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POTENTIAL OF PRODUCTION OF BIODEGRADABLE SURFACTANTS FROM SPIRULINA BIOMASS IN KAZAKHSTAN CONDITIONS

Abstract. The prospects of using Spirulina biomass in the process of creating a new class of natural non-ionic surfactants, such as alkyl amides (mono- or diethanolamides) of fatty acids, which are the most widespread and important class among nitrogen-containing nonionic surfactants are shown. The results of studies on quantitative growth and accumulation of biomass and its chemical composition are presented. The surface active properties of the isolated samples were studied. It was found that the biodegradable surfactants obtained from the Spirulina biomass could significantly reduce the surface tension of water.

Key words: biodegradable surfactant, microalgae, biomass, spirulina, properties.

Introduction. In the last decade, there has been a large surge in the interest given to production of biodegradable surfactants due to their advantages, mainly ecological ones. Moreover, their biological compatibility with living organisms and their non-toxicity makes them highly applicable in pharmaceutics, cosmetics and food industry as produce with high added value.

Synthetic surfactants from petrochemical materials is used in many industries, in most cases inflicting serious damage during their usage, both to the affected object and to the environment. This is way of developments, aimed the creation of non-toxic and biodegradable surfactants attract a lot of attention in the scientific circles. Particularly interesting are the biodegradable surfactants that consist of complex natural molecules of plant-based materials, that have high surface-active properties.

Although these natural compounds can not compete economically with their synthetic analogues due to their higher cost and deficiency of the original components, which provokes a disputable issue of their usability, the surfactants obtained from vegetable oils (rapeseed, olive and linseed) are nonetheless already available to consumers. It should be noted that the main problem here is the availability of competition with food products.

Thus, the search of new types of alternative resources for the production of biodegradable surfactants becomes very relevant. Great efforts have been put into perfecting the existing processes and the search for new means of fermentation of microorganisms (yeast fungi, bacteria, etc). However, the current progress in the creation of natural biodegradable surfactants (based on sugars, sterols and fatty acids) doesn't allow for their wide usage on a commercial scale. Main limiting factors are the high cost of production and the competitive struggle for the resources with the food industry. Usage of alternative, renewable resources that do not compete with food industry, such as microalgae, is, at this point, relevant and practically demanded. Among the available raw materials for the production of biodegradable surfactants, microalgae biomass have good potential due to the following reasons: higher growth rates, compared to the usual biomass sources; the ability to absorb gaseous CO_2 using solar energy during cultivation with various climates and on various territories; the ability to store solar energy in energy-rich compounds, such as lipids; lack of direct competition for land plots with food industry and the ability to use salt water sources.

The first such commercial production of betaine based on microalgae as an alternative to the plant-based amidopropyl betaine (figure 1) was created by the American branch of the BASF company (www.basf.us) under the commercial name Dehyton® AO45 based on the development of Solazyme (www.solazyme.com). Dehyton® AO45 is manufactured from microalgae made by Solazyme with the modern technology in a very short time.

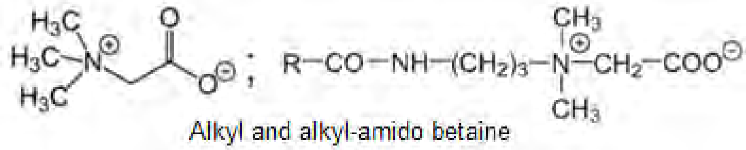


Figure 1 – Betaine molecule structure

The end goal of the current work is the evaluation of the possibility of creation of the material base for the biodegradable surfactants using Spirulina biomass as example in Kazakhstan-based conditions that do not compete with food industry, and synthesis of new derivatives of the biodegradable surfactants from the Spirulina biomass, and the evaluation of their surface-active properties, their potential.

As the object of research spirulina biomass has been chosen. The biomass was synthesized through autotrophic cultivation of a strain from the global culture collection located in the Norwegion Institute for Water Research. Spirulina is the most mass-produced among all of the industrially cultivated globally microalgae cultures.

