



## PRODUCTION OF BIOGAS USING MAIZE SILAGE SUPPLEMENTED WITH RESIDUAL GLYCERINE FROM BIODIESEL MANUFACTURING

TOMASZ POKÓJ<sup>1\*</sup>, ZYGMUNT M. GUSIATIN<sup>1</sup>, KATARZYNA BUŁKOWSKA<sup>1</sup>,  
BOGDAN DUBIS<sup>2</sup>

<sup>1</sup>Department of Environmental Biotechnology, University of Warmia and Mazury in Olsztyn,  
ul. Słoneczna 45G, 10-709 Olsztyn, Poland

<sup>2</sup>Department of Agrotechnology and Crop Management, University of Warmia and Mazury in Olsztyn,  
ul. M. Oczapowskiego 8, 10-719 Olsztyn, Poland

\*Corresponding author's email: tomasz.pokoj@uwm.edu.pl

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**Abstract:** The aim of this study was to investigate the influence of residual glycerine (5 and 10% w/w) from the biodiesel industry, used as a co-substrate, on biogas production from maize silage. The experiments were conducted in a laboratory-scale, single-stage anaerobic digester at 39°C and hydraulic retention time (HRT) of 60 d. Addition of 5% residual glycerine caused organic load rate (OLR) to increase to 1.82 compared with 1.31 g organic dry matter (ODM) L<sup>-1</sup>d<sup>-1</sup> for maize silage alone. The specific biogas production rate and biogas yield were 1.34 L L<sup>-1</sup>d<sup>-1</sup> and 0.71 L g ODM<sup>-1</sup> respectively, i.e. 86% and 30% higher than for maize alone. Increasing the residual glycerine content to 10% increased OLR (2.01 g ODM L<sup>-1</sup>d<sup>-1</sup>), but clearly decreased the specific biogas production rate and biogas yield to 0.50 L L<sup>-1</sup>d<sup>-1</sup> and 0.13 L g ODM<sup>-1</sup> respectively. This suggested that 10% glycerine content inhibited methanogenic bacteria and organics conversion into biogas. As a result, there was accumulation of propionic and valeric acids throughout the experiment.

### INTRODUCTION

Methane production through anaerobic digestion is currently one of the most intensively developed biofuel technologies. Since 2001 the total production of biogas in European Union (EU) countries has constantly increased at 2.7 million tonnes of oil equivalent per year [6]. The application of plant biomass for biogas production has assumed a growing importance. According to Amon et al. [2], in the EU the annual potential of anaerobically digested biomass is 1500 million tonnes, and half of this will be supplied by agricultural crops. Of various agricultural crops used in biogas production in Germany, a European leader in the utilization of biomethane technologies [21], maize is dominant due to its high biogas yield. From technological and economics points of view, in agricultural systems using plant biomass (i.e. grass, maize silage or fibrous plant residues) for biogas production, the application of other materials as co-substrates offer several advantages,

i.e. improved nutrient balance, optimized rheological qualities, and cost savings for farmers. For other reasons, the availability of some waste as co-substrates for biogas production in agricultural areas is crucial. Some substrates, including food industry waste (oils, whey, fruit and potato slops) show great potential for biogas production. Other substrates from breweries introduce risks of H<sub>2</sub>S production, chicken manure contains inhibiting NH<sub>3</sub>, whereas oils and fats can cause scum layer forming.

Among co-substrates, by-products from biodiesel production are relatively poorly recognized. In EU countries, therein Poland, the production of methyl esters as biofuels for the transport sector is showing tremendous growth. Biodiesel production for farmers' needs (as permitted by law from 2006 in Poland), usually uses simple systems without glycerine-refining devices. The glycerine phase contains residual alcohols, catalyst residues, carry-over fats/oils, and some esters. Their using for industrial purposes is limited, but they are not excluded from being processed into biogas. Up to now, residual glycerine has been used as a co-substrate during industrial and agricultural wastewater anaerobic treatment [1, 12, 17]. The use of residual glycerine to enhance biogas production from maize silage and changes in the digestion process performance has not previously been investigated. Glycerol, the main component of residual glycerine, is known as a readily digestible compound; however, its implementation in digesters at too high a concentration has negative effects on microorganisms growth due to increased osmotic pressure in bacterial cells.

In present study the effect of different glycerine contents in the feedstock containing maize silage on biogas production and digestion stability has been examined. The influence of residual glycerine on biogas production was assessed by comparing the biogas/CH<sub>4</sub> yield obtained experimentally with theoretical biogas/CH<sub>4</sub> potential, calculated from the elementary composition of the feedstock.

## MATERIALS AND METHODS

### *Substrate*

Maize silage, consisting of leaves, stalks, and cobs, was obtained from the Department of Agrotechnology and Crop Management, University of Warmia and Mazury in Olsztyn (north of Poland). The silage was chopped in a cutting mill (Retsch SM100, Germany) and passed through 1-mesh screen. The chaff was stored in plastic bags at 4°C. Directly before feeding into the digester, the maize was diluted with tap water to approximately 8% of dry matter (DM). Residual glycerine was supplied by a local biodiesel production plant in Lubomin (north of Poland). The characteristics of substrates are given in Table 1.

