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REVIEW

Use of microalgae biomass for production of granular nitrogen biofertilizers

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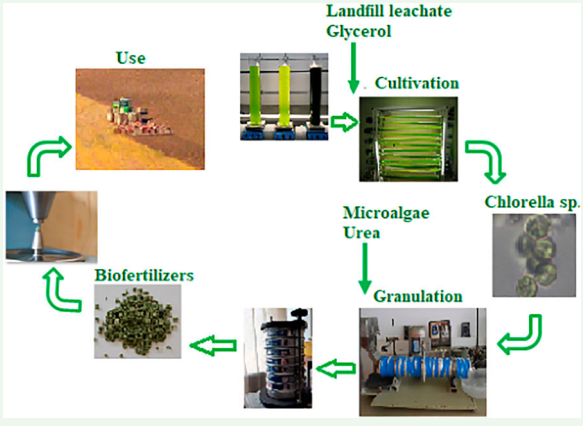
ABSTRACT

This article presents the results of optimizing microalgae *Chlorella* sp. cultivation to obtain a higher amount of biomass and to use cheaper suitable waste for algae cultivation. It has been found that the most suitable waste for growing microalgae is landfill leachate, which can replace the source of nitrogen in the cultivation medium. The largest concentration of microalgae *Chlorella* sp. biomass is obtained in the medium containing 0.08 $g{\cdot}L^{-1}$ nitrogen acquired from the landfill leachate. For fertilizer production, the microalgae biomass suspension was centrifuged and analyzed. The chemical composition of microalgae biomass was found to be sufficiently good to produce granular nitrogen fertilizer with bioactive materials because it contains primary nutrients (3.49% nitrogen, 2.10% phosphorus, and 0.50% potassium) along with secondary nutrients (13.42% calcium and 3.69% magnesium) and 75.33% organic matter and trace elements. Also, microalgae biomass does not contain any heavy metals. The use of a microalgae suspension allows to reduce the amount of moisture used in the production of fertilizers. If optimal conditions are chosen, it is possible to granulate bioactive nitrogen fertilizer that satisfies all the criteria.



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Introduction

As the world population grows and food resources and areas for crop production decline, and global environmental concerns intensify, the use of biofertilizers and enhancing plant nutrient efficiency are becoming increasingly important challenges (*1*).

The main elements needed by plants are nitrogen, phosphorus, and potassium. Due to the rapid dissolution and unbalanced use of fertilizers significant amounts of these nutrients get into groundwater. Nitrogen and phosphorus compounds, which cause eutrophication, are abundant in different types of wastewater and substrates obtained in biogas production (*2*). Scientific studies (*3*) have shown that microbiological products not only accelerate plant root development and nutrient uptake but also reduce the release of N_2O from concentrated nitrogen fertilizers.

Recently, there has been growing interest in using microalgae to satisfy various human needs. Microalgae biomass contains essential plant nutrients, various trace elements, and biologically active substances that promote a complete supply of nutrients to plants throughout their vegetation period (*4*,*5*).

Microalgae are attractive in that they do not require agricultural areas for cultivation, and they use carbon

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dioxide (6), nitrogen, and phosphorus compounds for biomass accumulation (7–10). In the presence of a light source, algae use carbon dioxide during their photosynthesis to produce their own cells (11).

Carbon dioxide plays a crucial role, as carbon accounts for the largest share of microalgal cells (45–50% of dry weight) (12,13). This ability of algae to convert carbon dioxide to biomass during photosynthesis contributes to reducing global warming.

As the range of new fertilizers expands around the world, there is an increasing focus on environmentally friendly bioactive fertilizers, which contain not only nutrients essential for plants but also trace elements and other substances that promote the growth or nutrient uptake. It is known that about 30–40% of fertilizers are lost due to the soil structure and meteorological and climatic conditions. Fertilizers, leached from the soil, pollute the environment and cause a number of ecological problems. In recent years, as raw materials of food deplete and there is an increasing need to protect the environment, the reuse of nutrients and products of biological origin for fertilization and increasing the efficiency of plant nutrients have become more important (1).

Microalgae biomass contains proteins, carbohydrates, and vitamins. This kind of biomass can be used in the production of 'green' fertilizers. The use of seaweed microalgae for these purposes is relatively wide (14). Such plants have been found to grow faster, be healthier, and more tolerant to stress (15).

Most commonly scientific studies investigate the influence of various bacteria (*Azotobacter chroococcum, Azospirillum lipoferum Bacilusmegatterium*, etc.) on plant growth (16,17), while the use of microalgae for fertilizer production has been studied to a lesser degree. Mulbry et al. (18,19) indicate that the slow release of nitrogen, phosphorus, and potassium is the characteristic of pure microalgae biomass, and this satisfies the needs of plants. Biomass contains trace elements, bioactive growth stimulants, phytohormones, vitamins, amino acids, and antifungals (20). Microalgae increase the plant growth rate and yield and improve their quality (4,5). The most commonly used fertilizers is dry microalgae biomass, and drying requires high energy consumption. If wet microalgae biomass (suspension) is used as a raw material for fertilizer production, this would reduce energy costs for drying and at the same time save a certain amount of water in the fertilizer granulation process. Such possibilities of using microalgae in scientific studies have not been examined and are not described in the scientific literature.

The aim of this study was to optimize the cultivation process of microalgae *Chlorella* sp. to obtain a higher biomass yield, to investigate the possibilities of using a microalgae suspension for the production of granular nitrogen fertilizers, and to evaluate the properties of the obtained fertilizers.

Materials and methods

Microalgae cultivation

The research was carried out using *Chlorella* sp. green algae belonging to the *Chlorella* family, the *Chlorella* genus. Microalgae were grown under myxotrophic conditions with nutrients of organic and inorganic origin in their growth medium. To optimize their growing conditions, microalgae were grown in 1 L flasks and glass cylinders at room temperature of 22 ± 2°C with magnetic stirrers and illumination with fluorescent lamps about 250 µmol/ms white cold light for 10 h per day. Illuminance was measured with a data logger (model LI-1400) on a LI-190SA Quantum sensor. Larger amounts of microalgae for fertilizer production studies were grown in a laboratory tubular 160 L reactor with four 40 L sections at room temperature, illuminated by fluorescent lamps and stirred with compressed air or a mixture of air and carbon dioxide. For control studies, microalgae were grown in universal nutrient medium BG11, which was prepared in the laboratory using distilled water and chemicals purchased from domestic chemical suppliers.

To assess the possibilities to use the liquid waste for microalgae cultivation, the nitrogen in the culture medium was replaced by the nitrogen present in the waste. Landfill filtrate containing 130 mg·L^{-1} nitrogen was used for this purpose. In this case, the cultivation media was prepared from all materials contained in the BG11 without the sodium nitrate as a nitrogen resource. The required amount of liquid waste was added to cultivation media as a nitrogen source. This amount was calculated according to the nitrogen content in the landfill filtrate. The amount of filtrate added to the BG11 medium was 0.1 g·L^{-1} to 0.15 g·L^{-1} in the nitrogen medium. The filtrate contains phosphorus compounds (25 mg·L^{-1} phosphorus), which are also required for the accumulation of microalgae biomass. As an additional source of organic carbon, technical glycerol, which is formed as a by-product in the production of biodiesel, has been added to the nutrient medium. The efficiency of its use was analyzed by adding 2–10 g·L^{-1} of technical glycerol to the cultivation medium.

