

The Influence of Anaerobic Digestion Effluents (ADEs) Used as the Nutrient Sources for *Chlorella* sp. Cultivation on Fermentative Biogas Production

Marcin Dębowski^{1,2} · Stanisław Szwaja¹ · Marcin Zieliński^{1,2} · Marta Kisielewska² · Ewa Stańczyk-Mazanek³

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Abstract

Purpose This study investigated the chemical characteristics and anaerobic digestion of *Chlorella* sp. microalgae cultivated on various anaerobic digestion effluents (ADEs) as a nutrient medium. *Chlorella* sp. was grown in anaerobically digested effluent of dairy wastewater (DW), municipal wastewater sludge (WS), maize silage and swine slurry, and cattle manure (CM).

Methods To evaluate the anaerobic biodegradability of harvested biomass, 20-days batch anaerobic digestion experiments were used.

Results It was found that a nutrient medium directly affected nitrogen concentration in the cultivated biomass, as well as the C/N ratio value which ranged 7.2–12.9. Higher C/N ratio of the *Chlorella* sp. cultivated on DW and WS significantly enhanced the methane production, which was 241 ± 5.5 mL CH₄/g VS and 267 ± 10.9 mL CH₄/g VS, respectively. The highest biogas production rate of 61.28 ± 2.7 mL/g VS·d and methane concentration in biogas of 69.7 ± 4.1 % were obtained during the digestion of *Chlorella* sp. biomass cultivated on WS.

Conclusions These results proved the applicability of ADEs as a nutrient medium for *Chlorella* sp. cultivation and the impact of a nutrient source on C/N ratio in harvested biomass, which subsequently affected the biogas/methane yield.

Keywords Microalgae · C/N ratio · Methane · Liquid digestate · Nutrient medium

Introduction

Biogas production via anaerobic digestion (AD) has rapidly developed in recent years [1, 2]. Besides renewable energy, biogas plants also produce large amount of liquid anaerobic digestion effluents (ADEs) which may lead to oversupply of ADEs in a short time. ADEs still have high chemical oxygen demand (COD) and they are rich in nitrogen and phosphorus, which excludes the possibility of these wastewaters discharge directly to the environment. Thus, a low-cost method to treat ADEs is needed. Considering both the characteristics of ADEs and nutritional needs of algae, it seems that ADEs may be a useful source of nutrients and microelements to ensure an intensive growth of microalgae biomass with simultaneous contaminants biodegradation [3–5].

Microalgae cultivation have nowadays gained high attention in the field of renewable energy because of their potential to produce large quantities of biomass, resistance to pollution, less water uptake and land requirement and higher bioenergy yield compared to terrestrial biofuel crops [6, 7]. Microalgae biomass can be converted into many biofuels such as biodiesel from cells lipids, hydrogen derived from photobiological processes, heat form direct combustion and biogas produced during anaerobic

✉ Marta Kisielewska
jedrzejewaska@uwm.edu.pl

¹ Faculty of Mechanical Engineering and Computer Sciences, Institute of Thermal Machinery, Częstochowa University of Technology, Armii Krajowej Str. 21, 42-200 Częstochowa, Poland

² Department of Environment Engineering, Faculty of Environmental Sciences, University of Warmia and Mazury in Olsztyn, Warszawska Str. 117, 10-720 Olsztyn, Poland

³ Faculty of Environmental Sciences and Biotechnology, Institute of Environmental Engineering, Częstochowa University of Technology, Brzeźnicka Str. 60a, 42-200 Częstochowa, Poland

digestion [8, 9]. Many studies have recommended AD of microalgae biomass as a profitable solution for biogas generation [10–12]. Recent studies on AD of algal biomass have reported the methane yield of 231 mL CH₄/g VS for *Navicula occulta*, 261 mL CH₄/g VS for *Scenedesmus* sp., 307 mL CH₄/g VS for *Chlorella vulgaris*, 350 mL CH₄/g VS for *Phaeodactylum tricornutum*, 280 mL CH₄/g VS for *Spirulina platensis* [13, 14]. Thus, this is evident that microalgae biomass have good methane potential and its AD can become commercially viable [15].

Coupling microalgae culture and ADEs treatment has been already explored by Cheng et al. [2] using ADE of swine manure, Morales-Amaral et al. [16] using centrate from AD, Yang et al. [17] using anaerobic digested starch wastewater, Park et al. [18] using ADE of livestock waste, Cai et al. [19] using ADE of municipal wastewater, Erkelens et al. [20] using microalgae digestate effluent. However, the effects of ADEs characteristics on microalgal growth and the subsequent AD of obtained biomass are still poorly studied [21, 22].

