ISSN 1563-034X • Индекс 75880; 25880

EXPO 2017
• Future Energy •
Astana Kazakhstan

KazNU Science • ҚазҰУ Ғылымы • Наука КазНУ

ӘЛ-ФАРАБИ атындағы ҚАЗАҚ ҰЛТТЫҚ УНИВЕРСИТЕТІ

КАЗАХСКИЙ НАЦИОНАЛЬНЫЙ УНИВЕРСИТЕТ имени АЛЬ-ФАРАБИ

AL-FARABI KAZAKH NATIONAL UNIVERSITY

ХАБАРШЫ

ЭКОЛОГИЯ СЕРИЯСЫ

ВЕСТНИК

СЕРИЯ ЭКОЛОГИЧЕСКАЯ

EURASIAN JOURNAL

OF ECOLOGY

1(50) 2017

UDC 602.3: 579.8; 606:622.75

B. Zayadan*[1], A. Sadvakasova[1], A. Usserbayeva[1], A. Bayzhigitova[1], F. Sarsekeyeva[1]

[1]Al-Farabi Kazakh national university,
Almaty, Kazakhstan
*E-mail: zbolatkhan@gmail.com

PROSPECTS OF USING CYANOBACTERIA FOR BIODIESEL PRODUCTION

Increasing growth of world costs on petroleum and growing deficiency of petroleum products lead to necessity of using alternative motor fuels. Thus, technology of using cyanobacteria as raw material for fuel production has one of the central place among tools of modern alternative energetics, because they are considered as more effective biological producers of natural hydrocarbons in the form of fatty acids. This allows observing it as a unique renewable biomass source, which is suitable for rapid conversion to biodiesel.

Nowadays, creation of such technology is actual and prospective; additionally it presents a great interest for development of alternative energetics. This article generalize literature data about problems of introducing the alternative "green" technologies and development on new field of industry – bioenergetics, about perspectives and possibilities of biofuel from photosynthetic microorganisms and their preference among conventional sources of raw material. There are also data of experimental works on screening of potential producers, optimization of cultivation of cyanobacteria for increasing the yield of end product. In addition, possible ways of increasing efficiency of their energetical using with the help of genetic engineering methods are reviewed. The main stages of technology of biodiesel fuel production from phototrophic microorganisms are described and results of scientific investigations in this area, conducted in the framework of scientific-research projects on the basis al-Farabi Kazakh National University are presented.

Key words: biofuels, biodiesel, microalgae, cyanobacteria, fatty acids, lipids.

Б. Заядан*[1], А. Садвакасова[1], А. Усербаева[1], А. Байжигитова[1], Ф. Сарсекеева[1]

[1]Әл-Фараби атындағы Қазақ ұлттық университеті,
Алматы қ., Қазақстан
*E-mail: zbolatkhan@gmail.com

Биодизельді жанармай алу үшін цианобактериаларды пайдаланудың болашағы

Мұнайдың әлемдік бағасының өсуі және мұнай өнімдерінің тапшылығының өсуі балама моторлы жанармайларды пайдаланудың қажеттілігіне алып келеді. Цианобактерияларды шикізат ретінде пайдалану технологиясы заманауи балама энергетикаға көшудің маңызды түрі болып табылады, себебі олар май қышқылдары формасындағы табиғи көмірсутегінің эффективті биологиялық продуценттері болып табылады. Бұл оларды жылдам биодизель өндіруге жарамды, универсалды, қалпына келуші биомасса көзі ретінде санауға мүмкіндік береді.

Қазіргі уақытта осындай технологияны дайындау өзекті, перспективті болып табылады және балама энергетиканың дамуы үшін үлкен қызығушылық туғызып отыр. Берілген мақалада балама «жасыл» технологияны енгізу мәселелері жайлы әдеби мағлұматтар берілген және өндірістің жаңа дамыған бағыты – биоэнергетика жайлы, фотосинтездеуші микроорганизмдерден биожанармай алудың мүмкіндіктері мен перспективалары және олардың дәстүрлі шикізат көздерінен артықшылықтары көрсетілген. Потенциалды продуценттердің скринингі бойынша тәжірибелік жұмыстардың мағлұматтары, соңғы өнімнің шығуын арттыру мақсатында, цианобактерияларды оптималды дақылдау жағдайлары көрсетілген. Сонымен қатар гендік инженерлік әдісті пайдалана отырып, олардың энергетикасын пайдаланудың эффективтілігін арттырудың ықтимал жолдары қарастырылады, және де фототрофты микроорганизмдерден биодизель алу технологиясының негізгі кезеңдері және әл-Фараби атындағы ҚазҰУ-дың негізіндегі ғылыми-зерттеу жобаларының шеңберінде өткізілетін ғылыми зерттеулердің нәтижелері көрсетілген.

Түйін сөздер: биожанармай, биодизель, микробалдырлар, цианобактериялар, май қышқылдары, липидтер.

Б. Заядан*[1], А. Садвакасова[1], А. Усербаева[1], А. Байжигитова[1], Ф. Сарсекеева[1]

Казахский национальный университет имени аль-Фараби,
г. Алматы, Казахстан
*E-mail: zbolatkhan@gmail.com

Перспективы использования цианобактерий для получения биодизельного топлива

Продолжающийся рост мировых цен на нефть и растущий дефицит нефтепродуктов приводят к необходимости использования альтернативных моторных топлив. Так, технология использования цианобактерий в качестве топливного сырья занимает одно из центральных мест среди подходов современной альтернативной энергетики, поскольку они являются наиболее эффективными биологическими продуцентами естественных углеводородов в форме жирных кислот. Это позволяет рассматривать их как универсальный возобновляемый источник биомассы, пригодной для быстрой переработки в биодизель.

В настоящее время создание такой технологии актуально, перспективно и представляет большой интерес для развития альтернативной энергетики. В данной статье обобщены литературные данные о проблеме внедрения альтернативных «зеленых» технологий и развития новой отрасли промышленности – биоэнергетики, возможностях и перспективе получения биотоплива из фотосинтезирующих микроорганизмов и их преимуществе перед традиционными источниками сырья. Приведены данные экспериментальных работ по скринингу потенциальных продуцентов, оптимизации условий культивирования цианобактерий с целью увеличения выхода конечного продукта. Также рассмотрены возможные пути повышения эффективности их энергетического использования методами генной инженерии. Рассмотрены основные этапы технологии получения биодизеля из фототрофных микроорганизмов и приведены результаты научных исследований в этой области, проводимых в рамках научно-исследовательских проектов на базе КазНУ им. аль-Фараби.

Ключевые слова: биотопливо, биодизель, микроводоросли, цианобактерии, жирные кислоты, липиды.

