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LASER PRETREATING OF CYANOBACTERIA BIOMASS TO PRODUCE LIPIDS AS A RENEWABLE ENERGY SOURCE

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Abstract

The composition of cyanobacteria in the Dnieper reservoir is important. At the same time, the development of cyanobacteria leads to the pollution of the Dnieper river waters. The extraction of the most valuable components of cyanobacteria from the reservoir and the valorization became one of the most urgent ecological and resource-saving tasks. The article proposes a method for intensifying the process of extracting lipids from the cellular components of cyanobacteria. A method of pre-treatment the cyanobacteria biomass with medium-intensity laser radiation has been developed. This method is used to destroy the cellular structures and isolate the lipid fraction from them. The total lipid content in the obtained organic fraction was determined. They were separated by thin layer chromatography. It was found that the use of laser pre-treatment of cyanobacteria biomass with subsequent extraction increases the yield of lipids. It is advisable to use the isolated lipids from the cyanobacteria biomass as a renewable source of raw materials to obtain a third-generation energy carrier, for example, biodiesel fuel.

Key words: biodiesel, cyanobacteria, extraction, laser radiation, lipids

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1. Introduction

Ukraine is an energy-deficient country and imports about 70% of its natural gas for its own consumption. At the same time, the energy intensity of the domestic economy is 3-4 times higher than the corresponding indicators of economically developed countries (Geletukha et al., 2013).

The use of renewable energy sources is one of the most important areas of Ukraine's energy policy. The energy strategy is aimed to preserve a traditional fuel and energy resources and improve the state of the natural environment. An increase in the use of renewable energy sources in the energy balance of Ukraine will increase the level of diversification of energy sources. This will help to strengthen the energy independence of the state.

The bioenergy industry in Ukraine has one of the greatest development potentials. This is due to the peculiarities of the climate, the potential of the agricultural sector and the availability of the necessary labour force. The most energy potential in Ukraine has the following types of biomass such as crops, wood waste, liquid fuels from biomass, the biological component of municipal solid waste, and biogas.

Receiving energy from renewable sources is an important environmental and economic task of Ukraine for today. This is due to the growing shortage of fuel resources.

The growth and development of microalgae is energetically provided by the process of photosynthesis. Photosynthesis is the conversion of solar energy into the energy of chemical bonds of organic compounds, mainly carbohydrates. The general equation of photosynthesis can be summarized as expressed by Eqs. (1-2):

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$$CO_2 + H_2O + n(hv) \rightarrow O_2 + (CH_2O) + 112\ kcal/mol \quad (1)$$

$$CH_2O \rightarrow 1/6\ C_6H_{12}O_6 \quad (2)$$

A huge amount of solar energy is supplied to the Earth's surface per year - approximately $5.7 \cdot 10^{24}$J. However, the effective concentration of this energy is negligible anywhere on Earth. Even at noon on a cloudless day, the maximum energy concentration is only about 1 kW/m^2. Therefore, the accumulation and transmission of solar energy requires significant costs. This limits its direct use as the main energy source. At the same time, photosynthetic organisms use this energy to fix large amounts of CO_2 (about $1.5 \cdot 10^{10}$ tons, for this purpose $3 \cdot 10^{21}$ J/year is taken). Plants and algae annually accumulate up to 10^{11} tons of cellulose and form $2 \cdot 10^8$ tons of free oxygen. Moreover, the only source of free oxygen on the Earth is photosynthesis.

Thanks to photosynthesis, algae produce about $1.8 \cdot 10^{11}$ tons of dry biomass per year (Blum et al., 2010). The development of bioenergy based on photosynthetic organisms such as microalgae has many significant environmental benefits. In particular, it helps to reduce greenhouse gas emissions (Blaga et al., 2018; Sorlini et al., 2018; Tymchuk et al., 2021). Under congenial conditions, the efficiency of photosynthetic conversion of solar light by microalgae reaches 5-6%. Pre-treatment a crop of 60 t/ha per year allows you to get 75,000 kWh of electricity.

The development of alternative energy technologies is based on the use of renewable resources, in particular, the energy of the Sun. Molecular hydrogen is such an environmentally friendly renewable energy carrier. Hydrogen is an environmentally friendly fuel. It has a high energy intensity, which is 3-5 times higher than that of gasoline and oil. In terms of energy, hydrogen has universal properties: it is a reducing agent, energy carrier and fuel. The need for high-energy and environmentally friendly fuels has led to the emergence of hydrogen energy. The rapid development of hydrogen energy makes it possible to assert that hydrogen is the fuel of the future.

