prone to agglutination.

For cultivation of microalgae under ordinary conditions (daylight), the light intensity was 2800 Lux and the temperature was 21.0 ± 1.0 ° C;

Infrared radiation (IR) or "thermal radiation" was realised using an IKZK infrared lamp at a voltage of 220 V, a power of 250 W, and an intensity of 14100 Lux. The temperature reached 28.0 ± 2.0 ° C due to the infrared radiation;

For ultraviolet (UV) radiation, an OUFD UV lamp 01 (wavelength 280-315 nm) was used for 3 hours on the first day of cultivation.

In addition, the culture was carried out under a fluorescent light (FL) of 2800 Lux intensity at a temperature of 21.0 ± 10 ° C;

Sample Preparation Concentration of the biomass: Chlorella sorokiniana suspensions obtained under various physical factors were concentrated by centrifugation at 6000 rpm for 10 minutes. The supernatant was removed using a syringe. The pellet was subjected to IR drying (T ° = 37 ° C) to constant weight and freeze-dried. (Figure 2)

*Figure 2 – Scheme of sample preparation*

In the obtained dry biomass, the content of carbohydrate contents were determined using the equipment of the laboratory of the Hamburg University of Technology.

The procedure for determining carbohydrates was carried out in accordance with the Dubois method: 50 mg of lyophilized microalgae powder was resuspended in 10 ml of distilled water using a high-speed homogenizer. The resulting suspension was filtered using a 0.45 μm syringe filter. An aliquot of the sample of 1 ml was mixed with 1 ml of a 5% solution of phenol and 5 ml of concentrated H2SO4. The resulting solution was immediately mixed and incubated at 30 ° C for 30 minutes. After the level of the obtained solution was adjusted to 50 ml with distilled water, a photometric determination was made at 480 and 490 nm for acidic and neutral carbohydrates.

The quantitative characteristics of acidic and neutral saccharides were carried out using calibration plots for alginate and dextran starting solutions, respectively.

**Results:**

*Table 1*

**Carbohydrate content in Chlorella Sorokiniana biomass under different cultivation conditions**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **№** | **Physical factors** | **Carbohydrate (total content)** | **Monosaccharides, mg/g** | **Oligo, polysaccharides, mg/g** |
| **%** | **mg/g** |
| 1 | Laser radiation (LR) | 39,04 | 390,40 | 180,25 | 210,15 |
| 2 | Fluorescente light (FL) | 37,26 | 372,57 | 170,30 | 202,27 |
| 3 | Infrared light (IR) | 34,03 | 340,31 | 140,22 | 200,09 |
| **4** | **Ultraviolet light (UV)** | **43,03** | **430,27** | **190,16** | **240,11** |
| 5 | Control sample (Day light) | 33,11 | 331,14 | 130,90 | 200,24 |

Based on the results described in the table above, the highest carbohydrate rates (total content) was measured in the sample of biomass grown under the influence of UV light. This could be the result of a protective reaction of the cells in response to the UV radiation.

The biomass obtained after laser exposure also showed a tendency to increase the overall concentration of carbohydrates.

Under IR light, biomass was characterized by the presence of "old cells" and contained more polysaccharides than monosaccharides.

In the control sample, there is a possible cell depletion. The development of a foreign microflora competing with Chlorella sorokiniana for the consumption of carbohydrates could be observed. In this biomass, there was a low content of monosaccharides: these populations have a predominant number of "old cells".

**Conclusion:**

This ongoing study will determine the optimization factors of Chlorella sorokiniana culture and the preliminary results presented above are encouraging.

This research was carried out within the framework of the federal program "Research and development in the priority areas for the development of the scientific and technological complex of Russia 2014-2020" on the theme "Development and implementation of bio- innovative technologies for the treatment of microalgae Chlorella sorokiniana and Lemna minor. " .21.0038). The unique identifier of the RFMEFI58717X0038 project.

**BIBLIOGRAPHY**

1. Imen Hamed The Evolution and Versatility of MicroalgalBiotechnology: A Review, Comprehensive Reviews in Food Science and Food Safety, Vol.15. 2016. P 1104.

2. Microalgal Cultivation in Secondary Effluent: Recent Developments and Future Work./ J. Lv, J. Feng, Q. Liu, & S. Xie // International Journal of Molecular Sciences 2017, T. 18, N° 1, P 79.

3. Abdo S. M., Ali G. H., F.K. El-Baz F.K. Potential Production of Omega Fatty Acids from Microalgae// International Journal of Pharmaceutical Sciences Review and Research, 2015, T. 34, N°2, P 210-215.

4. Chlorella for protein and biofuels: from strain selection to outdoor cultivation in a Green Wall Panel photobioreactor/ A. Guccione et Al. //Biotechnology for Biofuels. 2014, T. 7, P 84.

5. Wong K.H., Cheung P.C.K. Nutritional evaluation of some subtropical red and green seaweeds: Part I — proximate composition, amino acid profiles and some physico-chemical properties // Food Chemistry. - 2000. - V. 71, Issue 4. - P. 475-482.

6. Попов К. С., Арапов В. М., Полянский К. К. Хлорелла - перспективный ингредиент // Молочная промышленность. - 2009. - N 7. - С. 59-60. - Библиогр.: с. 60 (9 назв.). - ISSN 0026-9026

7. Belkoura M., Dauta A., Caractéristiques photosynthétiques de Chlorella sorokiniana Shihira & Krauss, en relation avec l'intensité lumineuse et la température. Corrélation avec le taux de croissance// Annls Limnol 1994. T. 30, N° 1, P. 3-9.

8. Еffect of laser radiation on the cultivation rate of themicroalga Chlorella sorokiniana as a source of biofuel/ N.A. Politaeva, Yu..A. Smyatskaya, V.V. Slugin, A. Toumi, M. Bouabdelli// Conf. Series: Earth and Environmental Science. 2018. P. 22-29.

9. Politaeva N.A., Bazarnova J.G., Boguk S.G. Innovative technologies secondary use of processed active source // Proceedings of the 2017 International Conference "Quality Management, Transport and Information Security, Information Technologies", IT and QM and IS 2017.- 2017. - № 8085865, P. 471-476.

10. Культивирование и использование микроводорослей Chlorella и высших водных растений ряска Lemnа / Политаева Н.А., Смятская Ю.А., Кузнецова Т.А., Ольшанская Л.Н., Валиев Р.Ш. // Саратов: ИЦ «Наука». 2017. С. 125.