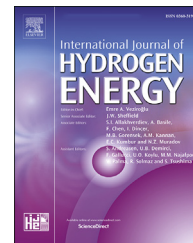


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Search for new strains of microalgae-producers of lipids from natural sources for biodiesel production

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ABSTRACT

Biomass of high-yielding strains of phototrophic microorganisms actively accumulating lipids is a promising non-traditional raw material for bioenergy including the production of biodiesel. In this study, we present results of searching for new strains of microalgae-producers of lipids from hot springs. Within the framework of research, the primary screening of water for the presence of lipid - accumulative microalgae was carried out with the help of qualitative reaction with lipid-specific dyes, as well as 5 axenic isolates of microalgae with stable growth were identified in the laboratory and their productivity and fatty acid composition were studied. The isolated strains were identified as *Chlorella vulgaris* sp-1, *Ankistrodesmus* sp-21, *Scenedesmus obliquus* sp-21, *Chlorella pyrenoidosa* sp-13 and *Chlamydomonas* sp-22. The obtained data showed that the isolated strains determined by biomass in the range 1.3 g/l to 1.81 g/l. As a result of the research, it was established that the highest content of lipids was observed in the strains *Chlorella vulgaris* sp-1 and *Scenedesmus obliquus* sp-21, which is 28.7 and 29.8% of the cell dry weight, respectively. The analysis of the fatty acid composition of the cells showed that the largest mass fraction of saturated and monounsaturated fatty acids was found in strain *Scenedesmus obliquus* sp-21 -

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61.9%. In the result, *Scenedesmus obliquus* sp-21 strain isolated from thermal sources was selected as a promising candidate for biodiesel production.

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Introduction

The last decade has been marked by an increasing interest in the development of technologies based on renewable raw materials including the production of eco-friendly motor fuel [1,2]. At the same time, in recent years, the bioenergy potential of photosynthetic microalgae has attracted increasing attention of biofuel producers, and the financing of research and development in this area is steadily growing [3,4]. The production of biodiesel from microalgae arouse interest due to the high lipid content, for instance, in the cells of strains *Botryococcus braunii*, *Dunaliella*, etc. under optimal cultivation conditions lipid quantity amounted up to 80% and yield of biomass and oil (lipids) exceeds the corresponding yield of terrestrial plants in ten times [5,6]. The technological advantages of microalgae cultivation allow them to successfully compete with land crops including food crops (use of land square, water resources, fertilizers). The possibility of growing microalgae on infertile recultivated lands, water areas, as well as adaptation of algae strains to growth in salt water and use of wastewater as sources of nutrients was proved [7]. The cultivation and energy use of microalgae, unlike traditional crops, does not increase the food problem. In addition, important indicators of the efficiency of the microalgae use are their high growth rate (including year-round), as well as lipid content in cells (up to 80%) higher than similar indicators of individual oilseeds (up to 40% in rape), which is the raw material for biodiesel and bio-oil [8]. Thus, it is believed that microalgae have significant potential to replace fossil energy raw materials in the future.

In this regard, the search for interesting strains of microalgae as a source of lipids is currently relevant. The review provides results of isolation of microalgae from natural sources and the study of their fatty acid composition in order to select promising strain for the biodiesel production.

Materials and methods

The objects of research were thermal springs of Uigur district, Almaty region, Kazakhstan. The sampling of water and algal bacterial mats was carried out in the spring-summer period 2017. Water samples were taken from 4 hot springs of the Uigur region (No. 1–49 km, No. 2–53 km, No. 3–73 km, No. 4–101 km along the Chunzha-Kalzhat) with the aim to isolate algologically pure microalgae cultures. Photos of sampling sites are given below (Fig. 1).

For the primary screening of lipid-containing microalgae, samples from natural sources were stained with lipid-specific Sudan dyes (Sudan B, Sudan III). To prepare the culture, the

collected material was sown (several cubic centimeters of water, green plaque, mucus, etc.) into flasks or tubes with a sterile liquid nutrient medium, and the liquid was transferred into flasks to make the occupied volume less than 1/3–1/4 of the flask volume. Flasks or tubes with sown material were placed on the glass above the frame with fixed fluorescent lamps; the illumination of the flasks was approximately $70\text{--}170\text{ }\mu\text{mol photons m}^{-2}\text{ s}^{-1}$.

Prata, 04, Tamiya and L-min nutrient media were used to obtain the cumulative protococcus cultures of unicellular algae [9]. Along with these media, the selection was carried out on more concentrated Meyers and Tamiya nutrient media [9]. The determination of cyanobacteria preliminary taxonomic affiliation was conducted according to Muzafarov, Tsarenko, Komárek and Gollerbach on the basis morphological characters [10–13]. To obtain the biomass of isolated strains, 1 ml of microalgae suspension was sown in the nutrient medium with initial concentration of one million cells/ml. The flasks with sowing material were placed under a constant light intensity and continuously purged with air by compressors. Cultivation was performed in such conditions for 14 days. The resulting suspension was washed with distilled water 3 times (to remove residual salt components of the nutrient medium) and centrifuged after each wash [14]. The concentration of biomass was conducted by centrifugation process. The algae biomass was dried until air-dry state in a drying cabinet at 45 °C. Lipid extraction from biomass was performed according to Folch method [15].

