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Biomass and Oil Production of Green Microalgae *Scenedesmus sp.* Using Different Nutrients and Growth

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Biofuel is mostly produced from oleaginous crops, such as rapeseed, sunflower, soybean. The search for new technologies and new feedstock for biofuel production is becoming an increasingly important issue for researchers. Special attention is turned to the raw materials which do not compete with food industry. Microalgae ability, due to their rapid growth, to accumulate oil, to treat wastewater and waste, seems to be a very attractive new object to be used for biofuel production. In this study we have investigated different microalgae growth conditions, including both autotrophic cultivation by means of nitrogen deprivation mode and mixotrophic cultivation by applying liquid waste and technical glycerol for determining the best growth and oil production conditions. It is found that applying nitrogen deprivation mode and mixotrophic growth conditions, microalgae Scenedesmus sp. have grown faster and accumulation of their oil has increased 10.88 times compared to that under autotrophic growth conditions using the usual amount of nitrogen. The highest biomass concentration (2.16 gL^{-1}) is obtained by adding 5 gL^{-1} glycerol into the growth medium, whereas the highest oil concentration (15.12 %) is reached when using 10 gL^{-1} technical glycerol. In addition, the elemental composition of microalgae biomass has been analyzed. Results indicate that the usage of glycerol for cultivation of microalgae increases C : N ratio to optimum (19.25) for biogas production, and algae biomass could be used for this biofuel production without adding any other co-substratum. The results of our study show that addition of cheap products: liquid waste and technical glycerol can effectively adjust the composition of microalgae biomass, making it more suitable for biofuel production.

Keywords: *Scenedesmus sp., microalgae, liquid waste, biomass, growth, technical glycerol, oil, triglyceride (TG), biodiesel, biogas.*

1. Introduction

Algae are big and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicellular forms. They are one of the oldest life forms in the world. Algae are used for many different purposes because of their ability to produce and accumulate a variety of useful materials. In industry and commerce algae are used as additive source of food and feed, as high-value derivatives such as antioxidants, cosmetics, natural dyes, and polyunsaturated fatty acids (Rosenberg et al. 2008). Recently, great attention has been turned to the investigation of a possibility to use algae for biofuel production.

The above mentioned products are produced by cultivating algae on diverse growth media containing minerals and organic substrates (Harun et al. 2010, Perez-Garcia et al. 2011). Considering that algae use nitrogen and phosphorus compounds as nutrients, NH_4^+, NO_3^-, NO_2^-, PO_4^{3-} contained in wastewater and liquid waste could be simultaneously used for production of algae cells and treatment of wastewater. Nitrogen and phosphorus, which algae extract from wastewater very effectively, are those of most

required elements for their growth (Makarevičienė et al. 2011). Algae are also characterized by being able to remove some organic pollutants and to dissolve volatile solids from wastewater (Prathima et al. 2012). Moreover, algae are excellently capable of biofixing carbon dioxide, and this feature can be well applied to reduction of pollutants concentration in the atmospheric air (removal of carbon dioxide from industrial flue gases), herewith preventing global warming.

Different conditions of algae cultivation have a considerable influence on their growth characteristics and composition of their cells. Mostly, algae are cultivated autotrophically (organic compounds of algae cells are produced during the photosynthesis process using inorganic carbon source: CO_2, Na_2CO3, $NaHCO_3$). Results of several studies have shown that better biomass (higher cell density) and lipid production of algae are achieved by cultivating algae under heterotrophic and mixotrophic conditions compared to an autotrophic mode of growth (Perez-Garcia et al. 2011). Heterotrophic growth means that algae grow using organic substrata (glucose, glycerol, acetate, wastewater, etc) as carbon source for their cells production in the dark, and under mixotrophic conditions algae combine the ability of photosynthesis and organic matter uptake. Growing under normal conditions algae cells contain protein, carbohydrate, and natural lipid (Xin et al. 2010). Many factors, such as nitrogen level in the growth media, organic carbon source, concentration of carbon dioxides, temperature, and salinity affect lipid accumulation in algae cells (Brennan and Owende 2010). Under normal growing conditions, algae produce fatty acids for esterification into membrane lipids, the majority of which are glycosylglycerides. Under unfavorable or stress conditions (nitrogen starvation, etc), a lot of algae synthesize and accumulate neutral lipids, mostly triglycerides (TG) (Hu et al. 2008).