EXPERIMENTAL PART

Cultivation of Spirulina microalgae. Biomass Spirulina was obtained by cultivation in nutrient medium on the basis of mineral water of hydrocarbonate nature using two kinds of photobioreactor. The laboratory photobioreactor was used for growth and multiplication of the microalgae in the standard Zarrouk medium with the purpose of their usage as stock culture in the inoculation process in an industrial nutrient medium, while in the tubular experimental and the pilot photobioreactors of the reservoir pond type we cultivated the required amounts of the biomass in the main nutrient medium from subterranean mineral waters of bicarbonate nature, obtained at the depth of 1800 meters, at the temperature of 75 °C.

To obtain the inoculant solution a sterile Zarrouk medium used with the following composition: compounds containing macroelements (g/L) – $NaHCO_3$, 16,8; K_2HPO_4, 0.5; $NaNO_3$, 2.5; K_2SO_4, 1.0; NaCl, 1.0; Na_2EDTA, 0.08; $FeSO_4 \times 7H_2O$, 0.01; $CaCl_2$, 0.04; $MgSO_4 \times 7H_2O$, 0.02; + distilled water and compounds containing microelements (g/L) – Na_2EDTA, 0.5; H_3BO_3, 2.86; $MnCl_2 \times 4H_2O$, 1.81; $ZnSO_4 \times 7H_2O$, 0.222; $CuSO_4 \times 5H_2O$, 0.079; MoO_3, 0.015; NH_4VO_3, 0.02296; $Co(NO_3)_2 \times 6H_2O$, 0.04398; $K_2Cr_2(SO_4)_4 \times 24H_2O$, 0.0960; $NiSO_4 \times 7H_2O$, 0.04398; $Na_2WO_4 \times 2H_2O$, 0.01794; $Ti(SO_4)_3$, 0.040).

To obtain biomass, at first a mother liquor of microalgae was prepared in a pilot pool-type photobioreactor, which is a specially assembled test-storage unit of a tubular photobioreactor (figure 2) with a volume of 50 to 100 liters of the suspension in a sterile standard Zarrouk solution. The laboratory photobioreactor was fed with carbon dioxide and air mixture using an aquarium compressor with an average airspeed of 4.5 L/min. The time of cultivation was up to 17 days, under natural lighting corresponding to the solar intensity of 400 MJ/m^3 or more, in the summertime at the temperature range 20–30 °C. Cultivation of microalgae by this system allowed the cultivation of reliable and resistant laboratory-based mononucleic culture, that could be used as stock solution during inoculation.

Figure 2 – Laboratory setup of the tubular photobioreactor

Inoculation of the mixture to the industrial nutrient medium was performed through a sequence of growing additions of hydrocarbonate water refined with the required biogenic elements on a series of photobioreactors with volumes of 0,5;1,0; 3 m^3, and the collection of the required amount of biomass was performed from the bioreactor with the volume of 15 m^3. Biomass was separated through the filtration of the mixture, and then washed with fresh water, then dried and grinded. For cultivation in the industrial photobioreactor we developed a special nutrient medium based on hydrocarbonate water of the subterranean flowing well with the addition of the required biogenic elements for the effective growth of the biomass. To keep the temperature of the liquid nutrient medium constant and stable, thermal energy of the geothermal waters was used, which allowed to provide a stable production of biomass – 5kg of dry biomass per month. Cultivation was performed in the patented by the authors: nutrient medium [1] and photobioreactor [2]. Thanks to the fact that the temperature of the process (its nutrient medium) lies in the 25–35 °C range, biotechnological regime can be supported using the alternative energy source – the source of the low-temperature geothermal water well. The spirulina biomass obtained this way was used as an object of research to obtain biodegradable surfactants.

Extraction of lipids from Spirulina biomass. The researched dry Spirulina biomass was thoroughly rubbed with quartz sand. Then it was mixed with the extracting mixture, 1:1 by mass. The obtained extract, after filtration, was collected into the measuring cup. Then, the methanol mixture was removed by evaporation.