To this end, a 10-fold volume of extractant $\text{CHCl}_3\text{:CH}_3\text{OH}$ (2:1) was added to the known biomass sample. The resulting mixture was resuspended and kept on a thermoshaker at 50 °C and 1400 rpm for 30 min. Then, the undissolved precipitate was separated by centrifugation at 13200 rpm for 5 min. After, 0.2 volume of 0.8% aqueous NaCl solution was added to separated supernatant. The obtained mixture was intensively resuspended and centrifuged at 13200 rpm for 5 min and aqueous phase was removed. The total lipid amount was determined calorimetrically by Agatova L.I. method with some modifications [16]. To quantify the lipids, 0.1–0.2 ml of the mixture was taken and evaporated in a boiling water bath. Then 0.2 ml of sulfuric acid was added, and the mixture was kept in the bath for 10 min. The tubes were cooled, 1 ml of phospho-vanilin reagent was added, the reaction mixture was thoroughly mixed and placed in a boiling water bath for 15 min. Then the tubes were cooled and colorimetricated on the KFK–3 device at $\lambda = 540\text{ nm}$, $l = 1\text{ cm}$. The measurement was taken against a blank sample. Then, lipid content was calculated according to the calibration curve. Finally, fatty acids were analyzed using gas-liquid chromatography with mass-spectrometer detector Agilent 5975S (Agilent Technology Systems, USA) [17].



Fig. 1 – Sampling sites, hot springs of Uigur district.

Results and discussion

In the framework of this research, the selection of lipid producers among microalgae from environmental objects included several stages: primary screening of lipid-accumulative microalgae in the studied water samples, isolation of pure microalgae cultures with high lipid content and high growth rate, and analysis of fatty acid composition of isolated microalgae strains.

Primary screening of lipid-accumulative microalgae

As known, lipids performing important functions in a living organism are divided into two large groups: polar (phospho- and glycolipids) and non-polar (neutral) lipids, among which the interest in triacylglycerides has significantly increased in recent time, due to their recognition as raw material to produce biofuels of the third and fourth generations.

The search for microalgae lipid producers included intracellular detection of lipids at the first stage and their future quantitative determination in the isolated pure cultures of microalgae. Intracellular detection of lipids was carried out by preliminary qualitative assessment of lipid content in microalgae from the studied samples. For this, the samples taken from natural sources were stained with lipid-specific Sudan dyes (Sudan B, Sudan III) which solubility in fat exceeds their solubility in solvent. The use of such dyes for qualitative determination of lipids in microalgae cells makes possible the identification of potential lipid-producers from natural sources without total isolation of all cultures, which significantly reduces the handling time.

Thermal springs were chosen as objects of the study. There are many hot springs associated with underground waters in Uigur district territory of Almaty region. Despite the high temperature (36–46 °C), the water in these hot springs also has a high salt content, i.e. they are related to so-called mineral springs. From the previous considerations, it must be pointed out that one of the features which allow algae to survive in extreme environmental conditions is the possibility of lipids accumulation in cells in unique volumes, compared

with other plant organisms. At the sampling time, the temperature of water ranged from 36 to 46 °C; the water pH was 6.7–7.3.

There are 16 species and varieties of microalgae were found in water samples, the most common are: representatives of green algae *Chlorella* and *Scenedesmus* genera; cyanobacteria: *Synechococcus*, *Nostoc*, *Phormidium*, *Oscillatoria*, *Anabaena*, *Spirulina*, *Shaeronostoc*, *Lyngbya*; diatoms: *Thalassiosira*, *Fragilaria*, *Zygnema*, *Navicula*, *Amphiprora*. The collection and analysis of water samples from different hot spring points showed that the representatives of filamentous cyanobacteria, diatom microalgae and unicellular green algae dominate in the communities of the studied natural sources.

As expected, the primary screening results of lipid-accumulative microalgae by staining with Sudan dyes showed the presence of potential lipid-producers in the studied samples taken from thermal sources. During the screening, the samples of microalgae positively colored and changed from orange to dark blue. The photomicrographs of the cultures colored with Sudan B and Sudan III preliminary related to green microalgae presented in Fig. 2.

Thus, because of microscopy of water samples stained with Sudan dyes, the promising lipid-accumulative microalgae cultures were detected. The next step was the screening of that samples on liquid and solid nutrient media for isolation of individual colonies, their subsequent cultivation on liquid nutrient medium to determine biomass productivity and lipid content.

Isolation of pure microalgae cultures from hot springs

In the result of sowing, 9 isolated microalgae cultures were obtained from the selected samples. From 9 cumulative cultures there are 5 axenic microalgae isolates distinguished by stable growth rate in laboratory conditions were obtained by multiple sowing on different selective nutrient media. A complete description of the obtained microalgae strains given below.

Culture TI-2 – unicellular green microalga related to *Chlorococconeae* class. Isolated from hot spring N^o 2. Cell size 2–3.5 µm in diameter, before division their sizes increase to

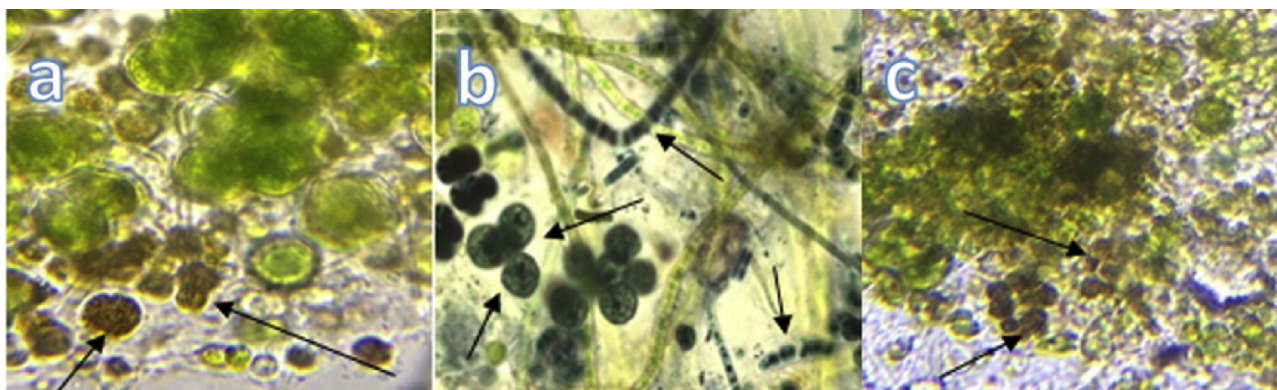


Fig. 2 – Photomicrographs of water samples from hot springs, arrows indicate microalgae cells colored by Sudan III (a, c) and Sudan B (b) dyes.