The property of algae to produce and accumulate triglycerides seems very attractive for scientists, who investigate new feedstock for biofuel production. The possibility to use wastewater or industrial by-products (for example, crude glycerol) and exhaust gases for biomass and lipid production of algae makes algae even more attractive and encourages researchers to investigate them as a potential for biofuel (biodiesel, biogas, etc) feedstock. Taking into account the possibility to use algae for biodiesel production, an important task faces researchers to adjust the mechanisms, which would enable algae to accumulate more triglycerides (oil), because it is not a structural but accumulative material.

This study is aimed at investigation of biomass production and lipid accumulation of green microalgae Scenedesmus sp. isolated from Lithuanian lakes by applying various cultivation (including autotrophic and mixotrophic growth) conditions. In consideration of the principles of sustainable development and cheap production of algae biomass, different cultivation conditions were capacitated by using nitrogen deprivation mode, liquid waste (the landfill filtrate and liquid fraction of digestate after biogas production), and technical glycerol as a secondary product of biodiesel production.

2. Methods

Microalgae strain and growth conditions

Microalgae Scenedesmus sp. isolated from Lithuanian lakes were cultivated under autotrophic and mixotrophic conditions in different media. For cultivation in laboratory condition, glass flasks with working volume of 3 L were used, the cultivation period from 20 to 30 days was chosen. The culture was grown at the room temperature under illumination of white fluorescent lamps for, approximately, 8 hours daily. Microalgae were hand-shaken several times a day to avoid sticking.

Autotrophic growth was carried out growing Scenedesmus sp. in modified BG11 medium, which contained the following: 750 mgL^{-1} $NaNO_3$, 40 mgL^{-1} K_2HPO_4, 75 mgL^{-1} $MgSO_4*7H_2O$, 36 mgL^{-1} $CaCl_2$, 3 mgL^{-1} citric acid, 3 mgL^{-1} ferric ammonium citrate, 1 mgL^{-1} EDTA (disodium salt), 20 mgL^{-1} Na_2CO_3 and 1 mlL^{-1} trace metal mix. This mixture contained 2.86 gL^{-1} H_3BO_3, 1.81 gL^{-1} $MnCl_2*4H_2O$, 0.222 gL^{-1} $ZnSO_4*7H_2O$, 0.390 gL^{-1} $NaMoO_4*5H_2O$, 79 mgL^{-1} $CuSO_4*5H_2O$, 49.4 mgL^{-1} $Co(NO_3)_2*6H_2O$. Nitrogen deprivation conditions were achieved by modifying the growth medium with different sodium nitrate amounts (1500, 750 and 375 mgL^{-1} $NaNO_3$ or 120, 60, 30 mgL^{-1} N).

Mixotrophic conditions for algae cultivation were achieved in two different ways: 1) by using liquid waste, such as landfill filtrate received from the Lapes landfill located near Kaunas (toxic properties of landfill filtrate were not analyzed), 2) by using liquid fraction of digestate after biogas production from JSC "Kurana" (Lithuanian producer of bioethanol and biogas) and technical glycerol purchased from JSC "Rapsoila" (Lithuanian producer of biodiesel). This glycerol contained 75-80 % of pure glycerol and its remaining part was impurities (methanol, free fatty acids, residue of catalyst, etc.).

Effect of liquid waste based cultivation medium on algal growth. Scenedesmus sp. was cultivated in four different media: control medium 1 - BG11 medium ((N concentration 0.1235 gL^{-1}); medium 2 - modified BG11 medium where N source was replaced with liquid fraction of digestate after biogas production (N concentration 0.0625 gL^{-1}); medium 3 - crude liquid fraction of digestate after biogas production (N concentration 0.625 gL^{-1}); medium 4 - modified BG11 medium where N source was replaced with the landfill filtrate (N concentration 0.15 gL^{-1}) .