Synthesis of methyl ethers of fatty acids. 2.25 moles (91 ml) of methyl alcohol and 0.25 moles (218.75 g) of Spirulina extraction lipid were placed in a 500 ml round-bottomed flask. To the reaction mass, 5 weight percent (14.5 g) of the prepared solid phase catalyst (KOH / activated carbon) was added. The reaction mixture was heated to 73 ° C. The heating rate was 1.2-1.3 ° C per minute. The reaction mixture was kept at this temperature for 8 hours. The catalyst was filtered off and washed with two 200 ml portions of methanol and reused. The reaction mass was cooled to 30 ° C and a heavier lower glycerin layer was separated. Excess methanol was distilled and regenerated.

Synthesis of methyl esters is carried out according to the inter-esterification reaction (figure 3).

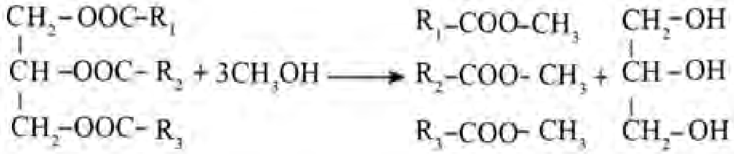


Figure 3 – Reaction of inter-esterification

Synthesis of pyrolysis lipids from biomass. Lipids from biomass were isolated by pyrolysis at temperatures up to 3000C and between 3000–4500C in a tubular quartz reactor in a current of inert gas of nitrogen. From the sample of

15.0 grams of biomass, the oil yield was 1.3 and 1.8 g. The obtained oil from the biomass was dissolved in hexane. The composition of the oil from the biomass was analyzed using gas-liquid chromotrophy (GLC) equipment equipped with a mass spectrometric detector.

Determination of fatty acid composition by chromatography-mass spectrometry method. Component compositions of the lipid obtained Spirulinae was identified by gas chromatography (GLC) AgilentTechnologies 5890N with a mass selective detector (mobile phase: chloroform: methanol = 1: 1, vol.%, HP-1 column type) by the procedure of [3] . The initial temperature of the column is 40 °C, the holding at the initial temperature is 1 min; programming temperature from 40 to 220 ^{o}C, speed 15grad/min, from 220 to 320 ^{o}C deg with the speed of 5 grad/min. Exposure at the last temperature of 15 minutes. The gas is helium osm. brand "5". The sample volume is 1 μl, the temperature of the evaporator is 280 °C.

Identification of the chromatograms was carried out manually by comparing the mass spectra of the test compounds with the library data of NIST05.

Determination of surface-active properties. The surface activity of the samples was evaluated on a KRUSS tensor K20 EasyDyne using the Wilhelm plate method. Surface and interfacial tension measurements were carried out at room temperature, or in the range of -10 to 100 °C using a thermostated jacket, the temperature of which is maintained by means of a circulating thermostat. At the same time, the surface tension of aqueous solutions was determined in the range of their concentration from 0.001 to 1 %.

RESULTS AND DISCUSSION

Quantitative indicators of growth and biomass accumulation were assessed by measuring the optical density of culture media on a Perkin Elmer lambda-35 ultraviolet spectrometer in the wavelength range from 420 to 650 nm. In this case, a maximum intensity of 420-680 nm characterizes chlorophyll, 490 nm beta carotenoids, and 620 nm phycocyanin. The obtained experimental data on the accumulation of biomass are shown in figure 4.

A common characteristic pattern is the S-shaped form of the growth-time curve, although the specific shape for each nutrient medium is quite different, due to the difference in the biogenic constituents of the components. At the same time, the growth of the microalgae biomass eventually leads to an increase in the cell concentration to a certain maximum culture density. This limiting density turned out to be 0.4 g/L and is apparently related to some limitations, including: the exhaustion of mineral nutrient elements, the decrease in the penetration of the layer of light into the stratum or the accumulation of metabolites in the nutrient medium, as well as by other physicochemical conditions of the environment.

The growth dynamics of the studied culture in both media have a typical S-shaped curve, which can be conditionally divided into 6 sections. Note that, in general, the process of accumulation of Spirulina biomass in the studied nutrient media is similar to that of any other microalgae [4].

