ЕҢБЕК ҚЫЗЫЛ ТУ ОРДЕНДІ
«Ә. Б. БЕКТҰРОВ АТЫНДАҒЫ
ХИМИЯ ҒЫЛЫМДАРЫ ИНСТИТУТЫ»
АКЦИОНЕРЛІК ҚОҒАМЫ

ҚАЗАҚСТАННЫҢ ХИМИЯ ЖУРНАЛЫ

ХИМИЧЕСКИЙ ЖУРНАЛ КАЗАХСТАНА

CHEMICAL JOURNAL of KAZAKHSTAN

АКЦИОНЕРНОЕ ОБЩЕСТВО
ОРДЕНА ТРУДОВОГО КРАСНОГО ЗНАМЕНИ
«ИНСТИТУТ ХИМИЧЕСКИХ НАУК
им. А. Б. БЕКТУРОВА»

3 (67)

ИЮЛЬ – СЕНТЯБРЬ 2019 г.

ИЗДАЕТСЯ С ОКТЯБРЯ 2003 ГОДА

ВЫХОДИТ 4 РАЗА В ГОД

АЛМАТЫ
2019

UDC 667.042.2

ZH. N. KAINARBAYEVA, A. M. KARTAY,
R. B. SARIYEVA, B. K. DONENOV, M. B. UMERZAKOVA

JSC «Institute of chemical sciencesnamed after A.B. Bekturov», Almaty, Kazakhstan

DEVELOPMENT OF ALTERNATIVE WAYS OF OBTAINING BIOMASS OF *ARTHROSPIRA PLATENSIS* SUITABLE FOR LARGE-SCALE PRODUCTION

Abstract. Comparative data on the determination of quantitative indicators for the process accumulation biomass of *Arthrospira platensis* depending on the nature of the used nutrient medium is considered. It is shown that using the alternative resources such as carbonat the source of the main nutrient element in the nutrient medium (raw natural soda and raw soda by absorbed flue gas CO_2) and geothermal hydrocarbonate water lead to increase on yield and decrease the cost of biomass produced, and at the same time to decrease greenhouse gas concentrations of CO_2 in the atmosphere. It is noted, that obtaining biomass at lower production costs is associated by decrease in the cost of mineral salts in the nutrient medium (about 23%), which makes the proposed method favorable for obtaining biomass on enlarge scale.

Keywords: microalgae, biomass, *Arthrospira platensis*, nutrient medium, cultivation, amassing, yield.

Due to the effects of global warming, special attention is now being paid to stabilizing or reducing the concentration of greenhouse gases in the atmosphere. Due to the increase in global overall emission level, deforestation and the intensive use of thermal energy, where the product of combustion of fuels carbon dioxide is one of the main greenhouse gases. Therefore, in recent years, great interest has been shown in the fixation of CO_2 by biological agents using microalgae to minimize the effect of this sequestration on the environment. In addition, microalgae are widely used both in laboratory and on an industrial scale for the production of high value-added molecules in pharmaceuticals, cosmetics, animal feed and food products, i.e. the main aspect of biofixation of CO_2 by microalgae is associated with the production of added-value molecules. One of them are natural biodegradable surface-active substances (surfactants). Interest in the production of biodegradable surfactants is caused not only by their ability to biodegrade, but also with biological compatibility with living organisms and low toxicity. Due to this, they have found wide application in pharmaceuticals, biomedicine, cosmetics and the food industry, and in most cases do no harm after use, both in relation to the object of exposure, and in relation to the environment. Of great interest are biodegradable surfactants consisting of complex natural molecules of plant matter, which have excellent surface-active properties. Although the use of traditional natural compounds is associated with problems caused by the competition with food products, the surfactants obtained from vegetable oils (rapeseed, olive and flax) are already available to consumers.

Therefore, developments aimed to create non-toxic and biodegradable surfactants which are not competing by production of food products are of great scientific interest [1-4].

