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ВОПРОСЫ ИНТЕГРАЦИИ ТЕХНОЛОГИЙ ОЧИСТКИ СТОЧНЫХ ВОД И ПРОИЗВОДСТВА ВОЗОБНОВЛЯЕМЫХ ИСТОЧНИКОВ ЭНЕРГИИ

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Работа посвящена исследованию объектов полезных для создания технологий, удовлетворяющих требованиям строящейся экономики замкнутого цикла, основанной на широком использовании возобновляемых ресурсов, максимальной переработке вторичного сырья, имеющей в качестве одной из задач переход от ископаемого топлива к использованию возобновляемых источников энергии. Проведено исследование возможности производства эфиров жирных кислот (ЭЖК) из возобновляемого сырья растительного происхождения - микроводоросли Chlorella vulgaris с использованием муниципальных сточных вод. Рассмотрены подходы к созданию технологий производства ЭЖК и перспективные пути совершенствования ключевых стадий таких производств. Показано, что муниципальные сточные воды при определенных условиях (температуре 10-32 °С, освещенности 7-14 клк) могут подвергаться очистке и одновременно служить питательной средой в процессе культивирования биомассы микроводорослей с содержанием липидов 10-18 % от сухого вещества. В зависимости от условий культивирования клетки штамма микроводорослей Chlorella vulgaris IPPAS C-2 снижают концентрацию катионов аммония, фосфат-анионов и общего микробного числа в муниципальных сточных водах на 93 – 95% (масс.), 90 – 97% (масс.) и 86 – 95%, соответственно. Проанализировано влияние технологических условий проведения процесса на скорость роста биомассы и жирнокислотный состав накапливаемых липидов. Экспериментально установлено, что триглицериды, О-диалкилмоноглицериды и жирные кислоты оказывают наибольшее ингибирующее влияние на микрофлору сточных вод. С использованием теории растворимости Ч. Хансена выбрана система полярного и неполярного растворителей, состоящая из этанола и петролейного эфира в соотношении (1:2) об., обеспечивающая эффективное проведение процесса экстракции. Получен выход эфиров жирных кислот $К_{ЭЖК}$ =45% при проведении реакции переэтерификации с использованием этанола в соотношении с липидами 6:1 (мол.) при температуре реакции 60 °С в присутствии щелочного катализатора – гидроксида натрия (3% от массы липидов).

Ключевые слова: биотопливо, экстракция, микроводоросли, сточные воды, липиды, жирнокислотный состав, эфиры жирных кислот

ON INTEGRATION OF WASTEWATER TREATMENT TECHNOLOGIES AND PRODUCTION OF RENEWABLE ENERGY SOURCES

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The work is devoted to the study of technological processes corresponding to the requirements of the developing closed-loop economy, which will be based on the wide use of renewable resources, maximum recycling of secondary raw materials, with the transition from fossil fuels to renewable energy sources as one of the objectives. A study of the possibility of producing fatty acid esters from renewable raw materials of plant origin - microalgae Chlorella vulgaris - with the use of municipal wastewater was carried out. Approaches to the development of technologies of fatty acid esters production and promising ways to improve the key stages of such production are considered. It is shown that under certain conditions (temperature of 10-32 °C, illumination of 7-14 klx) municipal wastewater can be treated and at the same time serve as a nutrient medium in the process of cultivation of microalgae biomass with lipid content of 10-18% of dry matter. Depending on the conditions of cultivation, the cells of the Chlorella vulgaris IPPAS C-2 strain of microalgae reduce the concentration of ammonium cations, phosphate anions and total microbial number in municipal wastewater by 93 - 95% (wt.), 90 - 97% (wt.) and 86 - 95%, respectively. The influence of technological conditions of the process on the biomass growth rate and fatty acid composition of accumulated lipids is analyzed. It is experimentally established that triglycerides, O-dialkylmonoglycerides and fatty acids have the greatest inhibiting effect on the microflora of wastewater. Using Hansen solubility parameters, a system of polar and non-polar solvents consisting of ethanol and petroleum ether in the ratio of 1:2 (vol). was selected, which ensures the efficient conduct of the extraction process. The yield of fatty acid esters of K_{FAE} = 45% was obtained in the course of transesterification reaction with the use of ethanol in the ratio with lipids 6:1 (mole) at the reaction temperature of 60 °C in the presence of an alkaline catalyst - sodium hydroxide (3% of the mass of lipids).