The current study evaluated the potential suitability of ADEs derived from different sources as a nutrient medium for *Chlorella* sp. cultivation and the subsequent biogas potential of harvested biomass. The selected ADEs used in the study for *Chlorella* sp. cultivation were anaerobically digested effluents of dairy wastewater, municipal wastewater sludge, maize silage and swine slurry, and cattle manure.

Materials and Methods

Experimental Design of Microalgae Cultivation

Microalgae biomass cultivation on ADEs was carried out in a laboratory scale with the use of vertical, closed photobioreactors (PBRs) of 7.6 cm diameter and 0.55 m height, with an active volume of 2.5 L. The PBRs were made of transparent glass. A 18 W cool-white fluorescent light (700 lx, Osram, Germany) with reflector was used as a constant light source. The supply of CO₂ ensured by continuous inflow of air (at 250 L/h), providing the

appropriate mixing of the cultivation medium and homogeneity of conditions in the entire PBR's volume. The temperature of the culture was 22.0 ± 2.0 °C. Proper thermal conditions were continuously monitored by temperature sensors inside PBRs.

Microalgae Inoculum and Cultivation

Algae of the genus *Chlorella* sp. (BA0103) used in the experiment originated from the Culture Collection of Baltic Algae Institute of Oceanography, University of Gdańsk, Poland. In all experimental variants, the initial algae biomass concentration in PBRs reached 250 ± 22 mg total solids (TS)/L.

Experimental variants differed in ADE used as a nutrient medium: variant I—anaerobically digested effluent of dairy wastewater (DW), variant II—anaerobically digested effluent of municipal wastewater sludge (WS), variant III—anaerobically digested effluents of maize silage and swine slurry (MS), and variant IV—anaerobically digested effluents of cattle manure (CM). Characteristics of the ADEs were shown in Table 1.

Before feeding to PBRs, the ADEs were centrifuged at 5000 rpm for 10 min (MPW-251, Donserv, Poland) and then autoclaved at 90 °C for 30 min to remove solid suspensions, obtain supernatant containing substances in the dissolved phase and hygienize the nutrient medium.

The criterion deciding about the applied ADEs volume was the initial total ammonia nitrogen (TAN) concentration in reactors used to grow *Chlorella* sp., which was set at 160 mg N-NH₄/dm³. In order to ensure the desired initial concentration of TAN and equal initial concentration of algae biomass, in all experimental variants the PBRs were fed with 1.54 L of *Chlorella* sp. biomass (with biomass concentration at 405 ± 31 mg TS/L) and filled up to the level of 2.5 L with ADE and deionized water. Inlet parameters of the cultivation medium were shown in Table 2.

The *Chlorella* sp. microalgae was grown until the biomass concentration in PBRs ca. 2000 mg TS/L. After a completed cultivation process, algae biomass was concentrated, separated and dehydrated in a sedimentation

Table 1 Characteristics of ADEs used in the study (mean ± SD)

Parameters	DW	WS	MS	CM
COD (mg O ₂ /L)	3300 ± 270	920 ± 62	7800 ± 390	6200 ± 430
TN (mg N/L)	460 ± 26	596 ± 77	1400 ± 210	1160 ± 90
TAN (mg N-NH ₄ /L)	410 ± 38	470 ± 46	1150 ± 180	910 ± 77
TP (mg P/L)	31 ± 3.4	29 ± 4.9	74 ± 5.7	61 ± 7.4
P-PO ₄ (mg P-PO ₄ /L)	27 ± 6.1	27 ± 2.6	49 ± 5.2	41 ± 5.9
pH	6.9 ± 0.3	7.0 ± 0.1	6.8 ± 0.4	7.0 ± 0.3

Table 2 Inlet parameters of the cultivation medium (mean \pm SD)

Parameters	DW	WS	MS	CM
COD (mg O ₂ /L)	1270.8 \pm 103.1	312.7 \pm 39.4	1089.4 \pm 99.5	1094.8 \pm 101.3
TN (mg N/L)	180.2 \pm 10.6	203.9 \pm 19.8	198.5 \pm 9.0	207.8 \pm 10.2
TAN (mg N-NH ₄ /L)	160.7 \pm 6.4	160.5 \pm 21.5	160.3 \pm 7.8	160.1 \pm 6.3
TP (mg P/L)	15.7 \pm 4.2	13.5 \pm 2.9	13.9 \pm 3.1	14.3 \pm 2.5
P-PO ₄ (mg P-PO ₄ /L)	14.0 \pm 2.7	12.6 \pm 2.5	10.4 \pm 1.8	10.8 \pm 2.0
pH	6.96 \pm 0.1	6.98 \pm 0.1	7.0 \pm 0.1	7.0 \pm 0.1

process and then through centrifugation (MPW-251, Donserv, Poland). Finally, it was subjected to chemical analysis and used as a substrate in fermentative biogas production.

