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GREEN ENERGY FROM MICROALGAE: USAGE OF ALGAE BIOMASS FOR ANAEROBIC DIGESTION

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Abstract

The microalgae biomass can be used for various types of biofuels, including biodiesel and biogas. The aim of this study is to investigate the possibilities of microalgae Scenedesmus sp. and Chlorella sp. (widespread in freshwater Lithuanian lakes) usage for biogas production. Microalgae were cultivated under mixotrophic conditions (growth medium BG11containing technical glycerol). In order to determine biogas yield and quality dependence on feedstock preparation, the analyses of biogas production have been performed with algae biomass prepared in different ways: wet centrifuged; wet centrifuged, frozen and defrost; dry not de-oiled and dry de-oiled. The highest biogas yield in both cases (Scenedesmus sp. – 646 ml/gDM and Chlorella sp. – 652 ml/gDM) was obtained from centrifuged, frozen and defrost biomass. Biogas yield was app. 1.46 times higher comparing to yield of biogas produced from wastewater sludge. Our results showed that different types of biomass preparation have no significant influence on quality of biogas.

Key words: *microalgae, biomass, biogas production, biogas quality.*

1. INTRODUCTION

Due to unique ability to produce and accumulate different useful materials under certain conditions microalgae such as *Chlorella, Scenedesmus, Spirulina, Haematococcus sp., Dunaliella, Arthrospira, Nannochloropsis, Tetraselmis, Botryococcus*, etc. are used for many purposes. Since their biomass is rich in various proteins, carbohydrates, fats, minerals, vitamins, carotenoids, algae are used in food and feed industry, for production of cosmetic and high-value products enriched with polyunsaturated fatty acids (omega-3), pigments, natural antioxidants (e.g. astaxanthin derived from *Haematococcus* (Lorenz & Cysewski 2000) (Spolaore et al. 2006).

Recently, more attention is focused on analysis of possibilities to use algae for biofuels production. Algae biomass can be converted into the energy by applying different methods, including thermochemical and biochemical conversions. Thermochemical energy production can be classified into gasification, pyrolysis and liquefaction. Biochemical conversion is subdivided in fermentation (product: ethanol, acetone, butanol), bioelectrochemical fuel cells (electricity), photobiological production and anaerobic digestion (Tsukahara & Sawayama 2005).

During anaerobic digestion organic matters are degraded by several types of microorganisms producing biogas in four stages: hydrolysis, fermentation, acetogenesis and methanogenesis. Certain conditions are required for action of anaerobic microorganisms: sufficient amount of moisture, nutrient materials, suitable temperature (process can occur in different temperature ranges: psychrophilic, mesophilic or thermophilic), pH, C/N ratio.

As feedstock for biogas production can be used various vegetable and animal organic materials, which are easily biodegradable. Among such materials can be number food waste, industrial by-products, silage, manure, algae, etc. For biogas production is suitable to use both residue of algae biomass after extraction of various compounds (e.g. extracted astaxanthin can be used as food colorant or for medicine purposes (Yuan et al. 1996; Guerin et al. 2003), oil - for biodiesel production (Huang et al. 2010), etc.) and concentrated in different ways pretreated biomass. In order to clear biomass pretreatment influence on biogas quantity and quality, different methods of biomass preparation are used. Process kinetic can be improved by drying concentrated biomass, affecting it chemically, using high temperature, high pressure homogenization and ultrasound (Mussgnug et al. 2010; Sialve et al. 2009; Park et al. 2013; Passos et al. 2013). Schwede et al. (2011) reported that using such biomass pretreatment modes like high temperature, microwave, French press, ultrasound and freezing, the highest biogas yield (app. 40 % higher compared to biogas yield produced from not pretreated biomass) was obtained when algae biomass was processed for 8 h at 100 °C temperature. Usage of microwave enables to increase biogas yield 33 %, and French press-30 %.

In order to use algae biomass for biofuels production it is very important to produce biomass as cheap as possible. Different algae species as growth medium can tolerate various waste and industrial by-products (Bhatnagar et al. 2011; Abreu et al. 2012).

Taking into account that algae growth conditions determine the composition of algae cells (Leadbeater 2006), and composition of algae biomass has influence on biogas production process, it can be stated that amount and yield of methane depends on feedstock growth conditions indirectly. The algae biomass during its growth time accumulates more nitrogen the C/N ratio in algae biomass is less, and this is the reason of greater emission of ammonium during biomass fermentation. Higher ammonium concentrations (1.7–14 gL^{-1}) under certain conditions are toxic for flora of anaerobic process, and that inhibits running of biogas production and methane yield (Sialve et al. 2009).