4–5 μm . Pyrenoids are invisible. Cells divide on 2–8, very rare 16 autospores. During the cultivation in the liquid medium cells do not precipitate and do not accrete on flask/tube walls. On the solid nutrient medium round smooth and convex colonies with even edges are created. Colony diameter is 3–4 mm, colored in dark green color. Strain is autotrophic, it grows well at 22–32°C on liquid and agar O4, TAR and Tamiya nutrient media. Systematic position of culture – Domain: Eucaryota, Kingdom: Plantae, Phylum: Phycobionta, Division: Chlorophyta, Class: Chlorophyceae, Order: Chlorococcales, Family: Chlorellaceae, genus *Chlorella*, species *Chlorella vulgaris* sp-1.

Culture B4 - unicellular green microalga related to *Chlorococcaceae* class. The culture was isolated from hot spring № 2. Young cells are spherical or slightly ellipsoid, ranging in size from 2 to 4 μm . Mature cells are spherical, 3–10 μm in diameter with thin membrane; chromatophore is wide, belt-shaped with pyrenoid, without flagella. Reproduction is only asexual. Reproduce by autospores emerging from the mother cell through the shell rupture. Cells divide on 4–16, often 4–8 autospores. The color is dark green; the cells are not precipitate, in the suspension evenly distributed. The cells slightly accrete on flask walls at 3rd day of cultivation. On depleted agar mineral medium, large convex colonies with smooth edges are formed at the 10th day under the light. The diameter of colonies is 1–3 mm, colored in dark green color, the size of cells is 3–7 μm . Strain is autotrophic, grows well at 22–30°C on liquid and agar O4 and Tamiya nutrient media. Systematic position of culture – Domain: Eucaryota, Kingdom: Plantae, Phylum: Phycobionta, Division: Chlorophyta, Class: Chlorophyceae, Order: Chlorococcales, Family: Chlorellaceae, genus *Chlorella*, species *Chlorella pyrenoidosa* sp-13.

Culture IS-6 – green volvox alga. The culture was isolated from hot spring № 2. Cells are oval, mobile, surrounded by pectin membrane, with two flagella of the same length located at the front end of the cell. Cell size is from 3 to 4 μm . Chlorophyll is concentrated in the chromatophore. Reproduces in asexual and sexual ways. Reproduces well on organic-mineral and organic nutrient media. Mesothermal. Avoids strong light and high temperature. During cultivation, living cells practically do not precipitate. In dormant state, the precipitation of cells starts after 6–7 days. On a solid medium, smooth green

shiny colonies of regular shape are formed. The strain grows well on liquid and agar synthetic L-min nutrient medium at 18–26°C. Systematic position of culture – Domain: Eucaryota, Kingdom: Plantae, Phylum: Phycobionta, Division: Chlorophyta, Class: Chlorophyceae, Order: Volvocales, Family: Chlamydomonadaceae, genus *Chlamydomonas*, species *Chlamydomonas* sp-22.

Culture IS-11 – green microalgae related to *Chlorophyceae* class. The culture was isolated from hot spring № 3. Cells are spindle-shaped consisting of coenobium forms. Coenobia consist of 2, 4 cells located side-to-side to each other and arranged in one row (sometimes 2 or 3 rows), without spikes. Each cell has an elliptic shape. The average cell size is 2.5–4 μm wide and 4.5–5 μm long. The surface of the cell is smooth without outgrowths and covered with a smooth membrane. Have a peripheral chromatophore with one pyrenoid. Reproduction is by autospores: 4, 8 or 16 spores formed in each cell which then joined into a new coenobium. Reproduce by aplanospores under optimal conditions. Photophilous autotroph, neutrophil, mesophile. It grows well at 22–28 °C on the liquid and solid Tamiya nutrient medium. On the liquid medium it grows homogeneously, on the solid medium it forms colonies of green color of regular shape. Systematic position of culture – Domain: Eucaryota, Kingdom: Plantae, Phylum: Phycobionta, Division: Chlorophyta, Class: Chlorophyceae, Order: Sphaeropleales, Family: Scenedesmaceae, genus *Scenedesmus*, species *Scenedesmus obliquus* sp-21.

Culture IS-7 – microalga of *Chlorococcales* order. The culture was isolated from hot spring № 3. The cells of the strain are unicellular, but can also form fan-shaped or ray-shaped cells connected in the central part. Cells are elongated, straight or slightly curved, gradually narrowed and pointed to the ends. The membrane is smooth, thin and colorless. Reproduction occurs by 2-4-8 - (16) autospores located in the maternal cell parallel to each other, released as a result of the rupture of the maternal cell. It grows well on liquid and solid Tamiya medium at 18–26 °C. Do not precipitate on the bottom when growing on a liquid nutrient medium, the flask wall does not overgrow. They form round, smooth and convex colonies with even edges on solid nutrient medium. The diameter of colonies is 2–3.5 mm, green colored. Systematic position of culture – Domain: Eucaryota, Kingdom: Plantae, Phylum: Phycobionta, Division: Chlorophyta, Class: Chlorophyceae, Order:

Table 1 – Total lipid content in the biomass of isolated microalgae strains.

Strains	Total lipid content, mg/ g	Biomass, g/ l
<i>Chlorella vulgaris</i> sp-1	287 ± 1	1.57 ± 0.2
<i>Chlorella pyrenoidosa</i> sp-13	73 ± 2	1.3 ± 0.1
<i>Chlamydomonas</i> sp-22	192 ± 1	1.3 ± 0.2
<i>Ankistrodesmus</i> sp-21	246 ± 1	1.79 ± 0.3
<i>Scenedesmus obliquus</i> sp-21	298 ± 2	1.81 ± 0.2

Sphaeropleales, Family: *Ankistrodesmaceae*, genus *Ankistrodesmus*, species *Ankistrodesmus* sp-21.

