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Microalgae Cultivation on Poultry Droppings Extract for Biodiesel Production

By Golub Natalia & Levtun Igor

University of León-Universidad de León

Abstract- *Background:* Variation of microalgae cultivation conditions affects biosynthetic processes, which leads to a change in the content and qualitative composition of lipids - the raw material for the biodiesel production. The cultivation of microalgae biomass requires mineral nutrition, to provide it natural sources of salts and chemically synthesized compounds are used. The use of agricultural waste will reduce the anthropogenic burden on the environment, reduce the biofuels cost and will help to solve the problems of alternative energy from renewable raw materials production. In this case, in addition to microalgae, it is possible to obtain biologically active substances that can be used as a feedstock for animals and poultry.

Objective: The purpose was to study the Chlorella vulgaris microalgae cultivation while using the extract from poultry droppings as a nutrient medium and its effect on lipid fraction content in the cells. The subject of the study is Chlorella vulgaris development and lipid accumulation in the cells.

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MICROALGAECULTIVATIONONPOULTRYDROPPINGSEXTRACTFORBIODIESELPRODUCTION

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Microalgae Cultivation on Poultry Droppings Extract for Biodiesel Production

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Method: Cultivation was carried out in photoreactors with an airlift system for mixing and feeding bubble air with additional CO_2. Observations and purity control of *Chlorella vulgaris* culture was performed by light microscopy, biomass growth – spectrophotometry, lipid yield – by chemical method, microalgae and lipids mass–gravimetric, lipid composition – by chromatographic method.

Results: It has been shown that using the poultry droppings extract as a nutrient medium for microalgae cultivation, the *Chlorella vulgaris* biomass yield increases up to 7 times in relation to cultivation on the Gromov medium. This increases the lipids content up 2 times. In relation to rapeseed oil, the concentrations of myristic, palmitoleic, stearic and linolenic acids are higher.

Keywords: cultivation, microalgae, lipids, biomass, Chlorella vulgaris, biodiesel.

I. Introduction

Increase in the diversity of energy sources creates conditions for countries to become less dependent on fossil energy resources. The greatest attention is attracted to the energy sources from renewable raw materials. One such source is microalgae, the high lipids content in which allows them to be used as raw material for biodiesel production. To cultivate microalgae, the salts of nutrient elements and CO_2 as a source of carbon are widely used. In industrial production, the use of mineral salts is significant, which leads to depletion of minerals and increase in the price of the final product. Depending on the nutrients form, the change in microalgae metabolism takes place, always in the direction of certain substances biosynthesis and that includes lipids. Therefore, the study of renewable raw materials (agricultural waste) usage as a source of microalgal nutrition, which also leads to increased lipid content in algae cells, is an urgent problem.

Such raw material is poultry waste (droppings), which contain all the necessary components for algae development. The lipids content and their qualitative composition is influenced by the nitrogen form introduced into the culture medium. Poultry waste contains a mixture of various nitrogen compounds - urea, amines, amino acids, nitrates and ammonia, the ratio of which depends on the storage conditions.

In paper [1], it has been shown that the addition of human urine or urea to standard Tamia medium increases the growth *Chlorella vulgaris* biomass by 20-30%. With the use of ammonium ions, it is necessary to double the amount of salt in relation to urea, as the consumption rate of such nitrogen form decreases. The highest growth rate of Scenedesmus sp. LX1 is characteristic for the higher ammonium ions in the nutrient medium. The increase in biomass decreases when ammonia is replaced by urea and nitrate [2]. In this case, the consumption of ammonium ions leads to a decrease in pH value and microalgae development inhibition.

In other works [3, 4], the results *Chlorella vulgaris* cultivation research for the use of poultry droppings extract for the further production of biodiesel are shown. The use of the extract increases the growth rate and lipid fraction content. Also, as a nutrient medium, extracts from various types of poultry wastes were used to grow *S. obliquus* HTB1. It has been shown that biomass growth depends on preliminary treatment of the droppings. In this case, the growth increases or decreases by 20% in relation to cultivation on the standard medium BG11 [5,6]

In work [7], extract concentrations from 5 to 20% of human urine, bird droppings, cow manure and urine were used as a nutrient source for microalgae *Chlorella singularis*, *Micractiniumpusillum* and *Chlorella sorokiniana* was used. It has been shown that *Chlorella sorokiniana* can grow in all types of medium. The maximum growth rate reached 140 ± 3.1 mg / L / day

Corresponding Author α: Environmental biotechnology and bioenergy Department, Igor Sikorsky KPI, Peremohy 37, corpus 4, Kyiv, 03056, Ukraine. e-mail: golubnb@ukr.net
Author σ: Environmental biotechnology and bioenergy Department, Igor Sikorsky KPI, Peremohy 37, corpus 4, Kyiv, 03056, Ukraine.