Therefore, the search for new types of alternative raw materials for biodegradable surfactants is becoming extremely important. However, the progress achieved in the field of synthesis of natural biodegradable surfactants (based on sugars, sterols and fatty acids) does not yet allow their widespread use on a commercial scale. The main limiting factors are the high cost and the presence, as noted above, of the competitive struggle with food products. The use of alternative renewable resources, such as microalgae, which do not compete with the food supply chain, is relevant and practically in demand. Among the available raw materials for the production of biodegradable surfactants, microalgae biomass has good potential for the following reasons: higher growth rates than terrestrial biomass sources; the ability to capture gaseous CO_2 when growing microalgae in a wide variety of climates and lands, converting it to organic matter using solar energy; the ability to store solar energy in energy-rich compounds such as lipids; lack of direct competition with agriculture and the ability to use salt water sources. That is why this approach to solving the problem should be considered as a promising method to counteract global warming and the energy crisis.

It should be particularly noted that with autotrophic photosynthesis of a microalgae culture using alternative methods, three tasks can be solved simultaneously: obtaining raw materials for the production of products with high added value, including biodegradable surfactants [4]; carbon sequestration (CO_2 biofixation) [5-7]; wastewater treatment [8].

Therefore, in order to create a raw material base for the synthesis of new biodegradable surfactants, work was started on obtaining biomass of *Arthrospira platensis* (the scientific name of Spirulina) in Kazakhstan using various alternative sources of nutrient medium and energy [9]. For the study, *Arthrospira platensis* biomass, produced by the method of its autotrophic cultivation, was used [10]. Since the use of distilled water in the production of *Arthrospira platensis* on a large scale is economically unjustified, distilled water was replaced by more affordable natural water, that does not contain impurities [9]. Considering that the standard nutrient medium for Arthrospira platensis is alkaline in nature, geothermal water from underground well No. 20a was used (Shauyelder group of geothermal waters located in the northwestern part of the Arys artesian basin in the district center of Shauyelder, Otyrar region of the Turkestan region, Kazakhstan). The study of the chemical composition and some characteristics of this water showed that underground water has a hydrocarbonate nature, and it may well be suitable for use as the basis of a nutrient medium for cultivation of *Arthrospira platensis* in industrial conditions.

It is known, that the nature of the nutrient medium is the main factor determining the process of forming the component composition of the resulting biomass and, as a result, its surface-active properties, which allows to purposefully carry out the biosynthesis of raw materials for the production of surfactants. The authors of [11] showed the ability to control the surface-active properties of the biomass extract of the microalgae *Arthrospira platensis*: complete exclusion of phosphorus and increased nitrogen content in the nutrient medium resulted in a biomass extract, which corresponds to a low level of surface tension (31.2 $mN{\cdot}m^{-1}$). In addition, as shown in [12], the biomass yield of microalgae depends on the composition of the nutrient medium. At the same time, as a result of the replacement of a part of Zarrouk nutrient medium with an organic extract of chicken manure (rich in nitrogen and phosphorus), productivity was increased and the cost of biomass was reduced.

There are known studies that aimed to increase the biomass yield and, consequently, reduce its cost by changing the composition of the nutrient medium using an integrated carbon capture system and algae production (Bicarbonate-based Integrated Carbon Capture and Algae Production System - BICCAPS), proposed by the authors [6] and successfully tested in [7]. It is shown that the absorption solution (the basis of a liquid nutrient medium) based on sodium carbonate can be recycled up to 7 times without reducing the productivity of microalgae.

In the present work, instead of pure sodium carbonate, natural raw soda was used (component composition,%: sodium carbonate hydrate $Na_2CO_3x7H_20$, 39.9; throne $Na_3H(CO_3)_2(H_2O)_2$, 23.5; thermonatrite $Na_2CO_3H_2O$, 24.4; burkeit $Na_6(CO_3)(SO_4)_2$, 12.3%), as it is a cheaper natural carbon raw material for growing microalgae, since its industrial production was introduced by quarrying. In addition, raw soda has high solubility in water. By absorbing carbon dioxide with a solution of this system, the raw soda is enriched with sodium bicarbonate due to the transfer of carbonate to bicarbonate. Further, its solution, enriched with bicarbonate, can be used in the process of cultivation of microalgae, which convert solubilized CO_2 into biomass [5-7]. At the end of this process, the biomass is removed by filtration from a solution of the nutrient medium, and the spent nutrient solution of soda, having a higher content of carbonate component, due to the extracted CO_2, is recycled to absorb CO_2. As a source of CO_2 a mixture of flue gas of power plants can be used.