Key words: extraction, microalgae, wastewater, lipids, fatty acid composition, fatty acid esters

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INTRODUCTION

Creating technologies that meet the requirements of the emerging closed-loop economy, based on the widespread use of renewable resources, maximum recycling of secondary raw materials, with the transition from fossil fuels to renewable energy sources as one of the objectives, is becoming increasingly important. One approach to this problem could be to develop third-generation CO_2-neutral biofuel technology

using renewable vegetable raw materials – microalgae biomass grown with wastewater [1].

The first stage of the biofuel production technology is the cumulative cultivation of microalgae, which is carried out in open, closed or mixed type reactors [2]. Depending on the microalgae strain, cultivation is carried out with the use of various artificial nutrient media (Tamiya, BG-11, TAP, A-5, etc.), at a temperature of 10-35 °C, barbotization of the suspension with a gas-air mixture containing 0.1-25% carbon dioxide, and illumination level of 2-40 klx. In the process of vital activity cells actively divide, accumulate exo- (lipids, phytohormones, vitamins, etc.) and endometabolites (proteins, carbohydrates, lipids, vitamins, antioxidants, etc.), and release oxygen. The concentration of biomass as a result of cultivation increases by 30-50 times compared with the initial concentration of cells [3-5].

The second stage of the technology is stress cultivation, which causes accumulation of lipids (or carbohydrates) inside the cells. It is carried out with the use of nitrogen- and phosphorus-depleted nutrient media, with changes in the level of illumination and temperature, allowing to accumulate fatty acids (C:12-C:22), which are promising raw materials for the production of biofuel. The concentration of intracellular lipids as a result of stress cultivation increases by 5-10 times in comparison with the lipid content in cells cultivated under optimal conditions [6].

One of the main problems of cumulative and stress cultivation is the significant costs of selection and preparation of appropriate nutrient media. The search for cheap analogues of nutrients, for example, wastewater, seems to be a promising way of solving this problem [7-10]. At the same time, the potential attractiveness of their use is complicated by the presence of the opportunistic and pathogenic microflora in them, which can affect the vital functions of microalgae.

There are many methods of cell and culture medium separation: centrifugation, sedimentation, ultrafiltration, flocculation, flotation with use of flocculants and coagulants [11]. However, each of these methods has its own drawbacks, which affect the cost of microalgae cells. The disadvantage of separation by centrifugation is the high energy consumption. The disadvantage of filtering is the need for frequent replacement of filters, membranes and long process times. In the process of sedimentation (gravitational deposition) the least amount of energy is spent, which is an undoubted advantage of this method. However, its long time and low degree of separation limits its industrial application. Electroflotation is associated with frequent electrode replacement and high energy consumption. The method of biomass concentration using flocculants is widely used, the implementation of this method is limited by the reduction of the target product yield as a result of cell adhesion and by the decrease of the contact surface area of the phases under the influence of the extractant. The analysis shows that centrifugation is the most widely used technology in biofuel production for cell concentration.

To increase the efficiency of lipid extraction from microalgae biomass cells, it is necessary to destroy their walls [12]. Cell disruption can be performed in one or more stages. When selecting methods of disintegration, it is necessary to take into account the structure of cellular membranes, their mechanical and biochemical characteristics. There are physical methods of cell disintegration (exposure to solids, microwave radiation, ultrasound, etc.) and chemical methods (exposure to chemical reagents, enzymes, antibiotics, etc.) [13, 14]. The choice of the cell disintegration method is determined by the structure and chemical composition of the cell wall of the microorganism and the target product. It appears to be a promising approach using a combination of chemical and physical methods of disintegration that do not provoke the destruction of target products – intracellular lipids, for example, the sequential treatment of biomass by enzymes and microwave radiation.

The process of extraction of lipids from disrupted microalgae cells is carried out using organic solvents (mixtures of polar and non-polar solvents) or using carbon dioxide in supercritical state [14]. The disadvantage of the first type of extraction is the need to separate organic extracting agents from the target product, so the use of carbon dioxide in the supercritical state is more promising, but at this stage of technology development the cost of the process using high pressure (100-250 atm) on an industrial scale remains high [15-17].

The final stage in the biofuel production process is lipid transesterification into fatty acid esters (FAEs). Chemical reactions at this stage take place between fatty acids of lipids and alcohol in the presence of a catalyst. The catalyst of this reaction can be homogeneous alkaline (potassium and sodium hydroxides) or acidic (HCl, BF_3, H_3PO_4, H_2SO_4) catalysts, as well as heterogeneous mesoporous catalysts (catalysts based on Na/γ-Al_2O_3, NaOH/γ-Al_2O_3 и Na/NaOH/γ-Al_2O_3, etc.). When using a homogeneous alkaline catalyst, alcoxide is formed in the process of interaction of alcohol with the base, which exposes the nucleophilic group of aliphatic acid to a nucleophilic attack.

