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Influence of Culture Conditions on the Growth and Fatty Acid Composition of Green Microalgae *Oocystis rhomboideus*, *Scenedesmus obliquus*, *Dictyochlorella globosa*

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***Abstract*—**Microalgae due to the ability to accumulate high levels of practically valuable polyunsaturated fatty acids attract attention as a promising raw material for commercial products. The features of the growth processes of cells green protococcal microalgae *Oocystis rhomboideus*, *Scenedesmus obliquus*, *Dictyochlorella globosa* at cultivation in different nutritional mediums were determined. For the rapid accumulation of biomass, combined with high productivity of total lipids fraction yield recommended to use the Fitzgerald medium (*Scenodesmus obliquus*, *Oocystis rhomboideus*) and/or Bold medium (*Dictyochlorella globosa*). Productivity of lipids decreased in sequence *Dictyochlorella globosa* > *Scenodesmus obliquus* > *Oocystis rhomboideus*. The bulk of fatty acids fraction of the total lipids is unsaturated fatty acids, which accounts for 70 to 83% of the total number of fatty acids. The share of monoenic acids accounts from 18 to 34%, while the share of unsaturated fatty acids - from 44 to 62% of the total number of unsaturated fatty acids fraction. Among the unsaturated acids dominate α-linolenic acid (C18:3n-3), hexadecatetraenic acid (C16:4) and linoleic acid (C18:2).

***Keywords*—**Fatty acids, lipids, microalgae.

I. Introduction

IN recent decades, growing interest to therapeutic potential of polyunsaturated fatty acids (PUFAS), which reflects trends in pharmacology, to find a «physiological» regulators and modulators of the complex biochemical systems. It is known that polyunsaturated fatty acids are essential for the growth and development of the body and plays an important role in the prevention and treatment of ischemic heart disease, hypertension, diabetes, rheumatoid arthritis and other inflammatory and immune diseases and cancer [1], [2]. Microalgae due to their ability to accumulate high level of PUFAS are attracting attention as a promising raw material for obtaining a number of commercial products [3]-[6]. It is known that the level of specific fatty acids depends on the physiological state of the culture associated with the phases of development, the intensity of growth, lipogenesis, conditions of cultivation, as well as the composition of the medium [7]-[9]. One of the biotechnological techniques to improve the content of lipids and practically valuable PUFAS in microalgae biomass, is the optimization of the conditions of their cultivation. Varying the cultivation conditions it is possible to change the physiological state of microalgae, configure their metabolism for synthesis of substances necessary for adaptation to new conditions. Thus, varying the cultivation conditions, we can find the optimal conditions for the accumulation of certain groups of lipids and fatty acids.

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II. Materials And Methods

The objects of study are three species of green algae (*Oocystis rhomboideus, Scenedesmus obliquus, Dictyochlorella globosa*) from the collection of the laboratory of the Institute of ecology problems of Al-Farabi KazNU, characterized the stable growth in laboratory conditions and a high content of lipids by the results of previous studies [10].

Algae cultivated in the accumulation mode, 16-hour photoperiod. Illumination on the surface of the solution was 120 $uM \cdot m^{-2} \cdot s^{-1}$. Medium temperature ranged 22-25°C. Fitzgerald medium, Bold Basal medium, Chu-10 medium, Elenkin medium, C medium, Gindak medium were used for cultivating of microalgae [11]. Volume of the medium in the flasks was 0.35 l at a height of 18 cm layer of solution. The dynamics of the biomass at the beginning and end of the experiment was determined by dry weight of inoculum and its culture, as well as an optical density of the culture in the first day (Df), and in the next day (Dn) at 750 nm. The increase of the cells concentration was determined by the method of direct calculation.

To determine the intensity of microalgae growth used Growth index (GI), which is calculated by the formula [12]:

$$GI=(Dn-Df)/Df \quad (1)$$

For the transition from the optical density units to absolutely dry weight (ADW) used the formula [12]:

$$ADW = 0.78 \bullet D_{750} \quad (2)$$

Relative growth rate (μ) was determined by the formula [12]:

$$\mu = \frac{\ln x_2 - \ln x_1}{t_2 - t_1}, \quad (3)$$

where x_2 and x_1 - biomass in the time t_2 and t_1, respectively.