Table 1. Main characteristics of maize silage and residual glycerine (standard deviations are in parentheses)

Parameter	Unit	Maize silage	Residual glycerine
Dry matter (DM)	g kg <sup>-1</sup>	425 (±28)	816 (±5.2)
Organic dry matter (ODM)	g kg <sup>-1</sup>	397 (±18)	740 (±0.9)
Chemical oxygen demand (COD)	g O <sub>2</sub> kg <sup>-1</sup>	1006 (±1.8)	2520 (±25)
Carbon	%	43.13 (±0.191)	55.93 (±0.099)
Hydrogen	%	7.12 (±0.021)	10.15 (±0.057)
Oxygen	%	48.88 (±0.318)	33.92 (±0.042)
Nitrogen	%	0.89 (±0.106)	0.06 (±0.002)
C:N	g g <sup>-1</sup>	48.46	932.17

Residual glycerine was marked by higher DM; however, the percentage of organic dry matter (ODM) was comparable to maize silage with 93 and 91%, respectively. The carbon and hydrogen content of glycerine was higher than that for maize silage, but nitrogen's – much lower. Consequently, the C:N ratio was about 19 times higher for residual glycerine compared to maize silage.

### ***Experimental set-up***

The experiments were conducted in laboratory-scale, single-stage anaerobic digester (CSTR), with the working volume of 6 L. The reactor was constructed of stainless steel and equipped with a propeller mixer with horizontal axis of rotation for continuous stirring of the mixed liquid at 50 rpm. Appropriately mounted valves enabled reactor feeding and biogas and digestate collection. The biogas was collected in Tedlar sample bags. The temperature was maintained at  $39 \pm 0.2^\circ\text{C}$  by a surrounding water jacket. The reactor was inoculated with anaerobic sludge originating from the municipal wastewater treatment plant in Olsztyn (north of Poland, population 175,000). Once daily each reactor was supplied with 100 mL of the feedstock after 100 mL of mixed liquid had been withdrawn. Therefore, a constant hydraulic retention time of 60 d was obtained.

The experiment was conducted continuously for 180 d in three successive runs differing in the residual glycerine content in the feedstock. During 1–60 d (run 1) maize silage alone was fed into the reactor; during 61–120 d (run 2) maize silage mixed with 5% (w/w) residual glycerine; and during 121–160 d (run 3) maize silage mixed with 10% (w/w) residual glycerine was used.

### ***Analytical methods***

Dry matter (DM) and organic dry matter (ODM), soluble chemical oxygen demand (COD), ammonia N, and Kjeldahl N were determined according to standard methods [3]. The pH was measured immediately after sampling using a pH meter (Hanna HI 221, USA). The total alkalinity was determined by titration to pH 4.3 with normalized 0.1 M HCl using a Schott titroline system. Analyses for COD, ammonia N and alkalinity were performed for filtered supernatant samples previously centrifuged at  $8693 \times g$  for 10 min.

Volatile fatty acids (VFAs) were analyzed using a gas chromatograph (GC, Varian 3800, Australia) equipped with a capillary column (FactorFour VF-1ms, 30 m  $\times$  0.25 mm i.d., 1.0- $\mu\text{m}$  film; Varian) and flame ionization detector (FID). Helium was used as a carrier gas with a flow rate of 1.0 mL  $\text{min}^{-1}$ . The samples were analyzed according to Gilroyed et al. [8]. Total VFA concentration was the sum of the concentrations of individual VFAs analyzed.

Biogas production was measured daily following standard methods [3] using apparatus consisting of a cylinder filled with a saturated solution of sodium chloride combined with an equalizing tank equipped with a side tube. The  $\text{CH}_4$  and carbon dioxide ( $\text{CO}_2$ ) contents were measured for samples collected in Tedlar sample bags using a GA 2000+ automatic analyzer (Geotechnic Instruments, UK). The volume of biogas and  $\text{CH}_4$  was corrected for standard pressure (101 kPa) and  $0^\circ\text{C}$ .

Organic dry matter removal efficiency, specific biogas production rate and  $\text{CH}_4$  content during runs 1–3 were compared using ANOVA and Tukey's test. Mean values were considered to be significantly different for  $p < 0.05$ .

## RESULTS AND DISCUSSION

**Characteristics of the feedstock and operational conditions**

Maize silage was marked by a low pH of 4.3. The addition of 5% (run 2) and 10% (run 3) residual glycerine increased pH to 5.3 and 5.7, respectively, but the mixtures still remained acidic (Table 2). Crop ensiling is based on the conversion of water-soluble carbohydrates into organic acids. Nkosi et al. [14] reported that during ensiling pH declined from 5.7 to  $< 4.2$  in maize water extract. The increase in pH for runs 2 and 3 probably resulted from the presence of a basic catalyst remaining in the glycerine phase after the oil transesterification.

