Pilot Production of *Spirulina* Biomass and Obtaining of Novel Biodegradable Surfactants

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**Abstract**

The research results describe the pilot production of microalgae biomass – *Spirulina*, especially in wintertime, using the geothermal energy of water to save the costs for heating of the pool photobioreactor and biomass drying box. For carrying out of the process a simplified nutrient medium consisting of geothermal water and salts: sodium bicarbonate, potassium nitrate, diammonium phosphate, and urea was developed. The conditions for the *Spirulina* biomass cultivation in wintertime were optimized. The technical and economic feasibility and conditions for large-scale production of *Spirulina* in Kazakhstan for commercial purposes are justified. It has been shown that the *Spirulina* biomass may serve as a feedstock for the production of biodegradable surfactants.

**1. Introduction**

*Spirulina* (*Arthrospiraplatensis*) is of particular interest among the well-known microalgae cultures as the largest-tonnage commercially cultivated microalgae in the world. *Spirulina* is a multicellular coiled filamentous microalgae. It is a set of blue-green filaments, consisting of cylindrical cells, arranged in unbranched filaments. The threads are mobile and slide along their axes. The spiral shape of the filaments is a generic feature of *Spirulina*, the parameters of the spiral are different in different species. The pitch and length of the spiral depend on the growing conditions [1].

By the content of vitamins and microelements, *Spirulina* surpasses many food products, of both plant and animal origin. It contains vitamins A, B (B1 B2, B3, B5, B6, B9, B12), E (tocopherol), C, minerals and trace elements: potassium, calcium, magnesium, zinc, manganese, phosphorus, iron, microdoses of iodine, selenium, rare metals; as well as more than 2000 enzymes in micro doses [2]. *Spirulina*’s biomass is very rich in amino acid compounds (65–70% protein compounds), the second leading component of its composition is carbohydrates (polysaccharides), in the amount of 7–8% there are lipids that can be used to create surfactants [3].

*Spirulina* is grown in open and closed photocultivators. There are projects for its cultivation in giant farms on the coast of the seas and oceans, where various renewable energy sources (solar ponds, solar collectors, etc.) serve as an energy source for servicing plantations [4].

Nowadays, surface-active substances (surfactants) are produced mainly from the petrochemical feedstocks, which are widely used in many industries, but in most cases their use produces a harmful effect on both the object of use and the environment. That is why the developments aimed at the creation of non-toxic and biodegradable surfactants are of big scientific interest. The biodegradable surfactants, consisting of complex natural molecules of the vegetable feedstock, with the excellent surface-active properties, are of great importance.

Biodegradable surfactants are the surfactants of the biological origin (or biosurfactants), representing amphiphilic compounds, formed mainly from the hydrophobic and hydrophilic fragments, which ensure an ability to reduce the surface and interfacial tension on the surface and at the interface boundary. Herewith, they possess the same features for reducing the surface and interfacial
tension, using the same mechanisms as the chemically synthesized surfactants from the petrochemical feedstocks. Biosurfactants can be defined as the surface-active biomolecules, produced by microorganisms with a wide range of applications.

An interest in biosurfactants has increased due to their various structural diversities, low toxicity, complete biodegradability, potential ability to function within the wide ranges of pH, temperature and salinity, as well as their increased selectivity and lower critical concentrations of micelle formation. Besides, their production is associated with the use of renewable sources, industrial wastes and industrial by-products [5]. The surfactants, produced by microorganisms, possess the following advantages: they are prone to decomposition by microorganisms and have significant specificity [6‒9].

Despite their significant advantages, the main drawback, which impedes their wide industrial application, is their expensive large-scale production, as they are synthesized by living organisms, such as plants (saponins), microorganisms (glycolipids), as well as the human organism (bile salts). However, a growing demand in the feedstocks, used as the food sources and the need for large areas of arable land for their production, limit the development of biosurfactants. The rapidly growing population of the planet and the predicted depletion of oil reserves dictate the need to find alternative ways for creating feedstocks for biosurfactants. The biore- sources of lipids such as vegetable oil, animal fat, and microalgal oil (lipids) are often discussed as a viable alternative feedstock for a wide variety of bioproducts, used in various industries. Biosurfactants are one of the main productions for industry branches, which can use the bioresources of lipids as the feedstocks.