To select the conditions under which the maximum concentration of microalgae biomass is obtained,

growth experiments were performed using the following nutrient medium composition:

- Control – BG11: nitrogen concentration – 0.12 g·L^{-1}.
- Landfill leachate + BG11: nitrogen concentration – 0.05 g·L^{-1}; 0.08 g·L^{-1}; 0.11 g·L^{-1}; 0.14 g·L^{-1}.
- Landfill leachate + BG11 + technical glycerol: nitrogen concentration is 0.14 g·L^{-1}; glycerol concentration: 2 g·L^{-1}, 4 g·L^{-1}, 6 g·L^{-1}, 8 g·L^{-1}; 10 g·L^{-1}.

Microalgae cultivation experiments were performed in triplicate under each test condition. For biomass content analysis, the microalgae suspension was mixed thoroughly before sampling. Biomass content analysis was performed for each sample separately, and the average of the measurements was taken as the final result.

For fertilizer production, the sample was prepared by growing microalgae biomass under optimal conditions in triplicate and forming a joint sample from an equal volume of suspension grown in separate reactors.

The concentration or mass gain of the microalgae was examined spectrometrically using a Lambda 25 UV/Vis spectrophotometer. The optical density of the microalgae suspension was measured at 750 nm. The biomass concentration in the sample was determined from its density values using a calibration curve. The calibration curve was constructed from the values of the microalgae concentration determined by the weight method and the optical density of the suspension determined by the spectrometric method.

To determine the concentration of microalgae by the weight method, the suspension was centrifuged for 10 min at 12,000 rpm, the biomass was washed with distilled water and dried at 105°C to constant weight. The microalgae biomass yield was calculated from the change in biomass concentration per time unit. Biomass yield (BI) is calculated according to the formula:

$$BI = \frac{X_1 - X_0}{t_1 - t_0}, \quad \text{g} \cdot (\text{L} \cdot \text{day})^{-1} \tag{1}$$

where X_1 and X_0 are biomass concentration (g/L) in days t_1 and t_0, respectively.

For fertilizer production, the microalgae biomass (MABM) suspension was centrifuged using a Heraeus Multifuge X3R centrifuge for 20 min at 12,000 rpm.

Microalgae biomass analysis

Total amide nitrogen (N) content was quantified according to the Kjeldal method using Vapodest 45s Gerhardt mineralizator and Vapodest 45s Gerhardt distillation system with automatic titration function. The result with 0.1% accuracy is therefore equal to the sum of the two measurements mean, when the difference between them is less 0.3%.

Phosphorus (P_2O_5) concentration was determined according to the method of spectrophotometric analysis (*20*) using UV-VIS T-70 spectrophotometer. The analysis was performed under the following conditions: 10.0 mm cell and wavelength $\lambda = 450$ nm (accuracy ±0.004 Abs). Based on the flame photometric method, potassium concentration in test samples was measured by using a flame photometer Jenway PFT-7.

Concentrations of Fe, Mn, Cu, Cr, Ni, Zn, and Pb were assessed by the atomic absorption spectroscopy method (AAS) using 'Perkin Elmer' Analyst 400 analyzer. A mixture of acetylene gas (7.5 L/min) and air (10 L/min) was used to atomize samples.

Chemical analysis was performed using chemically pure or pure analytical reagents, along with the standardized methodology (*21*) and appropriate laboratory devices. Each sample was analyzed in duplicate or triplicate or even more times (depending on the requirements for methodology or repeatability of measurements). All values presented in this article have been calculated as an arithmetic mean with standard error.

Test samples were analyzed using X-ray diffraction (XRD) method with Bruker AXS D8 Advance device (accuracy of the measurement $2\theta = 0.01°$). Parameters for obtaining results are as follows: radiation – CuKα, filter – Ni; detector movement step 0.02°; intensity measurement time in steps 0.5 s; anodic voltage Ua = 40 kV; and strength of the current I = 40 mA. Thermal analyzer 'LINSEIS STA PT-1000' (Germany) was used for simultaneous thermal analysis (STA). DSC–TGA parameters are as follows: temperature increase rate, 10°C/min; range, 25–300°C; crucibles, extruded aluminum; standard, empty crucible; atmosphere in the furnace, 20 mL/min N_2, and sample weight, 1.6 mg. Measurement accuracy was ±3°C. The infrared spectrum (FT–IR) of a test sample was registered with Perkin Elmer FT–IR spectrophotometer Spectrum GX. A pellet for analysis was made of an extruded mixture of optically pure, dry KBr, suitable for analysis, and the test sample. In pursuance of making a pellet under presented experimental conditions, 2 mg of the test sample and 200 mg KBr were mixed. The analysis was performed in two stages. First, the background absorption spectrum of KBr was obtained. Then, during the second stage of analysis, the spectrum of a mixture composed of KBr and test material was recorded.

Biofertilizer production and analysis

Laboratory drum granulator, which is a reduced prototype of an industrial drum granulator (*22*), was used to

obtain granular nitrogen fertilizer. The raw material mixture was composed of pure crystalline urea, which is the main constituent, and MABM as a bioactive additive.

The moisture content in raw materials and the final product was determined by the thermogravimetric method (*23*), using the electronic KERN MLS_N moisture analyzer (when test sample mass is higher than 1.5 g and operating temperature ranges between 40°C and 160°C, measurement accuracy is 0.01%). RETSCH's woven wire sieves with aperture sizes ranging from 0.2 mm to 7.0 mm (*24*) and electronic scales WPS 210/C Kern ABJ (accuracy – 0.001 g) were used to estimate granulometric composition of the final product. The bulk density of the granular fertilizer was measured according to the gravimetric method (*25*) with electronic scales WPS 210/C Kern ABJ (accuracy, 0.001 g). HANNA instrument pH 211 microprocessor pH meter with a glass electrode with an accuracy of 0.01 was used to determine the pH value of the 10% fertilizer solution. The granule static strength was measured with ИПГ-2 apparatus (measuring range, 5–200 N; margin of error, ±2.00%; when operating temperature is 20 ± 5°C) (*26*). Investigation of granules moisture of the same sample was performed three to five times and for granule static strength investigation, 20 separate granules (the same size) were used. The arithmetic mean of the determined values is presented in this study. To evaluate results, the standard error (SE), standard deviation (SD), and confidence interval (CI) at 95% probability were calculated. Statistically analytical data were analyzed by using MS Excel data analysis (ANOVA, descriptive statistics) tools, calculating a range of statistical parameters for every data set.

Results and discussion

Efficiency of landfill leachate as a nitrogen source for microalgae cultivation

To evaluate the suitability of landfill leachate for replacing mineral nitrogen in the microalgae cultivation media, studies were carried out on the growth of microalgae in BG11 cultivation medium, where the nitrogen resource was the landfill leachate. Figure 1 shows the growth dynamics of microalgae biomass using different amounts of landfill leachate in the cultivation media. The concentration of nitrogen ranged from 0.05 g·L^{-1} to 0.14 g·L^{-1}. For comparison, the growth dynamics of microalgae biomass in a conventional BG11 cultivation medium with a nitrogen concentration of 0.12 g·L^{-1} was observed.