Table 2. Main characteristics of feedstock in runs 1–3 (standard deviations are in parentheses)

Parameter	Unit	Run 1	Run 2	Run 3
<i>Feedstock liquor:</i>				
pH	pH	4.3 ( $\pm 0.16$ )	5.3 ( $\pm 0.12$ )	5.7 ( $\pm 0.15$ )
Volatile fatty acids (VFAs)	mg L <sup>-1</sup>	1457 ( $\pm 150.2$ )	1697 ( $\pm 180.1$ )	1784 ( $\pm 155.6$ )
Ammonium nitrogen	mg NH <sub>4</sub> -N L <sup>-1</sup>	134.0 ( $\pm 10.93$ )	126.0 ( $\pm 9.84$ )	110.6 ( $\pm 11.56$ )
<i>Feedstock:</i>				
Dry matter (DM)	g L <sup>-1</sup>	84.0 ( $\pm 7.94$ )	119.4 ( $\pm 3.89$ )	149.9 ( $\pm 8.17$ )
Organic dry matter (ODM)	g L <sup>-1</sup>	78.4 ( $\pm 7.37$ )	112.9 ( $\pm 2.32$ )	129.6 ( $\pm 6.67$ )
Kjeldahl nitrogen	g N kg <sup>-1</sup>	2.42 ( $\pm 0.20$ )	2.05 ( $\pm 0.25$ )	1.71 ( $\pm 0.14$ )

In all the runs, VFAs were present in the liquid phase. Their concentrations increased in accordance with the glycerine content of the feedstock. Residual glycerine contains methyl esters at 18–20% concentration [17]. Esters undergo auto-oxidation at different rates depending upon the number and position of the double bonds and results in formation of a series of by-products, e.g. acids, aldehydes, ketones and lactones [19]. This explains the higher concentration of VFAs in runs 2 and 3 compared to maize silage alone.

The addition of residual glycerine to the maize silage clearly increased the organic matter concentration (ODM) in the feedstock; approximately 44% and 65% higher with 5% and 10% glycerine content, respectively, compared to controls. Kjeldahl N concentration was highest in run 1 and lowest in run 3, with an analogous effect for ammonium N.

The experiments (runs 1–3) were carried out at constant HRT = 60 d; the different organic load rate (OLR) increased from  $1.31 \pm 0.12$  to  $2.01 \pm 0.11$  g ODM L<sup>-1</sup>d<sup>-1</sup> due to increased residual glycerine concentration in the feedstock (Table 3).

Table 3. The organic load rate (OLR) during anaerobic digestion of maize silage and its mixtures with residual glycerine

Parameter	Unit	Run 1	Run 2	Run 3
Organic load rate:	g ODM L <sup>-1</sup> d <sup>-1</sup>	1.31 ( $\pm 0.12$ ) <sup>a)</sup>	1.82 ( $\pm 0.04$ )	2.01 ( $\pm 0.11$ )
– maize silage		1.31	1.20 (65.9) <sup>b)</sup>	0.80 (39.8)
– glycerine		0.0	0.62 (34.1)	1.21 (60.2)

<sup>a)</sup> standard deviation

<sup>b)</sup> percentage of OLR for single substrate in the feedstock mixture

The range of OLR used in present study was within typical values employed in biogas plants. According to Weiland [21], biogas plants that use energy crops as the main substrate are operated with OLR of 1–3 g ODM L<sup>-1</sup>d<sup>-1</sup>.

### ***Effect of residual glycerine supplementation on digestion performance***

Anaerobic digestion of maize silage and co-digestion of maize silage with residual glycerine were performed for 180 d, starting from fermentation of maize silage alone (run 1) and adding co-substrate after 60 d (run 2) and 120 d (run 3) of the experiment.

### ***Organic dry matter removal efficiency***

The ODM concentrations in the digestate ( $X_{\text{digestate}}$ ) and the efficiency of ODM removal ( $E$ ) in the runs are shown in Fig. 1.

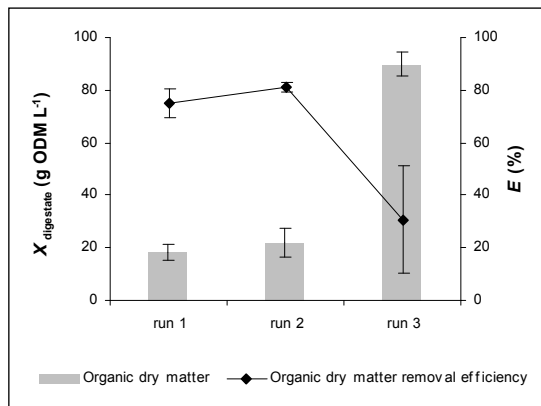


Fig. 1. Concentration of organic dry matter in the digestate (in g ODM L<sup>-1</sup>) and the efficiency of ODM removal ( $E$ ) during the digestion of maize silage (run 1) and maize silage mixed with 5% (run 2) and 10% w/w (run 3) residual glycerine (error bars represent standard deviations)

Using maize silage alone as a feedstock (run 1) gave high efficiency of organic dry matter removal at 74.9%. In run 2, despite increased OLR, due to 5% residual glycerine addition to the silage, there was a significantly higher ODM removal efficiency of 81.1% (Tukey's test at  $p < 0.05$ ).

