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Comparison of biogas production from anaerobic digestion of microalgae species belonged to various taxonomic groups

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Abstract: This study investigated the potential of three microalgae taxonomic groups of *Chlorophyta*, *Cyanoprokaryota* and *Bacillariophyceae* for biogas production. Biogas potential was assessed in mesophilic anaerobic digestion batch tests over a period of 20 days. The cumulative biogas yield (CBY) of *Chlorophyta* and *Cyanoprocaryota* was respectively 396.21 mL/g Volatile Solids (VS) and 382.45 mL/g VS. *Bacillariophyceae* digestion showed lower biogas production of 357.07 mL/g VS. The highest cumulative methane yield (CMY) of 241.25 mL CH_4/g VS was recorded for *Cyanoprocaryota* biomass, which was significantly higher ($p<0.05$) than the other two types of microalgae. The highest methane content in biogas of 63.08% was observed with *Cyanoprokaryota.* Chemical composition of biomass as well as biogas productivity are influenced by algal taxonomy.

Introduction

High fuel prices coupled with an increasing awareness of greenhouse gas emissions and global warming have promoted an interest in renewable energy sources based on biomass transformations. The use of agricultural derived biomass to produce biofuels may have negative impacts on the global supply and prices of foods (Johansson and Azar 2007) and lead to other problems, such as eutrophication, resource depletion, and reduced biodiversity due to current farming practices (Ward et al. 2014).

Microalgae appear to be an attractive alternative to typical energy crops because of their high photosynthetic effectiveness, potential to utilize CO_2 emissions, fast rate of growth, resistance to various types of contamination, and the fact that they can be cultured on areas that cannot be used for other purposes (Wirth et al. 2015, Yang et al. 2011, Mandal and Mallick 2009).

Nowadays microalgae are clearly one of the most promising sources for new-generation biofuels, whereas anaerobic digestion to produce methane is a feasible way to gain bioenergy from microalgae biomass (Wirth et al. 2015, Montingelli et al. 2015, Ward et al. 2014). The use of algal biomass for biogas generation has been studied since 1957 (Golueke et al. 1957), but relatively few investigations of the anaerobic digestion of microalgae have been done until recently (Wirth et al. 2015). Thus, a large-scale application of algae as a renewable source is not yet feasible because of economic and energy cost associated with cultivating and harvesting of microalgae (Lee et al. 2014).

The methane yield from anaerobic digestion of algae ranging from 140 to 360 mL/g VS fed the digester, which is comparable to the yield obtained with sewage sludge digestion of 190–430 mL/g VS (Wang and Park 2015). However, the intensity of biogas production is closely related to algal species and the growth conditions (Gerken et al. 2013, Mussgnug et al. 2010). A review of microalgae anaerobic digestion shows a wide range of methane yield ranging from 24 mL CH_4/g VS for the saline microalgae species *Dunaliella tertiolecta* to 587 mL CH_4/g VS for *Chlamydomonas reinhardtii* (Ward et al. 2014). Difficulties in anaerobic digestion of microalgae biomass may be attributed to several key factors, such as low concentration of digestible substrate, necessity of pretreatment of algae biomass, unbalanced nutrients in microalgae biomass, the cell wall degradability, the use of limited organic loading rates (OLRs) of the digesters as well as short term storage of biomass (Wirth et al. 2015, Montingelli et al. 2015, Klassen et al. 2015, Ward et al. 2014). It has been also postulated that biogas production cannot be predicted based on the taxonomic group of the algae that are used (Mussgnug et al. 2010), thus experiments must determine the quantity and composition of biogas that can be produced with the individual algal species as a substrate for anaerobic digestion (Ward et al. 2014).

The objective of the study was to determine how the use of the three microalgae taxonomic groups of *Chlorophyta*, *Cyanoprokaryota* and *Bacillariophyceae* affects the yield and the composition of biogas produced by anaerobic digestion.

Materials and Methods

Strains and growth conditions

Chlorella sp. MA10 and *Microcystis sp.* UTEX B 2678 were obtained from the own culture collection. *Cyclotella sp.* UTEX LB 2611 came from UTEX Culture Collection of Algae (University of Texas, Austin, USA).