Recently, biological methods for producing hydrogen have attracted considerable interest. In this respect, photosynthetic organisms deserve special attention, among which microalgae are the most promising. Their study reveals the relationship between photosynthesis and the process of hydrogen formation.

The ability of microalgae to produce molecular hydrogen through photosynthetic energy conversion is due to:

- the presence of an unlimited source of energy - sunlight;
- excess of photolysis substrate - water;
- high calorific value of hydrogen (29 kcal/g compared to 3.5 kcal/g for hydrocarbons);
- the ability to restore the process;
- photochemical transformation of water into hydrogen occurs at temperature without the formation of toxic intermediates. Therefore, one of the most promising areas to solve a number of global problems, including energy, is the biotechnology of microalgae (Cianchetta et al., 2019; Geletukha et al., 2014).

Biodiesel is an alternative fuel. It can be obtained chemically from raw materials of biological origin (for example, from unrefined vegetable oil). Unmodified biodiesel can be used in any diesel engine directly or in a mixture with petroleum diesel fuel (Mironenko et al., 2009). More often, the term "biodiesel" refers to the alkyl esters of fatty acids, which are formed during the re-etherification of lipids in vegetable oils or animal fats.

Biodiesel fuel is slightly inferior in calorific value to petro-diesel fuel. However, biodiesel more exceeds the fuel in terms of its lubricating properties. Due to this, the use of biodiesel extends the life of the engines. Biodiesel is an environmentally friendly fuel. When used, the level of harmful emissions is notably reduced. In particular, carbon dioxide (CO_2) emissions into the atmosphere are reduced by 60% compared to petro-diesel engines. When biodiesel enters water or soil, it undergoes a rapid biodegradation.

Biodiesel has been produced commercially since 1989. Its production volumes are growing rapidly. The European Biodiesel Council reported that in 2004 the growth of biodiesel production in the EU was 35% higher compared to the level in 2003. About 80% of European biodiesel is produced from rapeseed oil. For example, about a third of the rapeseed crop in 2004 was used specifically for biofuel production (Mironenko et al., 2009). According to Renewables (2013), the total production of ethanol and biodiesel from various types of biomass in 2012 reached about 3% of the total volume of motor fuels consumed in the world.

Plant oils of various origins can be used as starting crude for biofuel production. The cheapest plant raw material is palm oil due to the high yield of oil palms. Recently, microalgae have attracted a lot of attention as a potential raw material for the production of biodiesel. The yield capacity of microalgae is many times higher than the productivity of even the most productive plants, and the lipid content per unit of dry biomass is quite high. Therefore, now the microalgae are considered as the most promising starting crude for the production of biodiesel fuel (Zabarny et al., 2006).

Thus, the biomass of aquatic organisms is an important renewable natural raw material resource. Algae growth processes depend on climatic conditions and solar activity. This is manifested in the periodic occurrence of toxic phenomena of water "blooming" (Chizhevsky, 1976). A promising source of cyanobacteria is the Kremenchuk reservoir. The authors determined the amount of biomass during the collection of seston in “blooming” spots during the growing season (Nykyforov, 2010; Priymachenko, 1981). In Table 1 it is presented quantitative and qualitative chemical analysis of cyanobacteria biomass samples, which were taken from “blooming” spots, showed the presence of a number of valuable

organic substances (Nykyforov et al., 2018).

The resulting biomass of aquatic organisms is widely used as an organic ingredient in technologies for the production of biogas, medical products, food products, feed, biologically active additives, chemical products, polymers, and others. (Nykyforov et al., 2016; Shmandiy et al., 2010).

One of the components of the resource value of cyanobacteria biomass is the lipid fraction. The study of the lipid composition of blue-green algae was carried out by many scientists (Maslova et al., 2004; Sudyina et al., 1978; Sushchik et al., 2003). The lipid fraction is a part of the cell membrane complex and intracellular structures, which are represented by a dense, stable enough bipolar layer of phospholipids, etc. That is why the main task in the technology of lipid extraction from cyanobacteria biomass is the destruction of the integumentary structures of cell with the release of lipid fraction biomolecules. There are a variety of methods for the chemical extraction of lipids from the cells of various organisms, which include the use of volatile and/or toxic substances. To conduct such researches the certain laboratory conditions are required. The use of precursors requires obtaining a permit to work with them (Dvoretskiy et al., 2015). To maximize the extraction of lipids from the biomass of cyanobacteria it is necessary preliminary destruction of the complex chemical structures of their cellular components. Decomposition into simpler compounds is possible under the influence of environmental factors of various nature. The corresponding parametric characteristics are also important. Such procedures must be safe and easy to carry out. It is promising to use physical methods for the pre-treatment of cyanobacteria cell biomass in order to increase the extraction of primary metabolites. It is known the using of the physico-hydromechanical method to enhance the extraction of lipids from cyanobacteria.