The modern civilization require fuel in constantly increasing volume and today world supply of liquid fuel is almost fully dependent from petroleum (Posten 2009: 64–69). However, according to forecasts of specialists in the coming decades, further reduce of production of traditional energy sources, including oil production is expected (Слюсаренко 2010: 36-40). In this regard, there is growing interest in alternative renewable energy sources that can provide stable energy production for an indefinite period. According to forecasts of the International Energy Agency in 2030, oil consumption will reach 106 million barrels per day. At the same time, the cost of a barrel will grow to about $ 133 by 2035.

In this regard, the continued increase in world oil prices and the growing shortage of petroleum products lead to the need for alternative motor fuels (OECD/IEA 2011).

The last decade is characterized by a stable tendency to reduce the share of fossil hydrocarbon raw materials in the production of liquid motor fuels by replacing them with alternative raw materials of plant origin. The reason for this is a decrease in the explored reserves of high-quality «light» oil, the complexity of developing new oil fields and the depletion of old ones, which causes instability in the prices of carbon raw materials for the growing demand for oil and products of its processing. In addition, the burning of fossil fuels leads to the release of carbon dioxide (CO_2), the accumulation of which leads to global warming (Марков 2009: 83-90). According to the Organization for Economic Cooperation and Development, the International Energy Agency and the BCC Research agency, the projected level of biofuel consumption will reduce greenhouse gas emissions by approximately 2.1 gigatons (BCC research 2010). All this stimulates the introduction of alternative "green" technologies and the development of a new industry - bioenergy.

Depending on the type of raw materials used for production, biofuel is divided into four generations. The most widespread is biofuel of the first generation, for which agricultural crops are used as raw materials. In particular, sugarcane, corn, wheat are used for the production of bioethanol, while oil crops such as soybean, rape, oil palm, sunflower and others are used for the production of biodiesel. In the production of second-generation biofuels, the main part of the raw material base is non-food biomass, namely wood pulp (cellulose, lignin), wood processing waste and non-food residues of cultivated agriculture plants, and less valuable raw materials - straw (Naik 2010:578–597, Pahl 2010). Biofuel of the third generation, are obtained from microalgae, which can be cultivated in open water bodies or in closed photobioreactors. Compared with the production of biofuel of the first and second generations, the cultivation of microalgae is less energy-intensive, and also does not involve the use of agricultural land (Pr˘ibyl 2014, Chisti 2007, Schenk 2008). The fourth generation combines the properties of third generation biofuels primarily with the genetic optimization of their producers (Al-Thani 2012:427–440). To date, we are on the verge of creating and using fourth generation biofuels, which are based on rapidly growing, rapidly renewed and genetically modified sources - prokaryotic photosynthetic cells of cyanobacteria (Nozzi 2013). At the same time, it should be noted that biofuel research is not just a matter of finding the right type of biomass and converting it into fuel, but also finding environmentally and economically sound options for using by-products from biofuel production.

One of the most common types of biofuel is biodiesel, perspective for diesel engines of tractors and other agricultural machinery, trucks and cars (Erdrich 2014:128). Biodiesel has become widespread in many countries of the world, such as Germany, Austria, Czech Republic, France, Italy, Sweden, USA, as well as other countries (fig. 1). The world's leading manufacturers of biodiesel fuel are companies such as ADM, Direct fuels, Iowa Renewable Energy, Canadian Bioenergy Corporation, Algal Biomass Organization (http://www.ebb_eu.org/stats.php). In a number of Russian regions, such as the Krasnodar Territory, the Lipetsk Region, the Altai Territory, the own programs have been developed - RusbioDiesel, the Association of Rapeseed Oil Producers, and Rapes Biodiesel, which promote the introduction of biofuel technologies (McKendry P 2002:37–46).

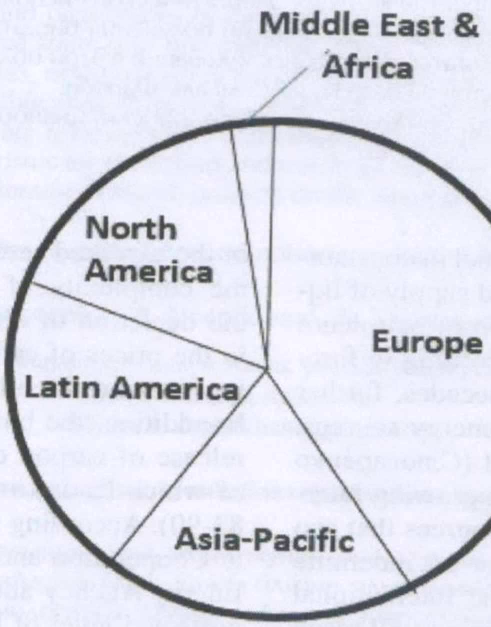

Figure 1 – Global biodiesel demand by region (http://www.ebb_eu.org/stats.php)

The active introduction of biodiesel fuel in the consumer market led to the need of introducing regulatory documents for this type of fuel – the national standards with requirements for the properties of biodiesel and its content in diesel fuel or biodiesel percentage of total diesel fuel consumption. Currently, the European Organization for Standards has developed standard EN14214, which regulates such parameters of biodiesel quality as density, viscosity, sulfur content, water, glycerin, sulphate ash, acid number, iodine number, etc. This standard allows the content of 5% biodiesel in an oil diesel. In addition, there are standards EN590 (or EN590: 2000) and DIN 51606. DIN 51606 is a German standard designed to be compatible with the engines of almost all leading automakers, so it is the most stringent. Most types of biodiesel produced for commercial purposes in the West correspond to it or even surpass it. So in the USA there is a standard - ASTM 6751, adopted in 2002, in Austria - ON C1191-1997, in Australia - FS (B) D-2003, in Sweden -SS155436-1996 g. (http://www.ren21.net/).

Such an established production is realized due to the significant advantages of biodiesel, unlike a conventional diesel: biodiesel in combustion is non-toxic and safe, because it has a high ignition temperature, it does not contain sulfur and carcinogenic benzene, and also reduces carbon dioxide emissions to the atmosphere. It has good lubricating properties, which significantly increases the life of the engine. Because raw materials for biodiesel production are renewable resources, the fuel spilled on the ground is decomposed by 90% by microorganisms in three weeks. So, for example, the production of biodiesel is easy to organize, for example, on the basis of a small farm (Knothe G (2010: 364–373). Rape is used mainly for raw materials in Europe, soybean in the USA, canola in Canada (canola variety), in Indonesia - palm, coconut, in Brazil - castor oil. Also waste vegetable oil, animal fats, fish oil, etc. are used (Bezergianni 2013:110–116). The existing technology for the production of diesel fuel based on vegetable oil, is concentrated mainly on biodiesel FAME (rapeseed oil).