Microalgae are currently considered as one of the most common alternative sources of biosurfactants. They do not require agricultural lands for growing the biomass – the feedstock for biosurfac- tants. The microalgae biomass can be cultivated on the non-arable lands, in the salty water medium, their mass cultivation does not come into collision with the food production [10, 11]. Their high photosynthesis indices, due to the simplified single-celled structure, allow them not only to serve as an efficient platform for carbon sequestration, but also to quickly accumulate lipids in their biomass (up to 77%). Even when using the conservative way of obtaining, microalgae still produce about 10 times more lipids per unit area of land than a typical ground oil-plant [12].

Biosurfactants are composed of complex molecules and cover a wide range of chemical structures such as glycolipids, lipopeptides, lipoproteins, neutral lipids, fatty acids and phospholipids. The chemical structure of the most thoroughly studied biosurfactants is provided in [13].

Fatty acid and phospholipid derivatives in the microalgae biomass lipids can act as surfactants. A strong decrease in the surface and interfacial tension has been observed, for example, for the branched fatty acids with the chain length of C12 – C14. Particular attention should be paid to fatty hydroxy acids, which are connected with amino acids and form the biosurfactant groups of lipoprotein acids, a striking example of which is a lipopeptide biosurfactant – Surfactin [13].

This research aims to study the conditions for large-scale obtaining of the Spirulina biomass for its further use for the production of new representatives of biodegradable surfactants with good surface-active properties.

The use in this work of the single-celled Spirulina culture, obtained by the method of industrial biotechnology – autotrophic photosynthesis, as a renewable bioresource, is connected with the fact, that it is the microalgae biomass that can ensure a wide development of a new generation of biodegradable surfactants. Since the microalgae lipids have a much higher fraction of long unsaturated fatty acids as compared with the regular vegetable oils, it makes them undoubtedly attractive candidates for such developments, especially taking into account that their large-scale production does not interfere with the food crops harvesting. The use of the Spirulina biomass, cultivated in the conditions of Kazakhstan, using the alternative natural resources for the production of biodegradable surfactants, as well as the products, which do not come into collision with the food production, comprises the novelty of this research.

2. Experimental

Obtaining the Spirulina biomass: for large-scale production of the culture biomass, a system of photobioreactors was used, which was located in the immediate vicinity of a flowing well of geothermal water. The photobioreactors were located in a translucent greenhouse and represented a system of concrete pools with a curb height of at least 0.45 m, consisting of inoculation and production cultivators with forced circulation of the nutrient medium with the help of a paddle wheel or by way
of aeration with an air stream, supplied through an air compressor. The nutrient medium in the inoculation photobioreactor circulated from the pool through a system of tubines with the help of a water pump, and returned to the heated pool. The photosynthesizing surface of the pool made up 3 m², the volume was up to 1 m³. The photosynthetic surface of the tubular system reached 11.3 m², with the volume V = 0.34 m³. The photosynthetic surfaces for the production photobioreactors made up from 50 to 100 m², a system of heat exchangers, made of flexible metal-polymer pipes, were embedded in their bottom. For the optimal life support of the Spirulina (Arthrospira platensis) culture, the temperature of the liquid nutrient medium (30–34 °C) in the photobioreactor system was continuously maintained by regulating the supply of geothermal water to the heat exchange system. The temperature of the water was 75 °C, the total salt content was 3 g/l, and the flow rate was 5 l/s.

A certified strain of the Spirulina culture from the culture collection of the Norwegian Institute for Water Research under the number NIVA-BAC 428 was used as a producer. Photosynthesis of the culture was carried out at the nutrient medium temperature (30–34 °C) under the local conditions of natural illumination in the winter period.

Geothermal water of the hydrocarbon nature of the flowing well No. 20A, extracted at the depth of 1800 m (the Shauelder settlement, Turkestan region, Kazakhstan), served as the liquid basis of the culture medium for growing the culture. Accounting for the composition of geothermal water, which was related to the hydrocarbonate-sulfate-chloride type of waters with a total salt content of up to 3 g/l and pH of 7.72, the following mineral salts, required for growing the Arthrospira platensis culture, were introduced, g/l: sodium bicarbonate – 10.0; potassium nitrate – 2.0; diammonium phosphate – 0.12; urea – 0.02. Regardless of the quantity of salts, introduced in the enlarged batch, their specified concentrations are maintained.

