

## **Effect of different compositions on anaerobic co-digestion of cattle manure and agro-industrial by-products**

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**Abstract.** The present research is dedicated to the study of anaerobic co-digestion process of different biomass materials. Anaerobic co-digestion of digested sludge, grass silage, haylage and cattle manure was evaluated in mesophilic tank reactors in the lab-scale experiment. Twelve laboratory scale tank reactors (1.5 L) were used during the incubation period of 45 days. First triplet of reactors was fed with pure digested sludge and the other three with different mixtures having the volumetric ratios of 30/35/25/10, 40/30/20/10 and 50/25/15/10 for digested sludge/corn silage/grass haylage/cattle manure. Methane production was analyzed for all lab-scale reactors individually. The resulting specific methane production of above-mentioned batches was 336.34, 238.1 and 233.23 L<sub>STP</sub>[CH<sub>4</sub>] kg<sup>-1</sup>[TVS], respectively. Other results such as cumulative biogas and methane yield, volumetric biogas and methane yield, volumetric biogas and methane yield per day were also assessed. These results had the highest meaning when complex substrate had no more than 30% of inoculum.

**Key words:** batch reactor experiments, cattle manure, co-digestion, crop residues, volumetric ratios.

### **INTRODUCTION**

The need for energy to meet the social and economic needs of mankind is increasing. All societies need energy to meet basic human needs such a cooking, lighting, space comfort, mobility, communication, etc. (IPCC, 2012). Global energy demand raised ~2.7-fold from 5,000 Mtoe (million tonnes of oil equivalent) in 1971 to 13,276.3 Mtoe in 2016 (BP, 2017). At the present time, fossil fuels satisfy 80% of global energy consumption. Even if the current policy commitments adopted by countries on climate change and other energy-related issues are implemented, global energy demand is projected to grow by 40% in 2035, and the share of fossil fuels will still be 75%. (IEA, 2013). World proven coal reserves will be enough just over the next 153 years for use,

and oil and gas over ~52.5 (BP, 2017). These terms do not look promising indeed, and, thus, the need for alternative energy sources development becomes topical for mankind.

Recently, the use of RES (renewable energy sources), non-fossil energy resources in particular, has become an essential component of sustainable global energy strategy (Song et al., 2014). Renewable energy accounted for 19.3% of the global final energy consumption, and growth continued in 2016. In 2016 the renewable energy sector employed 9,8 million people. Renewable power generating sector showed its biggest growth in 2016, with an estimated 161 GW (gigawatts) of capacity added. ~9% of global total heat demand is supplied by renewable energy sector. In 2016, liquid biofuels provided around 4% of world road transport fuels. The use of biogas for the needs of the transport sector has increased significantly in the USA and continued to share in the fuel mix in European Union (REN21, 2017).

In 2015, world TPES (Total Primary Energy Supply) was 13,647 Mtoe, of which 13.4%, or 1,823 Mtoe (up from 1,784 Mtoe in 2014), was from RES. The share of solid biomass/fuelwood/charcoal is a biggest among RES, it provides 63.7% of global renewables supply. The hydro power is the second one, which represents 18.3% of renewable energy supply or 2.5% of world TPES. Biogases, liquid biofuels, solar, wind, geothermal, and tide constitute the rest of the renewables energy supply. Since 1990, RES have grown at an average annual rate of 2%, which is slightly higher than the growth rate of world TPES, 1.8%. Biogases had the third highest growth rate at 12.8%, followed by solar thermal (11.4%) and liquid biofuels (10.1%) (IEA, 2017).