Biogas/Methane Production from *Chlorella* sp. Microalgae Biomass

Algae biomass fermentation was conducted using respirometers (WTW, Germany) that consisted of reaction tanks with an active volume of 0.5 L coupled tightly with measuring devices recorded an increase of the partial pressure induced by biogas production. Pressure in the reaction tank was recorded every 24 h. The ideal gas equation was the basis for computing the volume of produced biogas in the respirometric tests. The volumes of biogas generated per normal conditions were computed on the basis of pressure changes inside the bottle headspace. Respirometric tests also provided grounds to determine the volumetric biogas production rate (VBPR), depending on the employed experimental variants. Reaction rate constants (k) were determined on the basis of obtained experimental data with the nonlinear regression method using the Statistica 10.0 PL (Statsoft, Inc.) application. A conformity index φ^2 was accepted as a measure of curve matching to experimental data, which enabled the reaction order and reaction rate constant k to be determined.

0.5 L reactors were filled with 200 mL anaerobic sludge originated from the closed fermentation tanks of a local municipal wastewater treatment plant. The concentration of volatile solids (VS) seeded into the reactor was 69.2 ± 2.8 % TS. In order to ensure anaerobic conditions inside the respirometers, they were blown through with nitrogen to remove atmospheric air at the beginning of the fermentation. The measurements were carried out at a temperature of 38 °C. In all technological variants, the initial load was 5.0 g VS/L. Tests were carried out for a period of 20 days.

The composition of biogas produced in the headspace of reactors 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 temperature of the injection and detector ports were 150 and 250 °C, respectively. Helium and argon were used as the carrier gases at a flow of 15 mL/min.

Analytical Methods

Total nitrogen (TN), total ammonia nitrogen (TAN), total phosphorus (TP), orthophosphate (P-PO₄), and chemical oxygen demand (COD) in ADEs and cultivation media were determined using a DR 5000 spectrophotometer with an HT 200 s mineralizer (Hach-Lange, Germany). The gravimetric method was used to determine total solid (TS) and volatile solids (VS) in samples of microalgae biomass and anaerobic sludge. In those samples dried at 105 °C, TC (total carbon), TOC (total organic carbon) and total nitrogen (TN) were determined by elementary particle analyser Flash 2000 (Thermo Scientific, USA). Total phosphorus (TP) in *Chlorella* sp. biomass was determined spectrophotometrically (DR 2800, Hach-Lange, Germany) using an ammonium metavanadate and molybdate after prior reaction of sample in acid medium. The pH was determined using a pH-meter (1000L, VWR, Germany).

Statistical Methods

Each experimental variant was conducted in three replications (both *Chlorella* sp. cultivation and anaerobic digestion). The statistical analysis of results was carried out with Statistica 10.0 PL package (Statsoft, Inc.). The hypothesis on distribution of each analyzed variable was verified with a Shapiro–Wilk W -test. One-way analysis of variance (ANOVA) was applied to determine the significance of difference between variables. Variance homogeneity in groups was checked with a Levene's test, whereas the significance of differences between the analyzed variables was determined with a Tukey RIR test. In all tests, the level of significance was adopted at $p = 0.05$.

Table 3 Chemical characteristics of *Chlorella* sp. biomass cultivated on different ADEs (variant)

Parameter	Variant			
	DW	WS	MS	CM
VS (% TS)	84.8 ± 3.9	87.2 ± 2.3	84.2 ± 0.8	86.1 ± 1.7
TN (mg/g TS)	41.7 ± 2.4	36.5 ± 4.8	57.2 ± 1.4	54.5 ± 2.3
TP (mg/g TS)	18.4 ± 1.3	17.1 ± 2.4	15.6 ± 2.6	16.4 ± 0.7
TC (mg/g TS)	511.6 ± 23.9	521.3 ± 17.2	482.4 ± 22.5	488.8 ± 33.4
TOC (mg/g TS)	449.4 ± 19.5	470.1 ± 31.4	412.1 ± 14.8	439.6 ± 27.6
C/N	10.8 ± 0.9	12.9 ± 2.4	7.2 ± 0.3	8.0 ± 0.9
pH	7.42 ± 0.2	7.66 ± 0.1	7.63 ± 0.1	7.84 ± 0.1

Results and Discussion

Composition Analysis of ADEs

ADEs used in the study contained organic matter, nitrogen and phosphorus which are essential for the growth of microalgae biomass (Table 2). The highest COD concentration in the culture medium of 1270.8 mg/L was noted with DW medium, while the lowest of 312.7 mg/L with WS medium (Table 2). TN, TAN and TP concentrations was on the similar level in all ADEs used as a nutrient medium (Table 2). The pH of ADEs was about of 7.0 (Table 2), which was within the optimal pH range of 6.0–8.0 for the *Chlorella* genus [2].