EXPERIMENTAL PART

Production of microalgae biomass: Arthrospira platensis biomass was obtained by the method of autotrophic photosynthesis in a pilot photobioreactor, which is a laboratory test-accumulative tubular installation (figure 1) in various nutrient media, which are based on the liquid nutrient medium, which are distilled water and underground mineral water of hydrocarbonate nature extracted from a depth of 1800 m with a temperature at the wellhead of 75°C, located in the settlement of Shauelder, Otyrar region, Turkestan region of Kazakhstan. Zarrouk solution was used as nutrient medium, minerals: macronutrients ($g{\cdot}l^{-1}$) - $NaHCO_3$, 8.0; KNO_3, 2.0; $(NH_4)_2HPO_4$, 0.12; NaCl, 1.0; carbamide, 0.02; Na_2EDTA, 0.08;

Figure 1 – Laboratory installation of tubular photobioreactor

+ distilled or geothermal hydrocarbonate water, as well as trace elements ($g \cdot l^{-1}$) - Na_2EDTA, 0.5; H_3BO_3, 2.86; $MnCl_2 \cdot 4H_2O$, 1.81; $ZnSO_4 \cdot 7H_2O$, 0.222; $CuSO_4 \cdot 5H_2O$, 0.079; MoO_3, 0.015; NH_4VO_3, 0.02296; Co $(NO_3)_2 \cdot 6H_2O$, 0.04398; $K_2Cr_2(SO_4)_4 \cdot 24H_2O$, 0.0960; $NiSO_4 \times 7H_2O$, 0.04398; $Na_2WO_4 \cdot 2H_2O$, 0.01794; $Ti(SO_4)_3$, 0.040). The growth time of the microalgae was 17 days under natural light conditions at an ambient air temperature of 20–30 °C, the medium was aerated with the help of fresh air or a mixture of CO_2 and air. Biomass was separated by filtering the suspension. Then it was washed with fresh water, dried and then subjected to grinding.

In our nutrient medium, commercial $NaHCO_3$ in Zarruk's environment was replaced with an equivalent amount of a solution of natural raw soda, and in another case, raw soda that previously subjected to CO_2 absorption (raw soda/CO_2) of the flue gas of a coal-fired power plant (Ekibastuz GRES-1, Kazakhstan). When carrying out cultivation on an enlarged scale, the temperature of the nutrient medium (25–30°C) is provided by an alternative source of energy – geothermal waters of underground wells.

Component composition of raw soda, %: sodium carbonate hydrate $Na_2CO_3 \cdot 7H_20$, 39.9; $Na_3H(CO_3)_2(H_2O)_2$, 23.5; thermonatrite $NA_2CO_3H_2O$, 24.4; Burkeite $Na_6(CO_3)(SO_4)_2$, 12.3%).

Quantitative indicators of growth and accumulation of Arthrospira platensis biomass in Zarruk medium and experimental medium based on bicarbonate water and raw soda were determined by measuring the optical density of culture media on a Perkin Elmer lambda-35 UV spectrometer in the wavelength range from 420 to 650 nm the peaks of the components of the biomass components, based on the absolute values of which were judged on the nature of growth and accumulation of biomass in suspension, the final concentration was determined by the gravimetric method after collecting b omassy.

Flue gas CO_2 absorption was performed with a solution of natural raw soda in the flue gas flow of Ekibastuz GRES-1. The process was monitored by measuring the concentration of CO_2 on the Polar gas analyzer at the inlet and outlet of the absorber (figure 2). The insert into the operating ducts of unit No. 5 was carried out directly on the site immediately after the electrostatic precipitator.