There are several processes for producing biodiesel. The most common is the process of transesterification, representing the reaction of oil or fat with a monobasic fatty acid alcohol, in most cases - methanol. Triacylglycerides are compounds of trivalent glycerol with three fatty acids. As a result, the reaction of transesterification of triacylglycerides with methyl alcohol (methanol) CH_3OH in the presence of a catalyst (NaOH) produces fatty acid esters (biodiesel), and a by-product – glycerin. In this case, for the production of raw materials for biodiesel from oilseed crops, large land areas are allocated, often using increased doses of chemical plant protection products, which leads to biodegradation of soils and a decrease in the quality of soils (Knothe 1999: 795–800).

In this regard, in recent years, the increasing interest of biofuel producers has attracted the bioenergetic potential of photosynthetic microorganisms, with funding for research and development in this area steadily growing (Greenwell 2010: 703-726). Moreover, in addition to obtaining fuel, they can serve as a means for removing carbon dioxide from the atmosphere. Algae have been cultivated in significant quantities since the 1950s for the needs of the pharmaceutical industry. The technology of using phototrophic microorganisms as fuel raw materials occupies one of the central places among the approaches of modern alternative energy (table 1). Studies have shown that phototrophic microorganisms outperform in the potential energy output of typical representatives of vegetable oil crops. Thus, the comparison of the yield of biodiesel from phototrophic microorganisms with the best oil crops of higher plants (Singh 2010:2596–2610) showed that the yield of biodiesel from it contain only 30% oil (m/m) and made up 58700 l/hectare, while for rapeseed and canola – 1190 l/hectare (Singh 2011:26–34); jatroph – 1892 l/hectare (Tamagnini 2007:692–720); For palm trees Pongamia – 2590 l/hectare (Brennan 2010: 557–577).

Table 1 – Comparison of some sources of biodiesel (mt-metric tons, ha-hectare, boe-barrel of oil equivalents)

Oil source	Biomass (mt/ha/yr)	Oil content (% dry mass)	Biodiesel (mt/ha/yr)	Energy content (boe/1000ha/day)
Soya	1-2,5	20%	0,2-0,5	3-8
Rapeseed	3	40%	1,2	22
Palmoil	19	20%	3,7	63
Jatropha	7,5-10	30-50%	2,2-5,3	40-100
Phototrophic microorganisms	140-255	35-65%	50-100	1,150-2,000

The production of biodiesel from phototrophic microorganisms is of increasing interest due to the fact that the content of lipids in some of them under optimal conditions of cultivation can be high up to 80%, and their biomass productivity exceeds the corresponding yield of terrestrial plants in ten times

(Chisti 2007, Richmond 2004). There are several aspects related to the production of biofuels from phototrophic microorganisms that are of interest to researchers and entrepreneurs around the world:

These organisms are able to use water as an electron donor for oxygenic photosynthesis.

They grow to a high density and have a high productivity compared to conventional terrestrial oil crops. Consequently, mass cultivation of photosynthesizing microorganisms for commercial production can be effective.

They are non-food raw materials and can be grown in areas that are unsuitable for agricultural use.

They can use a wide range of water sources (fresh, brackish, sewage, and seawater) and have simple requirements for growth: light, carbon dioxide, inorganic nutrients.

Their biomass can be used for the production of both biofuel and valuable by-products (Sarsekeyeva 2015: 329-340).

From photosynthetic microorganisms for the production of biodiesel fuel, microalgae and cyanobacteria are actively used. Among microalgae characterized by high bioenergetics potential the *Chlorella* sp. (20–50 % lipids), *Neochloris oleoabundans* (35–54 %), *Nannochloropsis* sp. (31–68 %), *Botryococcus braunii* (25–85 %) etc. are the best ones (Басова 2005, Sharma 2012). However, in practice, such high lipid yields are not achievable under real production conditions, in which cultivation is carried out in photobioreactors of various designs. The oil content, as a rule, increases when algae grow under stress, for example, if nitrogen or other nutrients are not sufficient, while algae use oil as a storage material (Wang 2009). However, most microalgae strains have a high content of unsaturated fatty acids, that does not allow to receive without additional processing on the basis of their lipids fuel with low cetane numbers and good resistance to oxidation. Too high cetane numbers increase the viscosity of the fuel at low temperatures. In principle, fatty acids with a chain length of 14-18 carbon atoms with one double chain are considered ideal for the production of biodiesel (Лось 2014).

Cyanobacteria, unlike microalgae, have a more suitable fatty acid composition, which makes them potential candidates for obtaining biodiesel fuel. Fatty acids, found in cyanobacteria, are represented by the following types: 14:0, $14:1\Delta^{9}$, 16:0, $16:1\Delta^{9}$, $16:2\Delta^{9,12}$, 18:0, $18:1\Delta^{9}$, $18:1\Delta^{11}$, $18:2\Delta^{9,12}$, $18:3\Delta^{9,12,15}(\omega^{3})^{13}$, $18:3\Delta^{6,9,12}$ и $18:4\Delta^{6,9,12,15}$ (Sarsekeyeva 2015:329-340, Лось 2014). So there is evidence that the strain isolated from Kharg Island, *Synechococcus* sp. ISC 106, was characterized by the highest accumulation of lipids in the cell, which reached up to 204.91 mg /l per day. At the same time, analysis of fatty acid composition of lipids of strain ISC 106 showed that the amount of myristic acid was 41.44%, palmitic acid -15.53%, stearic acid - 5.28%, and palmitoleic acid - 30.74%. According to their data, the strain *Synechococcus* sp. ISC 106 can be recommended as a raw material for biodiesel production (Kiaei 2015:236-245). The composition of the oils depends very much on the type and growth conditions of the cyanobacteria. When cells grow actively, their metabolism is accentuated by photosynthesis and biomass production. Fatty acids are synthesized, most often in polar lipids, such as phospho- and glycolipids, which are necessary for photosynthesis. Unfortunately, only 30 to 50 percent of polar lipids can be converted to fuel. But when cells experience metabolic stress, like lack of essential nutrients, including nitrogen, the metabolism reduces the rate of growth and is redirected primarily to the synthesis of carbohydrates and triacylglycerides. Thus, it is very important not only to select the right producer, but also to create the optimal conditions necessary for the synthesis of the producers we need.