The method is based on cavitation crushing of the solid phase of biomass, turbulization of the boundary diffusion layer of the liquid with cavitation bubbles. In this case, non-stationary conditions with a high value of the mass output coefficients arise. Under such conditions, structural and morphological changes in the microbial cell occur (Malovanyy et al., 2016).

At the same time, a decrease in the contrast of cells, disintegration and a damage of their walls one can observe. In particular, their loosening or resorption, a decrease in the clarity of the contours, a change in the shape of cells, their aggregation and mechanical destruction. The modes of pre-treatment the cavitation field are determined. To destroy the cell wall of cyanobacteria, the method of cavitation was used to form zones of high and low pressure in the medium. The energy of two types of cavitation was used: acoustic and hydrodynamic (Bligh and Dyer, 1959; Halim et al., 2013; Zagirnyak et al., 2017).

This hydrodynamic method for intensifying the process of lipid extraction from the cellular structures of cyanobacteria has a number of disadvantages: a) low intensity of the lipid extraction process (this is explained by the high density and high polymer strength of the cyanobacteria cell walls); b) a long duration of cyanobacteria biomass processing in the field of hydrodynamic cavitation; c) incomplete isolation of lipids of different nature from unicellular cyanobacteria.

Even after pre-treatment of cyanobacteria in the field of hydrodynamic cavitation, the mechanical destruction of their surface structures occurs partially. The lipid extraction process is running with low intensity. This is due to the fact that the cellular components and intracellular contents of cyanobacteria are protected by a dense cell wall. The authors studied the effect of low-intensity laser radiation on the hydrolytic activity of proton pumps of the vacuolar membrane.

Table 1. Lipid content in the biomass of representatives of some species and genera of microalgae, % of dry weight (Basova, 2005)

Class	***Species***	***Lipid content, %***
Cyanophyceae	Genus *Spirulina*	6.0-16.6
	Anabaena	2.0-18.0
	Oscillatoria	2.0-18.0
Chlorophyceae	*Chlorella* sp.	10.4-16.2
	Chlorella sp.CS-247	18.4
	Dunaliella salina	28.1
Dinophyceae	*Gymnodinium* sp.	13.0
	Symbiodinium microadriaticum	15.0
	Scrippsiella sp.	16.0
Chrysophyceae	*Emiliania huxleyi*	23.0
Prymnesiophyceae	*Tetraselmis chui*	13.9
	P. viridis	25.3
	I. galbana	26.6-30.0
Prasinophyceae	*Micromonas pusilla* CS-98	13.3
	Pycnococcus provasoli	16.7
	Nannochloropsis salina	16.9
Bacillariophyceae	*N. closterium*	24.2-27.8
	Navicula	35.0-44.0

The aim of the researches is to develop an effective physical method to intensify the extraction of lipids (up to 5% of the cell mass) from the cellular structures of cyanobacteria with their most complete extraction from the biomass (Ozolina et al., 1997)

2. Materials and methods

The method of cyanobacteria pre-treatment that is developed in this work, provides the implementation of several stages of biomass preparation and processing. The first stage is the collection of cyanobacteria biomass in commercially viable volumes in polysaprobic zones of natural and artificial eutrophic reservoirs (Fig. 1). For example, in the Kremenchuk reservoir, which is 2.250 km^2 of water surface area and 828 million m^3 of water volume in the shallow water (depth up to 2 m; 18.4% of the reservoir area), the seston in places of "blooming" was collected in an amount of up to 50 kg/m^3 that is $4.14 \cdot 10^7$ tons of biomass during the growing season (120 days). In terms of dry matter, it is $1.61–2.15 \cdot 10^5$ tons, including from 8.05 to 10.76 thousand tons of pure lipids from phytomass.

Fig. 1. Eutrophic section of the reservoir

The second stage is the intensification of the process of extracting the lipid fraction from cyanobacteria cells, which is solved by technical methods. The use of the method of extracting lipids from the biomass of cyanobacteria is based on the action of a focused laser pulse with an average power of 0.5 kW. Direct irradiation of the aqueous cell suspension was performed for 3–25 min. depending on the volume of the suspension.