Determination of selected microalgae cultures productivity by dry biomass yield, lipid content and fatty acid composition

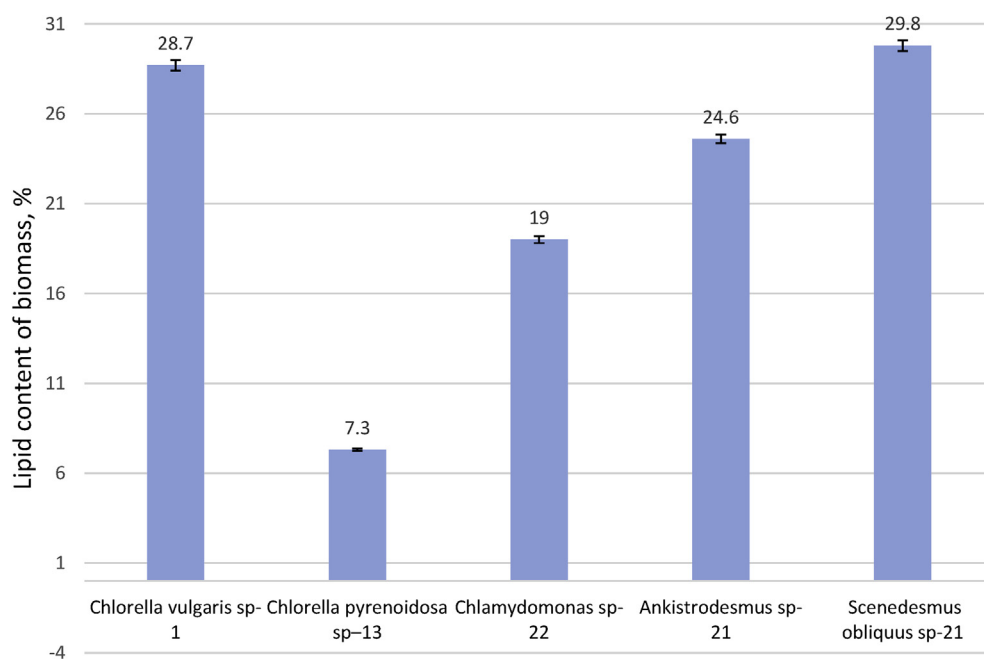
For the successful industrial mass cultivation of microalgae and use of selected strains in bioenergy, in particular, obtaining of biodiesel, the selection of highly productive species is required. As known, many of them are perspective producers of lipids and fatty acids, have a high growth rate on simple media [18]. Moreover, increased synthesis and accumulation of lipids are known to be species feature or even strain specificity [19]. As Takahashi et al. have shown in 1938, fatty acid composition of algae lipids distinguished by the presence of saturated and unsaturated compounds. For example, among the lipids of studied *Cystophyllum hakodatense* marine alga following acids were determined: myristic (4,5%), palmitic (18%), hexadecenoic (16%), hexadeuterated (7%), octadecenoate (39%), OCTA - decadienoate (3%), octadecatrienoic (1%), octadecatetraenoic (7%), docosadienoic (1%),

docosatrienoic (3%) acids [20]. In addition, planktonic algae contain a significant number of lipids, including both saturated and unsaturated fatty acids different in the position of double bonds in the carbon chain. The diatom alga *Skeletonema costatum* has a relatively high concentration of free fatty acids containing mainly 2, 3, 4 double bonds [21]. As given in references, lipid fatty acids of diatom algae have 2, 3, 4, 5, 6 double bonds with 18, 20 and 22 carbon atoms in the linear chain. The total lipid content reaches 30% or more of the dry weight of diatoms [22].

In cyanobacteria, the main components of lipids, as in other organisms of phytoplankton, are unsaturated fatty acids, mainly linoleic and linolenic. As for other less common microphytes, the bioproduction of lipids is the same intensity and direction. As an example, lipids of euglena algae contain the predominant amount of unsaturated fatty acids with hydrogen atoms' cis-configuration at double bonds, which is the main prerequisite for their inclusion in enzymatic processes such as prostaglandin biosynthesis. Investigation of lipid fractions of the microphyte *Euglena gracilis*, Hulanicka et al. (1964) identified the presence of the following unsaturated fatty acids: 7 hexadecene - 9-hexadecanoate, 7, 10-hexadecanoate, 4,7,10, 13-hexadecatetraenoic, 11, 14- eicosatrienoic, arachidonic, 11,14,17-eicosatrienoic, 8,11,14, 17-acetate-raenoic, 5, 8, 11, 14, 17-eicosapentaenoic [23].

Screening of isolated microalgae cultures was conducted based on comparative analysis of productivity, which included cultivation in the laboratory, quantification of dry weight, total lipid content and fatty acid analysis.