To date, significant experimental material has been accumulated to optimize the conditions of cultivation and extraction of lipids to increase the bioenergetic potential of cyanobacteria. At Anna University-BIT Campus (India), cultures of *Lyngbya* sp. and *Synechococcus* sp. were studied for possible biodiesel production, cultivating on various media such ASNIII, medium enriched by seawater and BG-11. An increase in the growth rates of these algae was observed in a medium enriched with sea water. Nitrogen depletion has less effect on the total chlorophyll content, while the lipid content of cultures *Lyngbya* sp. and *Synechococcus* sp. increased on 1,4 and 1,2% correspondingly. Increase in salinity from 0.5-1.0 M, increases the lipid content by 2.0% for the strain *Lyngbya* sp. and on 0,8% *in Synechococcus* sp. The results convincingly show that cyanobacteria can be used as a renewable energy source for the production of biodiesel (Selvan 2013: 262-268). In India, a study of lipids of freshwater cyanobacteria *Oscillatoria annae* BDU6, for the production of biodiesel. Effective extraction of lipids from O. *annae* was achieved by ultrasound in combination with organic solvents (50.9%). Effective transformation of triglycerides of the strain *O. annae* in biodiesel fuel was obtained by transesterification, mediated by alkali, with the release of biomass *O. annae*, 5,3 g/l dry weight/inoculate (Anbuselvan 2011: 959-967). There are reports on the study of accumulation of lipids in cells of *Synechococcus* sp., *Cyanobacterium aponinum* and *Phormidium* sp. On BG-11 medium. For *Synechococcus* sp. The content of C16 and C18 fatty acids made up 42,8% and 46,9%, respectively. For *C. aponinum* – 45,0% and 67,7%, and for *Phormidium* sp. – 38,2% and 90,6% respectively. The content of saturated fatty acids for *Synechococcus* sp., *C. aponinum* and for *Phormidium* sp. made up 74,5%, 77,9% and 84,7%, respectively. These fatty acids can be a promising raw material for the production of biodiesel (Karatay 2011).

At the same time, cyanobacteria are attractive as producers of biodiesel because they can easily be used as a suitable platform for genetic modification of their metabolic pathways. Cyanobacteria differ from microalgae and plants in that they are relatively easy to perform genetic manipulations. One of the ultimate goals of the genetic modification of cyanobacteria can be the production of such strains that are able not only to effectively assimilate atmospheric carbon and produce a large amount of raw biomass at the outlet, but also to store the desired end products within it that are most suitable for biofuel production. In addition, the genetic modification of cyanobacteria theoretically allows the production of strains capable of secreting metabolic products suitable for the production of biofuels (alkanes or free fatty acids) into the culture medium. Thus, the expensive stages of collecting and destroying the cells to extract the target products can be omitted, which seems economically more advantageous. It is these types of biofuels, whose production costs can be reduced by simplifying production technology, are currently considered to be the most attractive (Quintana 2011: 471–490). In Cho and Cronan's works, thioesterase I (encoded by the TesA gene) is a periplasmic enzyme at the C-terminus of the *E. coli* fatty acid synthase. As a result of the deletion, the TesA gene was modified in such a way that the mature enzyme was localized in the cytoplasm. The synthesis of thioesterase I (named as * TesA) in the cytoplasm leads to striking changes in the structure of the synthesis of fatty acids in E. coli. Compared to normal *E. coli* cells, the mutant strain synthesizes a huge amount of free fatty acids at all stages of growth. Moreover, the mutant protein * TesA redirects the synthesis of fatty acids into the culture medium (Cho 1995). This concept is implemented by the bioenergy company LS9 for biofuel production, using *E. coli* strain (Steen 2010). It will be more beneficial if we apply this concept to cyanobacteria, because cyanobacteria do not require an additional source of carbon, since the precursor for the synthesis of fatty acids, acetyl-CoA, comes directly from the Calvin's cycle. In addition, there is evidence that the genetic engineering strain E. coli, lacking the gene acyl CoA fatty acid synthase fadD, the amount of free fatty acids in the medium can reach 2.5 g per liter per day (Peralta-ahya 2012: 320-328). In case of *Synechocystis* or *Synechococcus* (fig.2), Mutant by the same gene, the concentration of free fatty acids in the culture medium reached -6.4 nmol and 8.4 nmol in ml (Kaczmarzyk 2010). Editing metabolic pathways of biosynthesis of fatty acids in cyanobacteria allows to increase the yield of free secreted fatty acids to 130 mg per liter of culture per day at a cell density of 0.23 g dry weight per liter (Liu 2011).

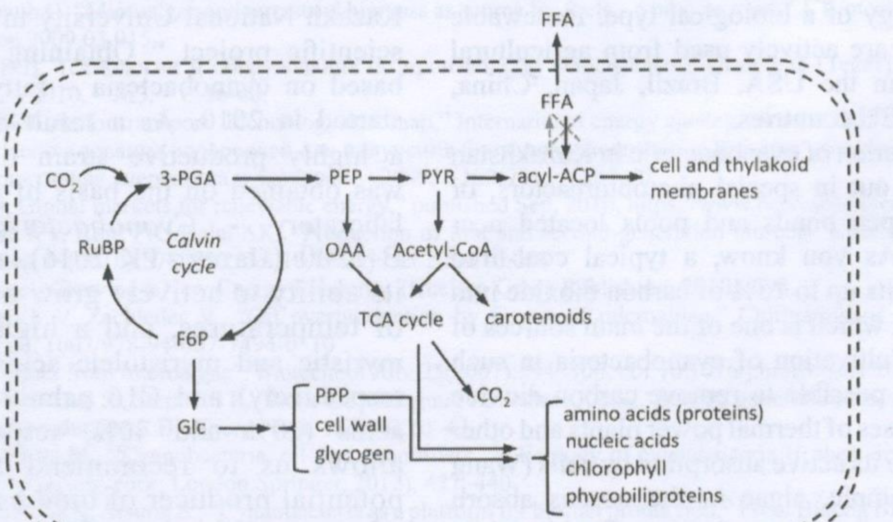

Figure 2 – Estimated scheme of metabolic pathways in genetically engineered cyanobacterial strains of allowing to accumulate free fatty acids inside of the cell with further escape into cultural (Anne 2012)

The creation of a new technology for obtaining biodiesel from the biomass of cyanobacteria, which actively produces fatty acids, is currently relevant, promising and of great interest for the development of alternative energy in the Republic of Kazakhstan. President of Kazakhstan Nursultan Nazarbayev signed the law "On state regulation of production and turnover of biofuel". The main objective of the law is to ensure environmental protection by reducing emissions of pollutants into the atmosphere through the use of biofuels, taking into account the country's food security issues (Указ Президента РК от 29.09.14 г).