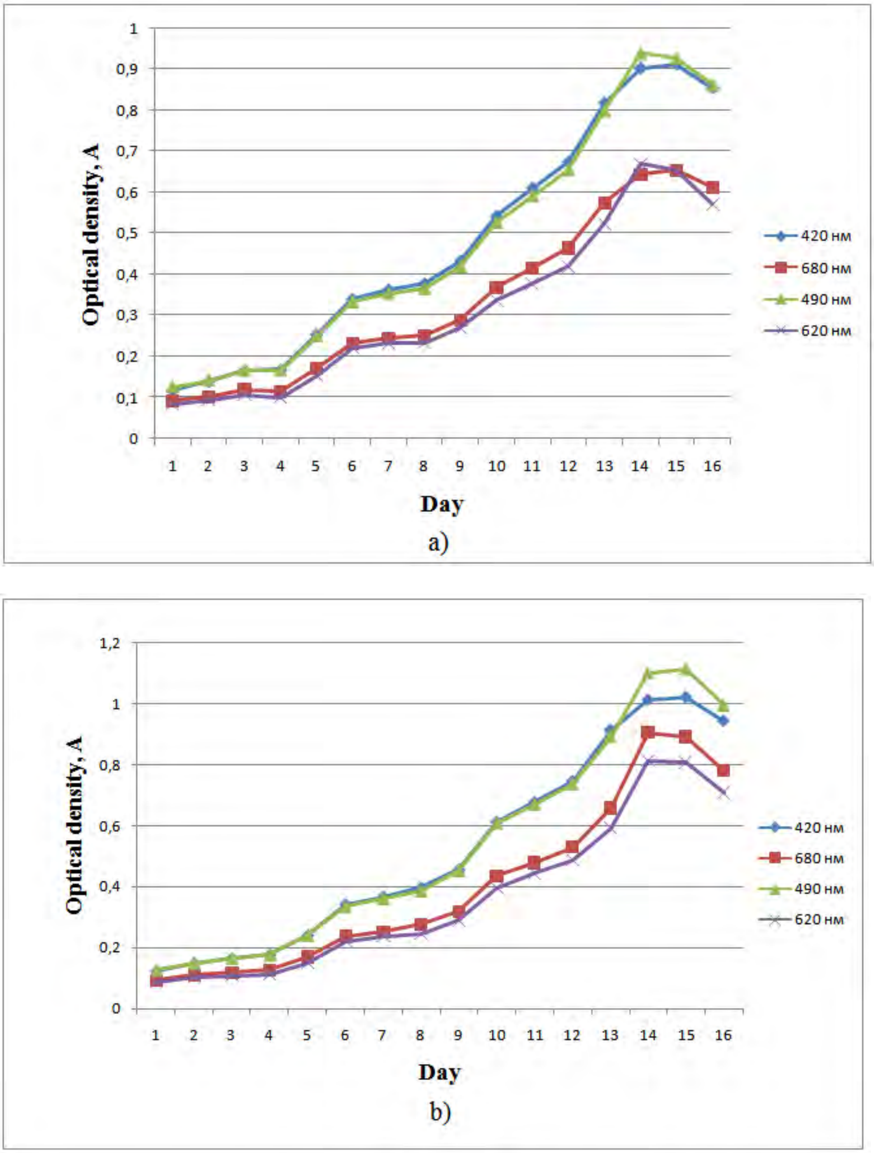


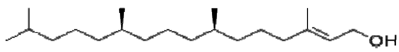
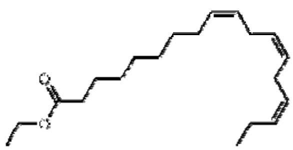
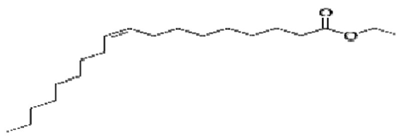
Figure 4 – Growth characteristics of biomass Spirulina in various nutrient media:
a) standard culture medium of Zarrouk;
b) commercial environment based on local bicarbonate mineral water

The composition of the nutrient media directly affects the component composition of the resulting biomass, which determines the final properties of the surfactant. This allows for directional biosynthesis. For example, the authors of [5] demonstrated the possibility of obtaining an extract of Spirulina biomass with specified surface-active properties.