Determination of the quantitative indicators of the growth and accumulation of the Spirulina biomass in the experimental nutrient medium on the basis of hydrocarbonate water was carried out by measuring its optical density on a PerkinElmer Lambda-35 UV spectrometer (USA) in the wavelength range of 420–650 nm, corresponding to the peaks of the constituent biomass components. The nature of the growth and accumulation of the biomass in suspension was judged by the absolute values of the peaks, the final concentration of the biomass was determined by the gravimetric method after collecting the biomass.

Preparation of the lipid fraction (oil): a weighed portion of the dry microalgal biomass under study was thoroughly triturated with quartz sand (in an amount of a triple volume in relation to the dry biomass), then an extracting mixture of methanol: chloroform = 1:2 mg·ml⁻¹ in the ratio of the weighed portion of the dry biomass: extracting solvent = 1 (mg·ml⁻¹), and vigorously mixed. The obtained extract after filtration was collected in a measuring tube. Then the solvent was removed by evaporation.

Synthesis of methyl esters of fatty acids: 91 ml (2.25 mol) of methyl alcohol and 218.75 g of extraction lipid were placed in a 500 ml round-bottom flask. 14.5 g (5% by weight) of a solid phase catalyst, KOH/activated carbon was added to the reaction mass. The reaction mixture was heated up to 73°C. The heating rate was 1.2–1.3 deg·min⁻¹. The reaction mass was kept at this temperature for 8 h. The catalyst was filtered off and washed with two 200 ml portions of methanol, and reused. The reaction mass was cooled down to 30°C and the heavier lower glycerol layer was separated. The methanol excess was distilled off and regenerated. The obtained methyl esters of fatty acids were analyzed by the methods of IR spectroscopy (infrared spectroscopy) and gas-liquid chromatography.

Synthesis of fatty acid amides: 7.5 g of methyl ethers of the Spirulina fatty acids and 1.65 ml of monoethanolamine was charged in a three-necked flask, equipped with a shutter and an electromechanical stirrer (for the continuous stirring of the reaction mixture in the course of the entire process), the reaction mixture was heated up to 100°C, and 0.01 g of NaOH catalyst was added at this temperature. Then the temperature was gradually raised up to 120°C and the reaction was carried out at this temperature for 3 h.

IR spectra were recorded in a solution of CHCl₃:CH₃OH=1:1 on KBr glasses on a Nicolet 5700 FT-IR spectrometer, manufactured by ThermoElectron Corporation (USA) in the wavenumber range 4000–400 cm⁻¹.

Evaluation of the surface activity of the studied samples was carried out using the Wilhelm plate method on a KRUSS Series K20 EasyDyne tensiometer (Germany). The measurements of the surface tension were carried out at 18–20°C, using a thermostatic jacket, whose temperature was maintained using a circulation thermostat. The surface tension of the aqueous solutions was determined in the range of their concentration from 0.001 to 1 wt.%.
3. Result and discussion

3.1. Enlarged production of Spirulina biomass

Earlier, we developed a method for the synthesis of the Spirulina biomass under the laboratory conditions, using distilled and natural bicarbonate water as a liquid basis of the nutrient medium [10, 14]. As a result of the conducted research [10], a nutrient medium based on geothermal bicarbonate water with natural raw soda was recommended instead of sodium bicarbonate for the large-scale growth of the microalgae biomass, leaving unchanged all other biogenic elements of the Zarruk composition, which provided the acceptable growth characteristics of the Spirulina biomass. The optimal final biomass content in the laboratory experiments was 3.79 g/l, the duration of cultivation made up 17 days in the summer period.