The lowest COD to TN ratio (COD/N) of 1.53 was found in WS. In MS and CM, COD/N ratio was slightly above 5, while the value of 7.05 was obtained with DW medium. The N/P ratio estimated was respectively 11.5, 15.1, 14.3, 14.5 in DW, WS, MS and CM. The optimal C/N mass ratio of microalgae is in the range 4–8 [23]. In turn, the N/P ratio of an algal cell is 7 [24]. This suggested that all the nutrient media were limited in phosphorus. Carbon limitation was found in WS. Many data indicate that that nutrient imbalance may limit the growth of microalgae [2, 3, 19]. The growth of *Chlorella* PY-ZU1 was almost doubled through the addition of phosphate, but adding phosphorus at higher concentrations may have an adverse effect due to higher cell osmotic pressure [2].

In all ADEs, the major component of TN was ammonia nitrogen at the concentration of about 160 mg/L. Ammonium is the preferred form of nitrogen for microalgal growth, but ADEs may contain high levels of total ammonia nitrogen (1000–3000 mg/L) which is toxic to microalgae strains at the concentration of above 100 mg/L have [1, 16]. Inhibitory thresholds depend on the microalgal species and cultivation conditions [3, 4]. It has been reported that TAN concentrations of 364 mg/L inhibited the growth of *Scenedesmus* sp. [25]. By contrast, Park et al. [26] found that the levels of *Scenedesmus* sp. inhibition were similar when TAN level ranged

200–500 mg/L. *Rhodobacter sphaeroides* and *Chlorella sorokiniana* were not inhibited by relatively high ammonia concentrations, while *Spirulina platensis* was completely inhibited at a TAN level of 400 mg/L [27]. Cai et al. [19] state, that ammonium at concentrations greater than 450 mg/L is often toxic to microalgae. According to Wang et al. [4], *Chlorella* sp. is highly ammonia nitrogen tolerant.

ADEs may also contain many other components like vitamins, amino-acids which benefited the growth of microalgae, but they also may contain heavy metals which can be toxic to microalgae [2]. However, heavy metals at trace concentrations may stimulate the growth of microalgae. *Chlorella* sp. is resistant to the action of heavy metals, owing to which it has been used for treatment of industrial wastewaters [28]. Anaerobic digestion effluent of swine and cattle manure, maize silage (MS and CM) or dairy wastewater (DW) contain too low concentrations of heavy metals to cause negative effect on microalgae growth [2]. Only anaerobically digested effluent of municipal wastewater sludge (WS) may contain metallic inhibitors to microalgae growth [16, 29].

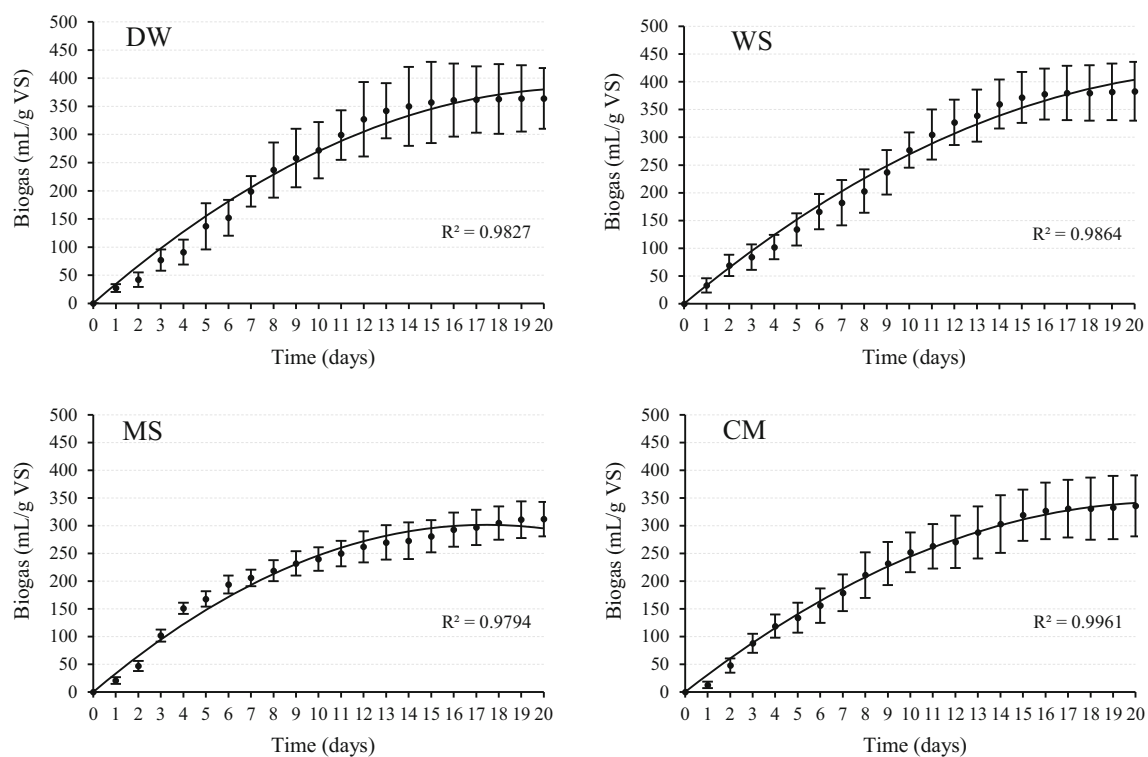
Composition of *Chlorella* sp. Microalgae Cultivated on ADEs