The microalgal species were cultivated in the three groups: *Chlorophyta* (*Chlorella sp.*), *Cyanoprokaryota* (*Microcystis sp.*) and *Bacillariophyceae* (*Cyclotella sp.*). Liquid algal cultures were grown photoautotrophically in continuous fluorescent white light with reflector (700 lux, Osram, Germany). The groups of microalgae were cultured in closed, vertical, tubular photobioreactors with an active volume of 2.5 L (inner diameter 76 mm and 550 mm height) made of transparent plexiglass. Compressed air was delivered continuously at 250 L/h from the bottom of the reactors upwards. This ensured appropriate mixing of the culture medium, homogeneity of conditions in the entire reactor volume and introduction of atmospheric CO_2 to the culture. The temperature of the culture was maintained at 22.0 ± 2.0°C. The nutrient medium for microalgae biomass cultivation was the mixture of liquid digestate, tap water and synthetic medium (Table 1). The liquid digestate was obtained from an agricultural biogas plant operated in a technical scale with maize silage and distillery stillage. Before using as a nutrient medium, digestate was centrifuged (Rotina 380, 3 min, 9000 rpm) and then pasteurized (30 min, 90°C), (Table 1). It constituted 10% of the active volume of the photobioreactor. The remaining part of the culture medium was tap water with synthetic medium composed of: KH_2PO_4 17.5 g/L, K_2HPO_4 7.5 g/L, $NaNO_3$ 25 g/L, $MgSO_4 \cdot 7H_2O$ 7.5 g/L, $FeSO_4 \cdot 7H_2O$ 5.0 g/L, $CaCl_2 \cdot 2H_2O$ 2.5 g/L, H_3BO_3 11.42 g/L, $MnCl_2 \cdot 4H_2O$ 1.44 g/L, $ZnSO_4 \cdot 7H_2O$ 8.82 g/L, $CuSO_4 \cdot 5H_2O$ 0.57 g/L, $Co(NO_3)_2 \cdot 6H_2O$ 0.49 g/L, $Na_2EDTA \cdot 2H_2O$ 0.5 g/L.

Algae cells were harvested by preliminary sedimentation followed by centrifugation (3000 rpm for 6 min). The content of total solids (TS) concentration was determined by drying of the cells at 105°C for 24 h. To determine VS fractions, the samples were subsequently incubated at 550°C for 5 h and the residual ash was determined by weighting. Any pretreatment method of harvested biomass was used before anaerobic digestion.

Anaerobic digestion and biogas analysis

Anaerobic digestion of algae biomass was conducted using respirometers (WTW, Germany) that consisted of reaction tanks with an active volume of 0.5 L coupled tightly with measuring devices which recorded an increase of the partial pressure induced by biogas production. Pressure in the reaction tank was recorded every 24 h. 0.5 L fermenters were filled with 150 mL anaerobic sludge originated from the closed anaerobic digestion tanks of a local municipal wastewater treatment plant. The characteristic of anaerobic sludge used in the study is presented in Table 2. In order to ensure anaerobic conditions inside the respirometers, they were flushed with nitrogen to remove atmospheric air at the beginning of the process. The measurements were carried out at a temperature of 35°C for 20 days, until the biogas yield results did not differ by more than 1%. In all technological variants, the initial load was 5.0 g VS/L. The experiments were carried out in four replications and results averaged.

The perfect gas equation was the basis for computing the volume of produced biogas in respirometric tests. The volumes of biogas generated per normal conditions were computed on the basis of pressure changes inside the measuring chamber. Respirometric tests also provided grounds to determine the biogas production rates. The endogenous production of the anaerobic sludge was removed from the calculations of biogas and methane productions of the tests. The composition of biogas produced in the headspace of respirometers was measured every 24 h using a gastight syringe (20 mL injection volume) and a gas chromatograph (GC, 7890A Agilent) equipped with a thermal conductivity detector (TCD). The GC was fitted with the two Hayesep Q columns (80/100 mesh), two molecular sieve columns (60/80 mesh) and Porapak Q column (80/100) operating at a temperature of 70°C. The temperatures of the injection and detector ports were 150°C and 250°C, respectively. Helium and argon were used as the carrier gases at a flow of 15 mL/min.