Effect of technical glycerol based cultivation medium on algal growth. Effect of glycerol on algae growth was investigated by adding 2 gL^{-1} glycerol during the stationary phase of algae growth and microalgae culturing in four different media: control medium 1 - BG11 medium; medium 2 - BG11 medium + 2 gL^{-1}

technical glycerol; medium 3 - BG11 medium + 5 gL^{-1} technical glycerol; medium 4 - BG11 medium + 10 gL^{-1} technical glycerol, adding glycerol during the lag phase (at the beginning of growing).

Estimation of liquid waste parameter

Main parameters (pollutants) of landfill filtrate and digestate such as total nitrogen (TN), total phosphorus (TP), and biological oxygen demand (BOD) were estimated according to the standard LST EN 25663:2000, spectrometric and electrochemical methods, respectively.

Biomass concentration

Sample with microalgae biomass in growth medium was centrifuged for 10 minutes at 12000 rpm, and washed with distilled water, and then dried in oven at the temperature of 105 ˚C to a constant weight. The dry biomass concentration BC (gL-1) was calculated using the following equation:

$$BC\ (gL^{-1}) = \frac{m_S \cdot 1000}{V_S} \qquad (1)$$

Where: m_S - dry weight of sample, g
V_S - volume of sample, ml.

Harvesting

Microalgae were harvested by centrifugation and as a result receiving paste, consisting of 10-20 % dry matter. The paste was stored in freezer. Before usage, microalgae biomass was defrosted and dried in oven at the temperature of 105 ^{0}C to a constant weight.

Lipid extraction from microalgae

Desiccated biomass of Scenedesmus sp. was milled with pestle to disrupt algae cells as far as possible. 0.1 g of dried sample was extracted with 20 ml n-hexane, purchased from POSH S.A. (Poland) using homogenizer. The sample was homogenized for 5 minutes at 15000 rpm and then placed into hot (55 ^{0}C) water bath. Such procedure was repeated three-times. Every time before homogenization 20 ml of n-hexane was added. After extraction, the solution was analyzed for lipid concentration applying the FTIR spectroscopy method.

Estimation of triglycerides (TG) amount

Extracted lipid can include neutral lipids, polar lipids, wax esters, etc (Hu et al. 2008). Our task was to determine triglyceride content and it was done by FTIR spectroscopy. Lipid solutions were diluted to 50 ml with hexane and analyzed. TG amount in algae was determined according to the carbonyl stretching absorption at the wave length of 1740 cmL^{-1}. Initially, the calibration curve was prepared by using algae oil solutions of a known concentration: (concentration of oil was the following: 0.01 gL^{-1}; 0.025 gL^{-1}; 0.05 gL^{-1}; 0.1 gL^{-1}; 0.15 gL^{-1}; 0.2 gL^{-1}; 0.4 gL^{-1}; 0.6 gL^{-1}; 0.8 gL^{-1}; 1 gL^{-1}). From this curve and data obtained by analyzing lipid extracts, the amount of triglyceride in test solution was determined and TG amount in dry algae mass was calculated.

Determination of elemental composition

Determination of elemental composition of microalgae was performed using CHNS-O Elemental Analyzer (Perkin Elmer 2400 Series). This analyzer uses a combustion method to convert the measured elements (C, H, N, S) to simple gases. These gases are measured as a function of thermal conductivity. Dry microalgae biomass was pulverized, weighed on thin foil (app. 2 mg), placed into elemental furnace, burnt in a pure oxygen environment at 975 ^{0} C, weight percent of each element was analyzed and calculated.

3. Results and Discussion

Biomass and lipid productivity of microalgae Scenedesmus sp. were investigated under different growth conditions. Microalgae growth and oil production were evaluated under autotrophic and mixotrophic conditions using liquid waste and technical glycerol. Information about liquid waste is given in Table 1.