Despite the fact that the intermediate stages of the process of etherification in the presence of acidic and alkaline catalyst are different, in both processes there is a nucleophilic attack with alcohol or alcoxide of the aliphatic acid carbonyl group. At the same time, since alcoxide possesses stronger nucleophilic properties than alcohol, the rate of transesterification in the presence of an alkaline catalyst is higher than in the presence of an acidic catalyst [18]. It is also possible to apply reusable heterogeneous mesoporous catalysts for the transesterification reaction [19]. The results of the study [20] showed that the highest biodiesel yield of 27% (lipid mass) was observed in the case of the transesterification reaction at 60 °C using a 6% alkaline catalyst, a reaction time of 4 h and a ratio of alcohol to biomass of 16:1 (vol./mass).

The presented analysis of the current state of the technology of production of third generation biofuel from renewable vegetable raw materials – microalgae biomass – allows us to conclude that the modernization of production stages is relevant to reduce the total cost of the project. In this regard, the study attempted to define the following:

1) conditions of cultivation (temperature, level of illumination) of the strain of lipid microalgae on municipal wastewater for maximum accumulation of biomass and intracellular fats;

2) kinetics of decrease in concentration of ammonium cations, phosphate anions and total microbial number in municipal wastewater during its treatment with the use of microalgae;

3) microalgae exometabolites suppressing the vital activity of waste water microflora;

4) solvents for effective extraction of lipids from microalgae cells;

5) conditions of the transesterification reaction, allowing to obtain the highest yield of fatty acid esters.

METHODS AND MATERIALS

To carry out the experiment, samples of municipal wastewater were used, taken after the sand traps (with the content of ammonium cations of 30-61 mg/l, phosphate anions of 10-38 mg/l, concentration of microflora 1-2.5 of million cell/ml). For the implementation of the wastewater treatment, a lipid-oriented strain *Chlorella vulgaris Beijer* IPPAS C-2 was selected, obtained from the Timiryazev Institute of Plant Physiology of the Russian Academy of Sciences.

Cultivation on wastewater was carried out within 10-12 days with the use of photobioreactor in volume of 5 liters (item 1 in Fig. 1), illumination was provided with the use of light-emitting diode tapes (item 2 in Fig. 1). Supply of nutrient medium (wastewater) was carried out with the help of container 6 in Fig. 1. The experiments were carried out under the following fixed conditions: 1) the seed was 5-10% of the total suspension volume; 2) the pH value was set within the range of 6.2...8.0; 3) the suspension was barbotaged by a gas-air mixture with the carbon dioxide content of 0.03% and the flow rate of 80 l/h for intensive mixing of suspension layers (items 3-5, 7 in Fig. 1); 4) the photoperiod was 24 h. The following cultivation conditions were used: sample No. 1 (t = 32 °C; I = 14 klx) – mode close to summer conditions; sample No. 2 (t = 15 °C; I = 7 klx) – mode close to autumn-spring conditions; sample No. 3 (t = 10 °C; I = 7 klx) – mode close to winter conditions.

Determination of the density (number) of algae cells in the culture fluid was carried out daily by direct count in the Goryayev chamber [21]. The kinetics of ammonium cations concentration in municipal wastewater was determined by the photometric method using Nessler's reagent [22]. Measurement of mass concentration of phosphate anions was carried out every day by the photometric method with reduction by ascorbic acid [23].

The determination of the total microbial number of aerobic and optional anaerobic heterotrophic microorganisms that use organic substances for nutrition and that form colonies on nutrient agar was carried out by direct seeding on solid nutrient medium [24].

The process of separation of treated wastewater and microalgae cells on the 10-12th day of cultivation was carried out in the field of centrifugal forces using a centrifuge SIGMA 2-16P, with the separation factor Fr = 1000 for 10 min (item 8 in Fig. 1).

Extraction of extracellular metabolites with antibiotic effect was carried out for 24 h in the dark with the help of a rotary mixer using petroleum ether as an extractant. Distillation of the extractant was carried out using rotary evaporator IR-1 M3 at a temperature of 85 °C. To determine the sensitivity of wastewater microorganisms to extracellular microalgae metabolites, the disc method was used [25].