Thus the 5% residual glycerine content in the feedstock was an appropriate co-substrate with a favorable effect on digestion performance. The enhancement of process efficiency by adding 5% glycerine phase (corresponding to 34.1% ODM) could result from its higher biodegradability compared to maize silage. Hongwei et al. [11] studied anaerobic biodegradability of 47 kinds of aliphatic compounds, i.e. carboxylic acids, esters, ketones, cycloalkanes, cycloalkenes, alcohols, and included glycerol. The batch experiments were conducted at 35°C for 50 d. Based on integrated assessment indices (IAI), tested compounds were classified into three groups: readily biodegradable (IAI > 1.0), partially biodegradable ( $0.3 < \text{IAI} \leq 1.0$ ) and poorly biodegradable ( $\text{IAI} \leq 0.3$ ). Glycerol had IAI = 1.44, and so was classed as readily biodegradable. The high biodegradability of glycerol after removing catalyst (KOH) by acidification with phosphoric acid, followed by vacuum distillation

with methanol and water, was also documented by Siles Lopez et al. [20]. Using granular sludge gave organics removal (expressed as COD) of about 100% [20]; implementation of non-granular sludge lowered substrate removal efficiency to 75%.

In contrast, an increase of glycerine content to 10% (run 3), brought about a strong decrease in the ODM removal efficiency to 30.6%. Hence, the majority of the organic matter was not converted into biogas, but remained in digestate, leading to an increase in post-methanation potentials. Since the OLRs of runs 2 and 3 did not significantly differ, reduction of organic dry matter removal efficiency was probably caused by the osmotic properties of glycerine phase. Glycerine penetrates bacterial cells by facilitated diffusion but cannot bring about an efflux of water from the cell; as a result, osmotic pressure rises and causes weakening of the membrane and cellular lysis depending on the resistance of the cell wall [18].

Zhang and Yang [24] showed the influence of glycerol concentration on propionic acid yield, and found no significant difference in productivities and yields between batch fermentation at glycerol concentrations of 20 and 40 g L<sup>-1</sup>. This suggested that glycerol osmotic pressure at such concentrations had a negligible effect on propionic acid fermentation kinetics. However, at glycerol concentration >60 g L<sup>-1</sup>, the cell growth was remarkably inhibited. In the present study there was no inhibition of the process at 5% (i.e. 50 g L<sup>-1</sup>) residual glycerine content in the feedstock, which confirmed a high efficiency of organic dry matter removal; however, increasing glycerine content to 10% significantly limited the organic degradation process.

### ***Stability of digestion performance***

Maintenance of an adequate buffering capacity and keeping the pH at a safe and stable level (Fig. 2) required addition of 5 M sodium hydroxide (NaOH) solution throughout the whole process.

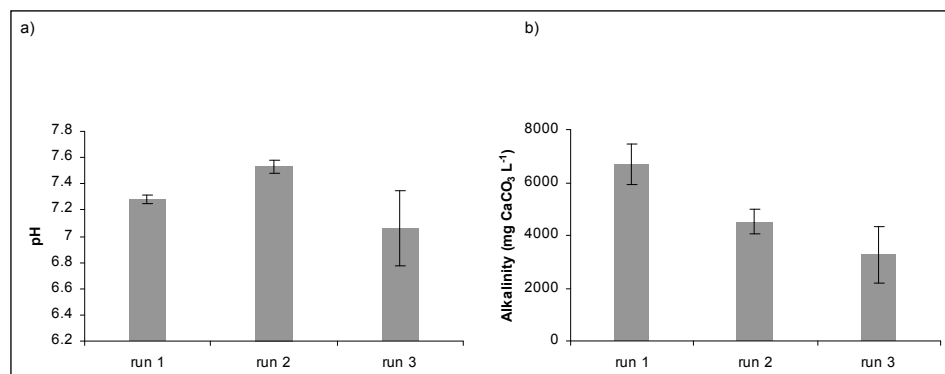


Fig. 2. Mean pH (a) and alkalinity (b) values after digestion of maize silage (run 1) and maize silage mixed with 5% (run 2) and 10% w/w (run 3) residual glycerine; (error bars represent standard deviations)

The pH of the digestate was approximately constant during experiments (Fig. 2), with a mean value of  $7.28 \pm 0.04$  using maize silage alone (run 1) and  $7.53 \pm 0.05$  using maize silage mixed with 5% glycerine phase (run 2). In the biogas digester fed with maize

silage containing 10% glycerine phase (run 3), pH averaged  $7.06 \pm 0.29$ , and was difficult to control despite adjustment with NaOH (shown by large standard deviations). These values remained within the optimal range for methanogens [7, 23].

The concentration of individual VFAs in the digestate, as well as specific biogas production rate and  $\text{CH}_4$  concentration, during anaerobic digestion of maize silage and its mixture with co-substrate are shown in Fig. 3.