Table 1. Main parameters of pollution in liquid waste

Type of liquid waste	Composition of liquid waste		
	BOD (mgO_2L^{-1})	TN ($mgN\ L^{-1}$)	TP ($mgP\ L^{-1}$)
Landfill filtrate	217	150	19
Liquid fraction of digestate after biogas production	9240	625	802

Effect of different nitrogen amounts on Scenedesmus sp. growth

The growth curves of Scenedesmus sp. in growth medium BG11 containing different initial nitrogen amounts are shown in Figure 1. For the first three days (during lag phase), the concentration of microalgae in all samples changed very little, microorganisms adapted to growth conditions. On the fourth growth day, a sharp increase in cell concentration of microalgae (exponential phase strongly expressed) was visible in growth medium containing 0.06 gL^{-1} nitrogen. The maximum concentration was achieved on the eighteenth day (at the beginning of stationary phase) of growth and reached 1.724 gL^{-1}. The highest biomass

concentration was reached in the growth medium containing 0.06 gL^{-1} N.

Exponential growth phases of other two growth media (containing 0.1235 gL^{-1} and 0.03 gL^{-1} N) were not so clearly framed at the beginning of cultivation as in the growth medium containing 0.06 gL^{-1} N. When growing microalgae in media containing 0.03 gL^{-1} N and 0.1235 gL^{-1} N, the best results were obtained at the beginning of stationary growth phase and were 1.579 gL^{-1} and 1.608 gL^{-1}, respectively. As results of the investigation show, at the start of cultivation and during cultivation differences of algae growth showed up with regard to different nitrogen amounts in growth medium, but at the end of cultivation these differences were not so observable (biomass production was insignificantly affected by nitrogen concentration).

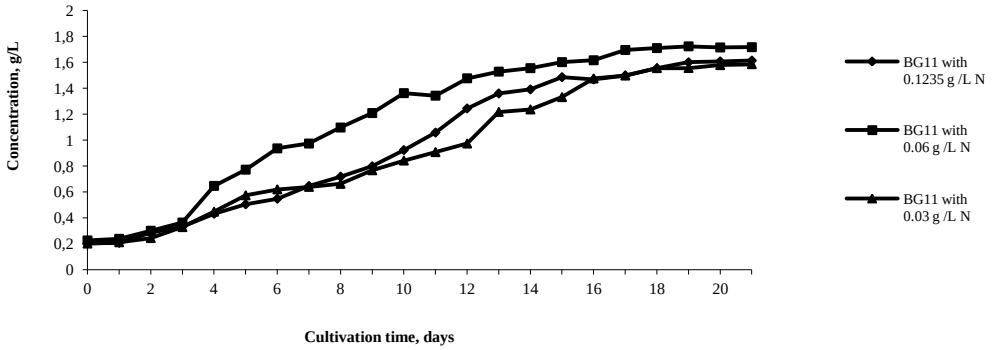

Fig. 1. Comparison of microalgae Scenedesmus sp. growth using different nitrogen amounts in growth media

Effect of liquid waste based cultivation medium on algal growth

Nutrients, such as dissolved inorganic and organic matter, are directly available for algae growth. Wastewater and liquid waste are good source of the above mentioned materials, because they are rich in dissolved organic materials, nitrogen, and phosphorus. We placed landfill filtrate and liquid fraction of digestate after biogas production as nitrogen source into the media with addition of other components from BG11. Changes in Scenedesmus sp. concentrations in four various growth media are shown in Figure 2. Results of the analysis show that during the first six days (lag phase) in all four media microalgae grew very similarly, their biomass concentration increased from 0.261 gL^{-1} to 1.302 gL^{-1}. At the end of the stationary phase in medium BG11+digestate containing 0.0625 gL^{-1} N, cell growth was significantly higher compared to other three media. The highest obtained biomass concentration was 2.040 gL^{-1}.

Biomass production in media BG11 containing 0.1235 gL^{-1} N and BG11+landfill filtrate containing 0.150 gL^{-1} N slightly differed in exponential phase, but in stationary growth phase the cell growth was almost the same, namely, dry biomass concentration was app. 1.800 gL^{-1} in both media. The reason of faster growth of microalgae in medium BG11+landfill filtrate containing 0.150 gL^{-1} N at the beginning of cultivation may be the presence of organic pollutants (BOD=217 $mgO2L^{-1}$) as organic carbon source. The result obtained at the end of cultivation could be explained that in both media there was almost the same concentration of nitrogen.