In laboratory studies, we used an LTI No 500 optical quantum laser generator. Its characteristics were: beam wavelength 1062 nm, pulse frequency 50–1000 Hz (Fig. 2). Under the action of laser irradiation of cyanobacteria biomass, it appears the phenomena of optical cavitation, thermal convection, and thermomechanical irreversible damage to cells - necrosis. The action of a laser pulse causes breaks in the phospholipid bi-layer. Structural defects are formed - hydrophilic pores. The pores take the size of the membrane thickness and cause its destruction.

The resulting necrosis of cyanobacteria cells is an irreversible process and is characterized by rupture of the cytoplasmic and intracellular membranes, which leads to the destruction of organelles, the release of lysosomal enzymes that promote the release of biomolecules into the extracellular space. Thus, the lipid fraction of the cells is almost completely removed from the cyanobacteria biomass.

Fig. 2. Installation of laser radiation

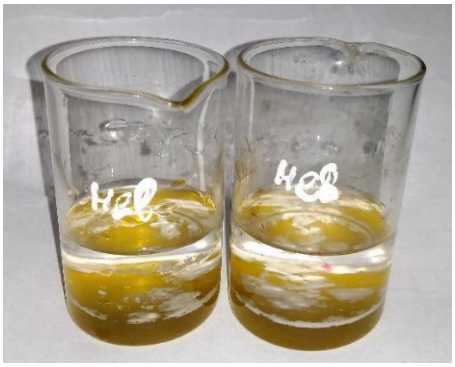
In the experiment, the laser treatment of cyanobacteria biomass continued until the biosubstrates were heated to a temperature of +46°C. This triggered the mechanism of photothermal destruction of the surface structures of cyanobacteria cells with the release of intracellular biomolecules (Fig. 3).

Fig. 3. Pre-treatment of cyanobacteria biomass with focused laser irradiation

At the same time, by controlling the pre-treatment time of biomass with focused laser radiation (up to temperatures from +43 to +60°C), you can achieve various effects, including an increase in lipid yield. The implementation of the developed method for extracting lipids from the biomass of the collected seston after pre-treatment of the substrate with continuous laser irradiation includes the third stage, which consists in further extracting the lipid fraction from the obtained biosubstrates by standard methods (Fig. 4).

After intensification of the process of extracting lipids from cyanobacteria cells by treating

their biomass with laser irradiation, further extraction of the lipid fraction from the biomass was carried out by chemical and physicochemical methods (Araujo et al., 2013; Kates, 1986; Zuorro et al., 2015).



a)

b)

Fig. 4. The obtained results of the experiment: a) – a sample obtained without laser pre-treatment; b) - a sample obtained after laser pre-treatment

To determine the total lipid content in the cyanobacteria biomass biosubstrates were dried to an air-dry state and ground in a mortar. The resulting crushed mass was transferred into a separatory funnel and poured with a hexane-water mixture in a 1:1 ratio in an amount of 100 cm^3. After shaking vigorously for 15 minutes the mixture was left to settle. In the lower part of the funnel, the aqueous part of the extract, containing insoluble remains of cyanobacteria cells, was intensively stained. The upper part of the funnel contained a non-polar liquid-phase mixture of hexane and extracted lipids of a weak green colour.

The aqueous solution was decanted, the mixture for mixing hexane was transferred to a porcelain dish and evaporated in a water bath. After evaporation of hexane, the amount of extracted lipids was determined gravimetrically.

At the next stage, lipids were separated by thin layer chromatography using the obtained lipid fraction (Kates, 1986; Shvets et al., 2013). To separate the mixture of lipids into individual components, plates with a thin layer of silica gel were used. Adsorption of lipids on the surface of silica gel grains occurs due to the formation of hydrogen bonds between polar groups of the sorbent and functional groups of lipids. Lipids containing ionic groups (phospho- and glycolipids) form strong bonds with the sorbent due to electrostatic interaction. The adsorption properties of silica gel are associated with the presence of silanol groups (Si–OH) on the surface of its particles, which normally contain a monolayer of water. In this regard, the plates were activated (30 minutes) at a temperature of 100–110 °C to remove water and increase the adsorption capacity of the silica gel. The studied mixture of lipids in an amount of 0.1 cm^3 was applied to the plates in the form of dots 0.2 cm in diameter at a distance of 1.5 cm from the lower edge of the plate and 1.0–1.5 cm from the side. The spacing between the points was 1.0 cm. As a control, standard lipid solutions were applied to 0.1 cm^3 dishes.