The chemical characteristics of *Chlorella* sp. biomass depending on the cultivation medium was investigated (Table 3). The harvested algae biomass was mainly composed of organic fraction (84.2–86.8 % TS). Even though carbon limitation was found in WS medium, *Chlorella* sp. biomass was characterized by a high VS and TOC content as well as C/N ratio, similarly to biomass grown on DW. The effect of different ADEs on organic microalgae components was slight. The average TOC/VS ratio was approximately 5 ($p > 0.05$). Our study found the impact of ADEs on TN concentration within *Chlorella* sp. biomass. Higher TN/VS ratio was observed in MS and CM, while lower ($p < 0.05$) in DW and WS. A higher C/N ratio ($p < 0.05$) within *Chlorella* sp. grown on DW and WS than on MS and CM was observed. No significant differences

Table 4 Biogas characteristic and biogas/methane yield from *Chlorella* sp. biomass cultivated on different ADEs (variant)

Parameter	Variant			
	DW	WS	MS	CM
CBY ^a (mL/g VS)	364 ± 54	383 ± 53	312 ± 31	336 ± 49
CMY ^b (mL/g VS)	241 ± 5.5	267 ± 10.9	183 ± 10.1	205 ± 3.7
BPR ^c (mL/g VS·d)	58.24 ± 4.1	61.28 ± 2.7	49.92 ± 1.3	53.76 ± 2.1
BPR constant (1/d)	0.18 ± 0.06	0.18 ± 0.02	0.15 ± 0.01	0.17 ± 0.01
CH ₄ (%)	66.4 ± 2.3	69.7 ± 4.1	58.9 ± 5.5	61.3 ± 1.8
CO ₂ (%)	33.5 ± 2.3	30.2 ± 4.1	41.0 ± 5.5	38.6 ± 1.8
H ₂ S (ppm)	1240 ± 291	1730 ± 357	3940 ± 454	3490 ± 303
H ₂ (ppm)	217 ± 27	401 ± 66	283 ± 79	168 ± 27
NH ₃ (ppm)	2120 ± 118	2041 ± 193	2736 ± 384	2412 ± 411

^a CBY cumulative biogas yield, ^b CMY cumulative methane yield, ^c BPR biogas production rate

**Fig. 1** Cumulative biogas yield of anaerobic digestion of *Chlorella* sp. grown on different ADEs

($p > 0.05$) were observed as regards TP/VS ratio and pH value in microalgae biomass.

Biogas/Methane Potential of *Chlorella* sp. Microalgae

The impact of chemical composition of *Chlorella* sp. biomass grown on ADEs of different characteristics was assessed in mesophilic fermentation batch tests over a period of 20 days.

The highest ($p < 0.05$) cumulative biogas yield (CBY) of 383 mL/g VS was observed in WS variant (Table 4;

Fig. 1), where the TOC/VS ratio and C/N ratio within *Chlorella* sp. biomass were greatest (Table 3). Less CBY was found in DW variant, while the lowest biogas yield ($p < 0.05$) were obtained during anaerobic digestion of *Chlorella* sp. cultivated on MS and CM media. Similarly, the cumulative methane yield (CMY) was significantly higher ($p < 0.05$) in WS and DW variants than that of MA and CM (Table 4). The highest daily biogas production over 40 mL/d was found with MS and DW media (Fig. 2).