Biomass of microalgae was accumulated in flasks with appropriate for each culture optimum temperature 22–28 °C and artificial illumination 70 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Lipid biomass for extraction was collected at the stationary growth phase of cultures after 14 days of cultivation. Since the

**Fig. 3 – Percentage of lipids in 1 g of biomass of microalgae isolated strains.**

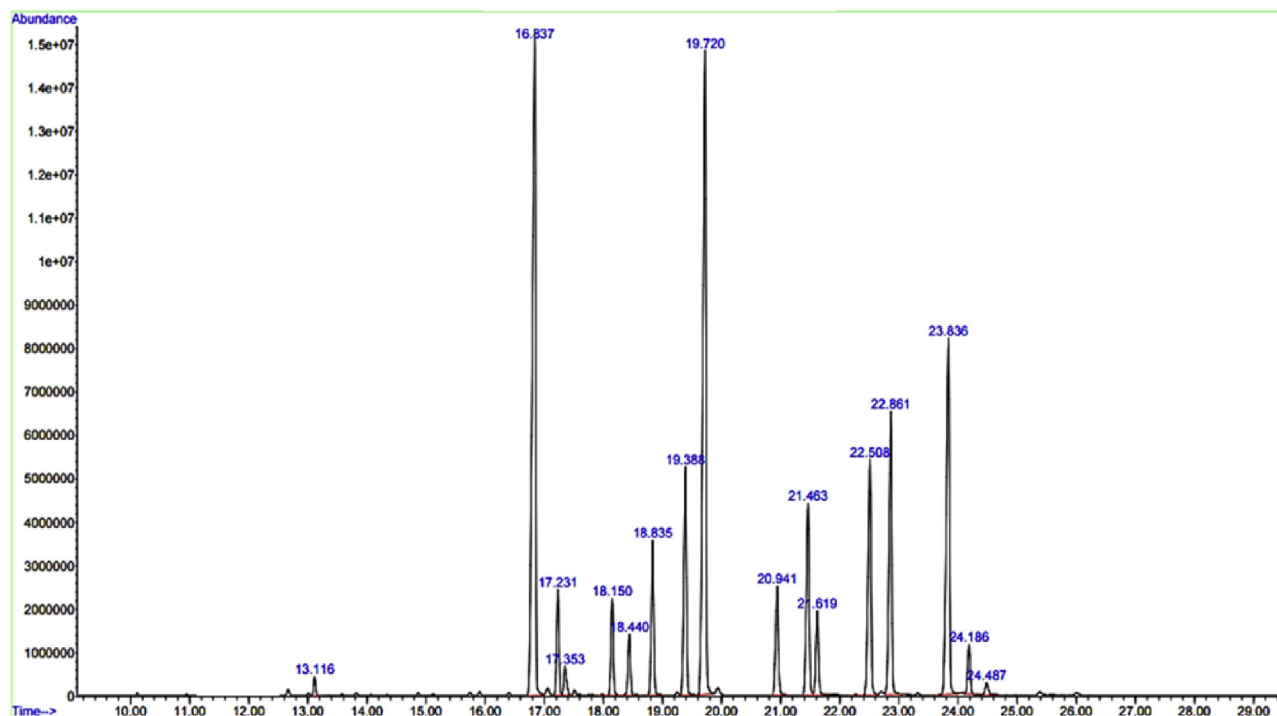


Fig. 4 – Chromatogram of fatty acids of *Chlorella vulgaris* sp-1 strain with the use of GCMS.

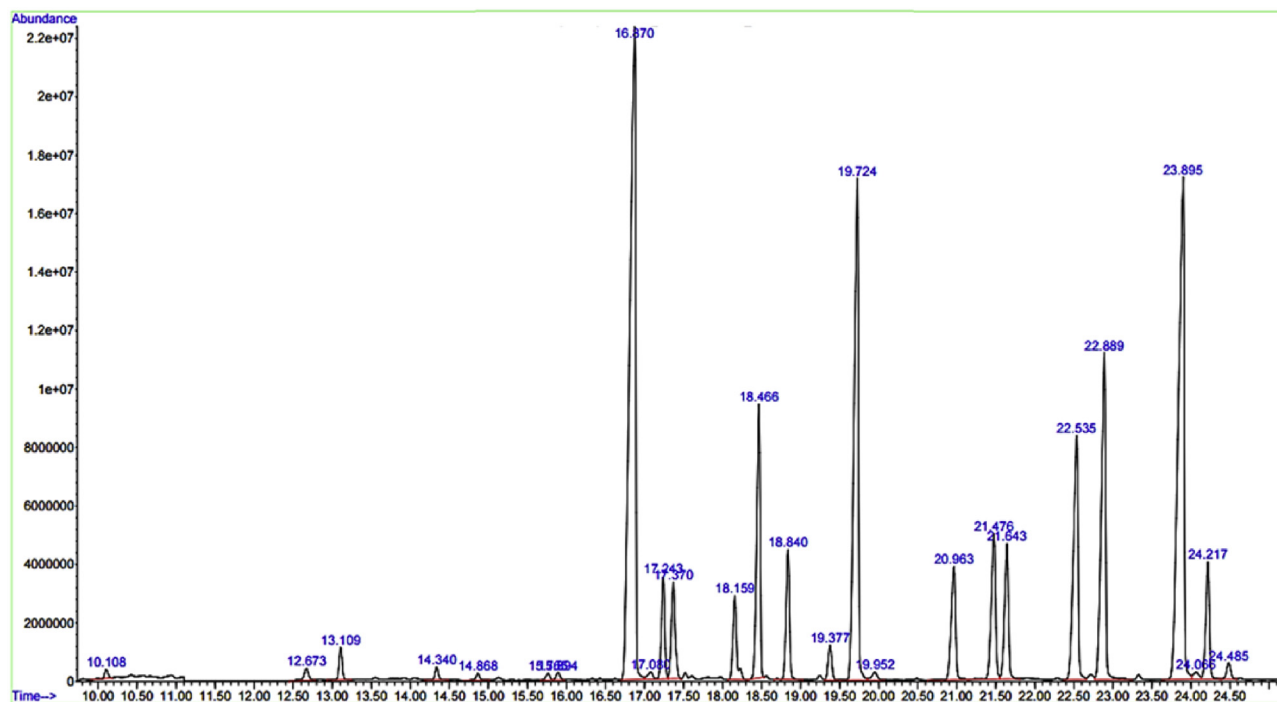


Fig. 5 – Chromatogram of fatty acids of *Ankistrodesmus* sp-21 strain with the use of GCMS.

biomass of the studied strains is different, we calculated the total lipid content in 1 g of dry weight. The amount of dry biomass and lipids given in Table 1.