The obtained results indicate that in the conditions of Kazakhstan it is possible to effectively cultivate Spirulina, suitable for the creation of biodegradable surfactants.

The chemical composition of the biomass oil obtained by extraction is determined, and results are given in table 1.

Table 1 – Chemical composition of the extraction oil of biomass from gas-liquid chromatography data

Compounds	Structural formula	Content, %
Hexadecane		1,17
Heptadecane		27,73
1, 19-Eicosadiene		1,90
n-Hexadecanoic acid		3,53
Hexadecanal		2,29
Hexadecanoic acid, ethyl ester		16,15
Phytol		3,74
9,12,15-octadecatrienoic acid, ethyl ester (z, z, z)		9,47
9,12-octadecadienoic acid, ethyl ester		5,79
Ethyl oleate		1,23

The reaction of the esterification of triglycerides (lipid fraction) with methanol was carried out in the presence of a catalyst KOH. The component composition of the fatty acid methyl esters obtained was analyzed by GC on an Agilent Technologies 6890N (USA) instrument equipped with a mass selective detector. Identification of the compounds obtained was performed using a gas-liquid chromatography database. The obtained spectra are shown in figure 5.

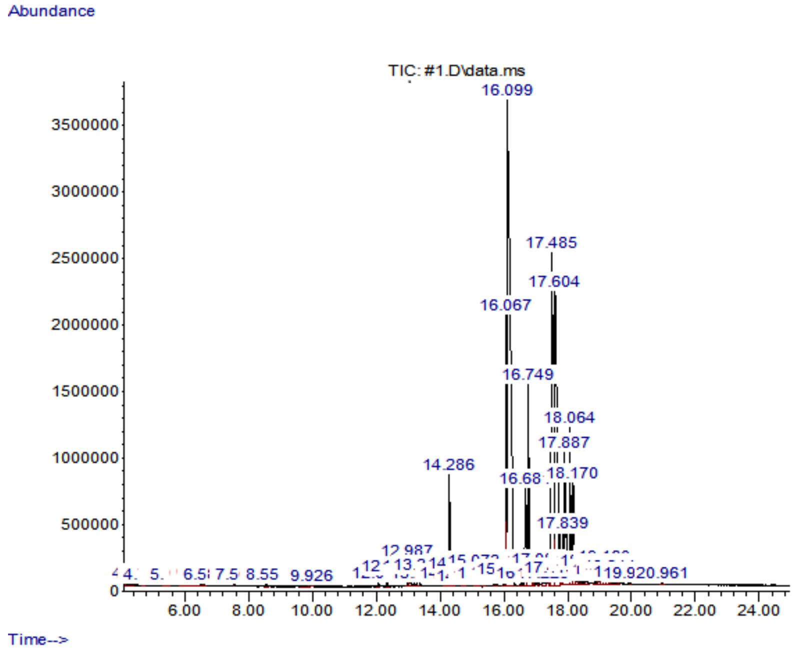


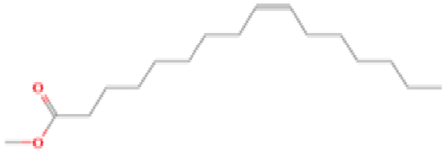
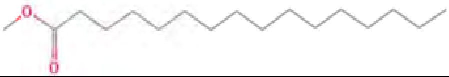
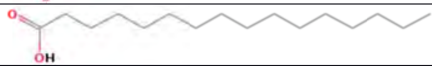
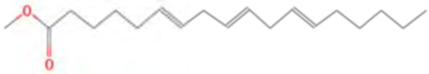
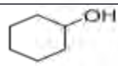
Figure 5 – Chromate-mass spectrometric data of biomass Spirulina

Based on the data of spectral studies, the chemical composition of the lipid constituents of the fatty acids of the Spirulina biomass is shown in table 2. It follows from the analysis of the table that the methyl lipid ester is mainly methyl fatty hexadecanoic acid.