Doubling time (d.t.) of cells' population was calculated by the formula [12]:

$$d.t. = \frac{\ln 2}{\mu} = \frac{0.693}{\mu} \cdot \quad (4)$$

Lipid extraction from microalgae cells was carried out according to modified method of Chi et al. [13].

Chromatographic separation of fatty acid esters was carried out at the gas chromatograph GC-2010 Plus, Shimadzu, Japan. Heptadecanic acid was added as the internal standard.

Esters of fatty acids have been identified according to the results of co-chromatography with authentic standards (Sigma Co., St Louis). Peaks, the area of which amounted to less than 0.1% of the total area of peaks, presented at the chromatogram in further analysis of the results were not taken into account [14].

III. Results and Discussion

6 nutrient mediums - Fitzgerald medium, Bold Basal medium, Chu-10 medium, Elenkin medium, C medium, Gindak medium were recommended for cultivation of green algae *D. globosa, O. rhomboideus, Sc. obliquus* (Table I) [11]. Mediums varied by composition and concentrations of various macro - and microelements, including the content of nitrogen and phosphorus, which primarily determine the microalgae growth characteristics.

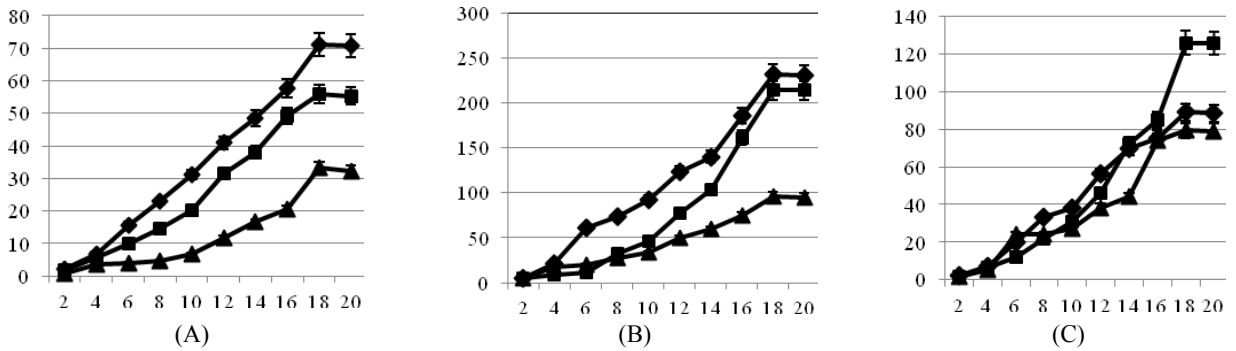
The initial density of inoculum in all variants of experience amounted to 1 $x10^6$ cells/ml. For all the studied mediums, except Gindak medium, used in ¼ dilutions, observed the growth of microalgae cultures. All the investigated strains of microalgae have been growing actively on the Fitzgerald medium. For example, in *D. globosa* after 10 days cumulative cultivation number of cells increased by 147 times and amounted 147.04 $x10^6$ cells / ml, for culture *Sc. obliquus* this value was 66.24 x 10^6 cells / ml.

After 10 days of cultivation intensity of the growth of the culture *D. globosa* and *O. rhomboideus* decreased on the mediums Fitzgerald > BBM > Elenkin > C > Chu-10 , *Sc. obliquus* - Fitzgerald > Elenkin > BBM > C > Chu-10.

The twentieth days of culture growth of *D. globosa* on the Fitzgerald medium has demonstrated high value of growth index, which is 1.3 times higher than that of culture growth on the BBM and was 2 times higher than the growth of the index that characterized the growth culture on Elenkin medium (Fig. 1). The growth indexes of *O. rhomboideus* culture characterized by close values on the Fitzgerald medium and BBM, while the growth index on the Elenkin medium was two times lower. In the culture of *S. obliquus* to 20th-day maximum of growth index was obtained on the BBM.