The data presented show that in the exponential growth phase, the growth of microalgae in different concentrations of nitrogen medium was slightly different.

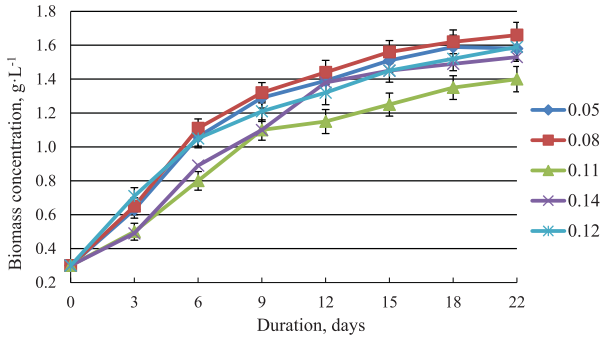

Figure 1. Growth dynamics of microalgae in nutrient medium with landfill leachate at nitrogen concentration of: 0.05 g·L^{-1}; 0.08 g·L^{-1}, 0.11 g·L^{-1}, 0.14 g·L^{-1}. Control BG11 medium with N concentration of 0.12 g·L^{-1}.

After 22 days, the highest biomass concentration was obtained by growing microalgae in the nutrient medium with landfill leachate at 0.08 g·L^{-1} nitrogen. It reached 1.66 g·L^{-1}, while the final microalgae biomass concentration obtained on BG11 culture medium for cultivation on day 22 was slightly lower at 1.59 g·L^{-1}. The maximum achieved biomass yield for cultivation using landfill leachate was 0.163 g·L^{-1} per day. The higher biomass content obtained compared to the cultivation of microalgae in BG11 medium can be explained by the fact that there were different nitrogen sources in the growth medium: ammonium and nitrate nitrogen, while BG11 medium consists only of nitrate nitrogen. Such trends have been observed by other authors who indicate that the use of mixed growing media results in high biomass yields (*27*).

At 0.05 g·L^{-1} nitrogen in the culture medium, the growth rate of microalgae was almost the same as in BG11 medium: at the end of the growth period, the concentration of microalgae biomass reached 1.58 g·L^{-1}. A nitrogen content of more than 0.08 g·L^{-1} in the nutrient medium containing the landfill leachate did not have a positive effect. The accumulation of microalgae biomass slowed down, and at 0.11 g·L^{-1} nitrogen, the final biomass concentration in the medium reached only 1.4 g·L^{-1}, and at an even higher nitrogen concentration of 0.14 g·L^{-1} in the medium, the microalgae biomass concentration only reached 1.53 g·L^{-1}.

The negative effect of the nitrogen content using landfill leachate compared to pure BG11 medium can be explained by the fact that all nitrogen in BG11 medium is in the nitrate form, which is well absorbed and tolerated by microalgae. Meanwhile, a relatively high amount of ammonium nitrogen is found in the landfill leachate. Its concentration was 98 mg·L^{-1}, while the total nitrogen concentration was 165 mg·L^{-1}. Some researchers (*28*,*29*) have also found the negative influence of ammonium nitrogen on the accumulation

of microalgae biomass. They reported that low concentrations of NH_4–N (up to 100 ppm) do not affect the growth rate of microalgae, while higher concentrations of nitrogen in the ammonium form (>200 ppm) reduce the biomass yield by up to 30%. Similar trends were observed by Park et al (2012) who investigated the possibility of ammonia removal using *Scenedesmuss* sp. microalgae (*30*). Anaerobic digestion effluent of livestock waste was found not to inhibit the growth of microalgae at up to 100 ppm NH_4 – N, but at concentrations of 200–500 ppm NH_4 – N in the culture medium, the concentration of microalgae decreased to 70%. These authors found that the negative effects of ammonium nitrogen can be reduced moderately by the degree of aeration of the nutrient medium, thus removing some of the ammonium from it due to stripping to the ammonia gas. Even better efficiency of nitrogen removal from liquid waste was found using a microalgae-bacterial consortium (*31*). Some authors point out that Ca and Mg ions in wastewater increase the efficiency of wastewater treatment and the yield of microalgae biomass (*32,33*).

In our case, inhibition of microalgae growth was observed in the growth medium at more than 0.11 g·L^{-1} total nitrogen or 0.065 g·L^{-1} ammonium nitrogen: biomass growth was inhibited by about 18–20%.

Summarizing the results, it can be stated that the landfill leachate can be used for the cultivation of microalgae biomass by replacing nitrate with nitrogen in the conventional growth medium, but the nitrogen concentration in the medium, which allows to obtain higher biomass content, should not exceed 0.08 g·L^{-1}.

Possibilities of using technical glycerol as a carbon source for microalgae biomass cultivation

A mycotrophic cultivation method can be applied to microalgae cultivation, where microalgae use both inorganic and organic carbon for photosynthesis and biomass storage. Refs. (*34–36*) found that the addition of a small amount of glucose, sucrose, or maltose to the microalgae culture medium increases the rate and the yield of microalgae biomass accumulation. A relatively high concentration of 0.33 g·L^{-1} biomass was obtained using glucose (*37*). Some authors have studied the efficiency of glycerol supplementation in microalgae cultivation and found that supplementing the microalgae growth medium with 10 g·L^{-1} glycerol can increase the growth rate of microalgae biomass up to 6.3-fold (*38*). To reduce the cost of growing microalgae biomass, it is necessary to look for cheaper sources of organic carbon. Technical glycerol, which is formed during the production of biodiesel, was chosen for our research. The amount of technical glycerol in Europe has increased significantly with the expansion of biodiesel production, but the market demand for refined glycerol has remained the same, leading biodiesel producers to look for new uses for technical glycerol. Most industries use pure glycerol for different purposes, which can be obtained by refining technical glycerol. This requires additional material and energy costs. The use of technical glycerol for microalgae cultivation would reduce the cost of microalgae cultivation and increase the profits of biodiesel production.

The glycerol was added to the cultivation medium with the landfill leachate (nitrogen concentration 0.08 g·L^{-1}). For comparison, tests were also performed using conventional microalgae cultivation medium BG11. The composition of technical glycerol was as follows: glycerol – 85.8%, water – 5.8%, free fatty acids – 1.1%, methanol – traces. Depending on the results of the researchers, the concentration of glycerol in cultivation media varied from 2 g·L^{-1} to 10 g·L^{-1}. Microalgae biomass accumulation was observed for 22 days (Figure 2).