Analytical methods

The content of TS and VS were determined according to the gravimetric method. Algae and sludge biomass samples dried at 105°C were also assayed for the contents of total carbon (TC), total organic carbon (TOC) and total nitrogen (TN) with the use of elementary particle size analyzer (Flash 2000, Thermo Scientific, USA). Total phosphorus (TP) and sugar content (saccharides) were determined using a spectrophotometer DR 2800 (HACH Lange, Germany). The content of total protein was estimated by multiplying the value of TN by 6.25. The concentration of lipids was assayed by Soxhlet's method using an extractor (Büchi, Switzerland). The pH of aqueous solutions of sludge and algae biomass was determined with a pH-meter (1000L, VWR, Germany).

Table 1. Composition of the nutrient medium used for cultivation of algae biomass

Parameter	Unit	Liquid digestate	Nutrient medium (tap water + synthetic medium + liquid digestate)
COD	mg O_2/L	6890 ± 431	793 ± 44
BOD_5	mg O_2/L	3192 ± 301	328 ± 33
TN	mg N/L	1385 ± 201	138 ± 19
TP	mg P/L	69 ± 13	6.4 ± 1.0
pH	–	7.0 ± 0.3	7.4 ± 0.2

Statistical methods

In respirometric assays, the quantity of biogas produced was calculated using the ideal gas law, which enabled the use of pressure changes inside the measuring tank for computation of the volume of biogas produced under normal conditions. Thus, the rate of biogas production (r) could be determined for each experimental variant. Reaction rate constants (k) based on experimental data were determined by non-linear regression using Statistica 10.0 PL software (StatSoft, Inc.).

The iterative method was applied, in which the function is replaced in each iterative step with a linear differential in relation to the determined parameters. The coefficient of convergence φ2 was adopted as the measure of the curve's fit (with determined parameters) to experimental data. This coefficient is the ratio of the sum square of deviations of the values calculated on the basis of the determined function from experimental values to the sum square of deviations of experimental values from the mean value. The convergence improves along with the lowering of the value of the φ2 coefficient. Such a fit of the model to experimental points was adopted in which the coefficient of convergence did not exceed 0.2. The results were processed statistically with the Statictica 12.0 PL package (StatSoft, Inc.). The hypothesis on the distribution of each analyzed variable was verified based on the W Shapiro – Wilk's test. One way analysis of variance (ANOVA) was conducted to determine the significance of differences between the variables. The homogeneity of variance in groups was tested with Levene's test, whereas Tukey's RIR test was used to determine the significance of differences between the analyzed variables. In all tests, differences were considered significant at $p = 0.05$.

Results

Characteristics of algae biomass

The algae selected for the study belonged to the taxonomic groups that are common in natural water reservoirs of the temperate zone, therefore they represent a selection of dominant strains. Three microalgal strains belonging to the three taxonomic groups of fresh water microalgae were selected: first group of *Chlorophyta* contained *Chlorella sp.* from the class *Trebouxiophyceae*, second group of *Cyclotella sp.* from the class *Bacillariophyceae* and the prokaryotic cyanobacterium *Microcystis sp.* (*Cyanoprocaryota*, class *Cyanophyceae*).

Table 3 shows the results of chemical characteristics of the three microalgal taxonomic groups. VS content as a proportion of TS ranged from 78.89% for *Bacillariophyceae* to 91.47% for *Cyanoprocaryota*. The difference in VS values for *Chlorophyta* and *Cyanoprocaryota* were not statistically significant, while for *Chlorophyta* and *Bacillariophyceae, and Cyanoprocaryota* and *Bacillariophyceae* were significant ($p < 0.05$). Ash content was significantly different ranging from approximately 8% to 21% in *Bacillariophyceae* biomass due to the presence of silicon.