The aim of this study is to investigate the possibilities of microalgae *Scenedesmus sp.* and *Chlorella* sp. biomass usage for biogas production. In order to estimate how algae growth conditions changes C/N ratio in algae biomass, autotrophic and mixotrophic growth conditions were used for algae biomass production.

2. MATERIAL AND METHODS

Microalgae *Scenedesmus sp.* and *Chlorella sp.* were isolated from Lithuanian lakes. Middle size of representative of *Chlorella* genus is 5-6 µm, while length of microalgae *Scenedesmus sp.* cell is app. 6 µm, and width 4 µm. Microalgae were cultivated under autotrophic and mixotrophic conditions. For batch cultivation in laboratory conditions glass flasks with a working volume of 3 L were used and duration of cultivation from 20 to 30 days was chosen. Cultures were grown at the room temperature under ~250 µmol photons m^{-2} s^{-1} illumination using white fluorescent lamps for 8-10 h daily. Light intensity was measured by data logger (model LI-1400) using LI-190SA Quantum sensor. Cultures were shaken to avoid sticking.

Autotrophic growth was carried out growing both microalgae species in modified BG11 medium, which contained the following: 750 mgL^{-1} $NaNO_3$, 40 mgL^{-1} K_2HPO_4, 75 mgL^{-1} $MgSO_4 \cdot 7H_2O$, 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} $MnCl_2 \cdot 4H_2O$, 0.222 gL^{-1} $ZnSO_4 \cdot 7H_2O$, 0.390 gL^{-1} $NaMoO_4 \cdot 5H_2O$, 79 mgL^{-1} $CuSO_4 \cdot 5H_2O$, 49.4 mgL^{-1} $Co(NO_3)_2 \cdot 6H2O$.

Mixotrophic conditions for algae cultivation were achieved by adding into the growth medium BG11 different concentrations (2, 5 and 10 gL^{-1}) of technical glycerol containing 75-80 % pure glycerol and remaining part were impurities (methanol, free fatty acids, residue of catalyst, etc). Glycerol was purchased from JSC "Rapsoila" (Lithuanian producer of biodiesel). Technical glycerol into the microalgae suspension was added during the first growth phase - lag phase.

Concentration of algae biomass was determined gravimetrically. Certain volume of microalgae suspension was centrifuged for 10 minutes at 12000 min^{-1}, washed with distilled water and then dried in oven at the temperature 105 °C to a constant weight. Using dry weight of biomass and volume of algae suspension biomass concentration was calculated.

Elemental composition of algae biomass was determined using CHNS-O Elemental Analyser (Perkin Elmer 2400 Series). Dry microalgae biomass was pulverized using mortar, weighed on thin foil (app. 2 mg), placed into elemental furnace, combusted in a pure oxygen environment at 975 °C, analyzed and calculated weight percent of each element.

De-oiled algae cake and algae biomass were used for biogas production. Oil from dry microalgae biomass was extracted applying solvent (using hexane) extraction by Soxhlet apparatus and used for biodiesel production. Residue of hexane from de-oiled algae cake was evaporated by heating of biomass.

The experiments of biogas production were carried out considering species of microalgae and method of biomass preparation. Microalgae biomass was prepared in different ways: wet centrifuged, wet centrifuged and frozen, concentrated and dried in dryer, and dried by lyophilisation. For biogas production was used both dry and wet biomass. Dry biomass of microalgae was prepared drying biomass in dryer at the 105 °C temperature and applying freeze drying (lyophilization) (SCANVAC Coolsafe freeze dryer). The initial freezing of algae biomass before biomass dryring in freeze dryer was done in refrigerator (to -18 °C). After that the moisture from algae biomass was eliminated in freeze dryer at the temperature lower than -40 °C by sublimation. Wet centrifuged and frozen biomass was prepared concentrated biomass freezing in refrigerator (to -18 °C). Before using for biogas production, biomass was defrostet.