Summary data on lipid content in cells in percentage presented in Fig. 3.

The lipid content in the cells of the isolated microalgae strains ranges from 7% to 30% of the dry biomass weight. The highest content observed in strains *Chlorella vulgaris* sp-1 and *Scenedesmus obliquus* sp-21 which makes up 28.7 and 29.8% of the dry cell weight, respectively. A good indicator of lipid

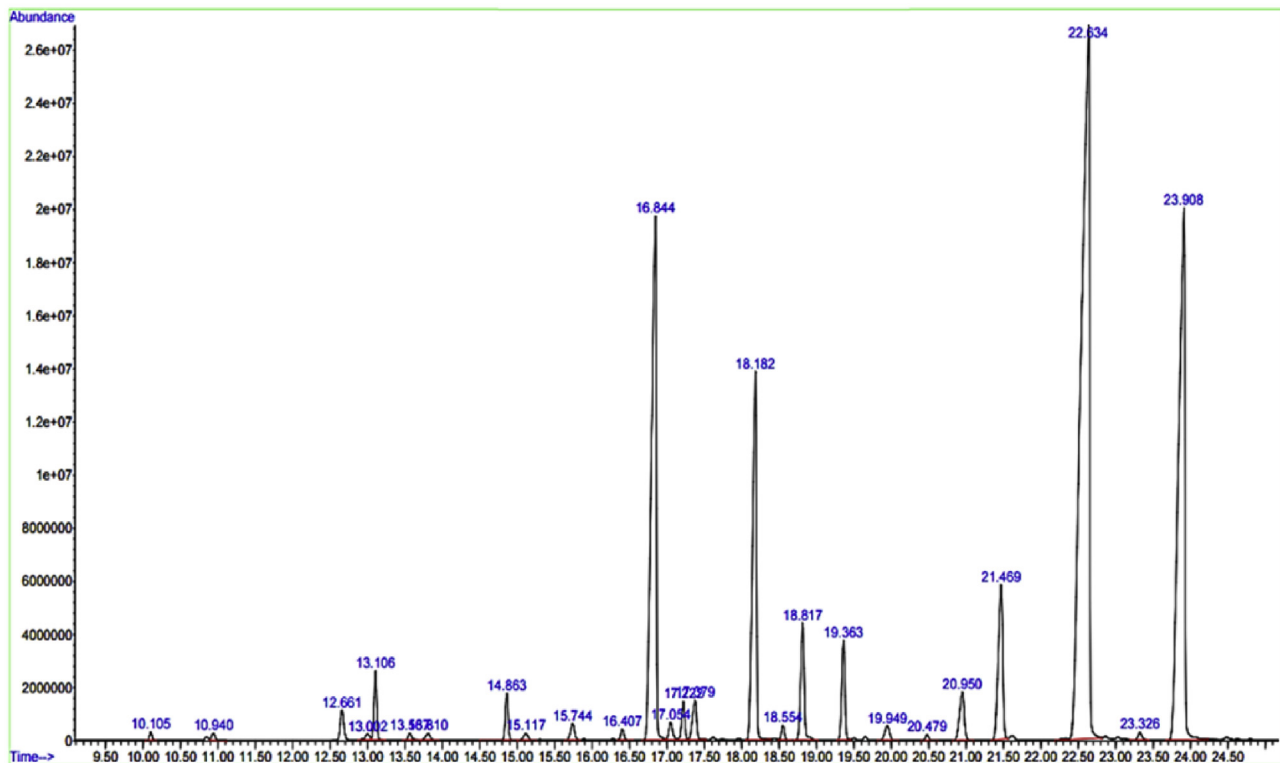


Fig. 6 – Chromatogram of fatty acids of *Chlorella pyrenoidosa* sp-13 strain with the use of GCMS.