3. Results and discussion

3.1. Thin layer chromatography results

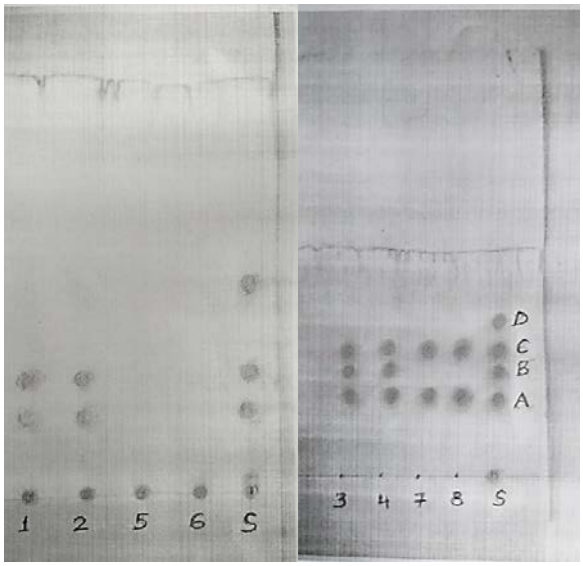
Plates with applied samples were placed in a chromatographic chamber with a pre-filled layer of 0.7-1 cm of solvent. The chamber was installed horizontally, tightly closed with a lid, and impregnated with solvent vapour. To separate the neutral lipids in samples No 1, 2, 5, 6, a mixture of hexane-diethyl ether-glacial acetic acid was used in the ratio 73:25:2 (first chromatographic chamber).

To separate the phospholipids in samples No 3, 4, 7, 8, a mixture of "chloroform-methanol-water" in a ratio of 65:25:4 was used (second consisting chromatographic chamber). The experiment was continued until the solvent front reached a level of 0.5-1.0 cm from the top edge. As a control, a standard chloroform-methanol mixture of lipids, of lecithin, fatty acids (palmitic, stearic, oleic), triacylglycerols, was used.

Iodine vapors were used to identify lipids (non-destructive reagent) - in samples No 1, 2, 5, 6. After drying in a fume hood, the plate from the chamber was placed in a desiccator with crystalline iodine for 5–10 minutes. The intensity of the spots varied, which, in our opinion, is associated with the possibility of the formation of internally complex compounds of the corresponding lipids with iodine molecules. For samples No. 3, 4, 7, 8, a solution of phosphoric-molybdic acid was used. The plate was irrigated with 5% phosphoric-molybdic acid in ethanol; after heating at a temperature of 80–90 °C, the lipid zones were stained dark blue against a yellow-green background. The results of lipid separation are recorded in the form of photographs (Fig. 5). In biomass pretreated with a laser, the lipid yield was 26.3% higher than in the untreated cyanobacteria substrate (lipid content, % of dry weight: 2.73 and 2.16, respectively). According to the data shown in Fig. 5, it was found that the lipids of cyanobacteria cells are mainly represented by the polar lipid fraction and, to a lesser extent, by the free fatty acid fraction.

Chromatograms were obtained after fractionation of lipids in the aforementioned systems. Lipid fractions were concentrated in a specific

sequence, starting from the initial line. It was found that the system "chloroform-methanol-water" in a ratio of 65:25:4 (Fig. 5 B) is more effective. Therefore, the calculation of *Rf* was carried out with this in mind. Fig. 5 shows that the main phospholipid is phosphatidylethanolamine, which is present in all samples, phosphatidylcholine and phosphatidylglycerol are not found in laser-treated cyanobacteria samples.



a) b)

Fig. 5. Thin layer chromatography: a) neutral lipids: S - reference sample; 1, 2 - samples of lipids with laser-processed biomass; 5, 6 - samples of lipids from untreated biomass;b) polar lipids (phospholipids): S - reference sample; 3, 4 - samples of lipids with laser-processed biomass; 7, 8 - samples of lipids from untreated biomass; A - phosphatidylcholine; B - phosphatidylethanolamine; C - phosphatidylglycerol; D - cardiolipin

3.2. Discussion of results

Analysis of the obtained results of chromatographic separation of lipids suggests that the content of lipids isolated from the cyanobacteria biomass, pretreated with a laser, ranges from 1.4 to 3.5%. When using biomass that was not treated with a laser, it was impossible to maximize the extraction of lipids. The strength of the cell wall of gram-negative cyanobacteria affects the completeness of their extraction. It is possible to increase the efficiency of extracting the lipid fraction from the biomass by the pre-treatment of cells with a laser.