Biomass energy is an encouraging resource for meeting upcoming needs for energy consumption. Sustainable bioenergy represents a huge potential for making a significant contribution to rural and economic development, enhancing energy security and reducing environmental impact. From biomass, modern energy carriers can be obtained that are clean, easy to use and have little or no connection with GHG (greenhouse gas) emissions. At present, various biomass conversion technologies are available or under development (Turkenburg et al., 2012). Carbonization, pyrolysis, gasification and AD (anaerobic digestion) are the main technologies by means of which biomass can be converted to useful renewable energy carriers such as biodiesel, ethanol, butanols, biomethane, hydrogen, DME (dimethyl ether) and other fuels and fuels additives. (Li et al., 2016). Pathways of biomass conversion as a transesterification or hydrogenation, fermentation or microbial processing, bio-photochemical and other biological and chemical routes also exist or under development (REN21, 2015). Among above-mentioned pathways AD (anaerobic digestion) technology is quite promising option, resulting in a useful energy carrier – methane. In short, AD is a microbial and biochemical process that results in the formation of a mixture of gases – biogas, mainly consisting of CH<sub>4</sub> and CO<sub>2</sub>. AD process consists of four steps; hydrolysis, acidogenesis, acetogenesis and methanogenesis (Hassan et al., 2017a). AD is divided into three categories of wet ( $\leq 10\%$ ), semi-dry (10–20%) and dry ( $\geq 20\%$ ) in accordance with feedstock total solid (TS) content. Dry AD is more favorable than wet AD, due to the smaller volume of the reactor and lower energy requirements for heating (Elsamadony & Tawfik, 2015). Feedstock for biogas production vary depending on the country. In Europe, biogas is produced from manure, agricultural waste and energy crops (accounting for 5.1 GW of power production capacity), landfill gas (1.4 GW), and smaller amounts of sewage sludge and other sources. In the Czech Republic growth of biogas manufacturers was particularly strong (+15%) in 2015 (REN21, 2015).

Animal manure as a substrate for AD is an easily available resource worldwide. However, sometimes the capital costs for industrial biogas plants are not justified because of low biogas yield from manure as a single substrate (Cavinato et al., 2010). Usually, manure has a low total solids (TS) concentration: approximately 7–9 & for cattle manure and 5–7 & TS for pig manure. Lignocellulose high content fraction contained in manure represents an extremely resistant to degradation fraction – fiber. A high fraction of fibers with high water content result in poor methane production of animal manure and it usually ranges from 10 to 20 m<sup>3</sup> CH<sub>4</sub> t<sup>-1</sup> [fresh weight]. Animal manure is considered to be the one of the most convenient substrate for initiating the fermentation reaction because it includes all the necessary microorganisms (Cavinato et al., 2010).

Co-fermentation of various substrates showed a better effect than mono-fermentation. Typically, each organic substrate rich in nutrients is required for anaerobic and aerobic bacteria growth. Nevertheless, the nutrient level differences are correlated with material age, species and growth conditions (Divya et al., 2015).

Co-digestion offers benefits such as: dilution of toxic compounds, increased biogas yield odor and pathogen reduction, enhanced nutrients balance, synergistic effect of microorganisms and increased weight of biodegradable organic substance (Sosnowski et al., 2008). Progressive studies of the C/N ratio (carbon to nitrogen ratio) optimization cases during co-fermentation conducted by Hassan et al. (2016), where co-digestion had enhanced the methane production from 31.49% to 85.11%. Rahman et al. (2017) showed that the best quality and a greater quantity of biogas can be produced as a result of the optimal selection of the C/N ratio of the complex substrate. Organic agricultural wastes have a large content of carbon and animal manure has a high content of nitrogen. Proper co-digestion of these two components increases biogas yield and improves the methane content (Bagudo et al., 2011; Rahman et al., 2017).

