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PROSPECTS OF BIODIESEL PRODUCTION FROM *NOSTOC LINCKIA* (ROTH.) BORN. ET FLAH. BIOMASS

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The work is devoted to the optimization of the process of obtaining purified lipids for the production of biodiesel from Nostoc linckia biomass. The cyanobacteria were cultivated in a photobioreactor on a medium of minimal mineral composition. The biomass was separated from the fugate and dried to a constant weight.

Different systems of polar and nonpolar solvents were tested for lipid extraction. The solvent system petroleum ether : butanol (1 : 2) was the most effective in the extraction of N. linckia lipids. The presence of phospholipids, free fatty acids, acidic substances, and waxes in the extract was checked. Depending on their presence, the mandatory stages of lipid refining were established. N. linckia biomass is a promising raw material for biodiesel production. The optimized methodology allows us to obtain purified lipids in the amount of 19.4% of the dry weight of the feedstock.

Keywords: Nostoc linckia (Bornet ex Bornet et Flahault, 1886), biodiesel, lipids, extraction, refining, microalgae

Introduction. In the light of recent events, the issue of energy independence of the country has become critical. Fossil fuels cannot ensure the stability of Ukraine's energy system, and nuclear power plants, which generate more than 50% of electricity in Ukraine, have begun to play a major role in the energy sector (Electricity..., 2021). Nevertheless, military operations pose a significant threat to nuclear energy and, as a result, to all adjacent territories. A recent trend has been the proliferation of solar and wind power plants, which allow covering electricity shortages when traditional power plants cannot cope with peak loads. The disadvantage of such sources is their dependence on weather conditions, such as wind speed and cloud cover. They require expensive additional equipment, such as battery arrays, which store energy when conditions are optimal for its production and release it in other cases. There is a need to find alternative energy sources that do not depend on weather conditions, can be decentralised to create security from hostilities, and are versatile and profitable in the conditions prevailing in the country.

Ukraine is an agrarian country, which means that bioenergy has great prospects for development in our realities. Large volumes of sunflower, rapeseed, energy willow and other energy crops have been grown in Ukraine and are still being grown to this day (Electricity..., 2021). Nevertheless, the hostilities have severely affected the area of fertile sown land, with most of the most fertile black soil in the east and south of the country under occupation, making it impossible to exploit (Ayolla et al., 2021; Ajala et al, 2015). In addition to the direct occupation, the problem is caused by land mines, mechanical and chemical pollution.

The main component for biodiesel production is purified vegetable or animal lipids and alcohols (methanol, ethanol), which are used in the process of transesterification (Lee et al., 2019). Sometimes bioethanol is used in the transesterification process, in which case biodiesel production becomes fully renewable and has no negative impact on the environment, although this method is very difficult due to the need to absolute bioethanol (Menegazzo and Fonseca, 2019). For efficient biodiesel production, the feedstock used to produce lipids must contain a high percentage of them, as all other biomass is of no use in biodiesel production. Among higher plants, the main feedstocks are rapeseed oil (84% of the market), sunflower oil (13%), soybean oil (1%), and palm oil (1%) (Chintagunta et al., 2021; Mobin et al., 2019). The search for promising producers is also underway among non-traditional raw materials, such as algae and cyanobacteria (Grama et al., 2022; Husnain et al., 2021; Heimann et al., 2016; Demirbas et al., 2011). The main requirement for such raw materials is the ability to synthesise and accumulate large amounts of lipids.

Microalgae can become a promising alternative source of raw materials in bioenergy (Pradana et al., 2020; Kligerman and Bouwer, 2015; Roberts et al., 2013; Mata et al., 2010). They do not require large areas of fertile land, can rapidly increase biomass using only minimal nutrient media without the need for fertilisers and additional nutrition, and have the ability to accumulate various organic compounds in high concentrations (Yan et al., 2016; Skjanes et al., 2013; Pulz and Gross, 2004). They can be grown in

bioreactors regardless of weather conditions and can be decentralised and protected from hostilities (Hoo et al., 2020). The products that can be obtained from microalgae raw materials are not limited to the prospect of electricity production, but can also be used in the operation of vehicles, medicine, and the feed industry (Camacho et al., 2019). Microalgae are capable of accumulating high concentrations of lipids, which are the raw material for biodiesel, the main competitor to traditional liquid fuels (Hasnain et al., 2023; Faried et al., 2017). Some strains of cyanobacteria can accumulate up to 80-95% of the lipid content in their cells (Hossain, 2019; Khan et al., 2017). Algae biodiesel is a third-generation biofuel produced by processing plant material. Algae is a very cheap and at the same time highly productive raw material. One hectare of algae can produce 30 times more biofuel than a hectare of soybeans (Hasnain et al., 2023; Kandasamy et al., 2022).