Our experimental findings of methane production (183–267 mL CH₄/g VS) from *Chlorella* sp. biomass cultivated on ADEs were similar to the values observed in the

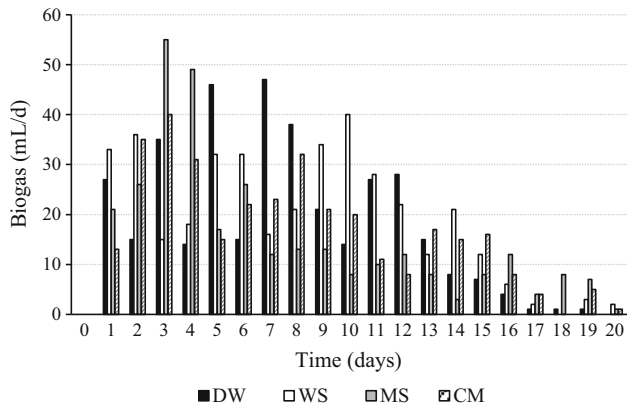


Fig. 2 Daily biogas production from *Chlorella* sp. grown on different ADEs

previous studies. Methane yield of *Chlorella* sp. microalgae anaerobic digestion ranged from 123 mL CH₄/g VS to 369 mL CH₄/g VS [7, 12, 30, 31]. In turn, methane yield of 317.31 mL CH₄/g VS was obtained with *Chroococcus* sp. by Prajapati et al. [15]. Comparing methane yields produced from algae biomass and other organic materials, it could be state that algae biomass is a good source to produce methane. Methane potential with the usage of the other organic materials is as follow: corn straw –216 mL CH₄/g VS, rice straw –178 mL CH₄/g VS, organic fraction of municipal solid waste –340 mL CH₄/g VS, fruit and vegetable wastes –430 mL CH₄/g VS, food waste with cattle manure –388 mL CH₄/g VS, poultry manure –195 mL CH₄/g VS [32], cattle manure –200 mL CH₄/g VS, sewage sludge 400 mL CH₄/g VS [33].