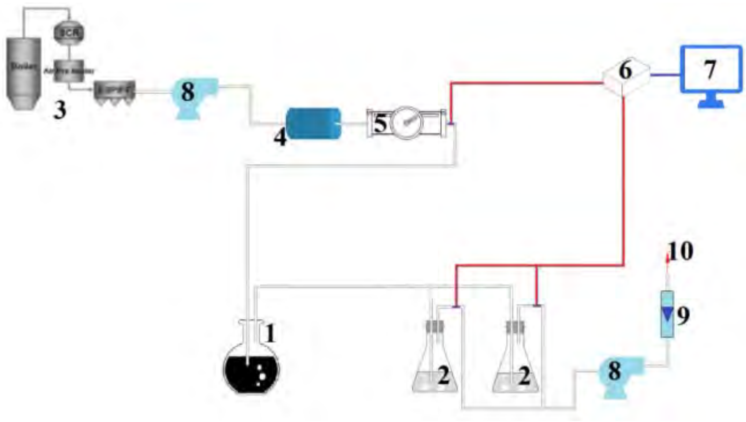

Figure 2 – Diagram of absorption of flue gas CO_2 by raw soda:
1 - buffer solution, 2 - absorber soda solutions, 3 - electrostatic precipitator, 4 - filter,
5 - gas / air flow meter, 6 - gas measuring point, 7 - computer, 8 - compressors (exhausters),
9 - rotameter, 10 - line of connection with the factory exhaust pipe

X-ray phase analysis of raw soda after absorption of flue gas CO_2 was conducted on D8 Advance (Bruker), α-Cu, pipe voltage of 40 kV, current of 40 mA. The processing of the obtained diffractogram data and the calculation of the interplanar distances were carried out using the EVA software. Sample decoding and phase search were performed using the Search/match program using the PDF-2 Powder Diffractometric Database.

RESULTS AND DISCUSSION

The potential of applicability of microalgae biomass for use as a raw material for the production of any valuable substances is determined by its effective productivity, which is ensured by optimizing the process of cultivation of microalgae cells [12]. One of the main parameters of this process is the nutrient medium; therefore, we carried out work to change its composition and its effect on the biomass yield. We have studied the nutrient medium in which commercial $NaHCO_3$ in the Zarrouk medium was replaced by natural raw soda, in the other case, raw soda/CO_2. It has been established that their use, with all other biogenic elements of Zarrouk medium composition unchanged in the nutrient medium both on the basis of distilled water and on the basis of geothermal bicarbonate water, is quite applicable, since such a replacement has a positive effect on the processes of biomass growth and formation of Arthrospira platensis (table 1).

As follows from this table, the growth characteristics of the samples studied by 2 times or more (taking into account the initial concentrations) exceed similar parameters in comparison with the case when baking soda is the main nutrient element, while the best results are obtained using raw soda and raw soda/CO_2 in distilled water.

Table 1 – *Arthrospira platensis* biomass cultivation conditions and yield

Nutrient medium			T_{medium}, °C	Biomass content, g·L^{-1}		Increase in biomass content	pH medium	
main biogenic element	liquid base	barbotage		start	end		start	final
Raw natural soda	Distilled water	Air	25.5	0.202	3.99	~ 20 times	9.5	10.5
Raw natural soda	Distilled water	air + CO_2		0.202	2.77	~ 14 times	9.5	9.2
Raw natural soda	Subterranean hydrocarbonate water	air		0.241	3.79	~ 16 times	9.2	10.4
Raw natural soda	Subterranean hydrocarbonate water	air + CO_2		0.241	3.65	~ 15 times	9.2	9.4
Raw soda/CO_2	Distilled water	air + CO_2	23.4	0.191	3.63	~ 19 times	9.4	10
Sodium hydrocarbonate*	Distilled water	air + CO_2	21.5	0.208	1.62	~ 8 times	8.6	9.1
Sodium hydrocarbonate *	Subterranean hydrocarbonate water	air + CO_2		0.208	1.6	~ 8 times	8.6	8.8

*Previously acquired data [9].