The data show that the addition of glycerol increased the yield of microalgae biomass in the growth medium where nitrogen resources were replaced by landfill leachate. As the technical glycerol content increased to 6 g·L^{-1}, the concentration of microalgae biomass consistently increased and the maximum concentration reached on day 22 was 1.95 g·L^{-1}. The highest yield of microalgae biomass in medium with glycerol medium was 0.139 g·L^{-1} per day. By further increasing the concentration of technical glycerol in the cultivation medium to 10 g·L^{-1}, the opposite effect was observed, and the concentration of microalgae at the end of cultivation was lower than when cultivating with 6 g·L^{-1} and 8 g·L^{-1} glycerol, but it remains higher than when cultivating microalgae without glycerol. The obtained data

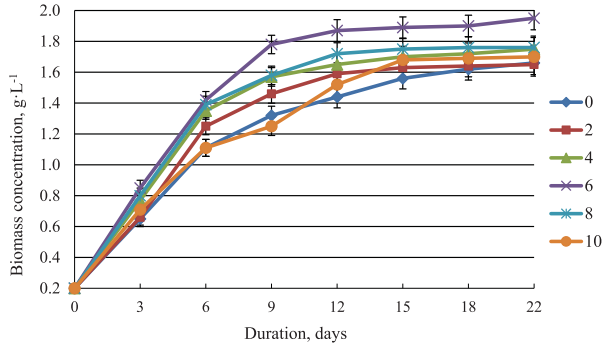

Figure 2. Growth dynamics of microalgae in nutrient medium with landfill leachate, when the concentration of nitrogen in the cultivation medium is 0.08 g/L and the concentration of glycerol is 0 g·L^{-1}; 2 g·L^{-1}; 4 g·L^{-1}; 6 g·L^{-1}; 8 g·L^{-1}, and 10 g·L^{-1}.

show that the addition of 6 g·L^{-1} technical glycerol increased the biomass yield by 17.5%.

Summarizing the obtained results, it can be stated that technical glycerol can be used in the heterotrophic microalgae cultivation process as a source of organic carbon. Its optimal concentration in the culture medium is 6 g·L^{-1}. This amount allows to increase the concentration of microalgae biomass in the cultivating medium from 17.5% to 20.6% in comparison with cultivation without the use of technical glycerol.

With higher glycerol content, the algal biomass yield decreases slightly. Compared to the results of the researchers who used pure glycerol for microalgae cultivation, it should be noted that a lower amount of technical glycerol was found to ensure the highest biomass growth.

Using pure glycerol, the maximum yield was obtained in the growth medium at 10% glycerol (*38*), while our results show that the optimal technical glycerol concentration is 6%, and higher amounts of technical glycerol result in lower microalgae biomass (MABM) yield. This could be explained by the negative impact of impurities in technical glycerol (free fatty acids, methanol, soap residue) on the development of microalgae.

Microalgae biomass suitability analysis

First, to validate microalgae biomass suitability for granular fertilizer production, the liquid phase content was measured. The analysis indicated that water content is very high and varies between 84% and 85%, which means that no additional irrigation of raw materials mixture is required for the granulation process. In the production of chemical fertilizer, the chemical composition and the concentration of inorganic elements are the most important issues. Hence, some of the chemical and instrumental analysis methods were used to determine the nutrient concentration in microalgae biomass suspension (MABM). As it can be concluded from the results of chemical analysis, dry matter (DM) of MABM (after decomposition with mineral acids) contains 3.49% nitrogen (N), 2.10% phosphorus (P_2O_5), 0.50% potassium (K_2O), 13.42% calcium (CaO), 3.69% magnesium (MgO), and 75.33% organic matter (C). Data obtained during the analysis show that the concentration of secondary plant nutrients (calcium and magnesium) is quite high; however, microalgae do not contain a high amount of primary nutrients. In spite of that, the nutrients listed earlier are the most essential for plant development and growth. Plants also require trace elements, just in significantly lower concentrations. The total content of trace elements and heavy metals was determined by using the AAS method. Obtained results indicate that MABM contain the following nutrients: 0.2615% Fe, 0.0237% Mn, 0.0142% Cu, and 0.0349% Zn. Furthermore, all tested samples contain 0.001% lead. The concentrations of inorganic elements determined in this study were compared with the results published by the other authors. It turned out that the results are almost the same as in other studies (*39,40*). Summarizing the data of chemical analysis of MABM (which had been dried at 60°C till the constant weight), it could be claimed that microalgae contain plant nutrients, which are soluble in mineral acid, and they could be used as a raw material for the production of fertilizers with bioactive materials.

To evaluate raw materials' thermal stability (based on industrial conditions of granular fertilizer production), the analysis was performed according to the STA method. The DSC curve presented in Figure 3(a) shows that urea melting and mass loss begin at a temperature of 131.5°C, i.e. when urea reaches its melting point at 132°C. A strong endothermic reaction is observed when urea starts to decompose at the temperature of 135.5°C. Ammonium and carbon monoxide gases are released by heating the urea above its melting point. These gases are produced in three stages over the corresponding temperature range. DSC curve shown in Figure 3 indicates two endothermic effects at 195.3°C and 238.7°C, which are followed by mass loss of 40.94% (TGA curve). These results are in agreement with the statement by Tischer et al. (*41*). According to the article by Tischer et al. , urea decomposes at higher temperatures in three stages of temperature range: at 190–250°C, 250–360°C, and 360–600°C, respectively.

The DSC curve (Figure 3(b)) shows that moisture residue in dry microalgae starts to evaporate at 33°C and gradually proceeds until the temperature of 160°C is reached. This process results in an endothermic effect of very low intensity (2 mV), and its peak is reached at the temperature of 66.4°C. Evaporation of moisture residue in microalgae is followed by mass loss of 4.3%, and those results correspond to the values measured according to the thermogravimetric methodology. At a given temperature range, there are no more distinct endothermic or exothermic effects. Nevertheless, the figure demonstrates that there is an increase in the DSK curve that is accompanied by a weight loss of about 14%. It is assumed that organic compounds would decompose due to an elevated temperature and finally burn down.

Regarding the limitations of nitrogen fertilizer production (due to urea decomposition at 80°C), data presented in the figure demonstrate that thermal changes