According to some reports, in Kazakhstan, oil emissions into the environment made up several million tons per year. In this connection, the issue of developing ecologically safe and economically justified measures aimed at intensifying the processes of biodegradation of hydrocarbons, cleaning and restoration of soil fertility is acute. The development of biotechnologies in the country is one of the priority directions, which is reflected in the strategy of Kazakhstan's entry into the list of the 50 most competitive countries in the world. For this purpose, namely, for an innovative and technological breakthrough, we need to accelerate the mobilization of scientific and technical potential, create conditions for the active introduction of scientific achievements in industry. It is the scientific and technological resources that should become the main factor of innovative development. According to the Kyoto Protocols, by 2020 Kazakhstan should transfer 20 percent of the energy sector to eco-friendly type (http://www.zakon.kz/). At present, 48 countries of the world have normatively consolidated and actively develop the production of "clean" energy of a biological type. Renewable energy sources are actively used from agricultural raw materials in the USA, Brazil, Japan, China, India, Canada, EU countries.

Mass cultivation of cyanobacteria in Kazakhstan can be carried out in special photobioreactors, or organized in open ponds and pools located near power plants. As you know, a typical coal-fired power plant emits up to 13% of carbon dioxide into the atmosphere, which is one of the main sources of air pollution. Cultivation of cyanobacteria in such ponds makes it possible to remove carbon dioxide from exhaust gases of thermal power plants and other industries due to its active absorption by cells (Wang 2008). For example, algae in the oceans absorb annually up to 2 gigatonnes of carbon dioxide, while terrestrial ecosystems absorb up to 1.5 gigatonons (Huntley 2007). In addition, the waste heat of a thermal power plant can cover up to 77% of the heat requirements necessary for the cultivation of cyanobacteria. This technology can be realized both in the northern regions of Kazakhstan with a sharply continental climate, and in the southern parts of the country.

Production of biodiesel is one of the most promising and profitable directions of bioenergy, it allows receive high profits, while maintaining a favorable ecological environment. Biodiesel production from cyanobacteria provides the highest, clean energy, because conversion of oil to biodiesel is much less energy-intensive than conversion to other fuels. The production cycle is practically non-waste, raw materials can be grown on the used land. After the production of biofuel, the oilcake remains, it can be used as animal feed and the glycerin phase remains, which after purification turns into pure glycerin. The profitability of this production is very high, the profit is the difference between the cost of raw materials and the amount received from the sale of fuel. Rentability of this type of business is very high, because the demand for biofuel increases day by day.

The economy of biodiesel production can be improved by advances in production technology. Different mutually complementary approaches to genetic and metabolic engineering will increase the productivity of cyanobacteria (Quintana 2011).

For the use of cyanobacteria for energy purposes, screening of oil-producing strains and the development of technology for their mass cultivation with an increase in the productivity of their biomass are required. Such works are actively conducted in the biotechnology laboratory of the Faculty of Biology and Biotechnology of al-Farabi Kazakh National University in the framework of scientific project " Obtaining of biodiesel fuel based on cyanobacteria – fatty acid producers" started in 2010. As a result of several studies, a highly productive strain of cyanobacterium was obtained on the basis of the biotechnology laboratory - *Cyanobacterium* sp. IPPAS B-1200 (Патент РК 2016), characterized by its ability to actively grow over a wide range of temperatures, and a high content of C14 myristic and myristoleic acids (30% and 10%, respectively) and C16 palmitic and palmitoleic acids (20% and 40%, respectively). Which allows us to recommend this strain as a potential producer of biodiesel. Technologies of mass cultivation of this strain have been developed (fig. 3) (Sarsekeyeva 2015, Патент 2016, Usserbaeva 2015, Sarsekeyeva 2014).

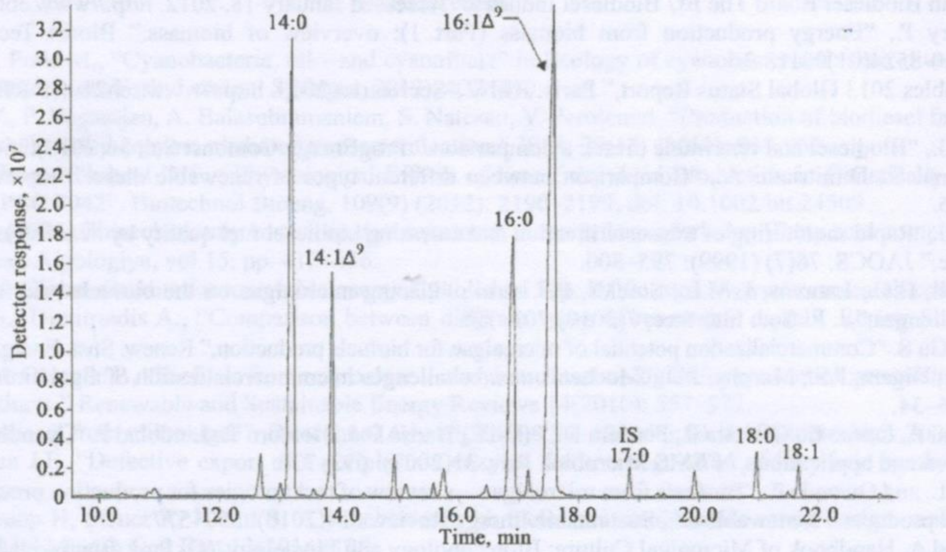

Figure 3 - Fatty acid composition of Cyanobacterium sp. IPPAS B-1200 by gas chromatography (Sarsekeyeva 2014)

In addition, the possibility of using a strain *Cyanobacterium* sp. IPPAS B-1200 In the purification of municipal wastewater with further use of the biomass for bioenergy purposes The biofuel technology developed within the framework of the project includes the growth of cyanobacterial culture, collection of biomass, extraction of lipid fatty acids from the strain Cyanobacterium sp. IPPAS B-1200, the process of transesterification, separation, recovery of alcohol and purification of the finished product. The resulting by-product, glycerine can be used in the food industry, in the production of soap and cosmetic products, thereby making biodiesel production waste-free technology.

Thus, ensuring the high efficiency of converting light energy into cyanobacterial biomass and achieving a high content of lipids, reducing the cost of additional energy to ensure their cultivation, optimizing the designs of bioreactors and a transesterification system to create a continuous production of biodiesel fuel, increases the possibility of producing renewable biofuels , which is environmentally and economically profitable for Kazakhstan.

References

1 Posten C., Schaub G. "Microalgae and terrestrial biomass as source for fuels – a process view," J. Biotechnol, 142(2009):64–69, doi:10.1016/j.jbiotec.2009.03.015

2 Слюсаренко В.В. Технологии и оборудование по производству биодизельного топлива [Текст]// Проблемы региональной энергетики. – 2010. – №3. – С.36-40.