In order to increase the yield of the target product – lipids, – the destruction of microalgae cell walls (item 9 in Fig. 1) was carried out by sequential treatment with a mixture of enzymes (Cellolux A - Protosubtilin g3x, taken in the ratio of 12 mg/ml : 4 mg/ml, the exposure time of 10 min at 55 °C) and microwave radiation (power 280-700 W, radiation frequency 2450 MHz, treatment time 30-40 s) [26].

Lipids were extracted from disintegrated microalgae cells in a stirrer apparatus (item 10 in Fig. 1) with the use of organic solvents at a temperature of 50 °C

and a ratio of microalgae biomass (g) : a mixture of solvents (ml) - 1:20. The ratio of organic solvents was selected using Hansen solubility parameters [29]. The qualitative composition of the extracted lipid fraction was determined by thin-layer chromatography [27], the analysis of lipid fatty acid composition was carried out using a gas chromatograph Crystallux-4000M.

Reaction of transesterification was performed in the reactor (item 11 in Fig. 1) with the use of alkaline catalyst and ethyl alcohol at a temperature of 60 °C.

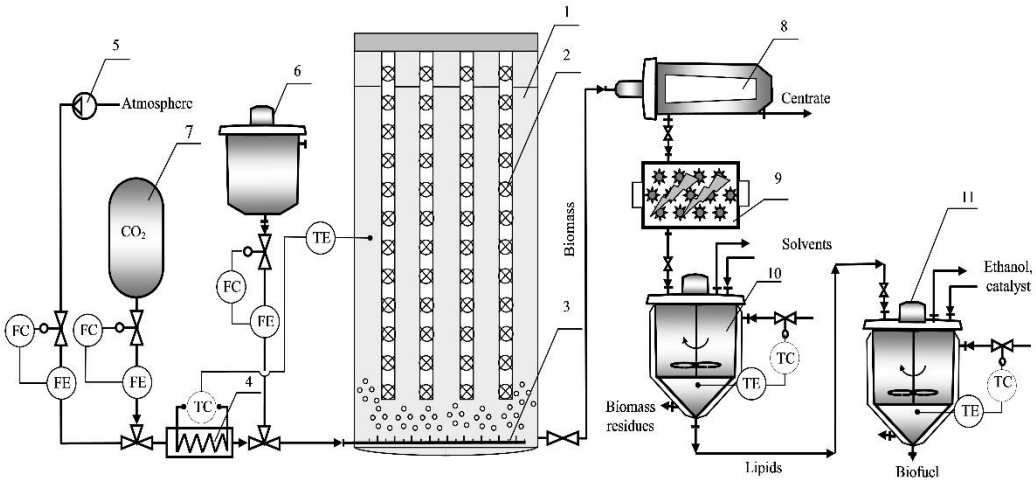

Fig. 1. Experimental installation scheme: 1 – photobioreactor, 2 – LED cable, 3 – a device for nutrient medium supply and bubbling, 4 – heating unit, 5 – compressor, 6 – nutrient medium container, 7 – CO_2 tank, 8 – centrifuge, 9 – desintegration unit, 10 – extractor, 11 – reactor, TE, FE – temperature and flowrate sensors, TC, FC – temperature and flow rate regulators

Рис. 1. Схема экспериментальной установки: 1 – фотобиореактор, 2 – светодиодные ленты, 3 - устройство для подачи питательной среды и барботажа, 4 – нагревательный элемент, 5 – компрессор, 6 – бункер с питательной средой, 7 – баллон с углекислым газом, 8 – центрифуга, 9 – дезинтегратор, 10 –экстрактор, 11 – реактор, TE, FE – датчики температуры и расхода, TC, FC – регуляторы температуры и расхода

RESULTS AND DISCUSSION

Cultivation stage. Experimental studies of *Chlorella vulgaris* IPPAS C-2 microalgae cultivation in municipal wastewater revealed that the maximum specific growth rate of microalgae cells μ_{max} was observed at a temperature of t = 32 °C and illumination level I = 14 klx (sample 1), which is explained by the fact that under these culturing conditions, metabolic reactions are actively taking place in the cells, providing microorganisms with substances and energy necessary for their vital functions (Table 1).