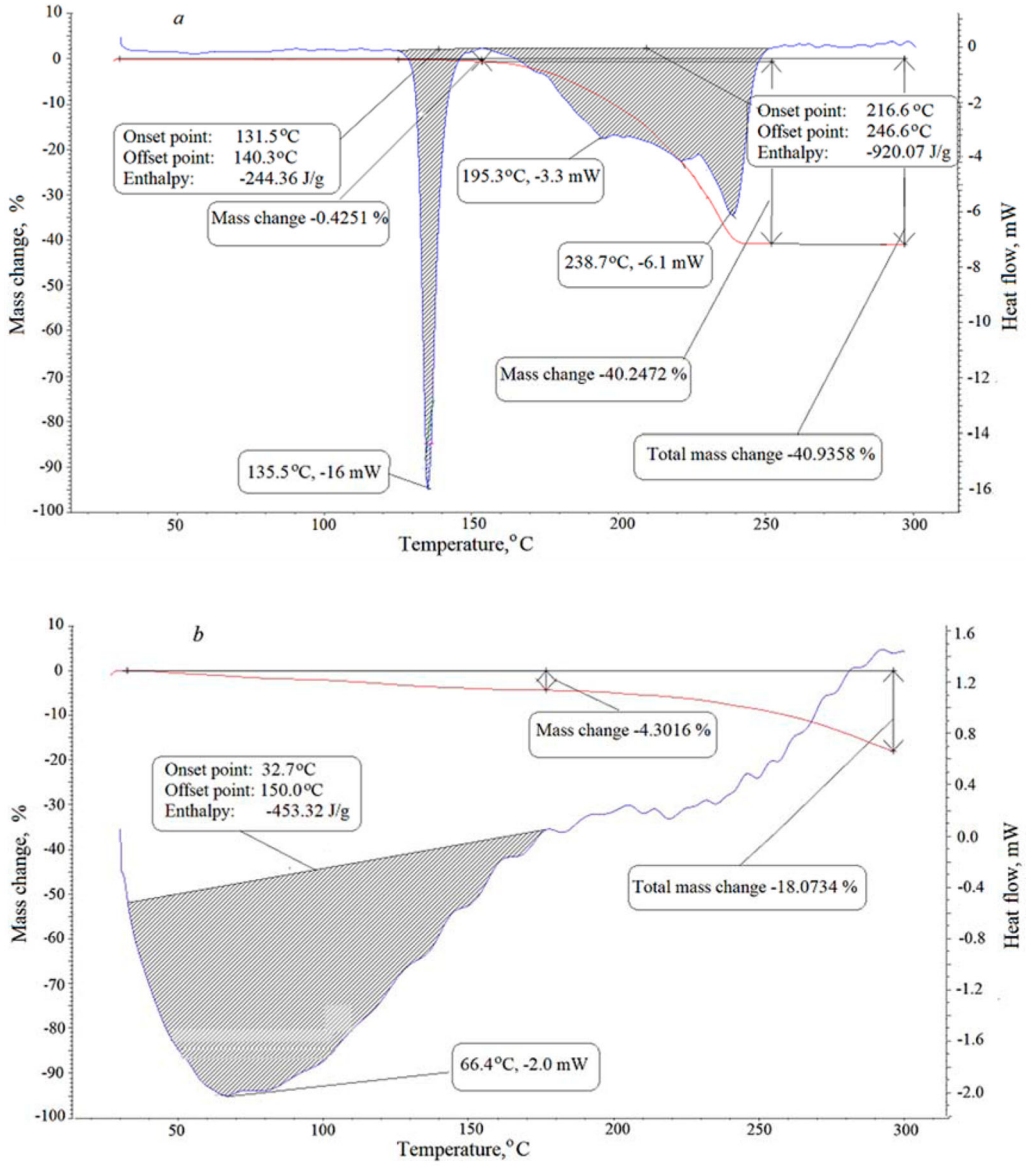

Figure 3. STA curves: *a* – urea; *b* – MABM (DM).

in MABM occur at higher temperatures. These results suggest that microalgae could be used as a raw material for the production of granular nitrogen fertilizer with microalgae biomass additive.

Production of granulated nitrogen fertilizer using microalgae biomass additive

Laboratory drum granulator was used to obtain granular nitrogen biofertilizer. Samples for granulation (Table 1) were prepared by mixing urea with different amounts of microalgae (7.5–10.0 g/50 g urea or 13.0–16.7%). The obtained granular product was dried until constant mass at 60°C. To evaluate its properties, the total nitrogen content was quantified; moreover, fractional composition, granule static strength, bulk density, pH of 10% solution, and moisture content were measured. As it is presented in Table 1, because of the excessive moisture content, no commercial fraction was obtained by using the mixture of 50 g urea and 10 g (16.7%) microalgae. It should be emphasized that all tested samples contain the same amount of nitrogen (values vary between 42% and 43%), which means that the addition of MABM does not affect fertilizer's nitrogen concentration.

Granule physical properties, such as bulk density and moisture content, in all studied cases are pretty much the same. From these results, it is clear that the addition of MAMB has no effect on any of those properties mentioned earlier.

The static strength of granules with a diameter of 2 mm to 3.15 mm ranges from 14.12 N/gran. to 19.17 N/gran. The static strength of granules with a

Table 1. Composition of raw materials and properties of the final product.

Sampl. no.	Raw materials, g		Properties of the final product							
	Urea, g	MABM, g (%)	N, %	Granule static strength, N/gran.		Bulk density, kg/m^3		pH of 10% solution	Moisture, %	
				>2 mm	>3.15 mm	>2 mm	>3.15 mm		>2 mm	>3.15 mm
1	50	10.0 (16.7)	No commercial fraction							
2	50	9.5 (16.0)	42.0	17.69 ± 1.2	24.23 ± 2.1	441.7	438.5	8.6	0.414 ± 0.004	0.559 ± 0.01
3	50	9.0 (15.3)	42.0	19.17 ± 2.0	20.09 ± 2.7	470.2	439.4	8.7	0.401 ± 0.005	0.413 ± 0.006
4	50	8.5 (14.5)	42.7	14.37 ± 1.8	15.67 ± 2.4	458.4	420.1	8.7	0.382 ± 0.005	0.485 ± 0.006
5	50	8.0 (13.8)	42.5	14.12 ± 1.8	17.93 ± 2.8	480.1	445.5	8.7	0.339 ± 0.005	0.384 ± 0.008
6	50	7.5 (13.0)	42.6	15.16 ± 2.1	18.79 ± 2.5	458.6	419.7	8.8	0.461 ± 0.004	0.499 ± 0.007

diameter of 3.15 mm to 5 mm ranging from 15.67 N/gran. to 24.23 N/gran. The total moisture content and bulk density measurements were calculated for different fractions: 2–3.15 mm and 3.15–4 mm, respectively. Results show that when urea mixture is granulated with MABM, values of strength and moisture content of granules with a larger diameter are slightly higher than those of granules smaller in diameter, while the bulk density of lager granules is lower. The results demonstrate that adding more MABM to the mixture of raw materials has little to no effect on pH values of 10% solution of the final product.

The granulometric composition of granules made of different amounts of MABM is presented in Figure 4. The analysis demonstrates that increasing the amount of MABM additive from 16.0% to 16.7% results in very large (>5 mm) granules, and only a small amount (2–3%) of fine and commercial fraction is formed.

Figure 5 shows how commercial fraction, i.e. medium-sized particles (2–3.15 mm and 3.15–4 mm), is dependent on MABM additive.

This study suggested that the highest amount of commercial fraction was obtained by a granulating mixture composed of 50 g of urea and 8.0–9.0 g (or 13.8–15.3%) of MABM, but it needs to be mentioned that the fraction of medium-size granules makes up only 39–46% of the total product. This is not a very good result, and such technology requires the return of small granules to the technological process, i.e., retour use.

In an attempt to see if some chemical reaction takes place between urea and microalgae by forming new compounds, instrumental analysis (FT–IR and XRD) of raw materials and granules was performed. To evaluate the granular product's thermal stability, a simultaneous thermal analysis (STA) was carried out. Figures 6–8 present the difference in the results of the instrumental analysis between the raw materials and granular product.