3 OECD/IEA "Biofuels for transport. Technology roadmap," International energy agency, (France 2011), 56.

4 Марков С.А. Использование водорослей для получения биотоплива и удаления избытка углекислого газа из атмосферы [Текст] //Альтернативная энергетика и экология. – 2009. – №2(70). – С.83-90.

5 "BCC research Global markets for renewable energy," published Sep, 2010, https://www.bccresearch.com/market-research

6 Naik SN, Goud VV, Rout PK, Dalai AK, "Production of first and second-generation biofuels: a comprehensive review," Renew Sustain Energ Rev 14(2010):578–597, doi:10.1016/j.rser.2009.10.003

7 Pahl G. Biodiesel. Growing a New Energy Economy. (Chelsea Green Publishing, 2010), 298.

8 Pr˘ibyl P, Cepa´k V, Zachleder V., "Oil overproduction by means of microalgae," Cultivation of cells and products, 1(2014):241–273, doi: 10. 1007/978-94-007-7494-0_10

9 Chisti Y, "Biodiesel from microalgae". Biotechnol Adv 25(2007):294–306, doi:10.1016/j.biotechadv.2007.02.001

10 Schenk P. Thomas-Hall S., Stephens E., Marx U., Mussgnug J., Posten C. et al."Second generation biofuels: high-efficiency microalgae for biodiesel production," BioEnergy Res. 1(2008):20–43.

11 Al-Thani R.F, Potts M., "Cyanobacteria, oil–and cyanofuel?" in Ecology of cyanobacteria II: their diversity in space and time, ed. Brian A. Whitton (New York; London:Springer, 2012), 427–440.

12 Nozzi NE, Oliver JWK, Atsumi S., "Cyanobacteria as a platform for biofuel production." Front Bioeng Biotechnol 1(2013):7, doi:10.3389/fbioe.2013.00007

13 Erdrich P, Knoop H, Steuer R, Klamt S, "Cyanobacterial biofuels: new insights and strain design strategies revealed by computational modeling." Microb Cell Fact 13(2014):128.

14 “European Biodiesel Board The EU Biodiesel Industry,” Accessed January 18, 2012. http://www.ebb_eu.org/stats.php.

15 McKendry P., “Energy production from biomass (Part 1): overview of biomass.” Biores Technol 83(2002):37–46, doi:10.1016/S0960-8524(01)00118-3

16 “Renewables 2013 Global Status Report,” Paris: REN21 Secretariat 2013. http://www.ren21.net/ REN21Activities/GlobalStatusReport.aspx.

17 Knothe G., “Biodiesel and renewable diesel: a comparison.” ProgEnergy Combust Sci, 36(2010):364–373.

18 Bezergianni S., Dimitriadis A., “Comparison between different types of renewable diesel”. Renew Sustain Energy Rev 21(2013):110–116.

19 Knothe G. “Rapid monitoring of transesterification and assessing biodiesel fuel quality by near-infrared spectroscopy using a fiber-optic probe,” JAOCS. 76(7) (1999): 795–800.

20 Greenwell, H.C., Laurens, L.M.L., Shields, R.J. et al., “Placing microalgae on the biofuels priority list: a review of the technological challenges,” J. R. Soc. Interface, 7(2010):703–726.

21 Singh J., Gu S. “Commercialization potential of microalgae for biofuels production,” Renew. Sust. Energ. 14(2010): 2596–2610.

22 Singh, A., Nigam, P.S., Murphy, J.D. “Mechanism and challenges in commercialization of algal biofuels,” Bioresour. Technol. 102(2011): 26–34.

23 Tamagnini P., Leitao E., Oliveira P., Ferriera D., Pinto F., Harris D.J., Heidorn T., Lindblad P. “Cyanobacterial hydrogenases: diversity, regulation and applications,” FEMS Microbiol. Rev. 31(2007): 692–720.

24 Brennan L. and Owende P., “Biofuels from microalgae—a review of technologies for production,processing, and extractions of biofuels and co-products.” Renewable and Sustainable Energy Reviews 14(2010): 557–577.

25 Richmond A. Handbook of Microalgal Culture: Biotechnology and Phycology, (Oxford: Blackwell 2004). 588.

26 Sarsekeyeva F., B.K. Zayadan, A. Usserbaeva, V.S. Bedbenov, M.A. Sinetova, D.A. Los, “Cyanofuels: biofuels from cyanobacteria: Reality and perspectives,” Photosynth. Res. 125(1-2) (2015):329-340, doi:10.1007/s11120-015-0103-3

27 Басова М.М., Жирнокислотный состав липидов некоторых видов микроводорослей [Текст]// Альгология. - 2005. - Т. 15, № 4. - С. 415-436.

28 Sharma, K.K., Schuhmann, H., and Schenk, P.M., “High lipid induction in microalgae for biodiesel production,” Energies, 5(2012):1532–1553.

29 Wang, Z.T., Ullrich, N., Joo, S., et al., “Algal lipid bodies:stress induction, purification, and biochemical characterization in wild_type and starch_less Chlamydomonas reinhardtii,” Eukaryot. Cell, 8(12) (2009):1856–1868.

30 Лось Д.А. Десатуразы жирных кислот[Текст] / Научный мир. – 2014. – 370 с. ISBN:978-5-91522-391-1

31 Kiaei, E., Soltani, N., Mazaheri assadi, M., Khavarinejad, R. A. and Dezfulian, M. “Screening of Cyanobacterial Strains as a Smart Choice for Biodiesel,” J. Appl. Environ. Biol. Sci., 5(8) (2015):236-245.

32 Selvan BK, Revathi M, Piriya PS, Vasan PT, Prabhu DI, Vennison SJ. “Biodiesel production from marine cyanobacteria cultured in plate and tubular photobioreactors,” Indian J Exp Biol. Mar. 51(3) (2013): 262-268.

33 Anbuselvan V., P. Nagarajan, A. Balasubramaniem, S. Natesan, V. Perulemal. “Production of biodiesel from cyanobacteria (Oscillatoria annae) by alkali and enzyme mediated transesterification,” JSIR, 70(11) (2011): 959-967.

34 Karatay S. E., G. Dönmez, “Microbial oil production from thermophile cyanobacteria for biodiesel production,” Applied Energy, 88(11) (2011):3632-3635.

35 Quintana N, Van derKooy F, Van de Rhee M.D, Voshol G.P, Verpoorte R. “Renewable energy from Cyanobacteria: energy production optimization by metabolic pathway engineering” Appl.Microbiol.Biotechnol., 91(2011):471–490.

36 Cho H, Cronan J.E, “Defective export of a periplasmic enzyme disrupts regulation of fatty acid synthesis,” Biol Chem., 270(1995):4216–4219.