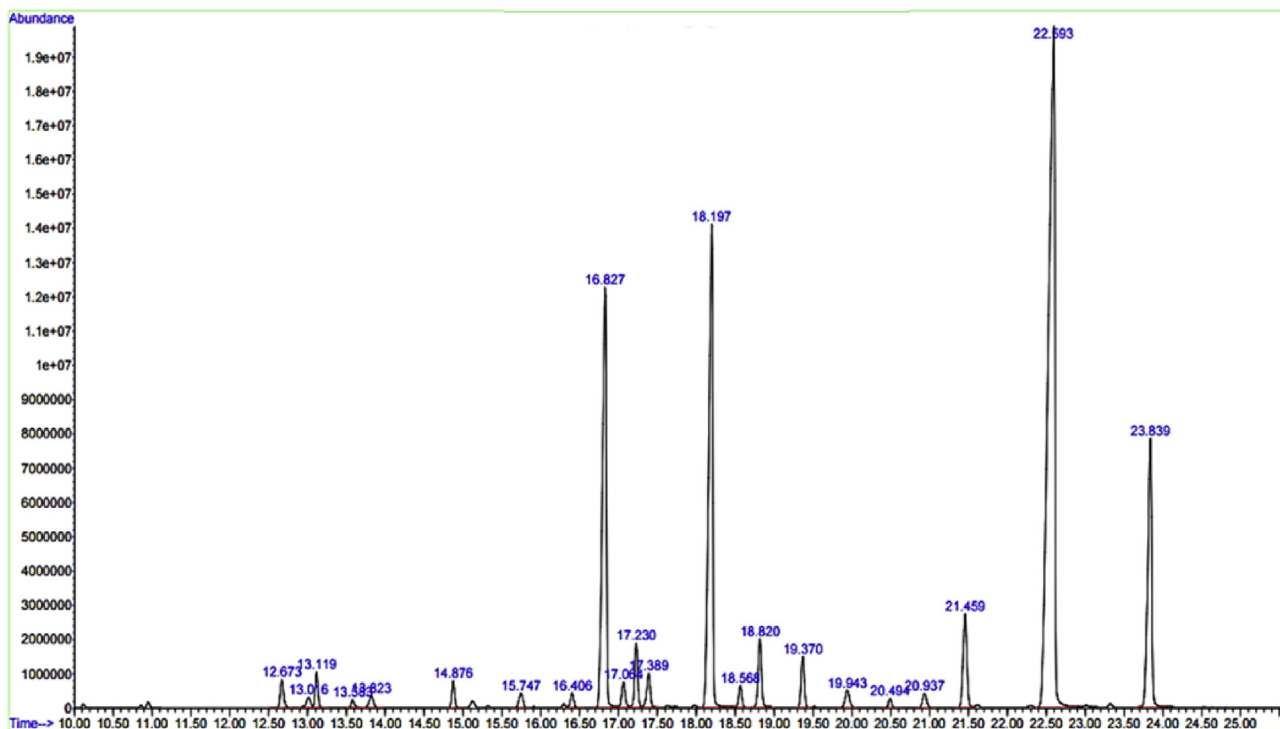


Fig. 7 – Chromatogram of fatty acids of *Chlamydomonas* sp-22 strain with the use of GCMS.

biomass accumulation was noticed in *Ankistrodesmus* sp-21 strain – 24.6%.

At the next stage of work, the analysis of fatty acid composition of the isolated strains was carried out by gas

chromatomass-spectrometry. In the result of studies, fatty acid chromatograms of investigated strains were obtained (Figs. 4–8).

The results of mass fraction determination of fatty acids in the cells of microalgae *Chlorella vulgaris* sp-1, *Ankistrodesmus*

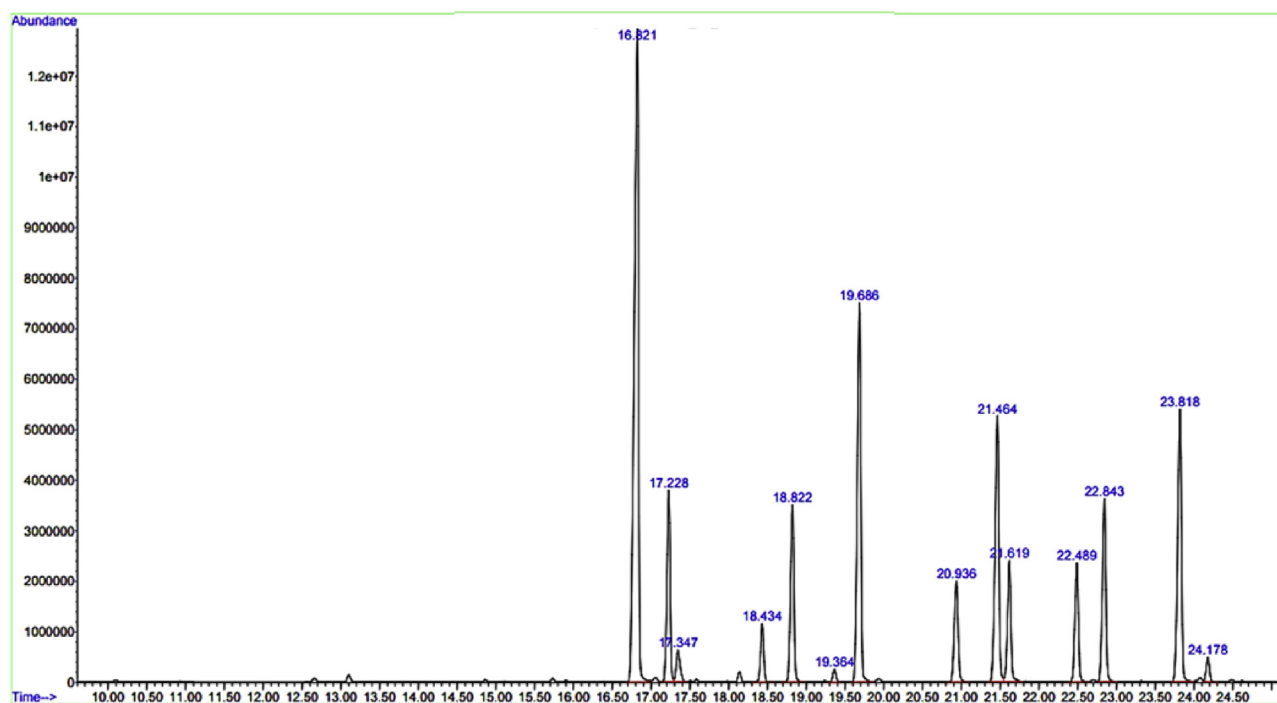


Fig. 8 – Chromatogram of fatty acids of *Scenedesmus obliquus* sp-21 strain with the use of GCMS.

sp-21, *Scenedesmus obliquus* sp-21, *Chlorella pyrenoidosa* sp-13 and *Chlamydomonas* sp-22 presented in Table 2.

As presented in Table 2, the fatty acid composition of total lipids of the studied strains included 17 fatty acids with a carbon chain length from 12 to 18 atoms. In the cells of *Chlorella vulgaris* sp-1, *Ankistrodesmus* sp-21 and *Scenedesmus*

obliquus sp-21 strains, palmitic acid dominates (16:0), which share was 26.9%, 27.1% and 33.9%, respectively. In addition, it should be noted that these strains are characterized by a sufficient amount of C16:4 and C18:3 fatty acids that are not synthesized in the cells of other studied strains. While in *Chlorella pyrenoidosa* sp-13 and *Chlamydomonas* sp-22 strains

Table 2 – Mass fraction of fatty acids in cells of isolated microalgae strains.