This work presents for the first time the results of the studies of the several batches of the large-scale cultivation of the microalgae biomass, carried out in the cumulative mode, under the conditions of photoperiod and intense illumination of the nutrient medium, corresponding to the natural conditions of the location of well No.20A in the winter period. The works have been carried out in a translucent greenhouse, using an open-type concrete photobioreactor with a forced paddle wheel to circulate the nutrient medium through a closed zigzag channel of the pool; the medium temperature has been maintained within 24 h due to the thermal energy of geothermal water. Since the Zarruk nutrient medium has a complex composition for use in industrial production, a simplified nutrient medium has been developed and applied for cultivation. Accounting for the composition of geothermal water, which is related to the hydrocarbonate-sulfate-chloride type of water with a total salt content of 3 g/l and pH of 7.72, the medium includes geothermal water of well No.20A and mineral salts: sodium hydrogen carbonate, potassium nitrate, diammonium phosphate, and urea. At the same time, the cultivation of the 1–4 batches of the studied culture biomass has been carried out, using only the newly prepared nutrient medium. The cultivation of the 5th and 6th batches has been carried out, using the nutrient medium, worked out in the previous batches, renewed by 1/3 with its fresh solution (the combined medium). The biomass collection has been carried out upon reaching the optical density value of the nutrient solution of no more than 2–3 cm, determined with the help of Secchi-Disk (Table 1). The data provided in Table 1 are the average values of the three repetitions of the large-scale cultivation of the Spirulina biomass, carried out in the cumulative mode with the purpose to determine the optimal conditions. The content of the final biomass in the nutrient solution, determined by the gravimetric method after collecting the biomass, has served as an optimization criterion. The discrepancy in the results of the repeated experiments has not exceeded 7–10%.

As can be seen from Table 1, the biomass yield in the course of the Spirulina cultivation during the daylight hours varies from 0.21 to 0.39 g/l. It appears that the minimum biomass content of 1 batch is connected with the suboptimal conditions of the photosynthesis process flow: the crop

| Name                                                                 | Conditions and results |
|----------------------------------------------------------------------|------------------------|
|                                                                      | 1 batch | 2 batch | 3 batch | 4 batch | 5 batch | 6 batch |
| The volume loaded in photobioreactor suspension of the culture in a nutrient medium, ton | 9.5      | 9.5     | 10.0     | 7.0      | 9.5      | 9.5     |
| Cultivation time, days                                              | 5        | 9       | 7        | 9        | 5        | 5       |
| Calculation of the final content of biomass in the nutrient medium, g/l | 0.21     | 0.37    | 0.27     | 0.39     | 0.26     | 0.32    |
| Costs of salts in the nutrient medium, USD                          | 102.02   | 102.02  | 107.77   | 75.44    | 37.72    | 37.72   |
| Dry biomass yield, kg                                               | 1.7      | 3.5     | 2.7      | 2.7      | 2.5      | 3.0     |
| Calculated cost of salts per 1 kg of dry biomass, USD               | 60.00    | 29.15   | 39.92    | 27.94    | 13.97    | 12.57   |

Table 1
Results of several cycles of enlarged cultivation of Arthrospirapatens in the cumulative mode in winter (the initial biomass concentration in all cases is 0.2 g/l)
maturation process has not been fully completed for 5 days. With an increase up to 9 days in the cultivation duration, the biomass content rises to an acceptable value, a further increase in time does not result in a significant change in the biomass quantity in the nutrient medium.

It has been revealed, that for obtaining one kilogram of the dry biomass, the costs for mineral salts have amounted to USD 28–60 for the 1–4 batches, and USD 12–14 for the 5–6 batches. A decrease in the specified costs in the case of the 5–6 batches can be explained by the use of a combined nutrient medium, as it has been indicated above. It should be noted, that the biomass, obtained using only the newly prepared nutrient medium, is intended for the food and medical production, and using a combined medium provides the biomass for cosmetic purposes. Besides, the obtained biomass can be used for the other purposes, in particular, the production of surfactants.

As a result of the conducted studies it has been found, that the cultivation of the Spirulina biomass and the costs for mineral salts, provided for the 4th and 6th batches, represent the optimal conditions (Table 1). In these cases, the better indices of the final biomass content in the nutrient solution and lower costs for mineral salts per 1 kg of the dry biomass are marked, which leads, in turn, to the lower prime cost of the biomass.

Drying of the biomass has been carried out on the racks of a specially assembled box, in which the thermal energy of geothermal water of the well serves as a coolant, which will allow the energy costs for this process flow to be excluded from the expense items.

It follows from the obtained experimental data, that growing Spirulina in the conditions of Kazakhstan for commercial purposes, using the alternative resources, such as the thermal energy of geothermal water and nutrient medium on its basis, is technically and economically expedient. Its use can significantly reduce the production cost of the biomass of this culture as a whole. In this regard, this process can be used for large-scale biomass production. Here we want to emphasize that the production of the Spirulina biomass in the climate of Kazakhstan, using the thermal energy of geothermal water, can be compared with the conditions of its cultivation in the countries, located in the tropical zones. The ambient temperature in the tropics is sufficient to maintain the temperature of the nutrient solution for growing the microalgae biomass, i.e. the costs for heating the nutrient medium are eliminated. Thus, the main manufacturing firms (companies) are as follows: Hainan Simai Pharmacy Co. (China); Earthrise Nutritional (California, the USA); Cyanotech Corp. (Hawaii, the USA); Myanmar Spirulina factory (Myanmar). They produce almost the entire global volume of Spirulina in the tropics, in the open pool-type photobioreactors.