A key factor in the relevance of biodiesel is the efficiency of its production. In order to be able to compete with conventional diesel, biodiesel production must be economical and efficient. Optimisation of all stages from the selection and production of raw materials to the purification of the final product is a priority area of development for biodiesel and bioenergy in general (Azadbakht et al., 2023; Menegazzo and Fonseca, 2019; Mobin et al., 2019).

The aim of the study was to optimise the process of biodiesel production from *Nostoc linckia* biomass and to assess the prospects of using this feedstock in bioenergy.

Materials and methods. The material of the study was the culture of *Nostoc linckia* (Bornet ex Bornet et Flahault, 1886) (Guiry and Guiry, 2020) grown in a photobioreactor in a medium of minimal mineral composition (artesian water). *Nostoc linckia* is a species of nitrogen-fixing cyanobacteria that forms filamentous structures. It is absolutely non-toxic, rapidly grows biomass and does not require complex post-cultivation methods of separation from the medium. The algae culture was grown for 21 days, after which the biomass was separated from the fugate by filtering through a planktonic net. The biomass was used to produce biodiesel. In preparation for the extraction, the biomass was dried in aluminium bins in an oven at ~110°C for 1-2 hours until it lost all moisture.

The classical scheme for producing biodiesel from plant material includes the following steps: lipid extraction, refining, esterification, purification, and production of marketable form. Depending on the type of feedstock used, the number of stages and their parameters will vary. The most critical are the choice of extractant, hydration, oil neutralisation and vinification in the presence of waxes.

A mixture of polar and non-polar solvents is classically used for the extraction of triacylglycerols. Typically, the ratio of polar to nonpolar solvents is 2x1 (Azadbakht et al., 2023; Nwokoagbara et al., 2015). We used butanol and a 5% NaCl solution as polar solvents, and ethyl acetate and petroleum ether as non-polar solvents. Butanol is butane alcohol, which is widely used in both food and industry as a food additive or solvent. Petroleum ether is a mixture of light saturated hydrocarbons of colourless colour, which are extracted from associated petroleum gases and light fractions of oil. It is a non-polar organic solvent. Ethyl acetate is a widespread organic solvent of a number of boats. It is often used in industry due to its low price and toxicity.

Lipid extraction from dried biomass was performed by maceration for 72 hours using experimental extractant mixtures. After extraction, the polar solvent fraction was removed. A paper filter was used to filter the oil. Hydration is carried out in the presence of phospholipids. Before the neutralisation step, the acid number of the oil was measured. The amount of alkali for neutralisation was calculated in relation to the acid number. The oil was winterised by freezing in a household refrigerator at a temperature of 4°C. In the presence of sediment, filtration was performed.

To carry out the alkaline esterification process, the oil is heated with the addition of alcohol in the presence of alkali as a catalyst. The purification stage involves separation from glycerol and separation from soapstock (a gel-like mixture of soap and water).

The amount of total lipids was determined by the photoelectrocolourimetric method using phosphovanillin reagent (Knight et al., 1972). The determination of total phosphorus was carried out according to the standard method of DSTU 7082:2009. The saponification number and acid number of the obtained lipid extract were also determined.

Statistical processing of these results was performed using conventional methods in Microsoft Excel. Differences in the results obtained are significant at a significance level of $p \leq 0.05$ by Student's test.

Results and discussion. For the study, the biomass of Nostoc linckia was grown in a photobioreactor using a medium of minimal mineral composition (artesian water) (fig. 1).

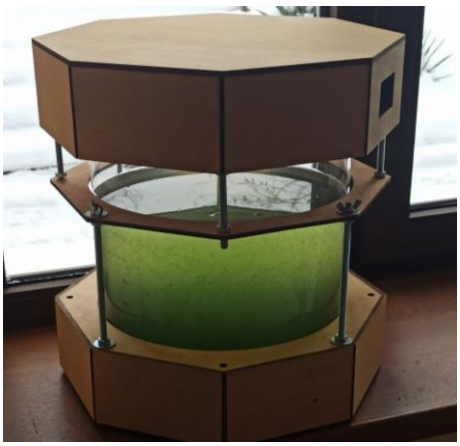

Fig. 1. N. linckia biomass obtained during cultivation in a photobioreactor

The grown biomass was subjected to extraction by maceration using the experimental extractant systems:

- petroleum ether : butanol (1 : 2);
- ethyl acetate : butanol (1 : 2);
- petroleum ether: 5% NaCl (1 : 2);
- ethyl acetate : 5% NaCl (1 : 2);

The resulting extracts were percentage fractionated to separate mechanical impurities and checked for total lipids. The results of the lipid concentration determination were compared with the goal of determining the most productive solvent system (tabl. 1).