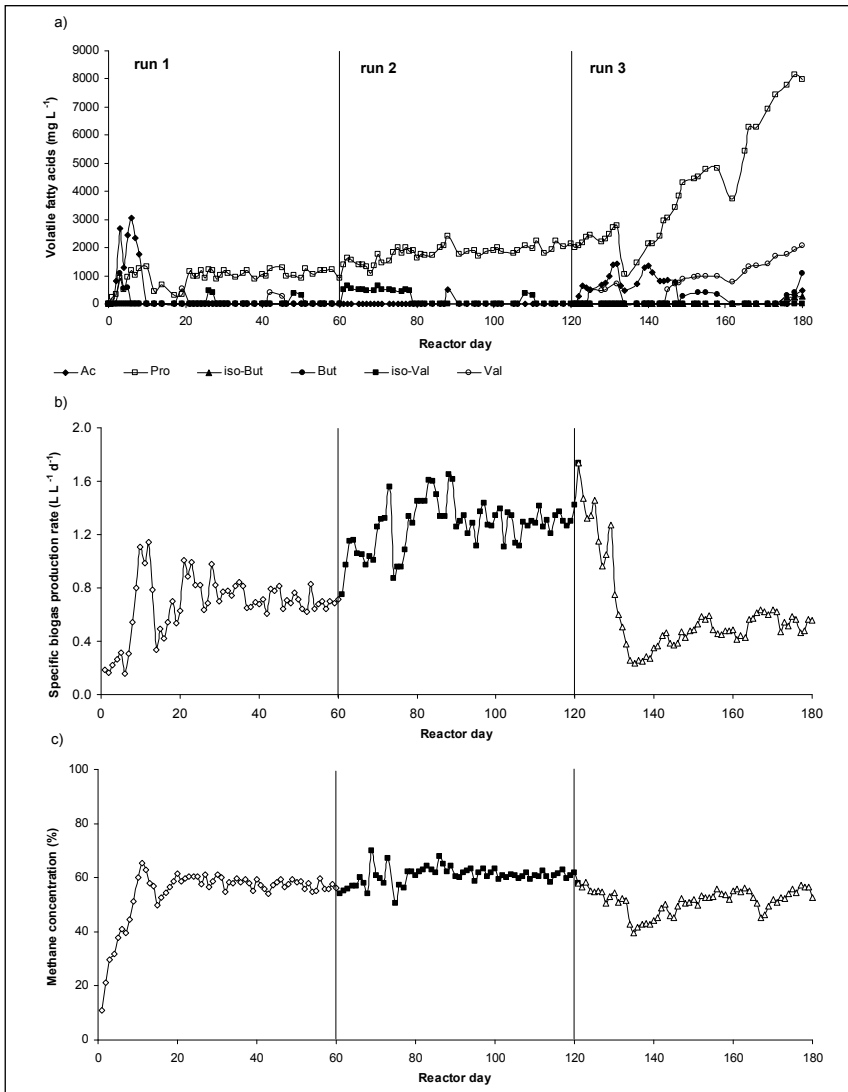


Fig. 3. Concentration of individual VFAs in the digestate (a); specific biogas production rate (b); and  $\text{CH}_4$  concentration (c) during mesophilic anaerobic digestion of maize silage and maize silage mixed with 5% and 10% w/w residual glycerine (Ac – acetic acid; Pro – propionic acid; iso-But – isobutyric acid; But – butyric acid; iso-Val – isovaleric acid; Val – valeric acid)

After commencing digestion of maize silage alone (run 1), acetic acid appeared in digestate liquor. From 10 d it was detected sporadically. Propionic acid was present at a mean concentration of  $1003.0 \pm 250.2 \text{ mg L}^{-1}$  throughout run 1. Specific biogas production rate was stable after 29 d of reaction and maintained an average of  $0.72 \pm 0.07 \text{ L L}^{-1}\text{d}^{-1}$  to the end of run 1.  $\text{CH}_4$  concentration during the stable phase averaged  $57.6 \pm 1.83\%$ . After addition of 5% (34.1% ODM) glycerine phase as a co-substrate, during the first 18 d of the experiment (61–78 d of reaction) propionic and isovaleric acids were detected in digestate liquor. The ratio of isovaleric to the dominant propionic acid ranged from 0.4 at the start to 0.25 at 18 d of run 2. Hill and Holmbert [10] showed that iso-acids are suitable indicators of the onset of digester instability, as confirmed in the present research. By 19 d (79 d of reaction), propionic acid dominated the digester at mean concentration of  $1933.4 \pm 183.4 \text{ mg L}^{-1}$ , and iso-valeric acid was not detected or appeared sporadically (Fig. 3a). This resulted from the adaptation of microorganisms to the glycerine content in the feedstock; after this, specific biogas production rate stabilized. Mean specific biogas production rate during 79–120 d of reaction was  $1.34 \pm 0.13 \text{ L L}^{-1}\text{d}^{-1}$  and  $\text{CH}_4$  concentration reached  $61.6 \pm 1.83\%$ . The results were significantly higher compared to those of maize silage alone (Tukey's test for  $p < 0.05$ ).

The increase of residual glycerine content to 10% (60.2% ODM) in run 3, had a negligible effect on propionic acid concentration but caused clear increases in concentrations of acetic and valeric acids, during the first 12 d (121–132 d of reaction). During this period there were significant decreases in specific biogas production rate from 1.73 to  $0.23 \text{ L L}^{-1}\text{d}^{-1}$  and  $\text{CH}_4$  concentration from 58.1 to 39.5%. This may be related to the inhibitory effect of 10% residual glycerine on methanogens, which cannot convert acetate into  $\text{CH}_4$ . After 133 d of reaction, there was an increase in propionic acid concentration; by 150 d, valeric acid was also found, and like propionic acid, its concentration tended to increase until the end of the experiment.