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and the lipids yield (45.5 ± 2.3 mg / L / day) was obtained using 20% bird droppings extract.

Among various waste-based mediums, the largest biomass yield was observed with algae cultivation on bird droppings extract [8].

The increased *Chlorella vulgaris* biomass growth observed with the use of poultry droppings extract can be explained by appearance of myxotrophic cultivation – droppings provide organic nutrients, inorganic carbon is introduced as a CO_2 [3,9,10]. In addition, as an inorganic carbon, gas emissions from production can be used [11,12].

This work is devoted to determine the influence of substances concentration extracted from poultry droppings.

II. Materials and Methods

Chlorella vulgaris ACKU 531-06 was provided from the collection of T. Shevchenko Kiev National University, Ukraine. Poultry droppings without litter were provided by the «Авангард» poultry farm, Ukraine.

Microalgae cultivation was conducted in photoreactors with volume of 1.3 dm^3 with illumination by light emitting diodes (color ratio of red / blue / green 2: 1: 1) for 12 hours light and 12 hours darkness during the day, with the temperature 20 ± 2 °C. Photoreactors contain an airlift system for environment mixing with air bubbling provided using compressor Resunair-pump AC-9601 (China). The bubbling speed is 100 dm^3 / h. As a source of carbon, in addition to the substances contained in poultry droppings, CO_2 was used from the gas cylinder, which was fed through the reducer system on daily basis (0.25 dm^3 CO_2/dm^3 of bubbling air).

As the control medium the Gromov medium № 6 was chosen [13].

Poultry dropping extract was prepared as follows. Weighed droppings (on a dry substance) were dissolved in 1 dm^3 of distilled water and infused 24 hours with stirring by magnetic stirrer MM-5 (Russia). The resulting solution was filtered and boiled for 1 hour. The extract was autoclaved for 1 ÷ 1.5 h. at a temperature of 120 ÷ 135°C with a pressure of 250 kPa.

The dry matter content in droppings was determined according to the standard method [14]. Humidity of the droppings fluctuated within 72 ± 2.2%. An aliquot of the solution was injected into a photoreactor.

The pH value, ammonium and nitrate ion content were determined using anИ-160 МИ (Russia) ionmeter.

Observation and control of *Chlorella vulgaris* culture purity was performed using the microscope TM XSP-139TP (Ulab, China) (increase from 40x to 1000x for visual observation).

The biomaterial concentration was measured on a spectrophotometer ULAB 102 (China) with a wavelength of 450 nm (D450) [15].

Allocation of algal cells from culture medium was carried out using a centrifuge ЦЛК-1 (Russia) at 2500 rpm. The precipitate was dried in a drying cabinet СШ-151 (Russia) at a temperature of 105°C to constant weight.

The lipids isolation was carried out according to Blay and Dyer's method [16].

The amount of biomass and lipids was determined using the analytical scales Ohaus Scout-Pro SPU 123 (China).

III. Results and Discussion

Droppings have a variable constituent's content. To find out the effect of the soluble compounds concentration in the droppings extract on biomass growth and the lipids accumulation in algae, as a standard the nitrogen content in nitrate ion and ammonium ion was used. It was presumed that the concentrations of other components varied according to changes in nitrogen concentrations. The content of nitrate and ammonium ions in the culture medium on a chicken droppings extract at the beginning of cultivation is shown in Table. 1. The samples given in the table correspond to the contents of the dry matter substance № 1 - 8 g/dm^3, № 2 - 5 g/dm^3, № 3 - 3 g/dm^3, №4 - 2 g/dm^3.

Table 1: Concentration of nitrate and ammonium ions in the culture medium in droppings extract

Ion	Sample			
	№1	№2	№3	№4
NO_3^-, mg/dm^3	157,9	101,1	54,3	36,7
NH_4^+, mg/dm^3	105,3	70,2	38,6	28,7

It is known [17] that microalgae biomass growth depends on the rational ratio of nutrients. Accumulation of lipids occurs under stress conditions, one of which is the reduction of nitrogen content in the medium. In addition to inorganic nitrogen, the nitrogen in the droppings contains urea, amino acids and amines, which can also be consumed by microalgae. Due to these compounds, the total nitrogen content in the solution increases by 8 ± 1%, which is about 11 mg/dm^3 of nitrogen.