AcoD of different substrates with animal manure can increase the biogas production from 25 to 400% (Shah et al., 2015) and also offers benefits for the management of animal manure and organic wastes (Li et al., 2013). The concept of AcoD of different substrates is not a new idea since it has been investigated for a number of combinations of organic waste. At present, many studies were reported about AcoD of animal manure and agriculture biomass that it is considered as the most substantial topic within research of AD. Hassan et al. (2017b) conducted AcoD by using goose manure with alkali solubilized wheat straw in order to optimize C/N ratio and organic loading rate regression. A significant enhancement of biogas yield was achieved by Wu et al. (2010) during co-digesting of swine manure with an external source of carbon – crop residues (corn stalks, oat and wheat straw). Another studies were carried out on AcoD of nitrogen rich substrate – chicken manure and corn stover (Li et al., 2014a) and organic fraction of municipal solid waste (Matheri et al., 2017). All the above-mentioned researches stated significant biogas production improvement as a result of AcoD. CM (cattle manure) is the most widespread substrate for AD which has been assessed over the last 3–4 decades and is described as an excellent ‘carrier’ substrate that used by industrial biogas plants as a base substrate for AcoD (Andriamanohiarisoamanana et al., 2016). Recent researchers reported significant biogas production enhancement when CM was digested with another co-substrate such a domestic food waste (Zhang et al., 2012), cheese whey (Comino et al., 2012), maize (Amon et al., 2007) and crop residues (Li et al., 2014b). But, despite the fact that AcoD has several advantages, this technology is

still a challenging biomass treatment process, and even two-stage reactor technologies cannot solve the optimization and stability problems (Hagos et al., 2017). The methodology of this study – BMP (biomethane potential) test is the same to above-mentioned studies and novelty lies in the screening of different composition of complex substrate and types of co-substrates.

There is a close link between the biogas development intensification and agriculture industrialization and modernization (Mao et al., 2015). In order to make AD technologies more attractive and profitable for agricultural sector, co-digestion of manure with come-at-able agricultural byproducts can increase methane yield. The objective of this study was to evaluate the methane production during batch-experiments of screening trials with the determination of the best complex substrate of compositions 30/35/25/10, 40/30/20/10 and 50/25/15/10 for digested sludge/corn silage/grass haylage/cattle manure, accordingly.

## MATERIALS AND METHODS

### *Feedstock sources*

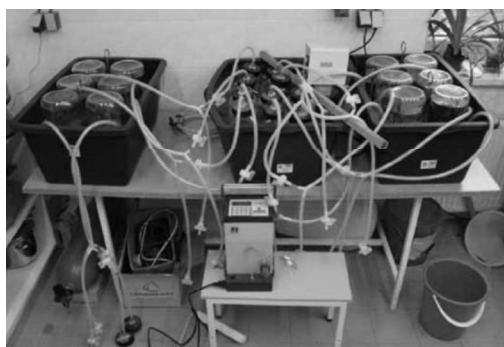
Three types of substrates were used in this study: corn silage (CS), grass haylage (GH), and cattle manure. CS and GH were the raw materials obtained from the crop rotation of the given farm situated in Mořina city, Czech Republic.

The digested sludge (DS) as an active inoculum used for starting up the fermentation was brought from large-scale BPP (biogas power plant) situated in Mořina city (Czech Republic), which operates at mesophilic mode and has 526 kW installed electric power. This BPS treats primarily cattle manure with some agricultural byproducts. The BPS operates at 41 °C. The effluent was transported in 20-liter plastic vessels. The temperature was always kept above the freezing point. In order to readapt the inoculum to 41 °C, ensure removing dissolved CH<sub>4</sub>, the inoculum was stored under anaerobic condition for 5 days in the incubator at the 41 °C.

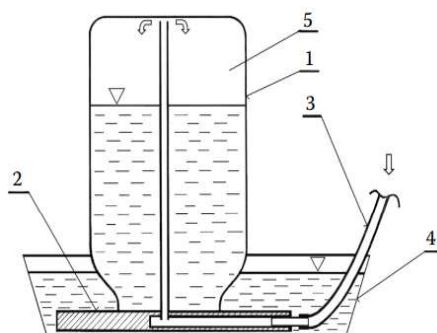
### *Experimental batch-up*

Wet anaerobic co-digestion investigation under mesophilic conditions for biogas production and biochemical methane potential (BMP) from CS, GH, CM and DS were carried out. The research was batchtled up using 12 single-stage lab-scale reactors (1,500 mL plastic vessels). The reactors were positioned into the reservoir with water thermostat (Fig. 1).