Table 1.

Amount of total lipids from N. linckia biomass using different solvent systems

Solvent system	Amount of total lipids, mg/g
petroleum ether : butanol (1 : 2)	320,5 ± 17,3
ethyl acetate : butanol (1 : 2)	218,3 ± 14,5
petroleum ether: 5% NaCl (1 : 2)	172,3 ± 9,32
ethyl acetate : 5% NaCl (1 : 2)	86,5 ± 4,81

During the extraction of lipids from *N. linckia* biomass using the experimental solvent systems, the highest content of unsaturated lipids was obtained using the petroleum ether : butanol (1 : 2) system. The lipid content was calculated at the level of 320.5 mg/g of dry raw material. Butanol proved to be more productive as a polar component of the solvent system; mixtures with its use had a higher lipid concentration than those with 5% NaCl. The most productive non-polar component, in turn, was petroleum ether. Normally, the mass fraction of lipids in this type of microalgae is about 20% (Cheban et al., 2020), but it can vary depending on the cultivation conditions. Growing microalgae in a depleted nutrient medium can increase the percentage of lipid accumulation in cells.

It is necessary to pay attention to the economic aspect of the selected extractants. Depending on their cost, the relevance of certain extractants may vary. To compare the performance of lipid extraction with their economic feasibility, we calculated the cost of the solvent systems used per 1 litre (tabl. 2).

Table 2.

Comparative cost of solvent systems used for lipid extraction of N. linckia

Solvent system	Cost, UAH/l
petroleum ether, butanol	190
ethyl acetate, butanol	116
petroleum ether, 5% NaCl	136
ethyl acetate, 5% NaCl	63

The highest extraction efficiency was shown by the mixture of petroleum ether and butanol, 32.05% of the weight of the mixture was lipids, but this mixture was also the most expensive at a price of 190 UAH/l. The second most effective was a mixture of ethyl acetate and butanol, 21.89% - 116 UAH/l. As for the mixtures where an aqueous solution of 5% NaCl was used as one of the solvents, they showed worse results. The mixture of petroleum ether with 5% NaCl not only showed lower extraction efficiency than the mixture of ethyl acetate and butanol, but also has a higher cost, which makes the use of this mixture inappropriate. Regarding ethyl acetate with 5% NaCl, this mixture showed the lowest percentage of lipid extraction, but also has the lowest price of all the experimental mixtures, however, the use of this mixture as an extractant is also not advisable, since the mixtures containing butanol showed a higher lipid yield per unit of value.

Several conclusions can be drawn from the results of the extraction step: the use of 5% NaCl as a polar component of the extractant is not advisable, butanol was more effective in all aspects; mixtures containing petroleum ether showed a higher percentage of lipid extraction than those containing ethyl acetate; all solvents chosen by us are less toxic than those used in the classical method of Bly and Dyer, namely chloroform and methanol. We recommend using the solvent system petroleum ether : butanol (2 : 1) for the production of biodiesel from *N. linckia*.

Refining optimisation aims to eliminate steps that would be irrelevant if microalgal biomass were used as a feedstock. Refining will ensure high quality of the final product, absence of mechanical and undesirable chemical impurities that may affect the operation of the fuel system, filters, engine and other systems.

The obtained extract was subjected to refining using the solvent system petroleum ether : butanol (2 : 1).

The refining process includes several stages: filtration, hydration, neutralisation, and vinification. The presence of all stages will depend on the

qualitative and quantitative parameters of the extract.

The filtration was carried out in a classical manner using paper filters. The next step was the hydration process - the removal of hydrophilic impurities (phospholipids, proteins, carbohydrates) (Azadbakht et al., 2023). The presence and amount of phospholipids in the extract is crucial for the hydration stage.

Phospholipids are determined by an arbitration method and compared to a standard. According to the standard, the proportion of phospholipids in biodiesel production should not exceed 10 ppm (tabl. 3).

Table 3.

Phospholipid content in the extract of N. linckia

Amount of phospholipids	Arbitrage values	Experimental values
	10 ppm	6,43 ± 0,31 ppm

Due to the low amount of phospholipids, it was decided to skip the hydration process, as it results in the separation of excess phospholipids.

For the next step, neutralisation, the pH, acid number and saponification number must first be determined. Neutralisation is the removal of free fatty acids and acidic substances. Based on the results obtained, we determined the amount of alkali required for neutralisation (Table 4).

Table 4.