Carbon content as TOC was similar for *Chlorophyta* and *Cyanoprocaryota* (over 430 mg/g TS), but was significantly lower ($p < 0.05$) for *Bacillariophyceae* (342.7 mg/g TS). Total nitrogen (TN) content in *Cyanoprocaryota* biomass was 58.07 mg/g TS and it was about 15 mg/g TS higher than noted in *Chlorophyta* and *Bacillariophyceae* biomass. In turn, the highest total phosphorus (TP) concentration was recorded for *Cyanoprocaryota*. The C/N ratio ranged from 8.5 for *Cyanoprocaryota* to 10.95 for *Chlorophyta*. Maximum concentration of lipids was recorded for *Chlorophyta* biomass, while protein and saccharides for *Cyanoprocaryota* biomass. The initial pH of algae biomass before anaerobic digestion was around 7.71–8.12.

Biogas potential of algae biomass

The possibility of using the fresh microalgae biomass belonging to the three microalgal taxonomic groups was assessed in mesophilic anaerobic digestion batch tests over a period of 20 days.

After 20 days of anaerobic digestion, CBY from *Chlorophyta* showed the highest production as 396.21 mL/g VS, while *Cyanoprocaryota* and *Bacillariophyceae* digestion showed lower biogas production of 382.45 and 357.07 mL/g VS, respectively (Figs. 1–3, Table 4). Statistical analysis showed that differences of

Table 2. Characteristics of anaerobic sludge used in the experiments

Parameter	Value			
	Mean	**Min.**	**Max.**	**Std. dev.**
TS(%)	3.81	3.60	4.02	0.21
VS (% TS)	68.46	65.93	70.99	2.53
TN (mg/g TS)	33.08	29.73	36.43	3.35
TP (mg/g TS)	1.66	1.43	1.89	0.23
TC (mg/g TS)	309.05	280.68	337.42	28.37
TOC (mg/g TS)	199.42	165.13	233.71	34.29
C:N ratio	9.34	9.42	9.26	0.08
pH	7.21	7.53	6.89	0.32
Protein (% TS)	20.67	17.90	23.44	2.77
Lipids (% TS)	3.12	2.88	3.64	0.51
Saccharides (% TS)	1.57	1.93	1.21	0.36

biogas production of *Chlorophyta*, *Cyanoprocaryota* and *Bacillariophyceae* were significant ($p < 0.05$). In turn, the highest cumulative methane yield (CMY) of 241.25 mL CH_4/g VS was recorded for *Cyanoprocaryota* biomass, which was significantly higher ($p < 0.05$) than the other two types of tested biomass (Table 4). Methane content in biogas produced from anaerobic digestion of *Cyanoprocaryota* was as high as 63.08% (Table 4). However, it was slightly lower for *Chlorophyta* and *Bacillariophyceae* (Table 4), but the differences were significant ($p < 0.05$).

Table 3. Characteristics of microalgae biomass (mean ± std. dev)

Parameter	*Chlorophyta*	*Cyanoprokaryota*	*Bacillariophyceae*
TS (%)	8.43 ± 0.59	7.19 ± 0.95	7.84 ± 0.45
VS (%TS)	87.12 ± 0.97	91.47 ± 0.92	78.89 ± 2.62
TN (mg/g TS)	43.37 ± 1.75	58.07 ± 5.67	43.41± 1.64
TP (mg/g TS)	19.96 ± 1.32	10.31 ± 0.97	13.39 ± 0.46
TC (mg/g TS)	474.80 ± 11.50	493.40 ± 17.10	391.30 ± 30.50
TOC (mg/g TS)	439.40 ± 27.27	434.30 ± 12.74	342.70 ± 19.55
C:N ratio	10.95 ± 0.39	8.50 ± 0.51	8.94 ± 0.42
pH	7.96 ± 0.29	8.12 ± 0.06	7.71 ± 0.30
Protein (% TS)	27.11 ± 2.72	36.29 ± 8.92	27.13 ± 2.62
Lipids (% TS)	14.19 ± 0.65	7.38 ± 0.37	8.01 ± 1.05
Saccharides (% TS)	39.77 ± 1.29	41.38 ± 0.36	35.33 ± 2.36