Using a more concentrated extract solution is not rational, since the color of the culture medium becomes brown, which may affect the passing of light energy specially in the middle area of the photoreactor and, accordingly, this will negatively affect photosynthetic processes.

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The culture was pre-cultivated on the Gromov medium № 6, which contains nitrate nitrogen. When *Chlorella vulgaris* culture is transferred to the medium with droppings extract, lag phase can be observed, in which the consumption of nitrate and ammonium nitrogen occurs. On the medium with droppings extract there is an increase in the exponential growth phase up to 8 days in relation to the control sample (Fig. 1).

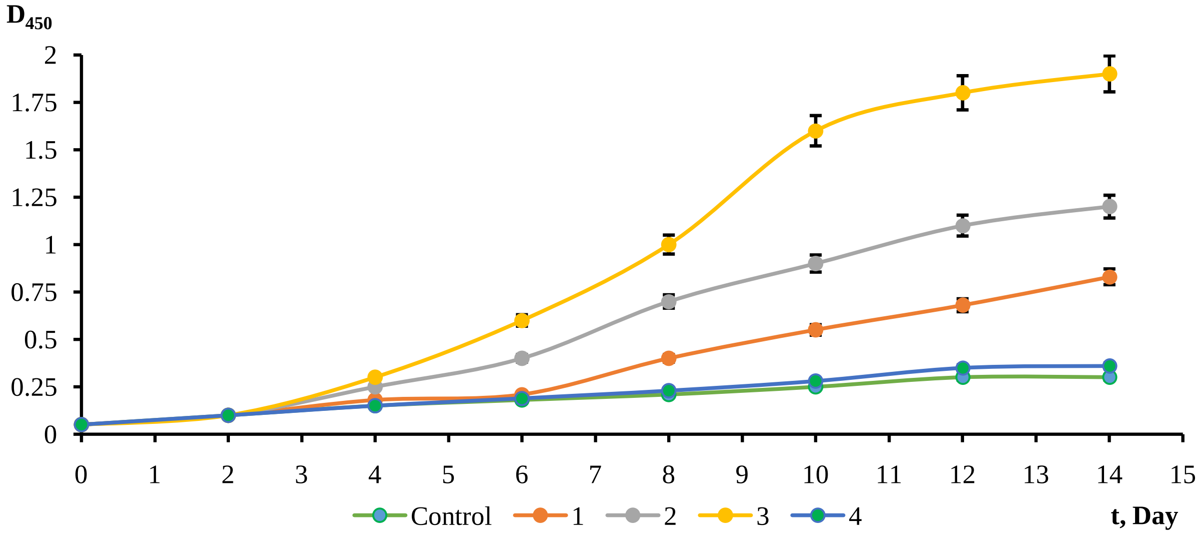

Fig.1: Changes in the optical density (D) of *Chlorella vulgaris* culture during cultivation (t) depending on the droppings content: 1 – 105 mg/dm^3, 2 – 70 mg/dm^3, 3 – 38 mg/dm^3, 4 – 28 mg/dm^3

In the exponential growth phase, the ammonium ion is primarily consumed (Fig. 2 from Table 4, for each sample), which confirms the data in the work [18]. NH_4^+ is the final product of nitrate recovery and functions as a reverse inhibitor that represses nitrate absorption. In the processes of metabolism, NH_4^+ is transformed into organic forms without altering the oxidation degree. Nitrate and urea require ATP energy to restore nitrogen. A slight increase in ammonium ions concentration in the nutrient medium observed during 4-5 day can be explained by the release of excess ammonium ions from the cells formed during urea assimilation from solution [19].

$$ATP + Urea + HCO_3^- \rightarrow ADP + P_i + Urea\text{-}1\text{-}carboxylate \quad (1)$$

$$Urea\text{-}1\text{-}carboxylate + H_2O \rightleftarrows 2\,CO_2 + 2NH_3, \quad (2)$$

since most species of *Chlorella* lack urease.