3.2. Obtaining biosurfactants from Spirulina biomass

As it is known [15], the most commonly used method for producing biosurfactants from the microalgae biomass lipids is the method through the formation of fatty acid methyl ethers (FAMEs) by re-esterification of lipid triglyceride with methanol. At the same time, the side interactions occur along with the main reaction. To maximally prevent them, we previously conducted studies to find the optimal conditions for obtaining FAMEs of the Spirulina lipid [10, 11].

It follows from the analysis of the experimental data on the determination of the composition of fatty acids of the Spirulina lipid fraction, including those provided in [10], that this fraction contains mainly saturated and unsaturated fatty acids with the hydrocarbon chain length of C16 and C18. These results are in good agreement with the data widely known in the scientific literature, pertaining to the fact that C16 and C18 are the most common microalgae fatty acids: palmitic acid (hexadecanoic, C16:0), stearic acid (octadecanoic, C18:0), oleic acid (octadecenoic, C18:1), linoleic acid (octadecadienoic, C18:2) and linolenic acid (octadecatrienic, C18:3). Other fatty acids, such as C14, C20 and C26–C32, have relatively low concentrations [16, 17].

The suitability of the Spirulina biomass as a feedstock for the production of biodegradable surfactants has been evaluated by the surface tension of the FAMEs and their modification products. It has been shown that the FAMEs, synthesized from the Spirulina lipids, obtained by the methods of extraction with an organic solvent and pyrolysis, have been able to reduce the water surface tension indices at their concentration of 1 wt.% from 72 mN·m⁻¹ down to 31 mN·m⁻¹ and 32 mN·m⁻¹, respectively [10].

These data are in good agreement with the fact, known in the literature, that the potential of microalgae biomass as a source of biosurfactants is ultimately determined quantitatively as the sum of their fatty acid components.
It is known that lipids represent a biological molecule, which is soluble in an organic solvent. Most lipids contain fatty acids, and they are usually divided into two groups according to their polarity: non-polar neutral lipids (acylglycerols, sterols, free fatty acids) and polar lipids (phosphoglycerides, glycosyl glycerides and sphingolipids). That is why, the component composition of the lipid compounds of the Spirulina biomass plays a dominant role for biosurfactants [3], since namely the lipid fraction is the main producer of glycolipids, phospholipids (polar groups of bio compounds) and neutral lipids.

The microalgae biomass, dependence on of the growth conditions, may contain 38–70% of protein, 13–25% of carbohydrates, 6–15% of lipids and 6–9% of minerals. They cover a wide range of biochemical compounds, such as polar lipids (glycolipids, lipopeptides, lipoproteins, phospholipids and neutral lipids (fatty acids) [18, 19], which are synthesized through the biological processes in the course of the autotrophic photosynthesis of the microalgae biomass.

A fatty acid molecule consists of a hydrophilic carboxylate group, attached to one end of the hydrophobic hydrocarbon chain. When the carboxylate end of a fatty acid molecule is bound to an uncharged head group (e.g., glycerin), a neutral lipid molecule (e.g., triacylglycerol) is formed. On the other hand, an association of a fatty acid molecule with a charged head group (for example, glycerol and a phosphate complex) forms a polar lipid molecule (for example, phospholipid) [16]. These types of lipids play different, but important roles in the production of biosurfactants.

When methanol is used for extracting lipids from the dry biomass, only the neutral lipids are extracted, and when a mixture of methanol and chloroform is used for the extraction, the polar lipids are also extracted. Namely, this circumstance explains the best surface-active properties of the FAME sample, obtained from the Spirulina oil by the extraction with a methanol/chloroform = 1/2 mixture, containing more polar lipids as compared with the oil sample, extracted with a methanol/chloroform = 1/1 mixture. In the first case, the water surface tension index reduces from 72 down to 23 mN·m⁻¹, in the second one – down to 31 mN·m⁻¹ (Table 2).