Table 1

Kinetic parameters of wastewater treatment with *Chlorella vulgaris* microalgae depending on the season of the year

***Таблица 1.* Кинетические параметры очистки сточных вод микроводорослями вида *Chlorella vulgaris* в зависимости от времени года**

Treatment conditions	Specific growth rate μ_{max}, day^{-1}	Rate of ammonium cations removal $\mu(NH_4^+)$, mg/(l·day)	Rate of phosphate anions removal $\mu(PO_4^{3-})$, mg/(l·day)
Sample 1	0.44	- 8.5	- 1.9
Sample 2	0.19	- 6.4	- 4.0
Sample 3	0.18	- 3.6	- 3.0

The rate of removal of ammonium cations also reached the highest values under the conditions of treatment for "sample 1", which is explained by the active metabolism of cells using ammonium cations for the biosynthesis of protein compounds. The highest rate of phosphate anions removal from the wastewater by microalgae was observed under conditions of treatment for "sample 2" (t = 15 °C; I = 7 klx), which can be explained by the fact that the temperature regime of cultivation during this period is stressful, so the cells actively accumulate phosphorus in the form of polyphosphates and "metabolic pool" (nucleic acids, phospholipids, phosphorus esters of sugars). Under the conditions of purification of "sample 3", cell metabolism slows down, therefore, the rate of removal of ammonium cations and phosphate anions decreases (Table 1).

The cells of the *Chlorella vulgaris* IPPAS C-2 strain effectively reduce the concentration of ammonium cations and phosphate anions in municipal wastewater by an average of 93-95% (wt.) and 90-97% (wt.), respectively. The maximum allowable concentrations of ammonium cations (MAC = 1.5 mg/l) and phosphate anions (MAC = 3.5 mg/l) have not been achieved for the "sample 3" treatment conditions, therefore, such water is not subject to discharge into the environment and requires additional treatment (Fig. 2, 3).

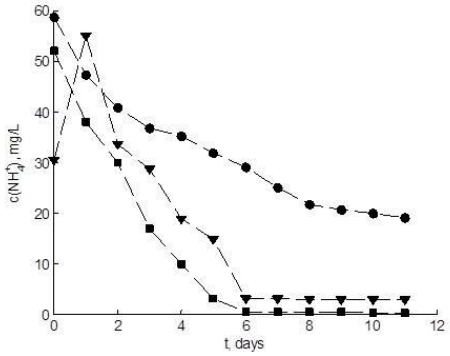

Fig. 2. Kinetics of changes in ammonium cations concentration: 1 – sample 1, 2 – sample 2, 3 – sample 3

Рис. 2. Кинетика изменения концентрации катионов аммония: 1 – образец 1, 2 – образец 2, 3 – образец 3

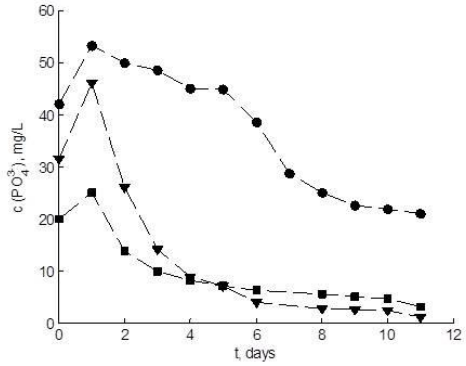

Fig. 3. Kinetics of changes in phosphate anions concentration: 1 – sample 1, 2 – sample 2, 3 – sample 3

Рис. 3. Кинетика изменения концентрации фосфат-анионов: 1 – образец 1, 2 – образец 2, 3 – образец 3

The use of microalgae such as *Chlorella vulgaris* for wastewater treatment for 11-12 days allows reducing the concentration of microflora in wastewater by 86-95% depending on the conditions of cultivation (temperature and illumination level) due to the release of exometabolites with antibiotic effect in wastewater [28]. By analysing the extract of microalgae cultural fluid, it was established that such exometabolites are: 1) triglycerides; 2) O-dialkylmonoglycerides; 3) fatty acids; 4) long-chain alcohols; 5) 1-O-dialkylglycerol esters. It was determined that O-dialkylmonoglycerides, fatty acids and triglycerides have the greatest inhibitory effect on the microflora of wastewater, and the value of this effect depends to a large extent on the intensity and time of light radiation: the antibiotic effect of these substances increases by an average of 2-5 times in comparison with the sample incubated in the dark.

Microalgae cultivation in municipal wastewater is considered stressful because of the imbalance in the ratio of macro- and microelements in their composition. A comparative analysis of the Tamiya medium, recommended (close to optimal) for the cultivation of *Chlorella vulgaris IPPAS C-2* strain, and the average composition of municipal wastewater (Table 2) shows that the lack of nitrogen-containing compounds and the excess of phosphorus-containing ones stimulates the accumulation of neutral lipids inside cells.