Subsequently, numerous volumetric ratios (batches) of substrates (DS/CS/GH/CM) were dosed into the reactors and closed tightly. Each batch had three replications. All the reactors were run at the same time under the temperature of 41 °C. The batches were blown out with nitrogen to remove air, which contains oxygen. The pH inside the digesters was not controlled during the experiment. The amount of evolved biogas was measured every day during the incubation period 45 days. The biogas was collected by the downward displacement method which is based on the water displacement principle (Fig. 2). Gradated transparent glass jar of 3 L were placed into distilled water basin. The bubbles of biogas produced pass through the water layer and push the water level down in the vessel (Wang et al., 2014).



**Figure 1.** Lab-scale reactors for biogas production inside reservoir with accurate water thermostat.



**Figure 2.** Equipment for measuring biogas volume based on water-column measuring system: 1 – glass jar; 2 – basic plate; 3 – biogas input; 4 – water basin; 5 – biogas produced.

Morina biogas plant at that time had problems with the production of biogas (high pH level about 8 and high concentration of ammonia nitrogen more than  $7 \text{ g L}^{-1}$ ). In order to stabilize the anaerobic digestion process in a lab-scale experiment different mixtures of DS, CS, GH and CM were used. Mixtures of DS, CS, GH and CM were poured into the reactors on a wet weight basis for attainment suitable total solid (TS) content of 8 & for each mixture except of single substrate blank (see Table 1). Three reactors were fed only with an (effluent) inoculum, i.e. used as blank. The biogas yield from the blanks was subtracted from other batches when calculated.

**Table 1.** Batch assay parameters

Trial	Incubation period, days	Batch digester volume, mL	Blend weight, g	Initial blend TS content, %	DS, %	CS, %	GH, %	CM, %
1	45	1,500	1,000	8.0	30	35	25	10
	45	1,500	1,000	8.0	30	35	25	10
	45	1,500	1,000	8.0	30	35	25	10
2	45	1,500	1,000	8.0	40	30	20	10
	45	1,500	1,000	8.0	40	30	20	10
	45	1,500	1,000	8.0	40	30	20	10
3	45	1,500	1,000	8.0	50	25	15	10
	45	1,500	1,000	8.0	50	25	15	10
	45	1,500	1,000	8.0	50	25	15	10
4	45	1,500	1,000	7.6	100	0	0	0
	45	1,500	1,000	7.6	100	0	0	0
	45	1,500	1,000	7.6	100	0	0	0

TS: Total solid; DS: Digested sludge; CS: Corn silage; GH: grass haylage; CM: Cattle manure.

### ***Analytical methods***

Characteristics of substrates were quantified using common procedures described by Standard methods for the examination of water and wastewater (APHA, 2005). In brief, TS was analyzed by filtering a well-mixed sample through a weighed standard glass-fiber filter and the residue retained on the filter was dried to a constant weight at  $104 \text{ }^\circ\text{C}$  for 40 minutes (total time to reach a constant mass of the sample). The residue

from first method was ignited to constant weight at 550 °C for 30 minutes. The remaining solids represent the fixed total, dissolved, or suspended solids while the weight lost on ignition is the total volatile solids (TVS). Characteristics of the influent substrates are shown in the Table 2.

**Table 2.** Characteristics of influent substrates

	Units	DS	CS	GH	CM
pH		7.90	3.80	4.73	7.00
TS (initial)	%, FM basis	7.60	27.40	26.50	5.50
TVS (initial)	%, TS basis	5.74	26.25	23.85	3.93
Ash	%, FM basis	1.68	1.47	1.19	1.15

TS: Total solids. TVS: Total volatile solids; FM: Fresh matter. DS: Digested sludge; CS: Corn silage; GH: grass haylage; CM: Cattle manure.

Methane and carbon dioxide content of the evolved biogas was analyzed by Aseko Gas Analyzer AIR LF which measures the composition of biogas with linear NDIR technology (nondispersive infrared sensor). This measuring method by absorption of infrared radiation is highly resistant and provides stable measured data. Measured components are: CH<sub>4</sub>, CO<sub>2</sub>, O<sub>2</sub>, H<sub>2</sub>S, H<sub>2</sub>, and NH<sub>3</sub>; gas flow – 0.5–0.7 L min<sup>-1</sup>; repeatability – ± 2–3% f.s. (full scale); response time – 90–20 s.