Prepared biomass (0.25 g dry mass (DM)) was mixed with 30 ml digestate after biogas production from wastewater sludge (JSC „Kauno vandenys"). Homogenised mass was placed into the plastic 100 ml syringes (biogas reactors). These biogas reactors with samples were placed on a thermostatically controlles laboratory shaker. Biogas production was carried out under mezophilic conditions at 37 °C temperature app. 25 days. Amount of produced biogas was measured keeping syringe in a vertical position every day. Composition of biogas was determined by gas chromatography using chromatograph „Clarus 580 GC" (Perkin Elmer) with thermal conductivity detector. As carrier gas was used helium and nitrogen. The samples were inserted manually with the help of loop injector. The experiments were carried out three times and mean values and standard deviation calculated.

3. RESULTS AND DISCUSSION

Biogas production from biomass of two microalgae species *Scenedesmus sp.* and *Chlorella sp.* was investigated in this study. De-oiled (after oil extraction) and not de-oiled (concentrated and in different ways pretreated) algae biomass was used for investigations. In order to clear how different algae growth conditions influence C/N ratio in algae biomass, two different algae biomass growth conditions (autotrophic and mixotrophic) were analyzed. Results of our investigations showed that addition of technical glycerol (cheap by-product of biodiesel production) into the growth medium stimulates not only algae growth (Fig. 1), but also increases C/N ratio in produced biomass (Fig. 2).

Results of algae cultivation experiments (Fig.1) showed that less amounts (2 and 5 gL^{-1}) of technical glycerol in growth media increased biomass production. The maximum biomass concentration (*Scenedemus sp.* - 2.16 gL^{-1}, *Chlorella sp.* - 1.92 gL^{-1}) was obtained in growth medium BG11 (0.12 gL^{-1} N) containing 5 gLl^{-1} glycerol. Increasing amount of glycerol (to 10 gL^{-1}) further, *Scenedesmus sp.* biomass concentration decreased to 1.27 gl^{-1}. *Chlorella sp.* differently responded to bigger concentration of glycerol in growth media. Biomass concentration of this algae species in growth medium containing 10 gL^{-1} technical glycerol was 1.72 gL^{-1} (biomass concentration in BG11 (0.12 gL^{-1} N) was just 1.58 gL^{-1}). Results of experiments showed that glycerol improved growth of investigated algae species, but *Scenedesmus sp.* was less tolerant for impurities (catalyst, methanol residues, free fatty acids, soaps) in technical glycerol.

For more effective biogas production process C/N ratio in algae biomass would be as higher as possible. Biogas production proceeds well than C/N ratio in feedstock ranges between 10 and 30 (optimal values are from 15 to 25) (Schnürer & Jarvis 2009). Increasing C/N ratio (from 10 to 30) encourages formation of fatty acids and stimulates methanogenesis, at higher ratio effectiveness of biogas process decreases.

Results of our analysis showed that cultivation of microalgae under mixotrophic conditions enables to increase C/N ratio to proper for biogas production. Increasing concentration of technical glycerol in growth medium from 2 to 10 gL^{-1} C/N ratio in algae biomass increases in both *Scenedesmus sp.* and *Chlorella sp.* cases (Fig. 2). By adding of 10 gL^{-1} technical glycerol C/N ratio in *Scenedesmus sp.* biomass increases 4.6 times (from 4.23 to 19.45) compared with C/N ratio in algae biomass produced under autotrophic conditions. Usage of additional organic carbon source *in Chlorella sp.* case enhances C/N ratio in biomass more than 2 times (from 6.19 to 13.28). Considering such results it can be stated that growing microalgae in media containing from 2 to 10 gL^{-1} (in *Scenedesmus sp.* case), and 10 gL^{-1} (in *Chlorella sp.* case) technical glycerol, produced biomass can be used for biogas production without adding any other co-substrata. Microalgae biomass produced under autotrophic conditions (with lower C/N ratio) could be used as co-substrata for biogas production together with feedstock containing high amount of carbon, thereby improving digestion process.

Initial investigations of biogas production were performed by using de-oiled microalgae biomass. The experiments were carried out considering species of microalgae. Results of analysis showed that big difference between biogas yields using microalgae *Scenedesmus sp.* and *Chlorella sp.* is not determined (Fig. 3). Biogas amount, obtained from microalgae *Chlorella sp.* at the end of investigations reached 376 mlg^{-1} DM, and biogas yield from *Scenedesmus sp.* was 338 mlg^{-1} DM. It is app. 10 % less biogas compared with biogas yield produced from *Chlorella sp.*

Comparing biogas yield, produced from above mentioned microalgae species with biogas yield from wastewater sludge, it was found that using dry de-oiled algae biomass is produced app. 15-24 % less biogas than using wet wastewater sludge. Different composition of feedstock could determine such result. Besides, less moisture amount could inhibit activity of bacterial biocenosis (Schnürer & Jarvis 2009).