Note that Spirulina biomass is very rich in amino acid compounds (65–70% protein compounds), the second leading component of its composition are carbohydrates (polysaccharides), and lipids occupy only 7–8%. Biomass microalgae also contains compounds such as glycolipids, phospholipids and neutral lipids [6, 7], in addition, sugars, sterols, terpenes and fatty acids, which serve as the main functional components of the composition of natural surfactants [8]. From each given group, in the future, the most suitable candidates for saccharides, peptides (amino acids) can be isolated as a starting material with significantly better hydrophilic properties for the production of biodegradable surfactants. As a hydrophobic compound from a lipid microalgae composition, one of the fatty alcohols can be selected. It is on this principle that a new 100% vegetable surfactant based on alkyl polyglucosides is created under the trade name EcoSense ™ from Dow Personal Care. This surfactant has the following superiority: excellent foaming, softness to the skin, does not contain preservatives, broad compatibility, easy biodegradation, low ecotoxicity.

Fatty acids are useful building blocks for the hydrophobic part of the surfactant molecule, since they contain a reactive carboxyl group. Simple alkenes are also of great interest, since it becomes possible to attach the units of the molecule

Table 2 – Products of the reaction of methylation of triglycerides

Compounds	Structural formula	Content, %
Heptodecane		2.915
Methyl 9-hexadecenoic acid		3.678
Methyl ester of hexadecanoic acid		33.979
n-hexadecanoic acid		2.569
Ethyl ester of hexadecanoic acid		6.227
The methyl ester of 6,9,12-octadecatrienoic acid		16.223
Methyl 9,12-octadecadienoic acid		13.428
Cyclohexanol		5.634
3-tridecene-1-yne		3.707
9,17-octadecadienal		2.497
Other		9.143

at the site of the double bond, and thus can be converted to good hydrophobic compounds.

The compounds obtained by us in terms of molecular structure are close to those promoted by the American company in the field of biodegradable surfactants Elevance Renewable Sciences Inc. 9-decenoicacid (9-DA). The company offers 9-DA () as a new special substance, which is a completely natural difunctional compound in the production of biodegradable surfactants. This comound allows us to create a technological platform for petrochemical products, acting as a building block, due to the presence of a difunctional group in the structure. This will significantly expand the market for innovation and the scope of their use.

Thus, the totality of the obtained data unambiguously testifies that Spirulina can be an effective renewable industrial raw material resource, which is an alternative to traditional types of plant raw materials, which give biopreparation lines, and not a separate, specific type of biodegradable products. And in our opinion, this is the main strength of Spirulina for the development of biodegradable surfactants.

Surface active properties of Spirulina samples were studied. The dependence of the surface tension on the surfactant concentration in the water solution is revealed. The results are represented in table 3.

Table 3 – Dependence of surface tension on concentration

Concentration of surfactant, mass.%	Name					
	Spirulina biomass extract	Methyl ethers of Spirulina fatty acids	Methyl ethers of Spirulina fatty acids (extraction methanol: chloroform)	Pyrolysis oils, temperature under 300 °C	Pyrolysis oils, temperature 300–450 °C	New nonionic surfactant based on amide of fatty acids Spirulina
	Surface tension (mN/m)					
0.001	56.1	63.6	64.2	69.3	44.1	59.2
0.01	49.8	61.5	43.8	65.8	43.5	41.4
0.1	45.5	54.1	37.2	61.7	35.4	31.0
1	**34.8**	**310**	**23.3**	**45.3**	**32.2**	**29.7**

As can be seen from this table, in the samples studied, the surface tension index decreases with increasing concentration. At the same time, the biomass extract obtained from methyl esters of fatty acids from spirulina triglycerides corresponds to lower values of the surface tension index (31.0 mN/m) compared to the pure extract (34.8 mN/m). This fact indicates that Spirulina is a suitable raw material for the production of a biodegradable surfactant, since according to [9] a good surfactant has the ability to reduce the surface tension of water from 72.0 to 35.0 mN•m^{-1}. By our amination of Spirulina fatty acids, a new nonionic surfactant with a surface tension of 29.7 mN/m (table 3) was obtained.