Compared to conventional substrates, such as glucose and other carbohydrates, glycerol has a much lower reducing state, which favors the production of more reduced metabolites (e.g. propionic acid) [4]. Conversion of glycerol to pyruvate would generate two moles of NADH per mole of glycerol. When pyruvate is converted to acetate, one additional mole of NADH would be generated, resulting in redox imbalance. However, only 1.44 moles of NADH would be consumed for each mole of pyruvate used in cell biomass production, causing accumulation of some NADH and a decrease in the  $\text{NAD}^+$  pool. Thus, cell growth on glycerol could be inhibited due to the higher NADH/ $\text{NAD}^+$  ratio. To maintain the redox balance in the cell, pyruvate must be converted to propionic acid, thus allowing consumption of the excess NADH.

Despite the increase of propionic acid content from 2150.6 to 7895  $\text{mg L}^{-1}$  during 140–180 d of reaction, specific biogas production rate and  $\text{CH}_4$  content was stable and reached a mean level of  $0.50 \pm 0.08 \text{ L L}^{-1}\text{d}^{-1}$  and  $51.9 \pm 3.47\%$ , respectively (Fig. 3b and c). However, the obtained values were significantly lower compared to runs 1 and 2 (Tukey's test for  $p < 0.05$ ). The results indicate that microorganisms adapted to high glycerine concentration through higher propionic acid production. The adapted biomass had acquired higher tolerance to the propionic acid as shown by stable biogas production by 140 d of reaction.

There are differences in the literature data concerning the influence of propionic acid on digestion processes. Concentrations of propionic acid  $>3000 \text{ mg L}^{-1}$  have

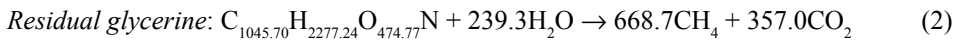
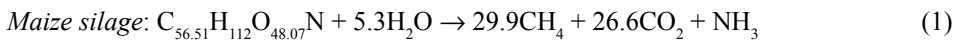


been previously shown to inhibit methanogenesis [5]; however, more recent studies by Pullammanappallil et al. [16] revealed that propionic acid was an effect rather than cause of inhibition of anaerobic processes – as also confirmed by the present research. Hajarnis and Ranade [9] examined the influence of propionic acid toxicity on methanogens and found that at propionic acid concentration of 5000 mg L<sup>-1</sup> and neutral pH, the CH<sub>4</sub> production was inhibited to 22–38% of control values; and a pH reduction drastically increased the inhibition. Pullammanappallil et al. [16] showed that propionic acid concentration up to 2750 mg L<sup>-1</sup> did not adversely affect CH<sub>4</sub> production even at pH <6.5. McCarty and Brousseau [13] reported that propionate concentration of up to 8000 mg L<sup>-1</sup> was tolerated in anaerobic digesters. Similarly, in the present study, due to microorganism adaptation, propionic acid at concentration around 7900 mg L<sup>-1</sup> and pH 7.06 ± 0.29 did not inhibit CH<sub>4</sub> formation; however, specific biogas production rate and CH<sub>4</sub> concentration were low.

To sum up, addition of glycerine phase to maize silage influenced both OLR and chemical composition of the feedstock. The experiments showed that specific biogas rate after the addition of 5% residual glycerine increased by approximately 86% in relation to maize silage alone that resulted from OLR increase. A higher residual glycerine addition (10%) slightly increased OLR, but the clear decrease in specific biogas rate was probably due to the inhibiting effect of glycerine phase.

#### ***Effect of residual glycerine supplementation on biogas potential***

In the present study, based on elementary composition of the feedstock, theoretical biogas and CH<sub>4</sub> potential (TBP and TMP, respectively; Table 4) were estimated and compared with biogas and CH<sub>4</sub> yield obtained experimentally. For calculations O'Rourke's equation [15] was applied. Assuming the elementary composition of the maize silage and residual glycerine (Table 1), equations (1) and (2) were formulated to calculate the theoretical number of moles of CH<sub>4</sub> and CO<sub>2</sub> produced from one mole of feedstock:



Theoretical biogas potential for maize silage amounted to 842.1 L kg<sup>-1</sup> ODM and 445.3 L CH<sub>4</sub> kg<sup>-1</sup> ODM. For residual glycerine, TBP and TMP were higher and amounted to 1155.6 and 761.1 L kg<sup>-1</sup> ODM, respectively. Supplementation of maize silage with 5% residual glycerine increased the TBP by 10% and TMP by 20% compared to maize silage alone, whereas at 10% glycerine content TBP and TMP were 17.5 and 38% higher, respectively.