The lowest cell growth of Scenedesmus sp. was in medium BG11+digestate containing 0.625 gL^{-1} N. The reason is not so clear, but a high concentration of nitrogen might inhibit cell division (Rise et al. 1994). Besides, the growth of algae may affect not only the amount of nitrogen, but also its form (nitrite, nitrate, ammonium, etc). It is known that liquid digestate is rich in ammonium, whose higher concentration could reduce microalgae growth. A very high concentration of organic pollutants (BOD=9240 $mgO2L^{-1}$) could block the growth of microalgae, too.

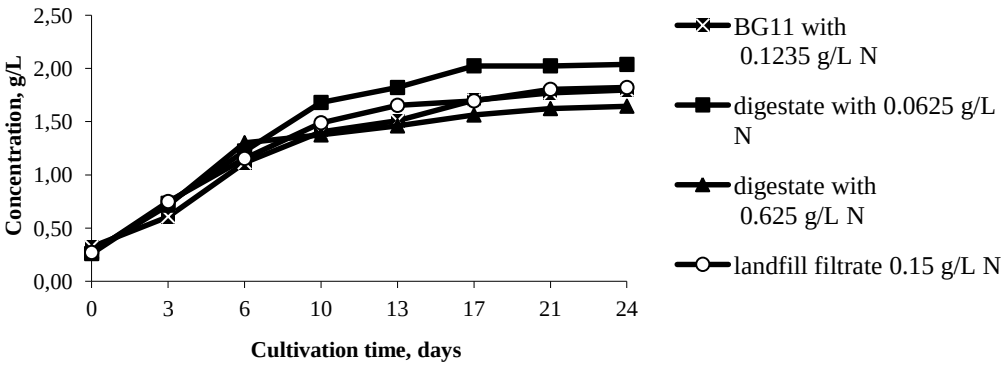

Fig. 2. Concentration changes of microalgae Scenedesmus sp. cultivated in liquid waste time-wise

Effect of technical glycerol based cultivation medium on algae growth

To improve biomass and lipid production, 2 gL^{-1} of technical glycerol was added into the conventional growth medium BG11 during stationary microalgae growth phase. Additional carbon source (organic carbon) caused an increase in algae concentration from 1.248 gL^{-1} to 1.922 gL^{-1}. In consideration of the initial investigation results, technical glycerol was used for further studies adding its various concentrations into growth medium at the beginning of cultivation. In this way, mixotrophic growth conditions were achieved. During the first seven days microalgae grew very similarly in all growth media containing different amounts of glycerol (Fig. 3). The fastest growth was fixed during exponential growth in the medium containing 5 gL^{-1} glycerol. The maximum concentration (2.16 gL^{-1}) was obtained in stationary growth phase. This concentration is 1.3 times higher than microalgae concentration in autotrophic control (BG11 containing 0.1235 gL^{-1} N), which was 1.67 gL^{-1}. Research results show that under mixotrophic conditions (using technical glycerol) Scenedesmus sp. grew much better compared to autotrophic control. The maximum dry mass concentration of microalgae in the sample containing 2 gL^{-1} glycerol in stationary growth phase reached 1.87 gL^{-1}. Better growth of microalgae, unfortunately, was observed not in all concentrations of glycerol. The growth of microalgae was lower in the growth medium containing 10 gL^{-1} glycerol. High concentration of glycerol (10 gL^{-1}) showed an inhibitory effect. The reason of growth inhibition could be the presence of impurities such as free fatty acids (Liang et al. 2010), residue of catalyst, soap. Soap containing medium leads to the lower biomass concentration compared to the soap-free medium (Pyle et al. 2008).