The stages of disintegration and extraction. Microalgae cells need to be disrupted to increase lipid yield. As a result of the complex treatment with a mixture of enzymes and microwave radiation, some of the cells are completely disrupted (lipids are located in the intercellular space), some of them die but retain their shape (lipids inside the cells) and some of them remain intact (lipids inside the cells). Lipids are in the cell in the form of droplets containing triacyl glyceride (TAG) molecules, non-polar lipids whose function is to store energy. This type of lipids can be extracted with the help of a non-polar solvent (hexane, petroleum ether, nephrase, etc.), due to the appearance of dispersion interaction between the molecules of the solvent and lipids. Also, lipid droplets containing proteins and polar lipids may be found in the microalgae cell; such droplets may be in the cytoplasm or in the composition of chloroplast or cell membranes.

Table 2

Comparison of the chemical composition of the Tamiya nutrient medium with municipal wastewater

Таблица 2. **Сравнение химического состава питательной среды Тамийя и муниципальных сточных вод**

	Nitrogen, mg/l	Phosphorus, mg/l
Tamiya nutrient medium	700	285
Wastewater	23.0-46.8	2.7-16.0

For the destruction and extraction of such protein-lipid complexes (PLC) it is necessary to use a mixture of polar and non-polar solvents [14]. In this connection, it is important to determine the ratio of polar and non-polar solvents. To make such a selection, Hansen solubility parameters can be used [29]. At the same time, it is important that the selected mixture of solvents allows both triglycerides and lipids included in protein-lipid complexes to be extracted. By means of chromatographic analysis it was determined that the composition of triacyl glycerides (TAG) of *Chlorella vulgaris* microalgae cells mainly includes fatty acids (% (wt.)): palmitic acid (C16:0) – 11.8; stearic (C18:0) – 17.9; oleic (C18:1) – 12.5; linoleic (C18:2) – 5.8; behenic (C22:0) – 5.3; erucic (C22:1) – 4.2. Taking into account chromatographic analysis and certain solubility parameters (δ – total Hildebrand solubility parameter ; δ_D – parameter of dispersion interaction of molecules, $MPa^{1/2}$; δ_P – parameter of induction interaction,

$MPa^{1/2}$; δ_H – parameter of orientation interaction, $MPa^{1/2}$) for some types of triacylglicerides [29] – triolein (δ_D = 16.4 $MPa^{1/2}$; δ_P = 3.2 $MPa^{1/2}$; δ_H = 4.1 $MPa^{1/2}$), tristearin (δ_D = 16.4 $MPa^{1/2}$; δ_P = 2.6 $MPa^{1/2}$; δ_H = 3.8 $MPa^{1/2}$) and trypalmitin (δ_D = 16.3 $MPa^{1/2}$; δ_P = 3.1 $MPa^{1/2}$; δ_H = 3.8 $MPa^{1/2}$), – the solubility parameters of a mean triacyl glyceride (TAG) of microalgae were calculated : δ_D = 16.4 $MPa^{1/2}$, δ_P = 3 $MPa^{1/2}$, δ_H = 4 $MPa^{1/2}$, R_0 = 4.7 (lipid solubility radius (R_0)).

Solubility parameters for a mean microalgae biomass lipid were determined with regard to the following considerations: microalgae lipids consist of phospholipids and glycolipids (53%), triglycerides (45%) and a small amount of free fatty acids (2%).

RED (Relative energy difference) – numerical representation of solubility of the target product in the solvent; according to Hansen's theory, the optimal solvent should have a minimum value of RED < 1.

The parameters of the mean triglyceride are defined above, and the solubility parameters of phospholipids and glycolipids are presented in the study [29]. The solubility parameters of the averaged fatty acid are defined as the arithmetic mean of the known solubility parameters: stearic acid, oleic acid and palmitic acid: δ_D = 16.3 $MPa^{1/2}$, δ_P = 3.12 $MPa^{1/2}$, δ_H = 4.9 $MPa^{1/2}$.