TABLE I
Number of Green Protococcal Microalgae Cells Growing on the Various Nutrient Mediums, after 10 Days of Cultivation

Species of microalgae	Number of cells per ml of medium					
	Fitzgerald medium	Elenkin medium	Bold Basal medium	C medium	Chu-10 medium	Gindak medium (¼)
D. globosa	147.0x10^6	109.2 x10^6	116.3 x10^6	54.28 x10^6	29.6 x10^6	0
O. rhomboideus	106.5 x10^6	33.42 x10^6	63.14 x10^6	12.6 x10^6	2.9 x10^6	0
Sc. obliquus	66.24 x10^6	44.72 x10^6	28.76 x10^6	13.12 x10^6	1.9 x10^6	0
Number of cells in the beginning 1 x10^6						



(A) (B) (C)

The horizontal axis - the time of cultivation (day), the ordinate axis - values of the growth index (conventional units),
-♦- Fitzgerald medium, -■- Bold Basal medium, -▲- Elenkin medium

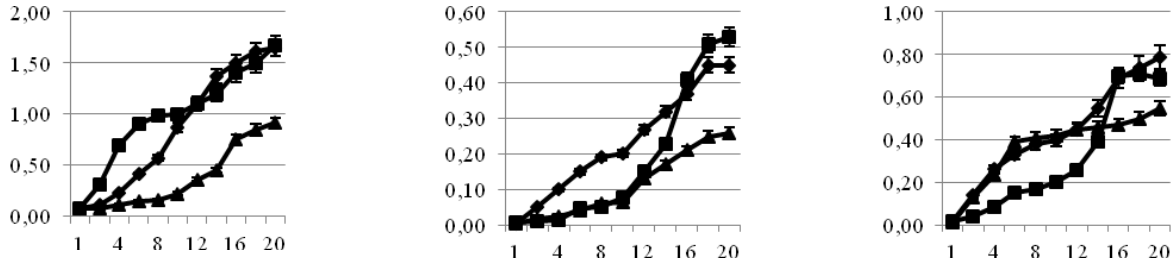
Fig. 1 The growth index of the culture *Dictyochlorella globosa* (A), *Oocystis rhomboideus* (B), *Scenedesmus obliquus* (C) at cultivation on various mediums

TABLE II
GROWTH CHARACTERISTICS OF GREEN MICROALGAE ON THE 20TH DAY OF CULTIVATION ON DIFFERENT NUTRIENT MEDIUMS

	Relative growth rate, per day			Doubling time, per day		
Species of microalgae	Fitzgerald medium	Bold Basal medium	Elenkin medium	Fitzgerald medium	Bold Basal medium	Elenkin medium
D. globosa	0.17	0.17	0.13	4.17	4.15	5.13
O. rhomboideus	0.25	0.24	0.21	2.79	2.94	3.33
S. obliquus	0.19	0.18	0.21	3.28	3.72	3.29

The growth indexes of cultures grown on Fitzgerald and Elenkin mediums were comparable and 1.5 times lower than those during the growth of culture on other medium. The maximum specific growth rate and the minimum time of doubling the cells of studied green microalgae cultures was noted at cultivation on the Fitzgerald medium (Table II).

Figures obtained by the growth of cells on the Bold medium take place an intermediate position between those obtained on the Fitzgerald and Elenkin mediums. Biomass yield, estimated by absolute dry weight of culture obtained from 1 litre of suspension to the 20th day of cultivation, testified that cultures *D. globosa* and *O. rhomboideus* grow effectively on the Fitzgerald and BBM mediums. Biomass of microalgae grown on the Elenkin medium was considerably lower (Fig. 2). Biomass of *D. globosa* culture with growth on the Fitzgerald medium did not differ from biomass culture grown on the Bold medium.