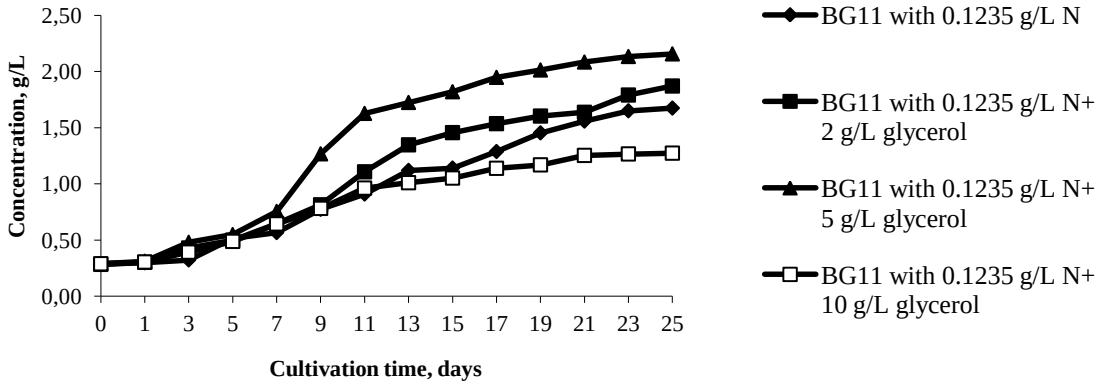

Fig. 3. Concentration changes of microalgae Scenedesmus sp. cultivated in glycerol of different concentrations time-wise

Similar concentrations of microalgae biomass were reached by some researchers investigating mixotrophic growth of green algae Chlorella vulgaris using glycerol. Kong et al. (2012) investigated cultivation of green microalgae Chlorella vulgaris in growth medium containing 1, 5 and 10 gL^{-1} glycerol. In the growth medium containing 5 gL^{-1} of glycerol the concentration of microalgae biomass increased to 2.13 gL^{-1} (in our study 2.16 gL^{-1}), however in contrast to our study, the growth medium with 10 gL^{-1} glycerol had 2.16 gL^{-1} biomass concentration, whereas biomass concentration of Scenedesmus sp. in present work in the growth medium containing 10 gL^{-1} of glycerol was only 1.27 gL^{-1}. The better growth of Chlorella vulgaris could be influenced by the absence of impurities in glycerol.

Effect of growth conditions on lipid accumulation of Scenedesmus sp.

The effects on lipid content of nitrogen deprivation under autotrophic conditions and glycerol under mixotrophic conditions were analyzed. We have investigated the accumulation of neutral lipid, in other words, the oil (triglyceride) with a goal to use it for further studies in the future. The investigation results are summarized in Table 2. They show that the lowest lipid content (1.39 %) based on dry biomass weight is obtained in the microalgae suspension, which was cultivated under autotrophic conditions using 0.1235 gL-1 of nitrogen. Applying stress conditions (nitrogen deprivation of 0.06 and 0.03 gL^{-1} N), microalgae began to accumulate oil. Stress conditions force changes in the metabolism of lipid. Synthesis of membrane lipid merges into the storage of neutral lipid (Hu et al. 2008). Decreasing the amount of nitrogen twofold, lipid content increased 5.83 times, from 1.39 to 8.1 % in dry weight and decreasing nitrogen content fourfold, an increase in lipid content was 8.6 times (11.95 % in dry weight). Reducing nitrogen content from 0.06 gL^{-1} to 0.03 gL^{-1}, there was a slight increase in lipid content..

Storage of neutral lipid in microalgae cells is improved by applying mixotrophic conditions (adding technical glycerol). The lipid content in microalgae cells increases by increasing the amount of glycerol

(Table 1.). Compared to autotrophic conditions, usage of glycerol for cultivation of microalgae has increased lipid content more than 10 times (from 1.39 % to 15.12 % in dry weight), but an increase in glycerol concentration in the growth media from 2 g L^{-1} to 10 g L^{-1} has raised lipid content in algae biomass only slightly (just 1.2 times). The highest lipid content (15.12 %) is obtained in the growth medium containing 10 gL^{-1}, the lowest lipid concentration (12.53 %) is observed in the growth medium containing 2 gL^{-1} glycerol.