Measured biogas/CH<sub>4</sub> yield (Y) was calculated as the slope of the regression line of accumulated biogas/CH<sub>4</sub> per accumulated added organic dry matter for the stable process (Fig. 4a–c). The process was considered to be stable by several weeks after feeding had started, when there were no operational disturbances. Experimental data showed process stability by 29, 19 and 20 d of runs 1–3, respectively, until the end of the experiment.

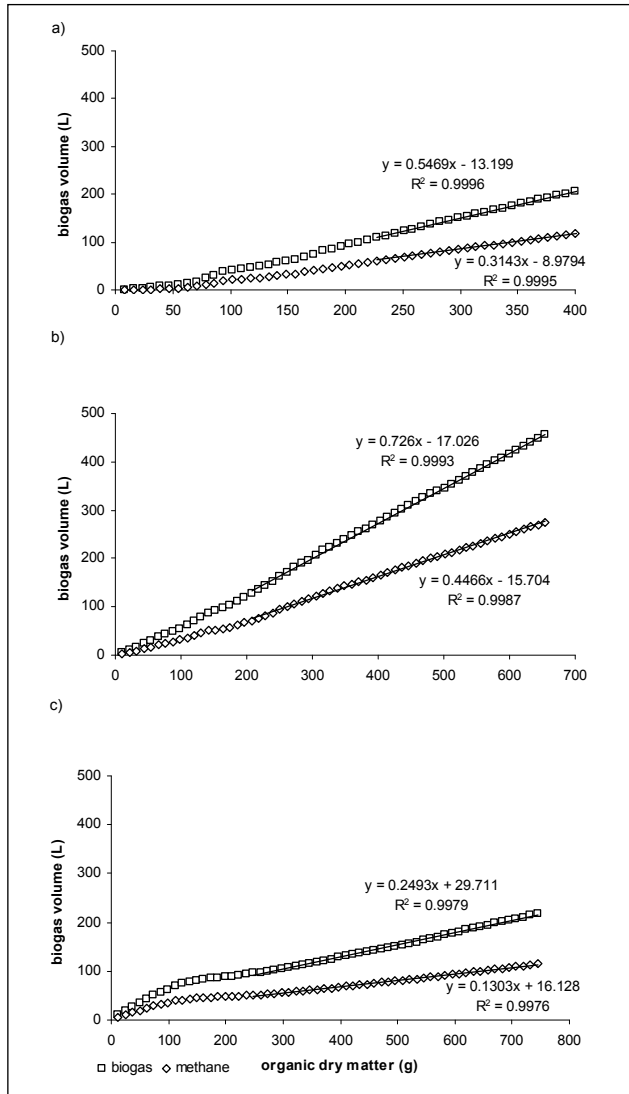


Fig. 4. Accumulated biogas and CH<sub>4</sub> production versus added ODM during mesophilic anaerobic digestion of maize silage (run 1) and maize silage mixed with 5% (run 2) and 10% w/w (run 3) residual glycerine

Supplementation of maize silage with 5% residual glycerine increased the measured biogas ( $Y_{\text{biogas}}$ ) and CH<sub>4</sub> ( $Y_{\text{methane}}$ ) yields compared to controls (maize silage alone); however, for 10% glycerine phase,  $Y_{\text{biogas}}$  and  $Y_{\text{methane}}$  were lower (Table 4).

For maize silage alone (run 1)  $Y_{\text{biogas}}$  and  $Y_{\text{methane}}$  values comprised 69 and 74% of TBP and TMP, respectively (Fig. 5). Similarly, higher  $Y_{\text{methane}}/\text{TMP}$  ratio in relation to  $Y_{\text{biogas}}/\text{TBP}$  was obtained for maize silage supplemented with 5% glycerine phase (run 2). According to Weiland [22], the real CH<sub>4</sub> content is generally higher in practice than the

Table 4. Feedstock load, and theoretical and observed potential of biogas and CH<sub>4</sub> for maize silage (run 1) and its mixture with 5% (run 2) and 10% w/w (run 3) residual glycerine

Parameter	Unit	Run 1	Run 2	Run 3
Feedstock load ( $F_{\text{added}}$ ):	g ODM d <sup>-1</sup>	7.84	10.90	12.40
– maize silage	% ODM	100	66.1	40.0
– glycerine	% ODM	0.0	33.9	60.0
Theoretical biogas potential (TBP):	L g <sup>-1</sup> ODM	0.80	0.88	0.94
– maize silage		0.80	0.52	0.32
– glycerine		0.0	0.35	0.62
Theoretical methane potential (TMP):	L g <sup>-1</sup> ODM	0.42	0.51	0.58
– maize silage		0.42	0.28	0.17
– glycerine		0.0	0.23	0.41
Measured biogas yield ( $Y_{\text{biogas}}$ )	L g <sup>-1</sup> ODM	0.55	0.73	0.25
Measured methane yield ( $Y_{\text{methane}}$ )	L g <sup>-1</sup> ODM	0.31	0.45	0.13