Figure 6(b) presents the results of X-ray diffraction analysis of microalgae that confirms algae's amorphous structure, i.e., there are no high-intensity peaks, which would indicate its crystalline nature. As expected, the X-ray diffraction pattern of granular product given in Figure 6(a) corresponds to a diffraction pattern of pure urea (Figure 6(c)). Values of interplanar distance (0.400;

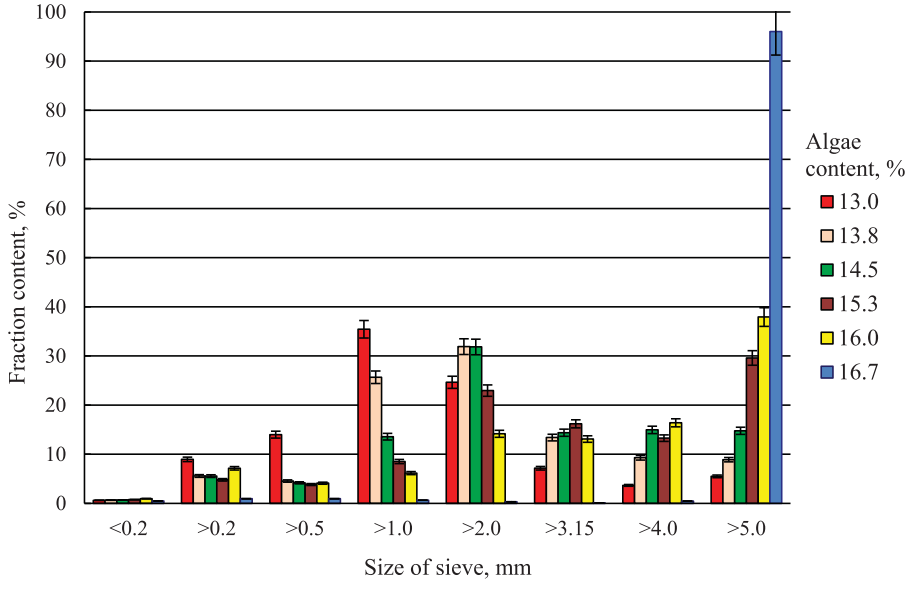

Figure 4. Granulometric composition of granules.

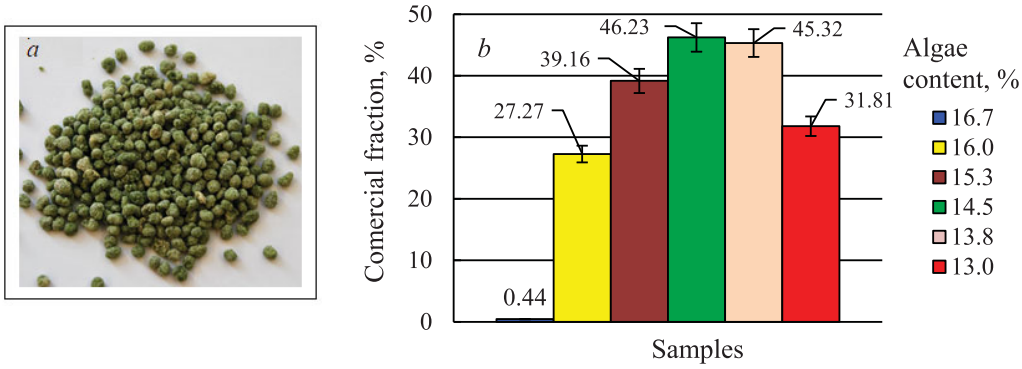

Figure 5. Commercial fraction (2–4 mm) of fertilizers: *a* – view of granules; *b* – quantity of commercial fraction.

0.362; 0.303; 0.281; 0.251; 0.241; 0.222; 0.192; 0.183; 0.167 nm) are equal in both figures, and only the intensity of the peak varies with respect to the sample. The findings of this analysis prove that there are no additional materials or compounds obtained during granulation.

The FT–IR spectrum of granular product (Figure 7(c)) was analyzed by comparing obtained results with raw material spectrum and data given in reference books (Figure 7(a,b)). Granular product spectrum is hardly distinguishable from urea, which is the main component of our fertilizer. The main difference between these two is the intensity of the peaks. Considering the amount of MABM in fertilizer, microalgae biomass's peaks of absorption in the spectrum are typical for the same functional groups (Figure 7(b)) and cannot be distinguished from the product spectrum due to low intensity and overlap. The functional groups were identified in the range of 400–4000 cm^{-1}.

There is an intensive peak at 3500–3200 cm^{-1}, which represents symmetric and asymmetric stretching of N–H bonds. The peak at 2927–2474 cm^{-1} can be attributed to the stretching of C–H bonds. An intensive peak at 1680 cm^{-1} matches the stretching of C–O bond. N–H bond can be confirmed by the intensive peak at 1620 cm^{-1}. One peak in the region of 1465 cm^{-1} corresponds to the symmetric and asymmetric stretching of C–N bonds. Peaks at 1152–1003 cm^{-1} indicate C–N bond. Less-intensive peaks at 780–720 cm^{-1},

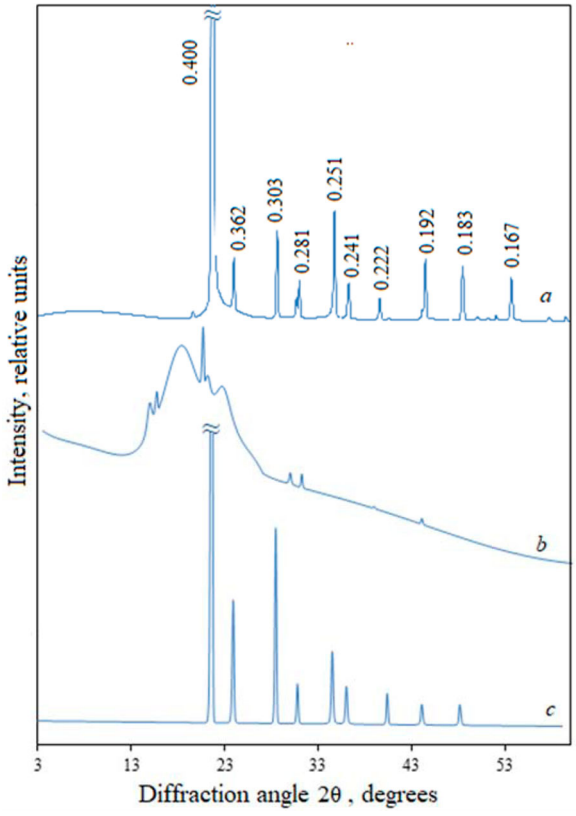

Figure 6. Curves of XRD analysis: *a* – urea; *b* – MABM (DM); *c* – granular fertilizers.

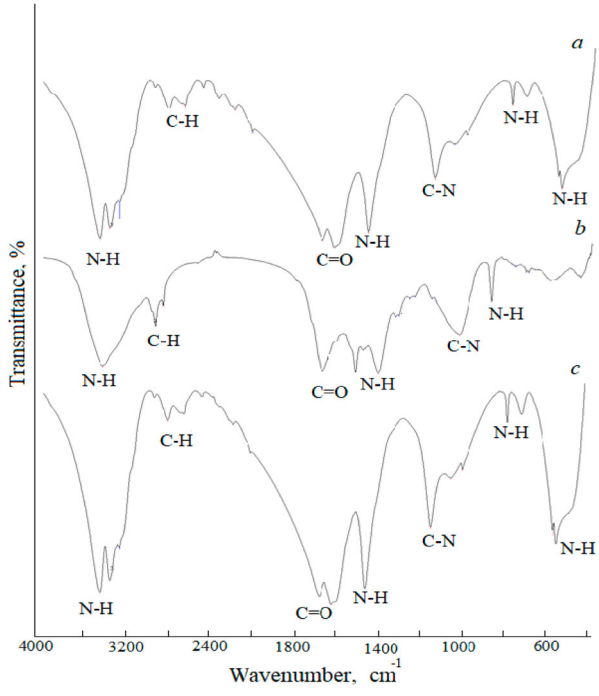

Figure 7. FT–IR analysis curves: *a* – urea; *b* – MABM (DM); *c* – granular fertilizers.