It can also be seen that in the case of enriched soda (raw soda/CO_2), the increase in biomass from the initial soda is larger compared to raw soda (19 and 14 times, respectively), which is apparently due to an increase in sodium bicarbonate content in raw soda as a result of its absorption of flue gas CO_2. An X-ray analysis obtained by X-ray phase analysis (figure 3) revealed that sodium bicarbonate in the raw soda/CO_2 is in the form of a trona — $Na_2CO_3NaHCO_3$, and its content is 52 %. This is due to the transformation of sodium carbonate (Na_2CO_3)

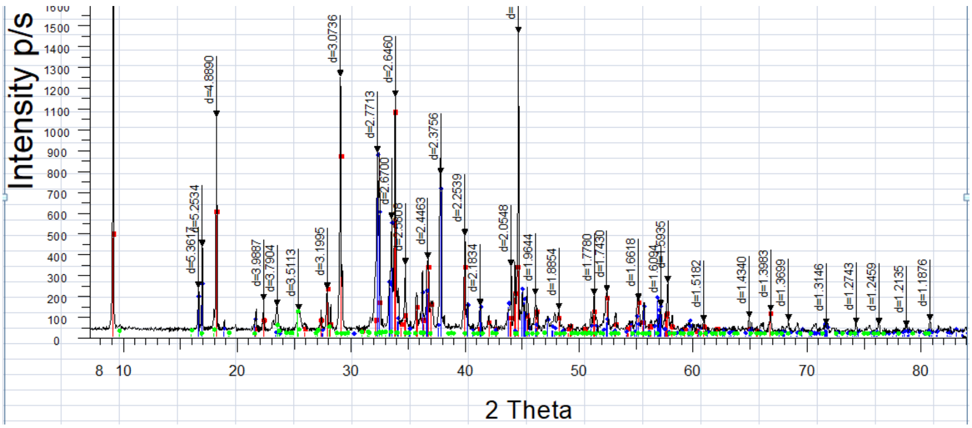

Figure 3 – X-ray pattern of natural raw soda after flue gas CO_2 absorption

raw soda after carbon dioxide absorption into sodium bicarbonate ($NaHCO_3$) according to the well-known equation: $Na_2CO_3 + H_2O + CO_2 \leftrightarrow 2NaHCO_3$. The proposed integrated carbon sequestration and algae production system (BICCAPS) based on sodium bicarbonate details the mechanism of transformation of sodium carbonate to bicarbonate when CO_2 is absorbed [5, 6].

From the data of table 1 it also follows that during the cultivation of microalgae using sodium bicarbonate as a source of nutrient, the pH gradually increases. This, according to [5], is due to the biological fixation of CO_2 in the form of carbon, biomass and the release of hydroxide ions [OH]-, which occurs as a result of photosynthesis. They further interact with unreacted sodium bicarbonate to form carbonate. The carbonate solution obtained in this way can be re-directed to the process of CO_2 absorption of flue gas (recycle), which leads to the formation of a technologically closed biomass cultivation cycle. As shown [7], such a nutrient medium can be recycled up to 7 times without reducing the productivity of microalgae.

Based on the above data, we can say that the developed nutrient medium for growing biomass Arthrospira platensis, in which natural soda or raw soda/CO_2 is taken as the main nutrient, can be used to cultivate microalgae biomass. At the same time, it can be obtained with lower costs, since the calculations made show that replacing marketable sodium bicarbonate in a nutrient medium by an alternative source, natural raw soda, will reduce the cost of mineral salts by 23% (table 2). In the case of raw soda/CO_2, the issue of environmental protection is also being addressed – the capture of CO_2 from the flue gas of a coal-fired power plant emitted into the atmosphere. As is known, at present, special attention is directed at stabilizing or reducing the concentration of greenhouse gases in the atmosphere, one of the main ones being carbon dioxide. In addition, additional savings in production costs will be obtained from the use of underground bicarbonate water instead of distilled water. Therefore, the proposed method of cultivation of biomass *Arthrospira platensis* can be considered suitable for release on an aggregate scale and the development on its basis of biodegradable surfactants.