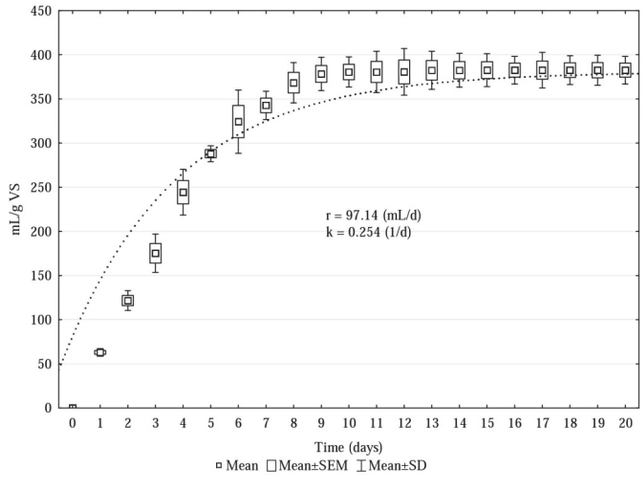

Fig. 1. Cumulative biogas yield of anaerobic digestion of *Chlorophyta* in the 20-day test

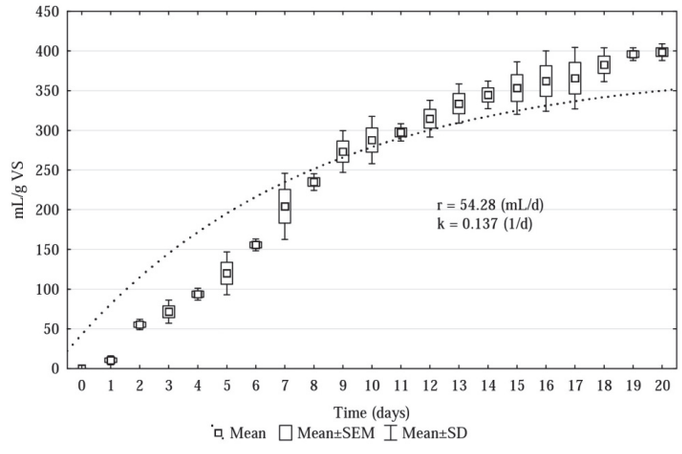

Fig. 2. Cumulative biogas yield of anaerobic digestion of *Cyanoprokaryota* in the 20-day test

During anaerobic digestion of *Chlorophyta* biomass, biogas production yield stabilized in 8 days (Fig. 1). Almost doubled time was needed for a stable biogas production from *Cyanoprocaryota* and *Bacillariophyceae* biomass (Fig. 2–3). Volumetric biogas production rate (VBPR) of 97.14 mL/d was recorded during anaerobic digestion of *Chlorophyta* biomass (Fig. 1), while it was on a much lower level ($p < 0.05$) of about 50 mL/d for *Cyanoprocaryota* and *Bacillariophyceae* biomass (Figs. 2–3).

The digestate characteristics depending on the taxonomic group of microalgae biomass was evaluated in Table 5. No significant drop in pH was noticed in digestates, which was still in the optimum range (pH 6.5–8.0) for methanogenesis.

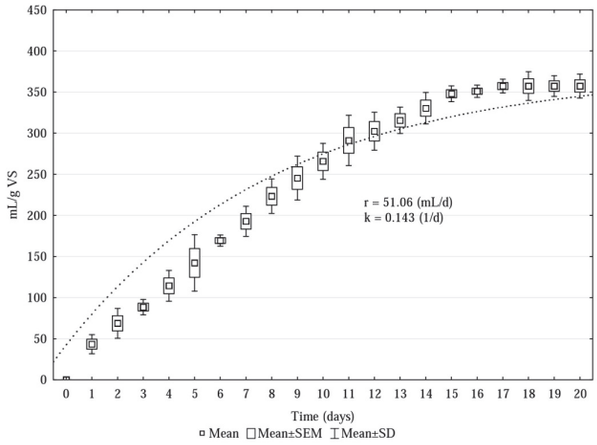

Fig. 3. Cumulative biogas yield of anaerobic digestion of *Bacillariophyceae* in the 20-day test