Currently, various directions of the synthesis of biosurfactants, representing the most important and industrially significant class of the nitrogen-containing nonionic surfactants, have become widespread. These products are widely used as components of detergents, foam stabilizers, cosmetic preparations, as well as dispersants, corrosion inhibitors, etc. In the years to come, a sharp increase in their application is expected, due to the low values of the hydrophilic-lipophilic balance, in the field of drilling oil and gas boreholes, in the technology for increasing oil recovery. It is well known that nonionic biosurfactants with improved characteristics are obtained by the ethoxylation of fatty acids. An introduction of ethylene glycols and amides into the reaction allows the analogs of ethoxylated biosurfactants to be obtained without

| Table 2 |
|-----------------|-----------------|-----------------|
| **Dependence of the decrease in surface tension of various mediums on the concentration of the biosurfactant obtained from Spirulina biomass oil** |
| **Biosurfactant concentration, wt.%** | **Surface tension, mN·m⁻¹** | **Amide of fatty acids** |
| **Surface tension, mN·m⁻¹** | **Methyl ethers of fatty acids** | **Amide of fatty acids** |
| **Methods of producing oils** | **Methods of producing oils** |
| Methanol: chloroform (1:1) extraction* | Methanol: chloroform (1:2) extraction |
| Nature of the biosurfactant research medium | Water | Water | Hexane |
| 0.001 | 63.6 | 64.2 | 36.6 | 28.3 |
| 0.01 | 61.5 | 43.8 | 31.7 | 26.0 |
| 0.1 | 54.1 | 37.2 | 28.3 | 20.3 |
| 1.0 | 31.0 | 23.3 | 20.0 | 10.4 |
| * – Data from [7], given for comparison |
the use of ethylene oxide [20, 21], for example, by the reaction of the interaction of vegetable fatty acids with monoethanolamine [20], according to the following scheme:

\[ \text{RCOOH} + \text{NH}_2(\text{C}_2\text{H}_4\text{OH}) \rightarrow \text{RC(O)NH(C}_2\text{H}_4\text{OH)} + \text{H}_2\text{O} , \]

where R is the fatty acid residue.

We have synthesized new types of nonionic biosurfactants from the modified fatty acids of the Spirulina oil and evaluated their surface-active properties [10]. The present work presents for the first time the study of the surface properties of the Spirulina fatty acid amides in hexane with a surface tension index of 18.0 mN·m⁻¹. At the same time, under the identical conditions (t = 18.7 °C), the surface tension of amides in water has been determined, whose value is lower than the value, provided in [10], since those measurements have been carried out at a different temperature. It is seen from Table 2, that a derivative of methyl ethers of the Spirulina oil fatty acids, modified with monoethanolamine, has an ability to reduce the water surface tension index at the concentration of 1 wt.% down to 20.0 mN·m⁻¹; and amide reduces the surface tension index of hexane down to 10.4 mN·m⁻¹. Moreover, amide reduces this index of water by 3.6, and of hexane by 1.7 times, which seems to be connected with the different polarity of the medium. Therefore, the amimated derivative is able to reduce the surface tension indices of both the aqueous and hydrocarbon media (hexane), which makes it possible to consider them as an effective biosurfactant in such areas as oil industry, oil trunk transportation.

It has been noted, that the fatty acid compositions of the microalgae biomass can be of significant practical interest as an efficient and valuable feedstock for the development and large-scale production of nonionic biosurfactants.

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References

[1]. A.A. Solov’ev, M.Ja. Ljamin, L.A. Koveshnikov, S.I. Zajcev, S.V. Kiseleva, N.I. Chernova, Algae energy. M.: MSY, 1997, 64 p. (in Russ.).
[2]. S.A. Kedik, E.I. Jarcev, N.V. Gul’tjaeva. Spirulina – food of the XXI century. M.: Farma Centr, 2006, 248 p. (in Russ.).
[3]. E.M. Radmann, E.G. de Morais, C.F. de Oliveira, K. Zanfonato, J.A.V. Costa, African J. Microbiol. Res. 9 (2015) 2283–2289. DOI: 10.5897/AJMR2015.7634G.
[4]. G.C. Zittelli, N. Biondi, L. Rodolfi, M.R. Tredici. Photobioreactors for Mass Production
of Microalgae. Handbook of Microalgal Culture: Applied Phycology and Biotechnology, Second Edition, Chapter 13. (Eds. A. Richmond, Q. Hu), USA, John Wiley & Sons, 2013, 225–266. DOI: 10.1002/9781185671666.ch13