As is known, Spirulina produces such compounds as glycolipids, phospholipids and neutral lipids, which are classified as biological surfactants. Here the situation is similar to the case of controlled biosynthesis, in which complete elimination of phosphorus and an increased nitrogen content in the nutrient medium lead to a mixture of biomass extract having a low value of the surface tension index (31.2 mN·m^{-1}).

Thermochemically obtained pyrolysis lipids Spirulina also have acceptable surface-active properties. Moreover, the pyrolysis lipid fraction obtained at temperatures above 300 °C, has better surface-active properties, as can be seen from the data in table 3.

Thus, the conducted researches established that in the conditions of Kazakhstan it is possible to effectively produce biomass of Spirulina, which does not compete with crop production.

The prospects of using Spirulina biomass in the process of creating a new class of natural non-ionic surfactants, such as alkyl amides (mono- or diethanolamides) of fatty acids, which are the most widespread and important class among nitrogen-containing nonionic surfactants possessing detergent and cleaning action, wetting and emulsifying properties , regulating and stabilizing foaming, which affect the viscosity increase. All this ensures their use in cosmetics, liquid detergent formulations, pharmaceutical emulsions as an additive to soaps, hair dyes, as raw materials for the production of other surfactant classes, as well as in the textile industry, plastic processing, flotation, dry cleaning and processing of metals. It has been found that biodegradable surfactants from Spirulina's obtained biomass have the ability to reduce the surface tension of water significantly more (up to 23 $mN{\bullet}m^{-1}$) than many good surfactants, which lower this figure to 35,0 $mN{\bullet}m^{-1}$.

Although the production of a completely natural non-ionic surfactant requires advanced technologies, it is already becoming a commercial reality. As a source of natural non-ionic surfactant, the raw material resources of the biomass Spirulina can contribute to the further development of the microalga industry, the potential market in Kazakhstan in the feed, salmon and poultry industries, as well as nutraceuticals in the food industry.

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Резюме

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Б. Қ. Доненов, М. Б. Өмірзакова

ҚАЗАҚСТАН ЖАҒДАЙЫНДА СПИРУЛИНА БИОМАССАСЫНАН
БИОЫДЫРАМАЛЫ ББЗ ӨНДІРУ ӘЛЕУЕТІ

Азотты қосылысы бар бейионогенді ББЗ класының ішінде кең таралған және маңызды (моно- неимесе диэтаноламиді) алкилоламидті май қышқылдары болып табылатын жаңа табиғи бейионогенді ББЗ класын құру процесінде Спирулина биомассасының келешекте пайда-лануы көрсетілген.

Зерттеу нәтижелсінде биомассаның өсуі мен жинақталуының сандық көрсет-кіштері мен оның химиялық құрамы анықталды. Бөлініп алынған үлгілердің беттік белсенді қасиеттері зерттелді.

Түйін сөздер: биоыдырамалы беттік-белсенді зат, микробалдыр, биомасса, спирулина, қасиеттері.

Резюме

Ж. Н. Кайнарбаева, А. М. Картай, Р. Б. Сариева,
Б. К. Доненов, М. Б. Умерзакова

ПОТЕНЦИАЛ ПРОИЗВОДСТВА БИОРАЗЛАГАЕМЫХ ПАВ
ИЗ БИОМАССЫ СПИРУЛИНЫ В УСЛОВИЯХ КАЗАХСТАНА

Показана перспективность использования биомассы Спирулины в процессе создания нового класса природных неионогенных ПАВ, таких как алкилоламиды (моно- или диэтаноламиды) жирных кислот, являющихся наиболее распространенным и важным классом среди азотсодержащих неионогенных поверхностно-активных веществ. Приведены результаты исследований по определению количественных показателей роста и накопления биомассы, ее химического состава. Изучены поверхностно активные свойства выделенных образцов.

Ключевые слова: биоразлагаемое поверхностно-активное вещество, микроводоросль, биомасса, спирулина, свойства.