Parameters of N. linckia extract before and after neutralisation

Parameters	Before neutralisation	After neutralisation
pH	6,13 ± 0,043	9,36 ± 0,18
Saponification number	536,06 ± 41,11	92,5 ± 4,3
Acid number	31,77 ± 2,02	0,689 ± 0,0276

Based on the results obtained, we calculated the amount of alkali to be used in the neutralisation stage. It is known that KOH or NaOH can be used for this purpose. Taking into account the cost of reagents and molar mass, we chose NaOH for the preparation of the initial solution. 25 ml of the extract weighing 18 g required 5 ml of 3M NaOH for neutralisation. In the process of neutralisation, a soap (soapstock) of gel-like consistency is formed. The addition of an aqueous alkali solution made it possible to combine the two stages of neutralisation and oil washing, i.e. to remove the soapstock remaining after neutralisation from the extract. After this stage, we repeated the determination of pH, acid number and saponification number (tabl. 3).

The removal of fatty and other acids during the neutralisation process led to a decrease in the acid and saponification numbers in the N. linckia lipid extract.

The next step is vinification - removal of waxes. The presence of waxes in biodiesel leads to clogging of the filter elements of the fuel equipment of the car during its operation, which is why the presence of waxes in biodiesel is not allowed. Typically, vinification is performed by freezing the extract at -12 to +8°C until wax crystals appear, after which the temperature is raised to +20°C to increase the size of the crystals and facilitate filtration (Pradana et al., 2020). We performed freezing at +4°C in a household refrigerator. The precipitate was practically absent, however, additional filtration was performed to finally isolate it.

At the end of all refining steps, a transparent purified lipid extract of *N. linckia* was obtained, free of substances that could reduce the commercial value of the final product (fig. 2).

Fig. 2. N. linckia extract after the refining process

Total lipids were determined in the refined extract. The obtained values were compared with the lipid content of the extract before the refining stage (tabl. 5).

Table 5.

Comparison of the amount of total lipids from N. linckia biomass before and after refining

Parameters	Before refining	After refining
Amount of total lipids	320,5 ± 17,3	189,6 ± 9,21

After the refining stage, a lower concentration of lipids was determined in the extract compared to their concentration before this stage. This may be due to the removal of free fatty acids during the neutralisation step, as well as to the ability of soapstock to mechanically capture a certain amount of neutral oil. The fat content in soapstock can reach 30% (20% in the form of mechanically captured neutralised oil and 10% in the form of soap) (Azadbakht et al., 2023; Pradana et al., 2020). The last step was the concentration of the extract by vacuum evaporation of the extractants to obtain pure lipids.

Thus, we have optimised the scheme of biodiesel production from *N. linckia* biomass. It includes the following steps: extraction using the petroleum ether : butanol (1 : 2) extractant system; filtering of the extract; hydration depending on the amount of phospholipids; neutralisation depending on the acid number; vinification in case of precipitate presence during freezing. The scheme involved allows to obtain purified lipids (biodiesel precursor) from *N. linckia* biomass, which make up 19.4% of the dry weight of the raw material.

Conclusions. The system of extractants petroleum ether : utanol (1 : 2) was the most productive and efficient in the extraction of lipids from *N. linckia* biomass. The yield of lipids in the crude extract was 32.05% in terms of dry weight of the raw material. The optimised scheme for the refining of lipids from *N. linckia* biomass includes the following mandatory procedures: filtration, neutralisation, and vinification. *N. linckia* biomass is a promising feedstock for biodiesel production. The optimised methodology allows obtaining purified lipids in the amount of 19.4% of the dry weight of the feedstock.

Interests disclosure. *The authors declare no conflict of interest*.

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ПЕРСПЕКТИВИ ОТРИМАННЯ БІОДИЗЕЛЮ ІЗ БІОМАСИ *NOSTOC LINCKIA* (ROTH.) BORN. ET FLAH.

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Робота присвячена оптимізації процесу отримання очищених ліпідів для виробництва біодизеля із біомаси Nostoc linckia. Ціанобактерії культивували в умовах фотобіореактора на середовищі мінімального мінерального складу. Біомасу відділяли від фугату та висушували до сталої маси.

Для екстракції ліпідів апробували різні системи полярних та неполярних розчинників. Система розчинників петролейний ефір : бутанол (1 : 2) виявилась найбільш ефективною при екстрагуванні ліпідів N. linckia. У екстракті перевіряли наявність фосфоліпідів, вільних жирних кислот, речовин кислої природи та восків. В залежності від їх присутності було встановлено обов'язкові етапи рафінування ліпідів. Біомаса N. linckia є перспективною сировиною для отримання біодизеля. Оптимізована методика дозволяє отримати очищені ліпіди у кількості 19,4% від сухої маси сировини.

Ключові слова: Nostoc linckia (Bornet ex Bornet et Flahault, 1886), біодизель, ліпіди, екстракція, рафінування, мікроводорості

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