37 Steen EJ, et al. “Microbial production of fatty-acid-derived fuels and chemicals from plant biomass,” Nature, 463(2010):559–562.

38 Peralta-ahya P.P., Zhang F., del Cardayre S.B., Keasling J.D. “Microbial engineering for the production of advanced biofuels,” Nature, 488(2012): 320-328.

39 Kaczmarzyk D., Fulda M., “Fatty acid activation in cyanobacteria mediated by acyl-acyl carrier protein synthetase enables fatty acid recycling,” Plant Physiol. 152(2010):1598-1610.

40 Liu X., Sheng J., Cutriss R., “Fatty acid production in genetically modified cyanobacteria,” Proc. Natl. Acad. Sci. USA, 108 (III) (2011): 6899-6904, doi: 10.1073/pnas.1103014108

41 Anne M. Ruffing, Howland D.T., “Physiological Effects of Free Fatty Acid Production in Genetically Engineered Synechococcus elongatus PCC 7942”, Biotechnol Bioeng. 109(9) (2012): 2190–2199, doi: 10.1002/bit.24509

42 О государственном регулировании производства и оборота биотоплива: Указ Президента РК от 29.09.14 г.- № 239-V// Республики Казахстан. Законы.

43 Для перехода на биотопливо в Казахстане имеются научные разработки, но нет финансирования [Электронный ресурс]. – Литер 2009. - Режим доступа: http://www.zakon.kz/151472-dlja-perekhoda-na-biotoplivo-v.html

44 Wang B., Li Y., Wu N., Lan C.Q., “CO_2 biomitigation using microalgae,” Appl. Microbiol. Biotechnol., 79(2008): 707–718.

45 Huntley M., Redalje D. “CO_2 mitigation and renewable oil from photosynthetic microbes: a new appraisal,” Mitigation and Adaptation Strategies for Global Change, 12(2007): 573–608.

46 Пат. Республики Казахстан на полезную модель «Штамм Cyanobacterium sp. IPPAS-1200 в качестве сырья для производства биотоплива» [Текст] / Сарсекеева Ф.К., Заядан Б.К., Лось Д.А., Садвакасова А.К., Усербаева А.А., Болатхан К.- №1750 от 30.09.2016

47 Usserbaeva A., Zayadan B., Sinetova M., Kozlova A., Mironov K., Kupriyanova E., Sidorov R., Los D. “Characterization of cyanobacterial strain IPPAS B-1200 with a unique fatty acid composition” Abstract book ECCO XXXIV European Culture Collections as tools in research and biotechnology Paris, (2015):74-75.

48 Sarsekeyeva F., A. Usserbaeva, B.K. Zayadan, Mironov K., Sidorov R., Kozlova A., Kupriyanova E., M. Sinetova, D.A. Los, “Isolation and characterization of a new cyanobacterial strain with a unique fatty acid composition,” Advances In Microbiology 4(15) (2014): 1033-1043.

References

1 Al-Thani R.F, Potts M., "Cyanobacteria, oil—and cyanofuel?" in Ecology of cyanobacteria II: their diversity in space and time, ed. Brian A. Whitton (New York; London: Springer, 2012), 427–440.

2 Anbuselvan V., P. Nagarajan, A. Balasubramaniem, S. Natesan, V. Perulemal. "Production of biodiesel from cyanobacteria (Oscillatoria annae) by alkali and enzyme mediated transesterification," JSIR, 70(11) (2011): 959-967.

3 Anne M. Ruffing, Howland D.T., "Physiological Effects of Free Fatty Acid Production in Genetically Engineered Synechococcus elongatus PCC 7942", Biotechnol Bioeng. 109(9) (2012): 2190–2199, doi: 10.1002/bit.24509

4 Basova M.M. (2005) Zhirnokislotniy sostav lipidov nekotoryh vidov mikrovodorosley [Fatty acid composition of lipids of some microalga species]. Algologiya, vol.15, pp. 415–436.

5 "BCC research Global markets for renewable energy," published Sep, 2010, https://www.bccresearch.com/market-research

6 Bezergianni S., Dimitriadis A., "Comparison between different types of renewable diesel". Renew Sustain Energy Rev 21(2013):110–116.

7 Brennan L. and Owende P., "Biofuels from microalgae—a review of technologies for production,processing, and extractions of biofuels and co-products." Renewable and Sustainable Energy Reviews 14(2010): 557–577.

8 Chisti Y, "Biodiesel from microalgae". Biotechnol Adv 25(2007):294–306, doi:10.1016/j.biotechadv.2007.02.001

9 Cho H, Cronan J.E, "Defective export of a periplasmic enzyme disrupts regulation of fatty acid synthesis," Biol Chem., 270(1995):4216–4219.

10 Erdrich P, Knoop H, Steuer R, Klamt S, "Cyanobacterial biofuels: new insights and strain design strategies revealed by computational modeling." Microb Cell Fact 13(2014):128.

11 "European Biodiesel Board The EU Biodiesel Industry," Accessed January 18, 2012. http://www.ebb_eu.org/stats.php.

12 Greenwell, H.C., Laurens, L.M.L., Shields, R.J. et al., "Placing microalgae on the biofuels priority list: a review of the technological challenges," J. R. Soc. Interface, 7(2010):703–726.

13 Huntley M., Redalje D. "CO_2 mitigation and renewable oil from photosynthetic microbes: a new appraisal," Mitigation and Adaptation Strategies for Global Change, 12(2007): 573–608.

14 Kaczmarzyk D., Fulda M., "Fatty acid activation in cyanobacteria mediated by acyl-acyl carrier protein synthetase enables fatty acid recycling," Plant Physiol. 152(2010):1598-1610.

15 Karatay S. E., G. Dönmez, "Microbial oil production from thermophile cyanobacteria for biodiesel production," Applied Energy, 88(11) (2011):3632-3635.

16 Kiaei, E., Soltani, N., Mazaheri assadi, M., Khavarinejad, R. A. and Dezfulian, M. "Screening of Cyanobacterial Strains as a Smart Choice for Biodiesel," J. Appl. Environ. Biol. Sci., 5(8) (2015):236-245.

17 Knothe G. "Rapid monitoring of transesterification and assessing biodiesel fuel quality by near-infrared spectroscopy using a fiber-optic probe," JAOCS. 76(7) (1999): 795–800.