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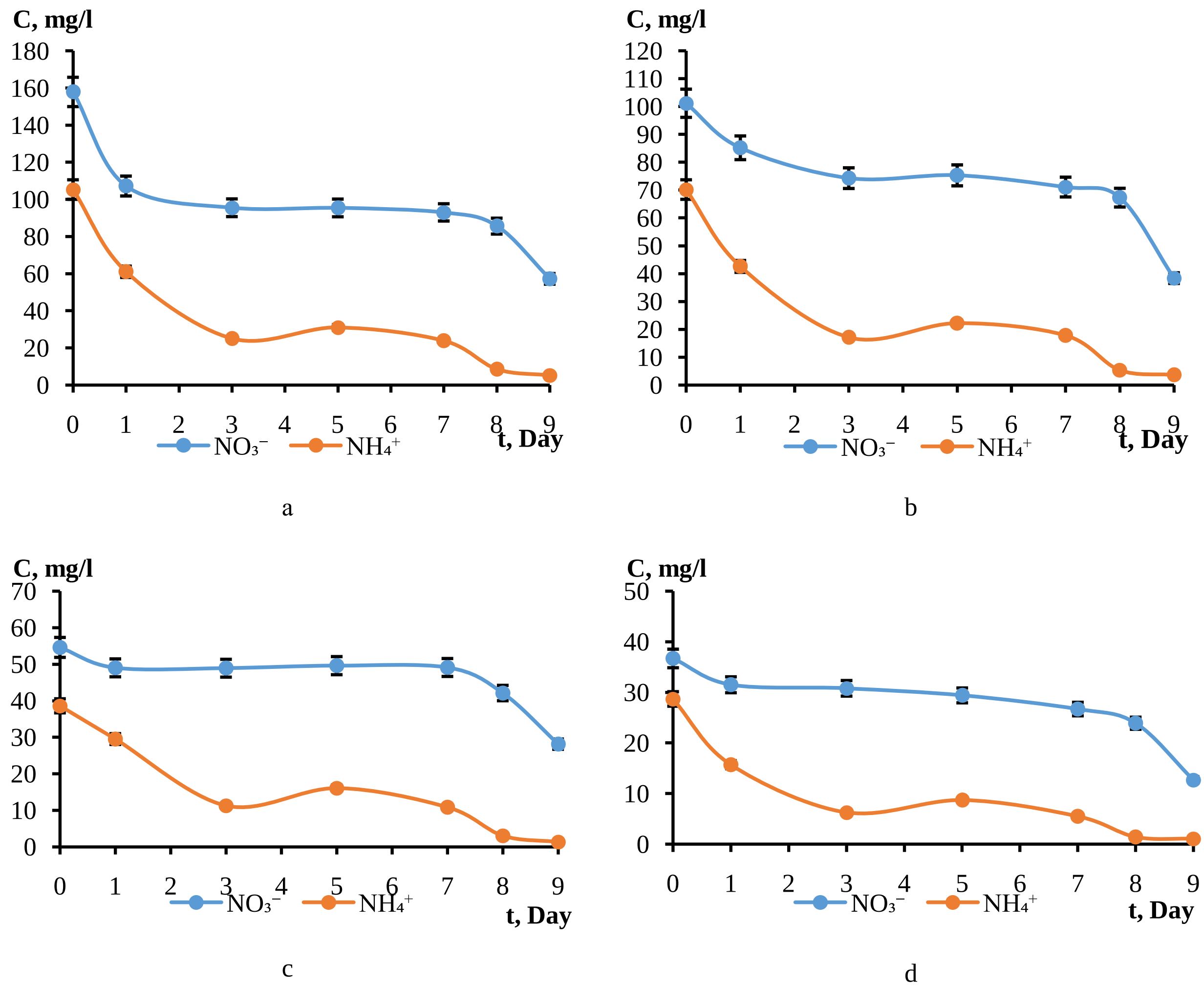

Fig. 2: Change in the nitrogen and ammonium content (C) ions during cultivation (t), depending on the droppings concentration on dry matter: a) 8 g/dm^3 b) 5 g/dm^3 c) 3 g/dm^3 d) 2 g/dm^3.

The initial pH value of the medium during the extract concentration in the culture medium also decreases from 8.3 to 7.4. For the Gromovmedium №6, the initial pH value is 7.2. During bubbling with CO_2 there is a decrease in pH value by 0,3 - 0,4 units. The pH value returns to the initial level within one cultivation day. In case of nitrate ions use as a nitrogen source (control), the pH value is increased up to 7.9 ± 0.2. With the use of the droppings extract, the pH value remains neutral for low concentrations (sample 3.4) and reduces down to 6.5 ± 0.3 with an increase of extract content (sample 1.2). Such change in pH value can be explained by acidification of the medium due to ammonium ions consumption, since during such biochemical process, the proton is released into the culture medium [20]. That is, for the use high extract concentrations, it is necessary to adjust the pH value of the medium.