Table 2 – Comparison of costs of mineral salts of traditional cultivation mode and alternative cultivation mode

Modes	Main salts used in the nutrient medium	Price of salts, KZT/g	Consumption norm, g/L	Cost, per liter, KZT	Total cost of per liter, KZT
Traditional cultivation	Sodium bicarbonate $NaHCO_3$	0,15	8	1,2	2,71
	Potassium nitrate KNO_3	0,67	2	1,34	
	Diammonium phosphate $(NH_4)_2\cdot 2HPO_4$	0,85	0,2	0,17	
Alternative cultivation	Raw natural soda (KNO_3 and $(NH_4)_2\cdot 2HPO_4$ no changes)	0,036	16	0,576	2,086 (decrease in cost 23%)

Thus, the growth characteristics of the biomass of *Arthrospira platensis* in various nutrient media were determined. The source of the main nutrient element carbon in the nutrient medium was varied. It was proposed to replace commercial sodium bicarbonate ($NaHCO_3$) in Zarruk's standard medium with an equivalent amount of a solution of natural raw soda, raw soda with absorbed CO_2 of the flue gas of a coal-fired power plant, and use geothermal hydrocarbonate water as a liquid nutrient medium to produce microalgae biomass. Such a replacement significantly affects the yield of biomass and allows you to get it with lower cost, suitable for release on an aggregate scale and the development of biodegradable surfactants. It was found that the replacement of sodium bicarbonate with raw soda will reduce the cost of mineral salts by 23 %. If we also take into account the additional contribution from compensation for reducing CO_2 emissions into the environment, as well as the use of underground hydrocarbonate water instead of distilled water, the savings increase. Due to that, there is both an economic and social effect.

For work on an enlarged scale, a pilot photobioreactor located in the immediate vicinity of the geothermal water well and operating on the basis of alternative resources is to be used. Those are, in addition to geothermal hydrocarbonate water - the liquid base of the nutrient medium, natural raw soda or raw soda/CO_2 – the main sources of nutrient, and the thermal energy of geothermal water used to ensure the temperature regime of the biotechnological process.

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Резюме

Ж. Н. Қайнарбаева, А. М. Қартай, Р. Б. Сариева, Б. Қ. Доненов, М. Б. Өмірзакова

КЕҢ КӨЛЕМДІ *ARTHROSPIRA PLATENSIS* ӨНДІРІСІНЕ ЖАРАМДЫ БИОМАССАЛАР АЛУДЫҢ БАЛАМАЛЫ ӘДІСТЕРІН ЖАСАУ

Қоректік ортаның табиғатына байланысты *Arthrospira platensis* биомассасының жинтық процесінің сандық көрсеткіштерін анықтау бойынша салыстырмалы деректер келтірілген. Баламалы ресурстарды пайдалану қоректік ортада көміртек элементінің негізгі биогенді көзі және геотермалды гидрокарбонатты су, өндірілетін биомассаның шығымының артуына және өзіндік құнының төмендеуіне, сонымен қатар, атмосферада парникті газдың CO_2 концентрациясының төмендеуіне әкеледі. Аз шығынмен биомассаны алу, қоректік ортаның минералды тұздарына жұмсалатын шығындардың азаюымен байланысты, бұл ұсынылып отырған әдіс ірі ауқымда биомассаны алуға қолайлы етеді.

Түйін сөздер: микробалдыр, биомасса, *Arthrospira platensis*, қоректік орта, өсіру, жинақтау, шығым.

Резюме

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

РАЗРАБОТКА АЛЬТЕРНАТИВНЫХ СПОСОБОВ ПОЛУЧЕНИЯ БИОМАССЫ *ARTHROSPIRA PLATENSIS*, ПРИГОДНЫХ ДЛЯ ИХ КРУПНОМАСШТАБНОГО ПРОИЗВОДСТВА

Приведены сравнительные данные по определению количественных показателей процесса накопления биомассы *Arthrospira platensis* в зависимости от природы использованной питательной среды. Показано, что использование альтернативных ресурсов: источника основного биогенного элемента углерода в питательной среде (сода-сырец и сода-сырец с абсорбированным CO_2 дымового газа) и геотермальной гидрокарбонатной воды, приводит к увеличению выхода и снижению себестоимости производимой биомассы и одновременно к снижению концентрации парникового газа CO_2 в атмосфере. Отмечено, что получение биомассы с меньшими затратами связано с уменьшением расходов на минеральные соли питательной среды (на 23%), что делает предлагаемый способ благоприятным для получения биомассы в укрупненных масштабах.

Ключевые слова: микроводоросль, биомасса, *Arthrospira platensis,* питательная среда, культивирование, накопление, выход.