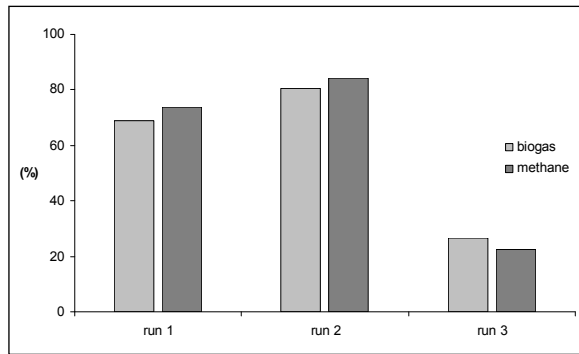


Fig. 5. Percentage of measured biogas yield ( $Y_{\text{biogas}}$ ) and CH<sub>4</sub> yield ( $Y_{\text{methane}}$ ) in relation to theoretical biogas potential (TBP) and CH<sub>4</sub> potential (TMP) during mesophilic anaerobic digestion of maize silage (run 1) and maize silage mixed with 5% (run 2) and 10% w/w (run 3) residual glycerine

theoretical values, due to part of CO<sub>2</sub> being solubilized in the digestate. In contrast, in run 3 the addition of 10% glycerine decreased both ratios, but  $Y_{\text{methane}}/\text{TMP}$  was lower than  $Y_{\text{biogas}}/\text{TBP}$ . This could be explained by the inhibitory impact of glycerine phase at 10% concentration leading to accumulation of propionic and valeric acids in the digestate and limited CH<sub>4</sub> production.

The supplementation of maize silage with 5% glycerine phase was beneficial in regard to biogas yield. Amon et al. [1] achieved analogous results in 1-L eudiometer batch experiments, in which they investigated the effect of increasing glycerine dosage (3, 6, 8 and 15%) on CH<sub>4</sub> yield during anaerobic digestion of a pig manure, maize silage and maize grain mixture (54:31:15 fresh matter basis). They found the highest specific biogas and CH<sub>4</sub> yields in feedstock enriched with 6% glycerine (0.68 and 0.44 L g<sup>-1</sup> ODM, respectively) – a result that corresponds to data obtained in the present study. At higher

concentrations the CH<sub>4</sub> yield diminished, achieving 0.37 L g<sup>-1</sup> ODM at 8% glycerine content. However, for all tested glycerine dosages they found increased specific biogas yield and CH<sub>4</sub> content in biogas compared to controls (basic mixture without glycerine). In the present research conducted in CSTR, at 10% glycerine phase in feedstock, there were decreases in specific biogas production rate. The discrepancy between these two sets of data could result from the use of a continuously operated reactor and distinct feedstock chemical compositions.

## CONCLUSIONS

Our results revealed that residual glycerine derived from biodiesel manufacturing had a favorable effect on anaerobic digestion of maize silage when it contributed 34.1% of ODM (5% w/w) in the feedstock. Compared to maize silage alone, there was higher specific biogas production (1.29 ± 0.09 vs. 0.72 ± 0.07 L L<sup>-1</sup>d<sup>-1</sup>) and measured biogas yield in relation to the TBP (81 vs. 69%). The increase of residual glycerine to 60.2% ODM (10% w/w) caused an accumulation of propionic and valeric acids in digestate liquor, and inhibitory effects were evident in the significant reduction in efficiency of organics consumption (as ODM) and, in consequence, a decreased specific biogas production rate.

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#### WYTWARZANIE BIOGAZU PODCZAS WSPÓLNEJ FERMENTACJI KISZONKI KUKURYDZY ZWYCZAJNEJ I FRAKCJI GLICERYNOWEJ Z PRODUKCJI BIODIESLA

W pracy badano wpływ frakcji glicerynowej w stężeniu 5 i 10% wag. na produkcję biogazu z kiszonki kukurydzy zwyczajnej. Doświadczenie prowadzono w skali laboratoryjnej, w układzie jednostopniowym. Hydrauliczny czas zatrzymania (HRT) i temperatura fermentacji wynosiły odpowiednio 60d i 39°C. Udział frakcji glicerynowej substracie w stężeniu 5% wag. spowodował wzrost obciążenia ładunkiem organicznym (OLR) do 1,82 g s.m.o./dm<sup>3</sup>·d w porównaniu do OLR podczas fermentacji samej kiszonki (1,31 g s.m.o./dm<sup>3</sup>·d). Jednostkowa szybkość produkcji biogazu oraz współczynnik wydajności biogazu wyniosły odpowiednio 1,34 dm<sup>3</sup>/dm<sup>3</sup>·d oraz 0,71 dm<sup>3</sup>/g s.m.o. i były o 86% oraz 30% wyższe w porównaniu do wartości uzyskanych dla samej kiszonki. Gdy stężenie frakcji glicerynowej w substracie wzrosło do 10% wag. (OLR = 2,01 g s.m.o./dm<sup>3</sup>·d) jednostkowa szybkość produkcji biogazu oraz współczynnik wydajności biogazu wyraźnie zmalały do 0,50 dm<sup>3</sup>/dm<sup>3</sup>·d i 0,13 dm<sup>3</sup>/g s.m.o. co oznacza, że w stężeniu 10% wag. frakcja glicerynowa miała inhibicyjny wpływ na wzrost metanogenów i konwersję substancji organicznych do biogazu. W rezultacie następowała kumulacja kwasów propionowego i walerianowego w wodzie osadowej.