Fig. 3 shows the biomass growth at different extract concentrations in the photoreactor. Microalgae growth in culture medium containing extract with the initial ammonium ions content of 54 mg/dm^3 exceeds the control 7 times (sample 3). Increase or decrease inextract content leads to a decrease in the production of microalgal biomass. This can be explained by a decrease in the light energy inflow due to an increase in the color intensity of culture medium when the extract content increases, and the presence of substances that at high concentrations inhibit the microalgae development. With a decrease in extract content the lack of nutrients decreases biomass growth.

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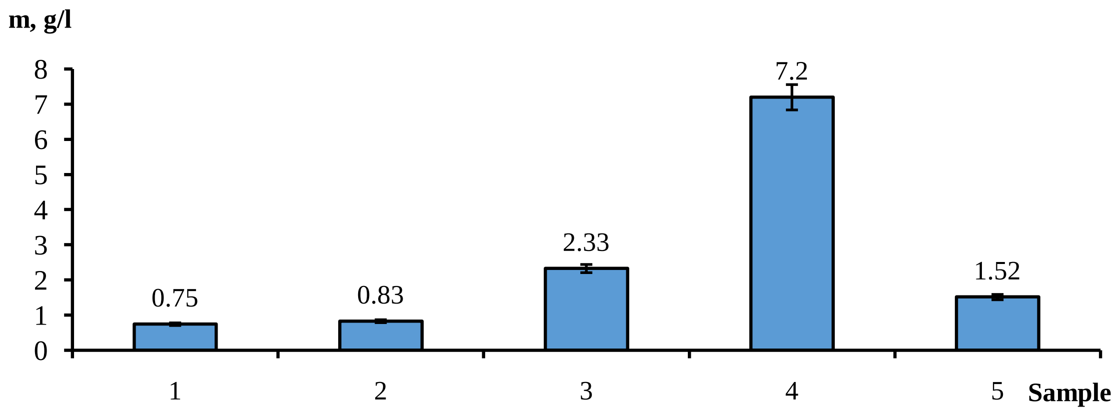

Fig. 3: Growth of *Chlorella vulgaris* biomass (m) at different concentrations of droppings dry matter: 1– control, 2 – 8 g/dm^3, 3 – 5 g/dm^3, 4 –3 g/dm^3, 5 –2 g/dm^3.

The time of *Chlorella vulgaris* culture cells generation is calculated according to the formulas:

$$g_k = \ln 2/\mu \quad (3)$$

$$\mu_{max} = \ln(X_t/X_0)/(t- t_0),$$

where X_0 and X_t are the biomass concentration at the beginning and at the end of the time t, respectively, μ is the exponential growth rate constant (specific growth rate), g is the time of cells generation.

For control it is 2.97 days, for specimens cultivated on the droppings extract - 2.67, 0.95, 0.31 and 1.48 days, respectively. That is, the use of the extract as a nutrient medium increases the cell division rate and at maximum biomass growth (sample№3) the cell doubling rate is 7.44 hours.

The increase in biomass growth rate in sample №3 in relation to control can be explained by a change in the cultivation conditions from autotrophic to myxotrophic. At the same time, there is a decrease in the energy consumption in the cells used on the cells constituents' biosynthesis due to the intake of both inorganic and organic components from the nutrient medium. It is known [18] that during the dark period glucose contained in the droppings, can induce the expression of two transport systems for the amino acids transfer into the cell. This provides cells with an additional source of nitrogen that does not require significant energy usage. The organic compounds contained in the droppings are digested throughout the whole cycle, both in the period of illumination and in the dark [18]. Therefore, the presence in the culture medium of organic and inorganic compounds contained in the droppings will contribute to increase of biomass biosynthesis rate.

Also, the presence of organic substances increases the digestibility of CO_2during the light period, which leads to increase in biosynthetic processes.

The use of an extract as a nutrient medium has the advantage in biomass production in relation to the Gromov № 6. The increase in biomass for samples goes as follows№ 2 – 5 by 11.4%, 208.4%, 860%, 103%, respectively.

The lipids content in dry biomass of *Chlorella vulgaris* at different extract concentrations is shown on Fig. 4. As can be seen from Fig. 4, the cultivation of *Chlorella vulgaris* in the extract containing medium leads to an increase in the lipid content up to three times in all samples in relation to the control. When the extract concentration decreases 4 times, the lipid content decreases by 11%. This can be explained by the influence of inhibitors, the ammonium nitrogen concentration, and change in pH value. The droppings extract contains organic substances that are used by the *Chlorella vulgaris* microalgae cells as a source of carbon in the mixotrophic cultivation, leading to a change in the metabolic pathways and carbon assimilation towards the lipid fraction accumulation [21].