Table 3

Selection of extractants based on Hansen solubility parameters

Таблица 3. **Подбор экстрагентов с помощью теории растворимости Ч. Хансена**

Solvent	RED for TAG	RED for PLC
Ethanol (E)	3.5	2.02
Petroleum ether (PE)	1.3	1.4
E/PE (4:1)	2.61	1.47
E/PE (2:1)	2.17	1.21
E/PE (3:2)	1.74	0.97
E/PE (1:1)	1.33	0.82
E/PE (2:3)	0.96	0.75
E/PE (1:2)	0.7	0.7
E/PE (1:4)	0.69	0.94
Chloroform	0.69	0.19
Methanol	4.41	2.67
Hexane	1.25	1.37
Isopropanol	2.72	1.52
d-limonene	0.43	0.48

Thus, the solubility parameters of the mean lipid will be approximately equal: δ_D = 16.2 $MPa^{1/2}$, δ_P = 3.6 $MPa^{1/2}$, δ_H = 4.14 $MPa^{1/2}$. In determining the solubility parameters of protein-lipid complexes (PLC), solubility parameters of such protein were used, which is similar in structure to the protein in the protein-lipid complex. The solubility parameters of ergosterol were used as the parameters of cholesterol solubility – the animal analogue of ergosterol: δ_D = 20.4 $MPa^{1/2}$; δ_P = 2.8 $MPa^{1/2}$; δ_H = 9.4 $MPa^{1/2}$; R_0 = 12.6. Taking into account the ratio of substances in the protein-lipid complex, the solubility parameters of the protein-lipid complex will be approximately equal: δ_D = 17.74 $MPa^{1/2}$, δ_P = $MPa^{1/2}$, δ_H = 6.69 $MPa^{1/2}$, R_0 = 7.1 [29]. Ethanol as the most widespread safe solvent was chosen as a polar solvent, and petroleum ether – as a non-polar solvent, because of its low boiling point and as requiring the least amount of energy for distillation. This solvent allows to extract lipids at a temperature of 45-50 °C, and exceeding this limit stimulates lipid peroxidation [30]. The final calculations on the selection of different types of solvents are shown in Table 3. The results of the calculations allow us to conclude that it is optimal to use a mixture of ethanol and petroleum ether, taken in a ratio of 1:2 (vol.).

Experimental study of the lipid extraction process from microalgae cells using a selected mixture of solvents showed that the maximum concentration of intracellular lipids was achieved by cultivation under "sample 1" conditions and amounted to (18±2) % (wt), after 7 days of stress cultivation (concentration of ammonium cations is below 22.5 mg/l). Under the conditions of cultivation for "sample 2" and "sample 3" the concentration of intracellular lipids decreased by 6% and 8%, respectively. At the same time, the ratio of saturated, monounsaturated and polyunsaturated fatty acids also changed significantly (Table 4). The increase in the level of mono- and polyunsaturated fatty acids during the cultivation of microalgae cells at a low temperature is explained by the need to maintain the working condition of the phospholipid bilayer.

Table 4

Fatty acid composition of microalgae lipids

Таблица 4. **Жирнокислотный состав липидов микроводорослей**

Fatty acids	Samples		
	1	2	3
(C16:0), %	11.8	9.3	7.1
(C18:0), %	17.9	3.4	2.4
(C18:1), %	12.5	24.7	32.0
(C18:2), %	5.8	11.0	19.4
(C22:0),%	5.3	4.1	4.1
(C22:1)	4.2	4.7	5.8
Saturated FA, %	35.0	16.8	13.6
Monounsaturated FA, %	16.7	29.4	37.8
Polyunsaturated FA, %	5.8	11.0	19.4

A decrease in the culturing temperature may cause the membrane to harden. To prevent this effect and to maintain the viability of microalgae cells, fatty acids containing double bonds and fewer carbon atoms are developed in lipids.

Transesterification stage. Comparison of the composition of the neutral microalgae lipids fraction (see Table 4 "Sample 1") with other oils showed that the qualitative composition of the neutral microalgae lipids fraction is similar to palm oil. At the same time, microalgae lipids contain 1.8 times less unsaturated fatty acids than soybean oil (biofuel feedstock in the USA) and 2 times less than rapeseed oil (biofuel feedstock in EU countries). The lower unsaturated fatty acid content will increase the shelf life of biofuels due to reduced oxidation of fatty acid ester molecules.