Fatty acids	Mass fraction of fatty acids, %				
	<i>Chlorella vulgaris</i> sp-1	<i>Ankistrodesmus</i> sp-21	<i>Scened. obliquus</i> sp-21	<i>Chlorella pyrenoidosa</i> sp-13	<i>Chlamydomonas</i> sp-22
1	2	3	4	5	6
12:0	0.1	0.5	0.2	0.3	0.2
14:0	0.6	0.9	0.4	1.8	1.3
16:0	26.9	27.1	33.9	19.3	17.8
16:1 Δ^7	3.0	2.7	3.6	1.4	0.5
16:1 Δ^9	3.0	2.5	7.7	0.9	2.2
16:2 $\Delta^{7,10}$	1.0	2.6	1.6	1.2	1.4
16:2 $\Delta^{9,12}$	2.8	2.0	0.4	10.9	21.4
16:3 $\Delta^{7,10,13}$	—	—	—	0.3	0.8
16:3 $\Delta^{4,7,10}$	6.9	0.8	—	—	—
16:4 $\Delta^{4,7,10,13}$	—	6.3	2.1	2.1	1.6
18:0	19.9	12.8	13.2	0.1	—
18:1 Δ^9	5.6	3.7	10.4	4.4	3.3
18:1 Δ^{11}	2.3	3.2	4.3	0.1	0.1
18:2 $\Delta^{9,12}$	7.0	6.7	4.3	36.7	39.7
18:3 $\Delta^{6,9,12}$	8.1	8.4	6.6	—	—
18:3 $\Delta^{9,12,15}$	11.4	17.2	10.6	20.6	9.8
18:4 $\Delta^{6,9,12,15}$	1.4	2.6	0.9	—	—
Amount of saturated fatty acids, %	30.7	31.2	38.0	22.8	19.8
Amount of polyunsaturated fatty acids, %	69.3	68.8	62.0	77.2	80.2
Fatty acid content, mg/g dry weight	90.2	92.2	93.0	82.4	84.2

C18:2 fatty acid was predominantly synthesized, which was 36.7% and 39.7%, respectively. These strains also contain relatively high amounts of C16:0, C16:2 and C18:3 fatty acids. It should be noted that the largest mass fraction of saturated and monounsaturated fatty acids was found in *Scenedesmus obliquus* sp-21 strain which amounted to 61.9%. In the cells of the studied strains, the remaining fatty acids were present in an insignificant amount. The highest quantity of fatty acids was found in cells of *Scenedesmus obliquus* sp-21 strain - 93.0 mg/g.

Our study reveals that despite insignificant difference in growth rate and biomass accumulation, microalgae indicated by various fatty acid composition. Microalgae strains that described by a high content of saturated and mono-unsaturated fatty acids with an average chain length from C14 to C18 have a great practical importance for biodiesel production [24], thus, for further experiments *Scenedesmus obliquus* sp-21 strain can be considered as a promising candidate for biofuel production. However, the perspectives of using *Chlorella vulgaris* sp-1 and *Ankistrodesmus* sp-21 green microalgae strains, whose metabolic characteristics also allow us to consider them as a feedstock for biofuel should be noted. In this case, it is possible to consider the possibility of obtaining biodiesel during cultivation of these strains in both monoculture and mixed culture. In the literature sources, there are a lot of information about the production of biodiesel and hydrogen by co-cultivation of individual strains of microalgae and cyanobacteria. Thus, in the work of Quanzhou Feng and his colleagues, the system of cultivation of filamentous cyanobacteria *Anabaena* sp. PCC7120 producing biohydrogen together with *Chlorella* sp. lipid-accumulating microalgae culture was presented. They have studied different cell ratios of these two cultures with joint cultivation, showed the advantage of mixed cultivation of strains to produce biodiesel. So, the cells of microalgae produced more saturated fatty acids in the association than in monoculture [25]. The use of isolated cultures for biodiesel production and their possible using in mixed culture require further research of their relationship, the conditions of their joint cultivation, the selection of cell ratios, etc. that is the subject of our further research.

Conclusions

As a key result, improving upon other works, we demonstrate analysis of hot springs of Uygur district (Almaty region), there are 16 species and following varieties of algae have been identified: common representatives of *Chlorella*, *Scenedesmus*, *Synechococcus*, *Nostoc*, *Phormidium*, *Oscillatoria*, *Anabaena*, *Spirulina*, *Sharonostoc*, *Lyngbya*, *Thalassiosira*, *Fragilaria*, *Zygnema*, *Navicula*, *Amphiprora* genera. Because of multiple transfers, five axenic strains were identified as *Chlorella vulgaris* sp-1, *Ankistrodesmus* sp-21, *Scenedesmus obliquus* sp-21, *Chlorella pyrenoidosa* sp-13, *Chlamydomonas* sp-22. Determination of the productivity of isolated microalgae cultures in terms of dry biomass yield, lipid content and fatty acid composition showed that the green microalga *Scenedesmus obliquus* sp-21 was defined by the highest biomass yield and total lipid content. Analyzing the results of experimental research of new microalgae strains isolated from hot springs, it can be concluded

with certainty that the fatty acid composition of algae is determined primarily by their species. Thus, as the result of analysis of algae fatty acid composition, there are 17 components were determined, 4 of them are saturated fatty acids. As known, among the saturated fatty acids palmitic and stearic acids are required for transesterification process in biodiesel production. The viscosity of liquid biodiesel increases with the length of the fatty acid chain, and their oxidative stability decreases. In the cell biomass, the increase of the unsaturated fatty acids is the optimal condition of high lipid accumulation [26]. Thus, biodiesel obtained from saturated and mono-unsaturated fatty acids with short carbon chain has higher quality. Among all the studied strains, the cells of *Scenedesmus obliquus* sp-21 culture contain a relatively high amount of saturated fatty acids, that allow us to consider it as a potential candidate for the preparation of raw materials for biodiesel.

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