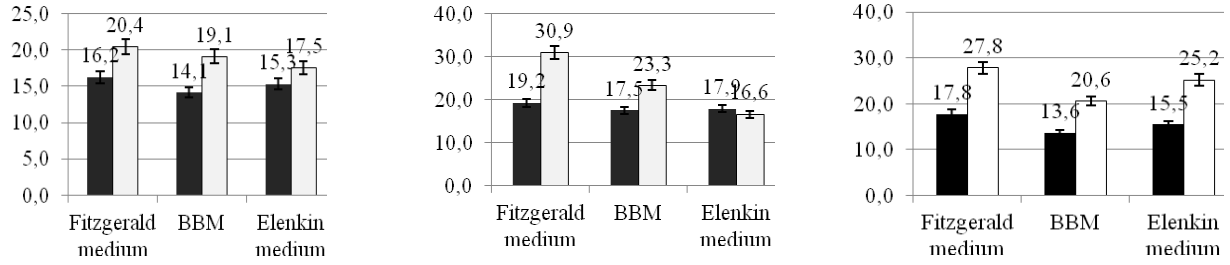


(A) (B) (C)

The horizontal axis - the time of cultivation (day), the ordinate axis - values of the dry weight (g · l^{-1}),
-♦- Fitzgerald medium, -■- Bold Basal medium, -▲- Elenkin medium

Fig. 2 Dynamics of biomass accumulation (absolute dry weight) of culture *Dictyochlorella globosa* (A), *Oocystis rhomboideus* (B), *Scenedesmus obliquus* (C) at cultivation on various mediums

According to the obtained data the most lipid weight calculated on the dry weight were characterized by algae growing on Fitzgerald medium. Content of lipids extracted from culture *D. globosa* grown on BBM and Fitzgerald medium was equal (Fig. 3), but in culture *Sc. obliquus* lipid content with growth on Fitzgerald medium was not significantly different from the lipid content in the growth culture on the Elenkin medium.



(A) (B) (C)

The horizontal axis - the time of cultivation (day), the ordinate axis - values of the lipids content (g · 100 g dry weight^{-1}),
white – on 5th days, black – on 20th days

Fig. 3 The content of total lipids of culture *Dictyochlorella globosa* (A), *Oocystis rhomboideus* (B), *Scenedesmus obliquus* (C) in different terms of cultivation and mediums

Productivity yield of lipids measured as mg of lipids · litre^{-1} · day^{-1} presented in Table III. According to the indicator of lipids productivity cultures were located in the sequence *Dictyochlorella globosa* > *Scenedesmus obliquus* > *Oocystis rhomboideus*. However, *Dictyochlorella globosa* was characterized by close lipids productivity at growth on Fitzgerald mediums and BBM, whereas for *Oocystis rhomboideus* and *Scenodesmus obliquus* high productivity of

lipids were obtained during the growth of cells on the Fitzgerald mediums.

TABLE III
PRODUCTIVITY OF LIPIDS AT GROWING OF GREEN PROTOCOCCAL MICROALGAE AT VARIOUS MEDIUMS

Species of microalgae	Lipids productivity, mg · litre^{-1} · day^{-1}		
	Fitzgerald medium	Bold Basal medium	Elenkin medium
Dictyochlorella globosa	347.2	326.6	111.4
Oocystis rhomboideus	234.0	187.0	19.1
Scenedesmus obliquus	306.6	252.0	103.0

Thus, it was established that for a fast accumulation of biomass green protococcal microalgae, combined with high productivity yield of total lipids recommended to use the Fitzgerald medium (*Scenedesmus obliquus*, *Oocystis rhomboideus*) and/or BBM (*Dictyochlorella globosa*).

At growth on Fitzgerald, BBM and Elenkin mediums trends in the content of fatty acids in the fraction of the total lipids extracted from cells of microalgae are the same (Table IV). In the composition of fatty acids of the total lipids fraction present acids C14-C24 series. The bulk of the acids are unsaturated fatty acids, which accounts for 74 % to 83% of the total number of fatty acids. The ratio of polyunsaturated/saturated fatty acids (PUFAS/SFAS) for all cultures is highly. The share of monoenic acids accounts from 18 to 34 %, while the share of unsaturated fatty acids - from 44 to 62% of the total number of unsaturated fatty acids fraction. There is a tendency expressed the increase in the proportion of unsaturated fatty acids and decreasing proportions of saturated fatty acids at a fraction of the total lipids for all studied mediums.