[5]. D.K.F. Santos, R.D. Rufino, J.M. Luna, V.A. Santos, L.A. Sarubbo, Int. J. Mol. Sci. 17 (2016) 401–417. DOI: 10.3390/ijms17030401

[6]. S. Akbar, N.H. Abdurahman, R.M. Yunus, F.F. Ayaz, O.R. Alara, Biotechnol. Res. Innov. 2 (2018) 81–90. DOI: 10.1016/j.biori.2018.09.001

[7]. S.S. Jha, S.J. Joshi, S.J. Geetha, Braz. J. Microbiol. 47 (2016) 955–964. DOI: 10.1016/j.bjm.2016.07.006

[8]. D.G. De Almeida, R.S. Da Silva, J.M. Luna, R.D. Rufino, V.A. Santos, I.M. Banat, L.A. Sarubbo, Front. Microbiol. 7 (2016) 1718. DOI: 10.3389/fmicb.2016.01718

[9]. S.J. Geetha, I.M. Banat, S.J. Joshi, Biocatal. Agricult. Biotechnol. 14 (2018) 23–32. DOI: 10.1016/j.bcab.2018.01.010

[10]. Zh.N. Kainarbayeva, A.M. Kartay, R.B. Sarieva, B.K. Donenov, M.B. Umerzakova, Russ. J. Appl. Chem. 92 (2019) 964–971. DOI: 10.1134/S1070427219070139

[11]. B.K. Donenov, K. Imanbekov, Zh.N. Kainarbayeva, A.M. Joldassov, A. Kozybayev Industrially important renewable hydrocarbons in the gulf of lake Balkhash, News of Kazakhstan Science [Novosti nauki Kazakhstana] 4 (2015) 55–73.

[12]. Z. Chen, L. Wang, S. Qiu, S. Ge, Biomed Res. Int. 2018, Article ID 1503126. DOI: 10.1155/2018/1503126

[13]. S. Kubicki, A. Bollinger, N. Katzke, K.E. Jaeger, A. Loeschcke, S. Thies, Mar. Drugs 17 (2019) 408–482. DOI: 10.3390/md17070408

[14]. Zh.N. Kainarbayeva, A.M. Kartay, R.B. Sarieva, B.K. Donenov, M.B. Umerzakova, Potential of production of biodegradable surfactants from Spirulina biomass in Kazakhstan conditions, Chemical Journal of Kazakhstan [Khimicheskii zhurnal Kazakhstana] 3 (2018) 133–144.

[15]. R. Halim, M.K. Danquah, P.A. Webley, Biotechnol. Adv. 30 (2012) 709–732. DOI: 10.1016/j.biotechadv.2012.01.001

[16]. H. Liu, W. Liu, Org. Geochem. 113 (2017) 17–26. DOI: 10.1016/j.orggeochem.2017.08.008

[17]. S. Ge, P. Champagne, W.C. Plaxton, G.B. Leite, F. Marazzi, Biofuel. Bioprod. Bior. 11 (2017) 325–343. DOI: 10.1002/bbb.1726

[18]. M.G. Morais, B.S. Vaz, E.G. Morais, J.A.V. Costa, Biomed Res. Int., 2015, Article ID 835761. DOI: 10.1155/2015/835761

[19]. S. Bellou, M. N. Baeshen, A. M. Elazzazy, D. Aggeli, F. Sayegh, G. Aggelis, Biotechnol. Adv. 32 (2014) 1476–1493. DOI: 10.1016/j.biotechadv.2014.10.003

[20]. I.E. Karpeeva, A.V. Zorina, Kh.S. Shikhaliev. Synthesis of sunflower fatty acid amides oils, Proceedings of Voronezh State University: Chemistry. Biology. Pharmacy [Vestnik VGU. Serija: Himija. Biologija. Farmacija] 2 (2013) 39–41 (in Russ.).

[21]. A.A. Gryneva, A.V. Zorina, N.V. Stolpovskaya, A.V. Falaleev, M.Yu. Krysin. Features of synthesis of diethylene glycol esters and fatty acids of vegetable oils, Proceedings of Voronezh State University: Chemistry. Biology. Pharmacy [Vestnik VGU. Serija: Himija. Biologija. Farmacija] 4 (2014) 17–21 (in Russ.).