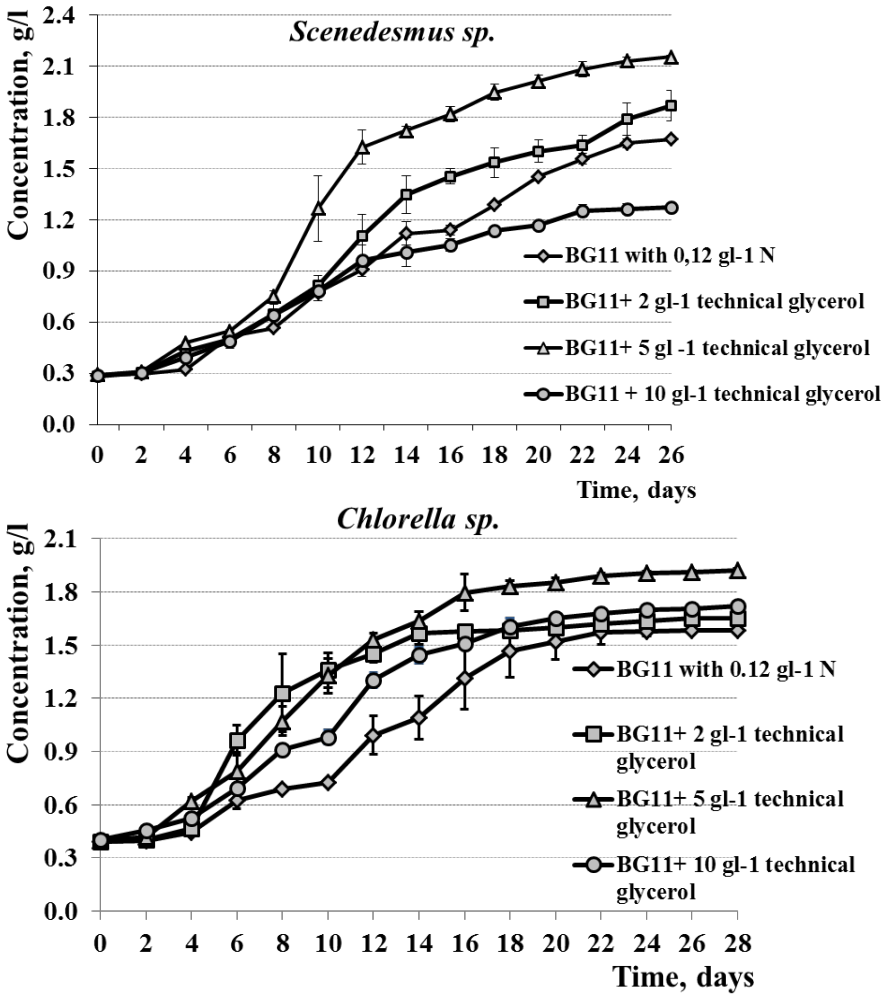


Figure 1. Microalgae growth kinetic in median with different addition of technical glycerol

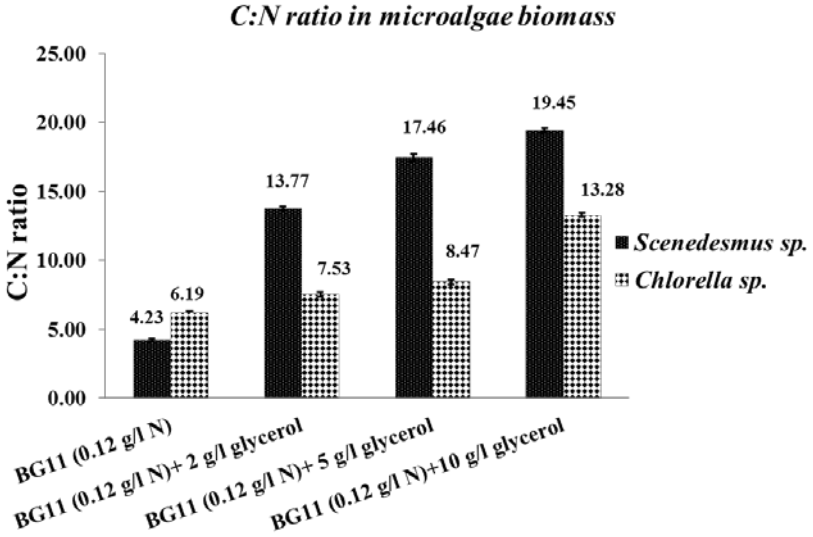


Figure 2. Carbon and nitrogen ratio in algae biomass produced under different conditions