Among of saturated fatty acids in all studied cultures at a fraction of the total lipids prevails palmitic acid.

Quantitative characteristics (% of the total content of fatty acids faction) of PUFAS of the cultures are in the following sequence: *Sc. obliquus* - C18:3n3 > C16:4 > C18:2 > C16:2 > C20:4n6 > C18:4; *O. rhomboideus* - C18:4 > C16:4 > C18:3n-3 > C16:2 > C20:4n6 > C20:3; *D. globosa* - C18:3n-3 > C16:4 > C18:2 > C16:3 > C16:2.

This results allow to conclude that among of the unsaturated acids dominates α-linolenic acid (C18:3n-3), hexadecatetraenic acid (C16:4) and linoleic acid (C18:2). By the physiological effect on the body linoleic acid is the main; its biological activity exceeds linolenic acid in 8-10 times. Linoleic, linolenic, hexadecadienic (C16:2) and hexadecatetraenic (C16:3) acids are essential and part of the vitamin F complex. It should be noted that linoleic acid, related to fatty acids family of n-6 (omega-6), is a structural component of cell membranes, regulates the metabolism of cholesterol, participates in the formation of tissue hormones, prostaglandins, is the biochemical precursor of linolenic and arachidonic acids. Under the influence of microelements, enzymes and vitamins in the body, it is converted into gamma-linolenic, from which synthesize prostaglandin E1, which, in turn, improves the immune system, reduces the risk of cardiovascular disease, reduces inflammatory processes, regulates the functioning of the brain and nervous system, normalizes the level of insulin metabolism. Linolenic acid belongs to fatty acids family of n-3 (omega-3), which are anti arrhythmic, hypocoagulating, anti-inflammatory and immunomodulatory effects. Inhibiting the activity of cyclooxygenase enzymes, lipoxygenase, protein kinases and phospholipases, which trigger tumor transformation, omega-3 fatty acids are a rather marked antitumor effect. Recent studies of hexadecadienic and hexadecatetraenic acids have shown their cytotoxicity to tumor cells [15]-[17].

TABLE IV
INFLUENCE OF NUTRIENT MEDIUM COMPOSITION ON THE PERCENTAGE OF FATTY ACIDS IN THE FRACTION OF TOTAL LIPIDS IN THE CULTURES OF GREEN MICROALGAE

Species of microalgae	Fatty acids	Fitzgerald medium		Bold Basal medium		Elenkin medium	
		Time of cultivation, day					
		5	20	5	20	5	20
Scenedesmus obliquus	saturated	22.4	20.0	21.4	18.1	21.1	23.0
	unsaturated	74.6	77.6	75.5	79.2	76.0	74.9
	polyenic	56.4	57.5	55.1	57.3	49.2	50.1
	monoenic	18.3	20.1	20.4	21.9	26.8	24.8
	not identified	3.0	2.4	3.1	2.7	2.9	2.1
	PUFAS/SFAS ratio	3.1	2.9	3.3	3.0	2.3	2.2
Oocystis rhomboideus	saturated	2.3	1.7	3.2	2.9	2.7	1.9
	unsaturated	74.5	81.1	76.5	77.8	74.9	78.8
	polyenic	44.1	46.7	46.3	47.9	45.1	47.9
	monoenic	30.4	34.4	30.2	29.9	29.8	30.9
	not identified	23.2	17.2	20.3	19.3	22.7	19.3
	PUFAS/SFAS ratio	19.3	28.0	14.5	16.5	16.7	25.2
Dictyochlorella globosa	saturated	15.5	13.5	12.3	10.9	13.8	14.1
	unsaturated	76.1	80.9	78.6	82.9	77.5	79.8
	polyenic	56.9	61.6	51.9	59.3	47.3	56.7
	monoenic	19.2	19.2	26.7	23.6	30.2	23.1
	not identified	8.3	5.6	9.1	6.2	8.7	6.1
	PUFAS/SFAS ratio	3.7	4.56	4.2	5.4	3.4	4.0

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