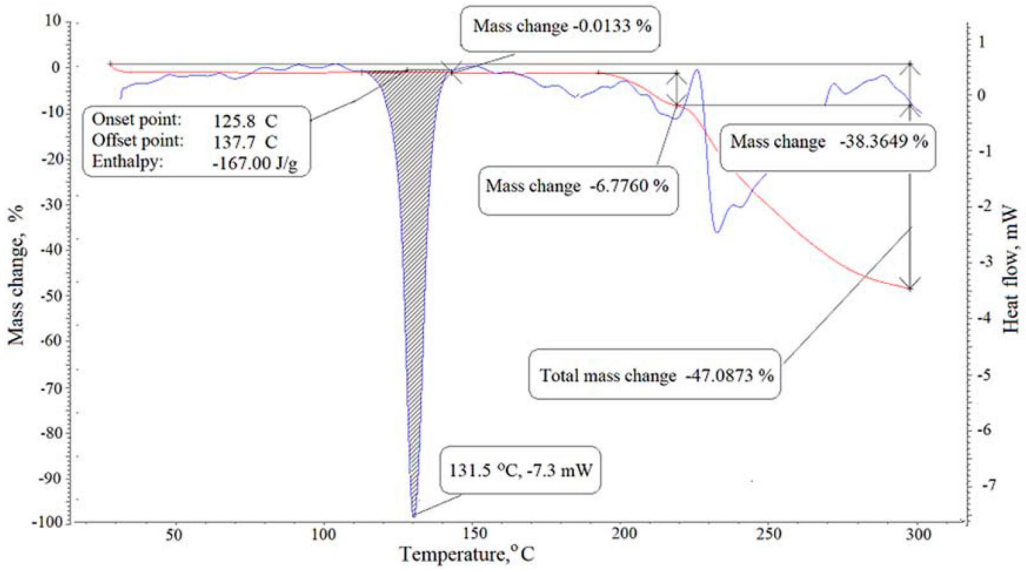

Figure 8. STA curve of granular fertilizers (Sample No. 4).

respectively, could be attributed to N–H oscillations. Peaks in the range of 570 cm^{-1}–560 cm^{-1} prove the presence of amine. FT–IR spectrum confirms the previous data of chemical and XRD analyses.

The analysis of the final product's thermal stability was performed at temperatures ranging from 0°C to 150°C (according to the conditions of planned industrial production). It has been proven that at this temperature range, the final product is thermally stable and total mass loss is less than 1% (0.78%). The endothermic effect at 134.2°C matches the temperature of urea's melting point (Figure 8).

The evidence from this study implies that raw materials used for granulation are stable up to the temperature of 100°C. Due to this issue, during the granulation process and drying stage, proper temperature range should be considered. In summary, it could be stated that by mixing urea with microalgae biomass as a bioactive additive grown in the medium of landfill effluent and technical glycerol, it is possible to produce granular fertilizer corresponding to all requirements.

Conclusions

The cost of microalgae cultivation can be reduced by the supplementing growth medium with landfill leachate, liquid waste containing nitrogen compounds, and technical glycerol, a by-product of biodiesel production, containing organic carbon. After 22 days of cultivation, the largest microalgae *Chlorella* sp. biomass concentration of 1.66 $g{\cdot}L^{-1}$ is achieved in the nutrient medium in the presence of 0.08 $g{\cdot}L^{-1}$ nitrogen obtained from the landfill leachate. Technical glycerol, a by-product of biodiesel production, accelerates the process of microalgae biomass accumulation and increases its yield by 20.6%. The optimal concentration of technical glycerol in the culture medium is 6 $g{\cdot}L^{-1}$. Also, obtained results have led us to conclude that the chemical composition of microalgae biomass is sufficiently good to produce granular nitrogen fertilizer with bioactive materials. It should be emphasized that microalgae contain primary nutrients, which are soluble in mineral acid (3.49% N; 2.10% P_2O_5; 0.50% K_2O) along with secondary nutrients (13.42% CaO and 3.69% MgO) and trace elements (0.2615% Fe; 0.0237% Mn; 0.0142% Cu; 0.0349% Zn). It needs to be mentioned that MABM does not contain any heavy metals. The use of microalgae allows reducing the amount of moisture used in the production technology. If optimal conditions are chosen, it is possible to granulate bioactive nitrogen fertilizers containing 42–43% N that meet the requirements for fertilizers. Results show that granule bulk density, moisture content, and pH of a 10% solution do not depend on the amount of MABM additive. The static strength of the granules ranges from 14.12 N/gran. to 19.17 N/gran. for granules with a diameter of 2–3.15 mm in and from 15.67 N/gran. to 24.23 N/gran. when the diameter of the granules is 3.15–4 mm.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

[1] Sutton, M.A.; Bleeker, A.; Howard, C.M. et al. *Our Nutrient World: The Challenge to Produce More Food and Energy with Less Pollution*. Global Overview of Nutrient Management. Centre for Ecology and Hydrology, Edinburgh on behalf of the Global Partnership on Nutrient Management and the International Nitrogen Initiative, 2013. http://nora.nerc.ac.uk/id/eprint/500700/1/N500700BK.pdf.

[2] Singh, A.; Verma, P. *J. Cleaner Prod.* **2017,** *167*, 473–483. DOI:10.1016/j.jclepro.2017.08.106.

[3] Calvo, P.; Watts, D.B.; Kloepper, J.W.; Torbert, H.A. *Can. J. Microbiol.* **2016,** *62* (12), 1041–1056. DOI:10.1139/cjm-2016-0122.

[4] Kumari, R.; Kaur, I.; Bhatnagar, A.K. *J. Appl. Phycol.* **2011,** *23*, 623–633. DOI:10.1007/s10811-011-9651-x.

[5] Coppens, J.; Grunert, O., Van Den Hende, S., et al. *J. Appl. Phycol.* **2016,** *28*, 2367–2377. DOI:10.1007/s10811-015-0775-2.

[6] Zhao, B.; Zhang, Y.; Xiong, K.; Zhang, Z.; Hao, X.; Liu, T. *Chem. Eng. Res. Des.* **2011,** *89* (9), 1758–1762. DOI:10.1016/j.cherd.2011.02.018.

[7] Mata, T.M.; Martins, A.A.; Caetano, N.S. *Renew. Sustain. Energy Rev.* **2010,** *14* (1), 217–232. DOI:10.1016/j.rser.2009.07.020.