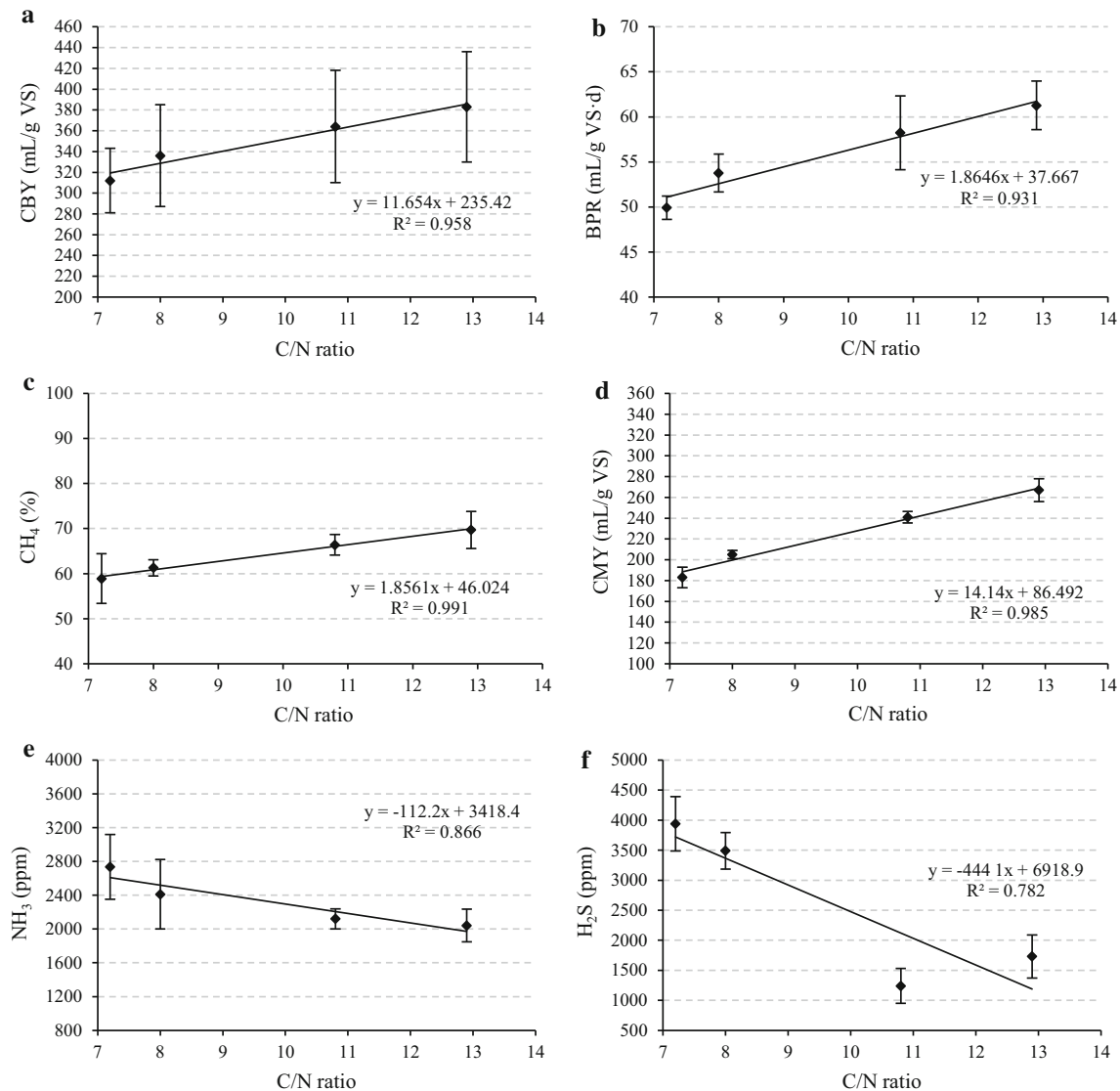


Fig. 3 Correlation between the C/N ratio and **a** cumulative biogas yield (CBY), **b** biogas production rate (BPR), **c** CH₄ content in biogas, **d** cumulative methane yield (CMY), **e** NH₃ content in biogas, **f** H₂S content in biogas

Table 5 A RiR Tukey statistical test to find the difference between different biogas production from various media (values in italics indicate significant differences at $p \leq 0.05$)

	MS	CM	DW	WS		MS	CM	DW	WS
CBY (mL/g VS)					BPR (mL/g VS·d)				
MS		0.8743	0.0707	<i>0.0248</i>	MS		<i>0.0173</i>	<i>0.0013</i>	<i>0.0083</i>
KM	0.8743		0.3873	0.1996	KM	<i>0.0173</i>		<i>0.0394</i>	<i>0.0077</i>
DW	0.0707	0.3873		0.9048	DW	<i>0.0013</i>	<i>0.0394</i>		0.0573
WS	<i>0.0248</i>	0.1996	0.9048		WS	<i>0.0083</i>	<i>0.0077</i>	0.0573	
CH ₄ (%)					CMY (mL/g VS)				
MS		0.6462	<i>0.0126</i>	<i>0.0037</i>	MS		<i>0.0207</i>	<i>0.0073</i>	<i>0.0003</i>
KM	0.6462		<i>0.0372</i>	<i>0.0187</i>	KM	<i>0.0207</i>		<i>0.0092</i>	<i>0.0016</i>
DW	<i>0.0126</i>	<i>0.0372</i>		0.0959	DW	<i>0.0073</i>	<i>0.0092</i>		<i>0.0127</i>
WS	<i>0.0037</i>	<i>0.0187</i>	0.0959		WS	<i>0.0003</i>	<i>0.0016</i>	<i>0.0127</i>	
NH ₃ (ppm)					H ₂ S (ppm)				
MS		0.1056	<i>0.0419</i>	<i>0.0374</i>	MS		0.0872	<i>0.0001</i>	<i>0.0002</i>
KM	0.1056		0.0677	0.0562	KM	0.0872		<i>0.0001</i>	<i>0.0002</i>
DW	<i>0.0419</i>	0.0677		0.3382	DW	<i>0.0001</i>	<i>0.0001</i>		<i>0.0037</i>
WS	<i>0.0374</i>	0.0562	0.3382		WS	<i>0.0002</i>	<i>0.0002</i>	<i>0.0037</i>	
CBY (mL/g VS)					BPR (mL/g VS·d)				
MS		0.8743	0.0707	<i>0.0248</i>	MS		<i>0.0173</i>	<i>0.0013</i>	<i>0.0083</i>
KM	0.8743		0.3873	0.1996	KM	<i>0.0173</i>		<i>0.0394</i>	<i>0.0077</i>
DW	0.0707	0.3873		0.9048	DW	<i>0.0013</i>	<i>0.0394</i>		0.0573
WS	<i>0.0248</i>	0.1996	0.9048		WS	<i>0.0083</i>	<i>0.0077</i>	0.0573	
CH ₄ (%)					CMY (mL/g VS)				
MS		0.6462	<i>0.0126</i>	<i>0.0037</i>	MS		<i>0.0207</i>	<i>0.0073</i>	<i>0.0003</i>
KM	0.6462		<i>0.0372</i>	<i>0.0187</i>	KM	<i>0.0207</i>		<i>0.0092</i>	<i>0.0016</i>
DW	<i>0.0126</i>	<i>0.0372</i>		0.0959	DW	<i>0.0073</i>	<i>0.0092</i>		<i>0.0127</i>
WS	<i>0.0037</i>	<i>0.0187</i>	0.0959		WS	<i>0.0003</i>	<i>0.0016</i>	<i>0.0127</i>	
NH ₃ (ppm)					H ₂ S (ppm)				
MS		0.1056	<i>0.0419</i>	<i>0.0374</i>	MS		0.0872	<i>0.0001</i>	<i>0.0002</i>
KM	0.1056		0.0677	0.0562	KM	0.0872		<i>0.0001</i>	<i>0.0002</i>
DW	<i>0.0419</i>	0.0677		0.3382	DW	<i>0.0001</i>	<i>0.0001</i>		<i>0.0037</i>
WS	<i>0.0374</i>	0.0562	0.3382		WS	<i>0.0002</i>	<i>0.0002</i>	<i>0.0037</i>	