It is very important to know not only the lipid content, but also effectiveness of microalgae growth. Better accumulation of oil, but slower microalgae growth could give lower oil yield compared to the faster-growth of microalgae that accumulates less amount of oil. Results of our study show that the microalgae in growth medium containing 10 gL^{-1} of glycerol accumulated the highest concentration, but in that medium the microalgae growth was observed to be the lowest, if compared to other growth media containing glycerol (Fig. 3). In consideration of this fact, we have calculated tentatively that cultivation of the same amount of microalgae in the growth medium containing 5 gL^{-1} of glycerol during the same time period will produce 1.5 times more oil compared to the microalgae cultivation in the growth medium containing 10 gL^{-1} of glycerol.

As shown in Table 1, some organic pollutants may influence the lipid accumulation in algae cells. When microalgae were cultivated in liquid waste, namely, in landfill filtrate, microalgae biomass contained 9.56 % of lipids. Compared to the autotrophic growth, in that case accumulation of lipid was 6.88 times higher. Summarizing, it should be mentioned that addition of organic substrate into the growth medium not only leads to the better growth of microalgae in some cases, but also enhances the better storage of oil in microalgae cells.

Table 2. Comparison of triglyceride content cultivating microalgae under different conditions

No.	Growth medium	Concentration of TG, % by dry weight of biomass
1	Growth medium BG11 (0.1235 gL^{-1}) (autotrophic growth)	1.39
2	Modified growth medium BG11 (0.06 gL^{-1}) (autotrophic growth)	8.1
3	Modified growth medium BG11 (0.03 gL^{-1}) (autotrophic growth)	11.95
4	Modified growth medium BG11 (0.1235 gL^{-1})+2 gL^{-1} glycerol (mixotrophic growth)	12.53
5	Modified growth medium BG11 (0.1235 gL^{-1} N)+5 gL^{-1} glycerol (mixotrophic growth)	13.47
6	Modified growth medium BG11 (0.1235 gL^{-1} N)+10 gL^{-1} glycerol (mixotrophic growth)	15.12
7	Landfill filtrate (mixotrophic growth)	9.56

Effect of growth conditions on elemental composition of microalgae

Growth conditions of microalgae affect not only the growth of algae, lipid accumulation, but also some other parameters. One of them would be elemental composition of algae. Microalgae cells consist of carbon, hydrogen, nitrogen, oxygen, etc. Biomass of microalgae contains app. 50 % by dry weight (Miron et al. 2003). Results of our study have confirmed this fact. The elemental composition of microalgae Scenedesmus sp. cultivated under different conditions is shown in Table 3. We see that carbon content in microalgae biomass ranges between 44.63 % and 47.71 %, whereas nitrogen amount balance - between 2.33 and 11.29 %. It should be noted that the lowest carbon and nitrogen (C:N) ratio 4:23 was obtained, when microalgae were cultivated under conventional autotrophic conditions (sample with growth medium BG11 (0.1235 gL^{-1} N), while under mixotrophic conditions the C:N ratio reached the maximum (19.45) (sample with modified growth medium BG11 0.1235 gL^{-1} N)+10 gL^{-1} glycerol). The reason of such results may be that under active photosynthesis process algae have the highest nitrogen concentration (Jimenez and Xavier Niell 1991). Other researchers published that microalgae Chlorella growing under mixotrophic conditions consumed O_2 and produced CO_2 (Yang et al. 2000). Based on these results it can be stated that mixotrophic conditions reduce photosynthesis intensity and the C:N ratio is increased. The results of our investigations have confirmed this fact. Growing Scenedesmus sp. on organic substrate (technical glycerol) the C:N ratio was the highest.

In order for use of biomass of microalgae for biofuel, namely, for biogas production, the C:N ratio is a very important parameter. It is desirable that this ratio should be as high as possible. Some publications say that biogas production works well when the values of the C:N ratio vary between 10 and 30, with an optimum between 15 and 25 (Schnürer and Jarvis 2010). At a higher ratio in substrate the biogas production effectiveness decreases. Our results show that for biogas production it is preferred to cultivate microalgae under mixotrophic conditions alternating with autotrophic growth conditions.