Earning a lipid fraction from the biomass of cyanobacteria after pre-treatment by laser, which increases the yield of lipids with biosubstrates during their further extraction to 25.2–32.8%, is the result of the application of the developed method of cyanobacteria cells pre-treatment. The application of this method of extracting lipids from cyanobacteria using a laser allows:

• maximally extract the lipid fraction from the cyanobacteria biomass;

• to reduce the duration of biomass pretreatment;

• to increase the surface of mass transfer in the substrate for further extraction of lipids in the implementation of biological or chemical technologies;

• to exclude or minimize the number of labour-intensive operations.

Compared to other physical analogues, the method of extracting lipids from cyanobacteria biomass using a laser is more efficient, requires less time, is technologically uncomplicated, consumes less energy, etc. The pre-treatment of cyanobacteria biomass with a laser promotes the destruction of the surface structures of bacterial cells, an increase in the yield of lipids, provides a more complete use of their bioenergetic potential and the ecological and economic feasibility of implementing the method of extracting lipids from the cyanobacteria biomass.

The main part of lipids containing cells of gram-negative cyanobacteria is represented by phospholipids, glycolipids, lipopolysaccharides and other complex organic compounds. In the total content of cells, phospholipids predominate, but lipopolysaccharides are characteristic of the lipid fraction, which are localized on the outer side of the membrane of gram-negative bacteria and make up about 50% of the dry mass of the membrane.

Fatty acids in cyanobacteria cells are found mainly in the composition of acyl lipid molecules. The chain length of fatty acids is from 10 to 20 carbon atoms, while acids with 15, 16, 18 and 19 carbon atoms predominate. Saturated C acids contain: lauric (C12: 0), palmitic (C16: 0), stearic (C18: 0). And of unsaturated acids, palmitoleic acid (C16: 1), cis-vaccenic acid (C18: 1) are present.

Thus, the implementation of the developed method for the isolation of lipids from cyanobacteria using a laser has significant energy and resource-saving sectoral effects with the further use of the obtained raw materials:

1) the use of free renewable natural raw materials - cyanobacteria biomass as a substrate for the production of lipids;

2) ensuring a more complete use of the regenerative energy potential of the biomass of cyanobacteria due to the high yield of lipids from cyanobacteria cells after their laser irradiation;

3) obtaining a natural, environmentally friendly lipid extract for use in various biological or chemical technologies;

4) introduction in the field of renewable energy sources of the preparatory stage of the previous laser irradiation of the substrate-raw material – cyanobacteria biomass in the process of energy production;

5) restoration of the structural and functional organization of littoral ecosystems disturbed as a result of eutrophication (restoration of gas balance, hydrochemical regime of spawning of ichthyofauna, reduction of water toxicity, etc.), by removing the biomass of cyanobacteria in commercially viable volumes from polysaprobic zones, natural and artificial eutrophic reservoirs.

Thus, the introduction of the developed method of pretreatment of cyanobacteria biomass as a raw material for technologies in various industries will provide three functions: energy, environmental, resource saving.

4. Conclusions

The developed scheme of preliminary pre-treatment with laser radiation forms the instrumental basis for an environmentally friendly method to intensify the process of extracting the lipid fraction from the biomass of cyanobacteria as organic raw materials for use in biological and chemical technologies in various sectors of the economy of the country.

The extraction of cyanobacteria from water bodies improves the ecological situation in the region. Cyanobacteria, collected during the growing season from the water area of the Dnieper reservoir cascade, are valuable raw materials. The biomass, which was pre-treated with a laser, gave the lipid yield on 26.3% higher compared to untreated cyanobacteria substrate. Lipid content, in % of dry weight: 2.73 and 2.16, respectively.

According to the results of chromatographic analysis of the lipophilic fraction of cyanobacteria, it has been established that among selected lipids, a group of phospholipids predominates, a certain number of which increases after irradiation of the biosubstrate with a laser. One of the ways to use the selected lipids is to obtain energy from cyanobacteria, the raw material of the third generation, biodiesel.

Taking into account the ecological situation during the period of "blooming" of cyanobacteria, their extraction and pre-treatment solves environmental and energy issues.

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