The CH₄ concentration was similar in the biogas produced from the digestion of *Chlorella* sp. cultivated on DW and WS medium ($p > 0.05$) (Table 4). Biogas produced in MS and CM variant characterized by a high H₂S and NH₃ concentration (Table 4). It was indicated that ammonia gas within the digester may have much more inhibitory effect on methanogenic bacteria than the aqueous ionised form of ammonium [23]. This may explain the lowest CMY in MS variant, where the gaseous NH₃ concentration was the highest.

The overall biogas production rate (BPR) of 61.28 mL/g VS·d in WS variant and 58.24 mL/g VS·d in DW variant were found ($p > 0.05$), which was higher ($p < 0.05$) than in MS and CM variant (Table 4). Lower values have been reported by Prajapati et al. [30]. They achieved the rates of biogas production determined for 30 days of anaerobic digestion of the three species of *Chlorella* ranged 11.02–17.35 mL/g VS·d.

Our study found a strong correlation between the C/N ratio in microalgae biomass and biogas/methane yield (Fig. 3; Table 5). Similarly, the rate of biogas production, methane and content H₂S in biogas were strictly dependent on C/N ratio (Fig. 3; Table 5). The C/N ratio strongly affects the anaerobic digestion thus it should range from 20 to 30 [34]. Low C/N ratio leads to increase ammonia nitrogen liberation and accumulation that may inhibit methanogens [12]. Moreover, ammonia nitrogen increases the pH value in the anaerobic reactor, while the mesophilic digestion is severely inhibited if the pH value rises above pH 8.3 [35]. A long HRT during AD of microalgae biomass can increase nitrogen release [12]. The relationship between the substrate C/N ratio and apparent released ammonia was investigated by Hikada et al. [31]. They found that microalgae biomass consisting mainly of *Chlorella* sp. released low ammonia, because microalgae contained some non-biodegradable organic residues.

In most cases, microalgae biomass usually contains high amounts of proteins, which is reflected by low C/N of 10 or below. Hidaka et al. [31] reported C/N ratio ranged 2.5–5.3 for *Chlorella* sp. cultivated on filtrate from dewatering of anaerobically digested sludge. TOC/TNK ratio of *Chlorella vulgaris* cultivated on synthetic anaerobic digestate was estimated at 6 [12]. In our study, C/N ratio of *Chlorella* sp. was higher and ranged from 7.2 to 12.9. Zhong et al. [36] suggested an appropriate C/N ratio of 20 for co-digestion microalgae with corn silage. Similarly to our study, Zhao et al. [37] reported an effective methane production from algae biomass having low C/N ratio ranged from 6.8 to 14.8.

Conclusions

Our study found that microalgae were successfully cultivated using various types of anaerobic digestion effluents. Characteristics of the nutrient source used in *Chlorella* sp. cultivation had a direct effect on C/N ratio in harvested biomass, which was subsequently influenced the biogas yield. The highest C/N ratio was observed when *Chlorella* sp. was cultured on anaerobically digested effluent of municipal wastewater sludge, while the lowest on anaerobically digested effluents of maize silage and swine slurry. A strong correlation between the C/N ratio value in microalgae biomass and biogas/methane production and rate were observed. It was also demonstrated high biogas production rate of 61.28 mL CH₄/g VS from anaerobic digestion of *Chlorella* sp. cultivated on effluent from anaerobic digestion of municipal wastewater sludge.

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