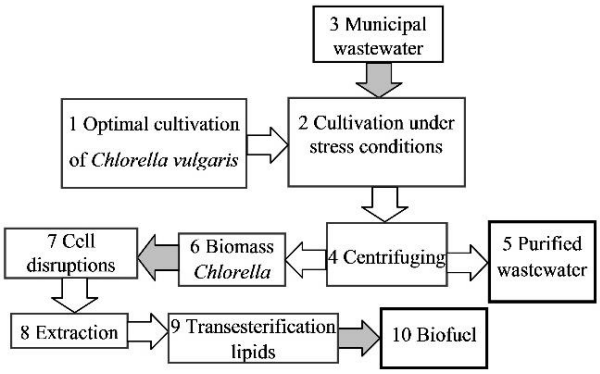

Fig. 4. Integrated technology of wastewater treatment and renewable energy sources production

Рис. 4. Схема интегрированной технологии очистки сточных вод и получения возобновляемых источников энергии

As a result of experimental studies it was found that the highest yield of FAE K_{FAE} = 45% was achieved by esterification reaction with the use of ethanol in the ratio with lipids 6:1 (mole) at a reaction temperature of 60 °C in the presence of an alkaline catalyst – sodium hydroxide (3% of the mass of lipids).

Based on the results of the conducted studies the following integrated flowchart of municipal wastewater treatment for the purpose of obtaining biofuel can be suggested (Fig. 4): at the first stage (stage 1) cumulative cultivation of microalgae on Tamiya medium is carried out within 8 days at the temperature of 30 °C, illumination level of 100 μmol photons/(m^2·s) at aeration of suspension by gas-air mixture with carbon dioxide content 0.03%, then (stage 2) the obtained biomass is cultivated in municipal wastewater under stressful conditions (nutrient deficiency) to stimulate the accumulation of lipids inside the cells (8-9 days). Cell biomass is separated from the treated wastewater by centrifugation with Fr = 1000 separation factor within 10 min (stage 4). Wastewater is further treated (at the concentration of ammonium cations (NH_4^+), phosphate anions (PO_4^{3-}) and the number of total coliform bacteria above 1.5 mg/l, 3.5 mg/l, and 5 CFU/ml, respectively or is immediately discharged into a water body (stage 5). Microalgae cells are disrupted in order to intensify the process of lipid extraction by a mixture of enzymes (Cellulux A - Protosubtilin g3x, taken in a ratio of 12 mg/ml : 4 mg/ml, treatment time of 10 min at 55 °C) and exposure to microwave radiation (power 280-400 W, frequency 2450 MHz for 30-40 s) [26]) – stages 7 and 8. After that, the extracted lipids are converted into fatty acid esters (third-generation biofuel), reacting in a transesterification reaction with ethanol taken in the ratio with lipids 6:1 (mol) at a reaction temperature of 60 °C in the presence of an alkaline catalyst – sodium hydroxide (3% of the mass of lipids) – stage 9.

CONCLUSIONS

The analysis of the current state of wastewater treatment with the use of microalgae and of technology of the third generation biofuel production has shown that the integration of these two technologies is promising as it meets the requirements of the emerging closed-loop economy, aimed at the widespread use of renewable resources and maximum recycling of secondary raw materials.

It has been determined that municipal wastewater can be a nutrient medium for the cultivation of microalgae of the *Chlorella vulgaris* IPPAS C-2 strain at a temperature of 10-32 °C, illumination level of 7-14 klx.

It has been established that the cultivation of *Chlorella vulgaris* IPPAS C-2 microalgae on municipal wastewater is equivalent to stress conditions, thus making it possible for the microalgae biomass to accumulate intracellular lipids. Their amount increases by 2-5 times and this constitutes up to 10-18% relative to the biomass grown on standard laboratory nutrient media.

It was found that, depending on the conditions of cultivation, the cells of *Chlorella vulgaris* IPPAS C-2 reduce the concentration of ammonium cations, phosphate anions, and the total microbial number in municipal wastewater by 93-95% (wt.), 90-97% (wt.), and 86-95% respectively.

Experiments have shown that triglycerides, O-dialkylmonoglycerides and fatty acids have the greatest inhibitory effect on the microflora of wastewater.

Theoretical research with the use of Hansen solubility parameters has proven that the optimal system of polar and non-polar solvents, providing for efficient extraction process, consists of ethanol and petroleum ether in the ratio of 1:2 (vol.).

As a result of experimental studies it was found that the highest yield of fatty acid esters K_{FAE} = 45% was achieved by esterification reaction with the use of ethanol in the ratio with lipids 6:1 (mole) at a reaction temperature of 60 °C in the presence of an alkaline catalyst – sodium hydroxide (3% of the mass of lipids).

According to the results of theoretical and experimental studies, the following scheme of integrated wastewater treatment and renewable energy sources production (Fig. 4) can be proposed. A distinctive feature of this scheme is the possibility of using municipal wastewater as a nutrient medium for stress cultivation, stimulating the accumulation of intracellular lipids with fatty acid content C12-C22.

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