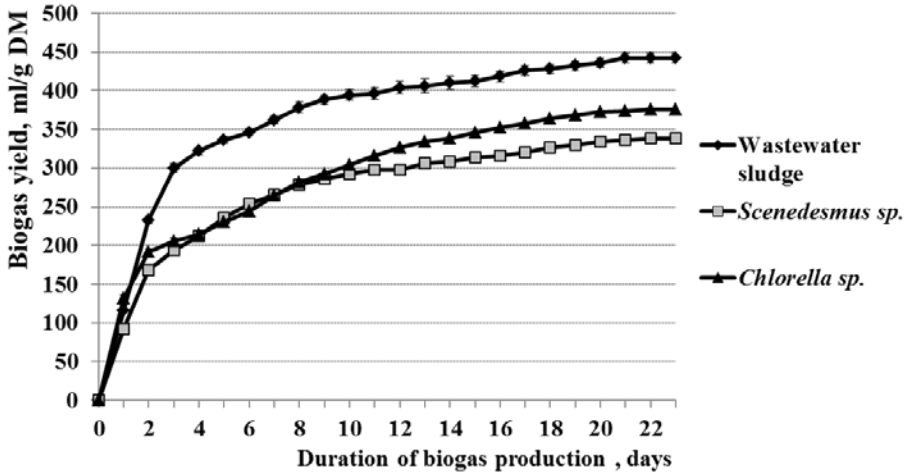


Figure 3. Biogas yield from different microalgae species

Preparation of feedstock can have influence on decomposition of organic materials under anaerobic conditions and biogas production. For biogas production studines biomass of microalgae Scenedemus sp. and *Chlorella sp.* was prepared in different ways. Biomass pretreatment methods involved concentration, concentration and freezing, concentration and drying at 105 C° temperature and drying by lyophilisation. Results of this study showed that the best quantitive parameters of biogas production were achieved using wet biomass (Fig. 4). Algae biomass pretreatment in different ways has significant influence on biogas yield. In both *Scenedesmus sp. and Chlorella sp.* cases, the highest biogas yield (646 mlg^{-1} DM using *Scenedesmus sp.*, 652 mlg^{-1} DM using *Chlorella sp.*) was obtained from wet centrifuged, frozen and defrostet biomass. This biomass pretreatment method increased biofas yield for 21 % in the case of *Scenedesmus sp.*, and for 22 % in *Chlorella sp.* case. Such increasing of biogas yield can be explained so that during freezing cellulose containing walls of microalgae cells were disrupted. After disruption of cellulose containing and resistant walls (Okuda 2002) organic materials become available for anaerobic microorganisms.