Table 4. Biogas characteristic and biogas/methane production depending on taxonomic composition of algal biomass

Parameter	Unit	*Chlorophyta*		*Cyanoprokaryota*		*Bacillariophyceae*	
		Mean	Std. dev.	Mean	Std. dev.	Mean	Std. dev.
CBY[a]	(mL/g TS)	345.18	31.61	349.83	12.05	281.69	11.24
	(mL/g VS)	396.21	30.94	382.45	9.24	357.07	2.20
CMY[b]	(mL/g TS)	206.18	32.44	220.67	18.82	162.90	16.01
	(mL/g VS)	220.53	33.38	241.25	17.97	206.49	12.37
CH_4	(%)	59.73	2.43	63.08	3.10	57.83	3.09
CO_2	(%)	49.66	2.17	36.08	2.87	41.80	2.84
H_2S	ppm	1439	477	2014	763	921	773
H_2	ppm	612	206	1007	238	872	375
NH_3	ppm	3967	983	5399	1305	1906	199

[a] CBY: cumulative biogas yield, [b] CMY: cumulative methane yield

Table 5. Digestate characteristics (mean ± std. dev)

Parameter	*Chlorophyta*	*Cyanoprokaryota*	*Bacillariophyceae*
TS (%)	3.92 ± 0.93	3.31 ± 0.78	2.99 ± 0.85
VS (%TS)	68.83 ± 3.17	69.94 ± 1.92	63.38 ± 1.27
TN (mg/g TS)	35.09 ± 2.03	41. 11 ± 3.14	37.48 ± 1.69
TP (mg/g TS)	4.27±1.38	4.03 ± 0.75	3.75 ± 0.24
TC (mg/g TS)	328.15 ± 41.74	372.09 ± 30.26	380.99 ± 18.38
TOC (mg/g TS)	215.18 ± 29.96	229.73 ± 31.11	233.07 ± 22.06
C:N ratio	9.37 ± 0.61	9.07 ± 0.07	10.16 ± 0.11
pH	7.48 ± 0.19	7.01 ± 0.32	6.82 ± 0.38
Protein (% TS)	14.43 ± 1.27	16.94 ± 1.97	19.68 ± 1.05
Lipids (% TS)	3.02 ± 0.45	3.14 ± 0.73	3.03 ± 0.33
Saccharides (% TS)	1.51 ± 0.51	1.33 ± 0.14	1.15 ± 0.27

Discussion

The anaerobic digestion of *Chlorophyta* and *Cyanoprocaryota* showed similar CBY and CMY, but the higher methane content in biogas produced from anaerobic digestion of *Cyanoprocaryota* resulted in a higher CMY. The lowest biogas/methane potential was recorded for *Bacillariophyceae* biomass.

The results were different from those presented by Zamalloa et al. (2012) who investigated the feasibility of anaerobic digestion of *Scenedesmus obliquus* (*Chlorophyta*), *Phaeodactylum tricornutum* (*Bacillariophyceae*) and *Spirulina platensis* (*Cyanoprokaryota*). After 30 days of fermentation, they achieved the highest methane production yield with *Bacillariophyceae* biomass, then with *Cyanoprokaryota*, while the lowest methane yield with *Chlorophyta*. Anaerobic digestion of various taxonomic groups of microalgae was also investigated by Mussgnug et al. (2010). They concluded that biogas production potential was strongly dependent on the algal strain used, whereas no correlation was found between the taxonomic groups and the biogas yield. The highest and the lowest biogas production were recorded during anaerobic digestion of algae that were phylogenetically fairly closely related (both belonging to the class *Chlorophyceae*).