18 Knothe G., "Biodiesel and renewable diesel: a comparison." ProgEnergy Combust Sci, 36(2010):364–373.

19 Liu X., Sheng J., Cutriss R., "Fatty acid production in genetically modified cyanobacteria," Proc. Natl. Acad. Sci. USA, 108 (III) (2011): 6899-6904, doi: 10.1073/pnas.1103014108

20 Los D.A. (2014) Desaturazy zhirnyh kislot [Desaturases of fatty acids]. Nauchniy mir, P.370.

21 Markov S.A. (2009) Ispolzovanie vodorosley dlya polucheniya biotopliva I udaleniya izbytka uglekislogo gaza iz atmosfery [Potential of using microalgae for biofuel production and CO_2 removal from atmosphere]. Alternative Energy and Ecology, vol.2, no 70, pp. 83-90.

22 McKendry P., "Energy production from biomass (Part 1): overview of biomass." Biores Technol 83(2002):37–46, doi:10.1016/S0960-8524(01)00118-3

23 Naik SN, Goud VV, Rout PK, Dalai AK, "Production of first and second-generation biofuels: a comprehensive review," Renew Sustain Energ Rev 14(2010):578–597, doi:10.1016/j.rser.2009.10.003

24 Nozzi NE, Oliver JWK, Atsumi S., "Cyanobacteria as a platform for biofuel production." Front Bioeng Biotechnol 1(2013):7, doi:10.3389/fbioe.2013.00007

25 OECD/IEA "Biofuels for transport. Technology roadmap," International energy agency, (France 2011), 56.

26 O gosudarstvennom regulirovanii proizvodstva I oborota biotopliva [On state regulation of production and turnover of biofuel] Ukaz Prezidenta Respubliki Kazakhstana ot 29.09.14 г. № 239-V.

27 Pahl G. Biodiesel. Growing a New Energy Economy. (Chelsea Green Publishing, 2010), 298.

28 Peralta-ahya P.P., Zhang F., del Cardayre S.B., Keasling J.D. "Microbial engineering for the production of advanced biofuels," Nature, 488(2012): 320-328.

29 Posten C., Schaub G. "Microalgae and terrestrial biomass as source for fuels –a process view," J. Biotechnol, 142(2009):64–69, doi:10.1016/j.jbiotec.2009.03.015

30 Pr˘ibyl P, Cepa´k V, Zachleder V., "Oil overproduction by means of microalgae," Cultivation of cells and products, 1(2014):241–273, doi: 10. 1007/978-94-007-7494-0_10

31 Quintana N, Van derKooy F, Van de Rhee M.D, Voshol G.P, Verpoorte R. "Renewable energy from Cyanobacteria: energy production optimization by metabolic pathway engineering" Appl.Microbiol.Biotechnol., 91(2011):471–490.

32 "Renewables 2013 Global Status Report," Paris: REN21 Secretariat 2013. http://www.ren21.net/ REN21Activities/GlobalStatusReport.aspx. REN21.

33 Richmond A. Handbook of Microalgal Culture: Biotechnology and Phycology, (Oxford: Blackwell 2004). 588.

34 Sarsekeyeva F., A. Usserbaeva, B.K. Zayadan, Mironov K., Sidorov R., Kozlova A., Kupriyanova E., M. Sinetova, D.A. Los, "Isolation and characterization of a new cyanobacterial strain with a unique fatty acid composition," Advances In Microbiology 4(15) (2014): 1033-1043.

35 Sarsekeyeva F., B.K. Zayadan, A. Usserbaeva, V.S. Bedbenov, M.A. Sinetova, D.A. Los, "Cyanofuels: biofuels from cyanobacteria: Reality and perspectives," Photosynth. Res. 125(1-2) (2015):329-340, doi:10.1007/s11120-015-0103-3

36 Sarsekeyeva F.K., Zayadan B.K., Los D.A., Sadvakasova A.K., Usserbaeva A.A, Bolathan K. (2016) Shtamm Cyanobacterium sp. IPPAS-1200 v kachestve syrya dlya polucheniya biotopliva [Strain Cyanobacterium sp. IPPAS-1200 as a raw material for the production of biofuel]. Patent of the Republic of Kazakhstan №1750 от 30.09.2016.

37 Schenk P. Thomas-Hall S., Stephens E., Marx U., Mussgnug J., Posten C. et al."Second generation biofuels: high-efficiency microalgae for biodiesel production," BioEnergy Res. 1(2008):20–43.

38 Selvan BK, Revathi M, Piriya PS, Vasan PT, Prabhu DI, Vennison SJ. "Biodiesel production from marine cyanobacteria cultured in plate and tubular photobioreactors," Indian J Exp Biol. Mar. 51(3) (2013): 262-268.

39 Sharma, K.K., Schuhmann, H., and Schenk, P.M., "High lipid induction in microalgae for biodiesel production," Energies, 5(2012):1532–1553.

40 Singh J., Gu S. "Commercialization potential of microalgae for biofuels production," Renew. Sust. Energ. 14(2010): 2596–2610.

41 Singh, A., Nigam, P.S., Murphy, J.D. "Mechanism and challenges in commercialization of algal biofuels," Bioresour. Technol. 102(2011): 26–34.

42 Slusarenko V.V. (2010) Technologii i oborudovanie po proizvodstvu biodiselnogo topliva [Technologies and equipment for the production of biodiesel fuel], Problems of regional energy, pp.336-40.

43 Steen EJ, et al. "Microbial production of fatty-acid-derived fuels and chemicals from plant biomass," Nature, 463(2010):559–562.

44 Tamagnini P., Leitao E., Oliveira P., Ferriera D., Pinto F., Harris D.J., Heidorn T., Lindblad P. "Cyanobacterial hydrogenases: diversity, regulation and applications," FEMS Microbiol. Rev. 31(2007): 692–720.

45 Dlya perehoda na biotoplivo v Kazahstane imeutsya nauchnie razrabotki, no net finansirovaniya [There are scientific developments for switching to biofuel in Kazakhstan, but there is no financing]. accessed 2009. http://www.zakon.kz/151472-dlja-perekhoda-na-biotoplivo-v.html

46 Usserbaeva A., Zayadan B., Sinetova M., Kozlova A., Mironov K., Kupriyanova E., Sidorov R., Los D. "Characterization of cyanobacterial strain IPPAS B-1200 with a unique fatty acid composition" Abstract book ECCO XXXIV European Culture Collections as tools in research and biotechnology Paris, (2015):74-75.

47 Wang B., Li Y., Wu N., Lan C.Q., "CO_2 biomitigation using microalgae," Appl. Microbiol. Biotechnol., 79(2008): 707–718.

48 Wang, Z.T., Ullrich, N., Joo, S., et al., "Algal lipid bodies:stress induction, purification, and biochemical characterization in wild_type and starch_less Chlamydomonas reinhardtii," Eukaryot. Cell, 8(12) (2009):1856–1868.