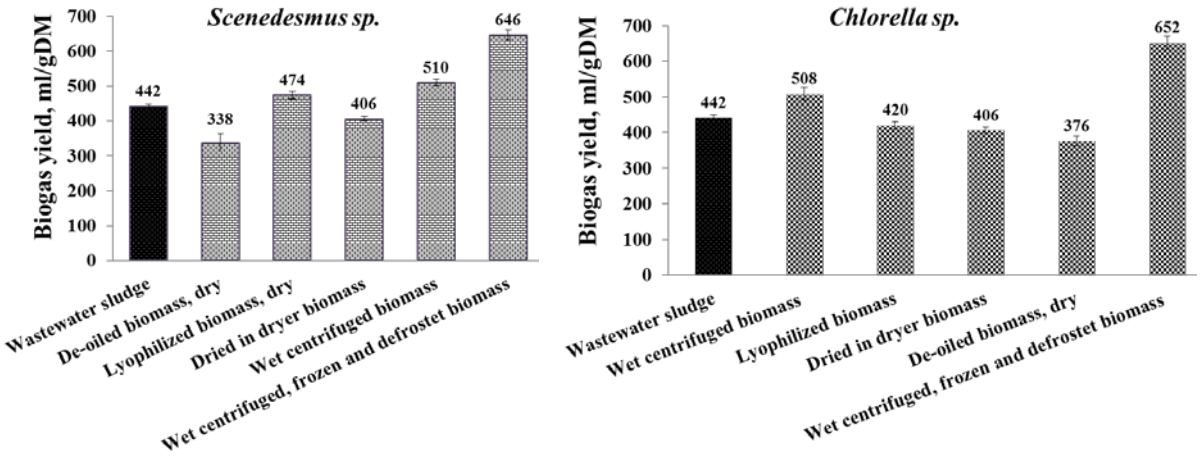


Figure 4. Dependence of biogas yield on the feedstock pretreatment method

The usage of wet algae biomass for biogas production allows expect higher biogas yield compared to biogas yield from dry biomass. Using in dryer dried biomass was achieved 20 % less biogas yield from *Scenedesmus sp.* and *Chlorella sp.* compared with biogas yield from wet concentrated biomass. As results of our study showed, biomass drying method has influence on effectiveness of biogas production also. From lyophilized *Scenedesmus sp.* and *Chlorella sp.* biomass is possible to obtain from 4 to 14 % more biogas comparing with dried in dryer

biomass. Such results could be explained so that during lyophilisation stable walls of cells are disrupted, and released organic materials are used in substrata being organisms. Oil extraction from algae biomass for biodiesel production decreases quantitative biogas parameters app. 8 % for *Chlorella sp.* and 16 % for *Scenedesmus sp.*

Comparing biogas yields produced from wet (only concentrated by centrifugation) algae biomass and wet wastewater sludge it could be stated that is possible to produce 1.15 time more biogas from algae biomass than from sludge.

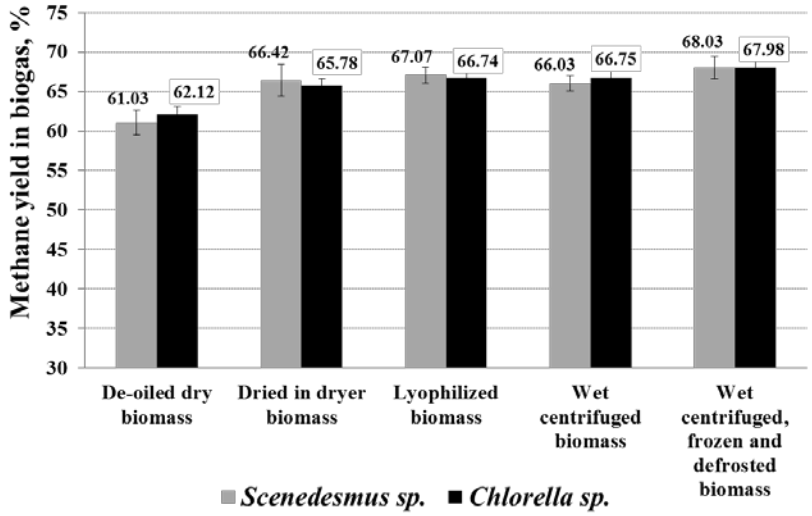


Figure 5. Qualitative parameters of biogas produced by applying different pretreatment methods

Results of analysis of biogas composition showe that different methods of biomass preparation do not have significant influence on biogas quality (Fig. 5). In all samples of biogas produced from in different ways prepared algae *Scenedesmus sp.* and *Chlorella sp.* biomass (excluding de-oiled biomass) methane (CH_4) content was higher than 65 % and ranged within 2 %. In the samples of biogas produced from de-oiled *Scenedesmus sp.* biomass CH_4 content was 5 %, and *Chlorella sp.* 4 % lower compared to biogas, produced from biomass containing oil. Content of CH_4 in biogas from wastewater sludge ranged from 63.29 to 64.77 %.

CONCLUSIONS

Summarizing the results of biogas production from microalgae biomass, it has to be noted that biomass of microalgae *Scenedesmus sp.* and *Chlorella sp.* is suitable to use for biogas production and can add potential of feedstock's for biogas production. Comparing biogas produced from wet microalgae biomass and wet wastewater sludge in point of quantity and quality, significant difference was not observed, only when wet frozen and defrostet algae biomass was used app. 1.47 times more biogas was produced comparing with biogas yield from wastewater sludge. Quality of biogas (CH_4 content 61-68 %) produced from microalgae biomass faces out quality of biogas produced from wastewater sludge (CH_4 63-67 %).

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