The biodegrability and the biogas production potential of individual species of microalgal biomass can be attributed to the structure of the cell walls (Roberts et al. 2016, Klassen et al. 2015, Mussgnug et al. 2010). All easily-biodegradable species of algae, that enabled to achieve high biogas production, were characterized by a lack of the cell wall, like *Dunaliella salina* (Sheffer et al. 1986), or their cell wall did not contain *hardly* anaerobically *degradable* cellulose and hemicellulose and was made of protein substances, such as *Chlamydomonas reinhardtii* (Miller et al. 1972), *Arthrospira platensis* (van Eykelenburg et al. 1980) and *Euglena gracilis* (Nakano et al. 1987). In contrast, *Chlorella kessleri* and *Scenedesmus obliquus* have hemicellulose-containing carbohydrate-based walls which make them tougher to digest (Mussgnug et al. 2010). Even more complex is the silica structure of the cell wall of *Bacillariophyceae* from *Ochrophyta* division (Hildebrand et al. 2012), which was also confirmed in this study. In turn, prokaryotic cyanobacterium from *Cyanophyceae* class ensured a high biogas productivity which was associated with a low amount of indigestible residues (Mussgnug et al. 2010). Gerken et al. (2013) noted that major changes in cell wall composition might depend on very small differences in growth conditions as well as on factors such as the culture age. According to Roberts et al. (2016), further work is needed on the likely impact both of growth conditions and of post-harvest storage and processing on algal biomass anaerobic digestibility.

However, the structure of algae cell wall is not the only factor affecting a degree of the cell disintegration and subsequent biogas production. Some microalgae can produce compounds which may have detrimental effects on anaerobic microbes by inhibition of the methanogens (Schlüter et al. 2008, Klocke et al. 2007).

The literature has shown that low C/N ratio in algae biomass may have inhibitory effect for the efficient methane production (Yen et al. 2007). During the study, C/N ratio for all biomass ranged from 8.50 to 10.95, which was well below optimal C/N ratio of 20–30 preferred for anaerobic digestion (Ward et al. 2014). If the C/N ratio is low, nitrogen will be liberated and accumulated in the form of ammonia that may inhibit methanogens. Moreover, ammonia nitrogen will increase the pH value in the anaerobic reactor. The mesophilic digestion is severely inhibited if the pH value rises above pH 8.3 (Seadi et al. 2008). Data showed that *Cyanoprokaryota* biomass represented the lowest C/N ratio of 8.50 and the highest pH of 8.12, while after anaerobic digestion there was the highest ammonia concentration in biogas composition. In spite of this, *Cyanoprokaryota* biomass gave the highest methane yield. Frear et al. (2011) showed similar lack of ammonia nitrogen inhibition during anaerobic digestion of manure at a C/N ratio of 11, which was a result of bacterial acclimation to high ammonia concentrations (Roberts et al. 2016, Calli et al. 2005). Zhao et al. (2014) also reported an effective methane production from algae biomass having low C/N ratio ranging from 6.8 to 14.7. Ammonia inhibition may be a risk in non-acclimatized systems at the moderate to high algal biomass concentrations required to reach high OLR (Yen and Brune 2007). Thus, it could be avoided by co-digestion algae biomass with substrates of low nitrogen content or by using a more dilute feedstock with a short HRT (Roberts et al. 2016, Klassen et al. 2015).

The cultivation conditions and algae species determine the protein, lipid and saccharides content in biomass (Wang and Park 2015), thus the difference in the algae characteristics will affect their digestibility and biogas yield. The theoretical methane yields of carbohydrates, proteins and lipids are 415, 496 and 1014 L/kg VS_{fed} at standard temperature and pressure conditions (STP, 273 K, 101.3 kPa) (Wang and Park 2015). According to Zhao et al. (2014) methane productivity from algae biomass was not related to microalgal species, but more to lipid content. Both *Chlorella sp.* and *Scenedesmus sp.* are able to undergo mixotrophic growth that allows higher lipids production (Bohutskyi and Bouwer 2012), thus during the study *Chlorophyta* biomass characterized by the highest lipid concentration of 14.19%. In contrast, the highest methane yield and methane concentration in biogas was not recorded with *Chlorophyta* but with *Cyanoprokaryota* biomass. In turn, *Cyanoprokaryota* has the highest content of protein and saccharides and the lowest lipid content. According to Ward et al. (2014) lipids can cause inhibition due to their intermediate products such as long chain fatty acids (LCFAs). However, Cirne et al. (2007) reported that the lipid concentrations of 18% in algae biomass did not inhibit anaerobic digestion