[8] Doan, T.T.Y.; Sivaloganathan, B.; Obbard, J.P. *Biomass Bioenergy* **2011,** *35* (7), 2534–2544. DOI:10.1016/j.biombioe.2011.02.021.

[9] Yoo, C.; Jun, S.Y.; Lee, J.Y.; Ahn, C.Y.; Oh, H.M. *Bioresour. Technol.* **2010,** *101* (1), S71–S74. DOI:10.1016/j.biortech.2009.03.030.

[10] Yeesang, C.; Cheirsilp, B. *Bioresour. Technol.* **2011,** *102* (3), 3034–3040. DOI:10.1016/j.biortech.2010.10.013.

[11] Zhao, Q.; Huang, H. *Trends Biotechnol.* **2021,** *39* (2), 112–115. DOI:10.1016/j.tibtech.2020.08.009.

[12] Sánchez Mirón, A.M.; Cerón Garcıa, C.; Contreras Gómeza, A.; Garcıa Camachoa, F.; Molina Grima, E.; Chisti, Y. *Biochem. Eng. J.* **2003,** *16* (3), 287–297. DOI:10.1016/S1369-703X(03)00072-X.

[13] Posten, C. *Eng. Life Sci.* **2009,** *9* (3), 165–177. DOI:10.1002/elsc.200900003.

[14] Temple, W.D.; Bomke, A.A. *Plant Soil* **1988**, *105*, 213–222. DOI:10.1007/BF02376785.

[15] Tarakhovskaya, E.R.; Maslov, Y.I.; Shisshova, M. *Russ. J. Plant Physiol.* **2007,** *54* (2), 163–170. DOI:10.1134/S1021443707020021.

[16] Mahfouz, S.A.; Sharafeldin, M. *Int. Agrophys.* **2007,** *21* (4). DOI:10.1055/s-2007-987419.

[17] Sumbul, A.; Ansari, R.A.; Rizvi, R.; Mahmood, I. *Saudi J. Biol. Sci.* **2020,** *27* (12), 3634–3640. DOI:10.1016/j.sjbs.2020.08.004.

[18] Mulbry, W.; Westhead, E.K.; Pizarro, C.; Sikora, L. *Bioresour. Technol.* **2005,** *96* (4), 451–458. DOI:10.1016/j.biortech.2004.05.026.

[19] Mulbry, W.; Kondrad, S.; Pizarro, C. *J. Veget. Sci.* **2007,** *12* (4), 107–125. DOI:10.1300/J484v12n04_08.

[20] Spolaore, P.; Joannis-Cassan, C.; Duran, E.; Isambert, A. *J. Biosci. Bioeng.* **2006,** *101* (2), 87–96. DOI:10.1263/jbb.101.87.

[21] Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilisers. *OJ L* 304, 2003.

[22] Paleckienė, R.; Sviklas, A.; Slinksiene, R.; Štreimikis, V. *Pol. J. Environ. Stud.* **2012,** *21* (4), 993–999. eISSN: 2083-5906. http://www.pjoes.com/Processing-of-Rape-Straw-Ash-into-Compound-r-nFertilizers-Using-Sugar-Factory-Waste,88831,0,2.html.

[23] Lithuanian Standard. Soil Improvers and Growing Media – Sample Preparation for Chemical and Physical Tests, Determination of Dry Matter Content, Moisture Content and Laboratory Compacted Bulk Density. LST EN 13040: 2008.

[24] Lithuanian Standard. Solid Fertilizers – Test Sieving. LST EN 1235:2002/A1:2005.

[25] Lithuanian Standard. Fertilizers – Determination of Bulk Density (loose) of Fine-Grained Fertilizers. LST EN ISO 7837:2001.

[26] Lithuanian Standard. CEN REPORT. Fertilizers – Crushing Strength Determination on Fertilizer Grains. LST CR 1233:2006.

[27] Zhu, L.; Li, S.; Hu, T.; Nugrohoc, Y.K.; Yin, Z.; Hu, D.; Chu, R.; Mo, F.; Liu, C.; Hiltunen, E. *Energy Convers. Manage.* **2019,** *201* (1), 112144. DOI:10.1016/j.enconman.2019.112144.

[28] Borowitzka, M.A. *Prog. Ind. Microbiol.* **1999,** *35*, 313–321. DOI:10.1016/S0079-6352(99)80123-4.

[29] Tam, N.F.Y.; Wong, Y.S. *Bioresour. Technol.* **1996,** *57* (1), 45–50. DOI:10.1016/0960-8524(96)00045-4.

[30] Park, J.; Jin, H.-F.; Lim, B.-R.; Park, K.-Y.; Lee, K. *Bioresour. Technol.* **2010,** *101*, 8649–8657. DOI:10.1016/j.biortech.2010.06.142.

[31] Biswas, T.; Bhushan, S.; Prajapati, S.K. *J. Environ. Manage.* **2021,** *286*, 112196. DOI:10.1016/j.jenvman.2021.112196.

[32] Chen, Z.; Qiu, S.; Amadu, A.A.; Shen, Y.; Wang, L.; Wu, Z.; Ge, S. *Bioresour. Technol.* **2020,** *297*, 122517. DOI:10.1016/j.biortech.2019.122517.

[33] Qiu, S.; Yu, Z.; Hu, Y.; Chen, Z.; Guo, J.; Xia, W.; Ge, S. *Water Res.* **2021,** *196*, 117038. DOI:10.1016/j.watres.2021.117038.

[34] Perez-Garcia, O.; Escalante, F.M.; de-Bashan, L.E.; Bashan, Y. *Water Res.* **2011,** *45* (1), 11–36. DOI:10.1016/j.watres.2010.08.037.

[35] Chen, C.Y.; Yeh, K.L.; Aisyah, R.; Lee, D.J.; Chang, J.S. *Bioresour. Technol.* **2011,** *102* (1), 71–81. DOI:10.1016/j.biortech.2010.06.159.

[36] Heredia-Arroyo, T.; Wei, W.; Ruan, R.; Hu, B. *Biomass Bioenergy* **2011,** *35* (5), 2245–2253. DOI:10.1016/j.biombioe.2011.02.036.

[37] Zhang, H.; Weiliang, L.Y.; Yang, W.; Shen, G. *Biomass Bioenergy* **2011,** *35*, 1710–1715. DOI:10.1016/j.biombioe.2011.01.002.

[38] Kong, W.B.; Yang, H.; Cao, Y.T.; Song, H.; Hua, S.F.; Xia, C.G. *Food Technol. Biotechnol.* **2013,** *51* (1), 62–69.

[39] Dineshkumar, R.; Narendran, R.; Jayasingam, P.; Sampathkumar, P. *J. Aquac. Mar. Biol.* **2017,** *5* (3), 00119. DOI:10.15406/jamb.2017.05.00119.

[40] Tibbetts, S.M.; Milley, J.E.; Lall, S.P. *J. Appl. Phycol.* **2015,** *27*, 1109–1119. DOI:10.1007/s10811-014-0428-x.

[41] Tischer, S.; Börnhorst, M.; Amsler, J.; Schoch, G.; Deutschmann, O. *Phys. Chem. Chem. Phys.* **2019,** *21*, 16785–16797. DOI:10.1039/C9CP01529A.