Table 3. Elemental composition of microalgae biomass

No.	Growth medium / Elemental composition (dry weight of biomass)	C, %	H, %	N, %
1	BG11 (0,.1235 gL^{-1} N)	47.71	7.88	11.29
2	BG11 (0.06 gL^{-1} N)	46.70	7.49	8.53
3	BG11 (0.03 gL^{-1} N)	46.54	7.54	4.52
4	BG11 (0.1235 gL^{-1} N)+2 gL^{-1} glycerol	45.72	7.59	3.32
5	BG11 (0.1235 gL^{-1} N)+5 gL^{-1} glycerol	45.57	7.00	2.61
6	BG11 (0.1235 gL^{-1} N)+10 gL^{-1} glycerol	45.33	7.04	2.33
7	Landfill filtrate with 0.15 gL^{-1} N	46.37	6.70	4.26
8	Liquid fraction of digestate after biogas production with 0.625 gL^{-1} N	46.10	6.69	8.02
9	Liquid fraction of digestate after biogas production with 0.0625 gL^{-1} N	44.63	6.66	8.72

4. Conclusions

Biomass and lipid production of green microalgae Scenedesmus sp. was investigated using different growth conditions (autotrophic and mixotrophic). Consumption of liquid waste and technical glycerol as a by-product of biodiesel production for cultivation of microalgae, which could be used for biofuel production, helps not only reduce pollution of the environment, but also makes the biofuel production sustainable.

As results of our study indicate, the best growth of microalgae is obtained under mixotrophic conditions by adding 5 gL^{-1} of technical glycerol into the growth medium. Similar results are achieved by cultivating microalgae in liquid waste, namely, liquid fraction of digestate after biogas production containing 0.0625 gL^{-1} N. In this case, the presence of organic carbon source (organic pollutants, glycerol) in the growth medium increases the growth of microalgae, just high concentration of organic carbon might inhibit the growth process. Mixotrophic conditions improve not only the growth of microalgae, but also accumulation of lipid. The maximum amount of accumulated lipid in microalgae is reached in the growth medium containing 10 gL^{-1} of technical glycerol. Usage of technical glycerol for improving lipid accumulation in microalgae cells is implemented to achieve desirable results compared to nitrogen deprivation mode. Besides, glycerol helps increasing the C:N ratio in Scenedesmus sp. biomass. In this case, biomass of microalgae becomes more attractive as feedstock for biogas production. Microalgae biomass with a lower C:N ratio could be used as co-substrate for biogas production together with materials containing high carbon amount, thereby improving the digestion process.

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Žaliadumblio *Scenedesmus sp.* biomasės ir aliejaus gavimas skirtingomis auginimo sąlygomis naudojant skirtingas maistines medžiagas

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Mikrodumbliai dėl greito augimo, gebėjimo kaupti aliejų, fiksuoti anglies dioksidą, taip pat dėl galimybės valyti nuotekas, tampa vis įdomesniu ir patrauklesniu objektu, kai jų biomasė panaudojama biodegalams gaminti. Siekiant nustatyti žaliadumblio *Scenedesmus sp.* optimalias augimo ir aliejaus kaupimo sąlygas, tirtos skirtingos auginimo sąlygos, taip pat ir autotrofinis auginimas, taikant azoto stygiaus metodą ir miksotrofinį auginimą, naudojant skystas atliekas ir techninį glicerolį. Nustatyta, kad, taikant azoto stygiaus metodą, miksotrofinėmis auginimo sąlygomis žaliadumblis augo geriau, o aliejaus kaupimas padidėjo dešimteriopai, palyginti su įprastinėmis autotrofinėmis auginimo sąlygomis. Didžiausia biomasės ir aliejaus koncentracija pasiekta auginant mikrodumblius terpėje su techniniu gliceroliu. Ištyrus mikrodumblio biomasės elementinę sudėtį, nustatyta, kad naudojant glicerolį, kai žaliadumblis auginamas, padidėja C:N santykis iki optimalaus biodujų gamybai. Gauti tyrimų rezultatai parodė, kad skystų atliekų ir techninio glicerolio naudojimas gali efektyviai pakoreguoti mikrodumblio biomasės sudėtį. Tuomet ji tampa tinkama biodegalams gaminti.