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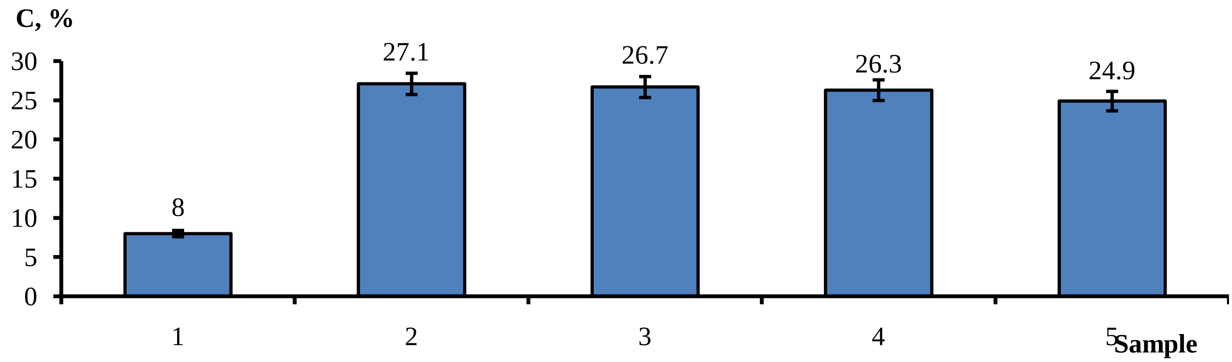

Fig. 4: The lipid fraction content (C) in dry biomass during microalgae cultivation with extract, at a different concentration of dry matter, g/dm^3:1 - control; 2 – 8; 3 – 5; 4 – 3; 5 – 2.

Based on the obtained data, it is rational to obtain biodiesel fuel from microalgae using the droppings extract obtained from 3 g/dm^3 of dry matter. Under these conditions, the lipids yield is 1.89 ± 0.09 g/dm^3 per week of cultivation. When decreasing or increasing the extract content, the lipid output per unit volume is reduced because the reduction of biomass growth.

Table 2 according to the chromatographic analysis shows a comparative description of lipid fraction composition obtained under optimum conditions from the *Chlorella vulgaris* biomass during cultivation on droppings extract. The use of the droppings extract increases the content of saturated fatty acids in the lipid fraction compared to rapeseed oil, which positively affects the production of biodiesel fuel.

Table 2: The content of fatty acids in the lipid fraction of Chlorella vulgaris microalgae cultivated on bird droppings extract

№	Acid	Content in lipids, %	
		Chicken droppings extract	Rapeseed oil [22]
1	Tridecanoic (13: 0)	7,34	-
2	Palmitic (16: 0)	42,2	5,2
3	Palmiotolein (16: 1ω9)	10,8	-
4	Stearic (18: 0)	11,57	0,5
5	Oleic (18: 1)	5,8	58
6	Linoleic (18: 2)	7,4	22,3
7	Linolenic (18: 3)	12,3	13,2

Thus, for the *Chlorella vulgaris* cultivation, it is possible to use bird droppings as a nutrient medium. Under these conditions, the biomass growth and the lipids content in the cells increases. An increase in the growth rate occurs due to mixotrophic cultivation conditions in the presence of organic carbon and nitrogen sources, which are digested by the cell. During the dark period, the amount of biomass that was formed during the illumination period is not reducing. It enables the possibility to use a periodic lighting regime, which will reduce energy costs and reduce the cost of final product. Increase in the content of saturated fatty acids in the lipid fraction makes it possible to use the resulting raw material for the biodiesel production for the application in standard technologies.

IV. Conclusions

The possibility of chicken droppings extract application for Chlorella vulgaris cultivation is shown. It has been established that the maximum biomass increase occurs while using 3 g/dm^3 of droppings dry matter. Under such conditions, the biomass yield increases up to 7 times in relation to culture grown on Gromov medium №6, and the lipids content is also higher up to 3 times.

It has been established that the application of droppings for cultivation increases the saturated fatty acids yield relative to rapeseed oil, which makes it possible to use the resulting oil for the biodiesel production.

Ethics Approval and Consent to Participate

Not applicable.

Human and Animal Rights

No Animals/Humans were used for studies that are basis of this research.

Consent for Publication

Not applicable.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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