The yields of methane production reached during the study are similar to the data reported in the literature. Cumulative methane yields in the 90-day biochemical methane potential test with the species *Navicula occulta (Bacillariophyceae)*, *Scenedesmus sp.* (*Chlorophyta*) and *Chlorella vulgaris* (*Chlorophyta*) were 231 mL CH_4/g VS, 261 mL CH_4/g VS, 307 mL CH_4/g VS, respectively (Roberts et al. 2016). The yield of methane production with *Phaeodactylum tricornutum* (*Bacillariophyceae*), *Spirulina platensis* (*Cyanoprokaryota*) and *Scenedesmus obliquus* (*Chlorophyta*) reached 350 mL CH_4/g VS, 280 mL CH_4/g VS and 210 mL CH_4/g VS, respectively (Zamalloa et al. 2012). The methane potential of *Chlorella vulgaris* achieved 150 mL CH_4/g VS and 240 mL CH_4/g VS after 16 and 28 days of anaerobic digestion, respectively (Ras

et al. 2011). A typical duration for a biochemical methane potential assay for slow-degrading substrates, including microalgal material which may be degraded over long periods, should be 20–40 days (Roberts et al. 2016).

Methane potential with the usage of other organic feedstock is shown in the Table (6) comparing methane yields produced from algae biomass and other organic materials. It could be noted that algae biomass is a good source to produce methane.

In conclusion, our data indicate that the algal taxonomy may determine the biogas and methane production. The cumulative methane production yield for *Microcystis sp*. (*Cyanoprocaryota*) was much higher than that estimated for *Chlorophyta* and *Bacillariophyceae* biomass. In turn, volumetric biogas production rate and cumulative biogas yield was the highest with a culture of *Chlorella sp.* (division *Chlorophyta)*. The lowest values of biogas production were found during anaerobic digestion of *Cyclotella sp*. (class *Bacillariophyceae*, division *Ochrophyta*) with the solid silica cell walls, which makes biomass recalcitrant to biodegradation. An efficient conversion of algae biomass into biogas is dependent on the complete disintegration of all cellular components (Klassen et al. 2015). The cell wall resistance can be overcome by enzymatic, physical or chemical pretreatment methods which are extremely expensive and energy intensive, and therefore they are not profitable for biofuel generation (Passos et al. 2014). Thus, harvesting and storage methods may act as pre-treatments to rupture algae cells and thus leading to a higher anaerobic digestibility, increasing methane yields or improving production kinetics (Roberts et al. 2016). A novel cultivation strategy with inherent nitrogen limitation has been also proposed by Klassen et al. (2015) to optimize C/N ratios for improving the subsequent anaerobic digestion process and increasing methane production.

Conclusions

In this work there was investigated the potential of three microalgae biomass representing the three taxonomic groups for biogas/methane production. It was found that biomass characteristics and biogas/methane yields are influenced by algal taxonomy. The cumulative biogas yield (CBY) and cumulative methane yield (CMY) were the highest for *Cyanoprocaryota* and *Chlorophyta* biomass while the lowest values were found with *Bacillariophyceae* biomass. It could be stated that cell wall composition of microalgae has a greater effect on biogas and methane productivities rather than low C/N ratio, which ranged from 8.5 to 10.95. The conducted research confirmed other studies that have shown the effect of the species composition of microalgae biomass on the biogas production efficiency.

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Table 6. Methane production from different organic feedstock

Feedstock	Methane production (mL CH_4/g VS)	Reference
Corn straw	216.7	Mao et al. 2015
Rice straw	178.3	Mao et al. 2015
Organic fraction of municipal solid waste	340	Mao et al. 2015
Fruit and vegetable wastes	430	Mao et al. 2015
Food waste with cattle manure	388.3	Mao et al. 2015
Poultry manure	195	Mao et al. 2015
Cattle manure	200	Seadi et al. 2013
Sewage sludge	400	Seadi et al. 2013
Grass silage and maize silage	<450	Seadi et al. 2013

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