## RESULTS AND DISCUSSION

### *Production and composition of biogas*

The results of this experiment are presented in the Table 3. According to displayed data all 3 batches had expressively increased overall methane yields compared with previously reported data when cattle manure was fermented alone. Particularly, Ashraf et al. (2016) have reported average methane yield from CM about 90–100 L[CH<sub>4</sub>] kg<sup>-1</sup>[TVS]. Also, Angelidaki & Ellegard (2003) and Zhang et al. (2013) published that average methane yield from CM is approximately 150–200 L[CH<sub>4</sub>] kg<sup>-1</sup>[TVS]. These values are less than the results obtained by this study twice in terms of methane produced per kg of TVS.

Both, the highest specific biogas production per kg of TVS ( $674.38 \pm 126.54$  L<sub>STP</sub>[CH<sub>4</sub>] kg<sup>-1</sup>[TVS]) and the highest specific methane production per kg of TVS ( $336.34 \pm 110.18$  L<sub>STP</sub>[CH<sub>4</sub>] kg<sup>-1</sup>[TVS]) were obtained when complex substrate had the ratio 30/35/25/10 for DS, CS, GH and CM, respectively. This is very similar to a previous study (Westerholm et al., 2012) when methane potential had been enhanced through co-digestion batch experiments of cattle manure with whole stillage – 460 L[CH<sub>4</sub>] kg<sup>-1</sup>[TVS] after 60 days of fermentation. However, biochemical methane potential exceeded some other reported co-digestion investigations as of Cagri et al. (2016) when cow manure and barley were digested at different ratio and from the best one (1/1) specific methane yield – 230 L[CH<sub>4</sub>] kg<sup>-1</sup>[TVS] was obtained.

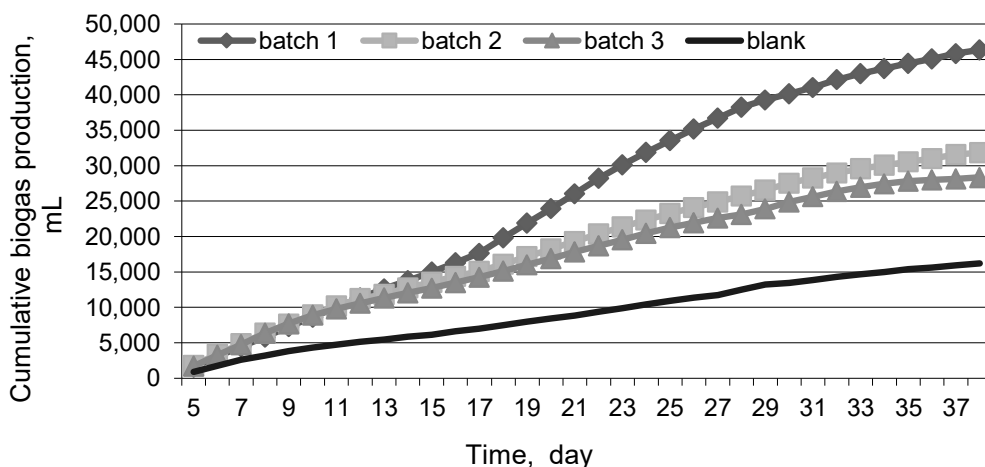
Other results in terms of biogas yield and composition such as: cumulative biogas yield (Fig. 3) and cumulative methane yield (Fig. 4); volumetric biogas and methane yield (L<sub>STP</sub>[biogas] L<sup>-1</sup>[wet weight]); volumetric biogas and methane yield per day (mL[methane] L<sup>-1</sup>[wet weight] d<sup>-1</sup>) also had the highest meaning when complex substrate

had no more than 30% of DS (see Table 3). Thus, increasing the volume of DS above 30% has not resulted in advanced methane yield.

**Table 3.** Chemical composition of the batches, biogas and methane yields obtained during incubation period – 45 days (mean  $\pm$  standard deviation of 3 determinations)

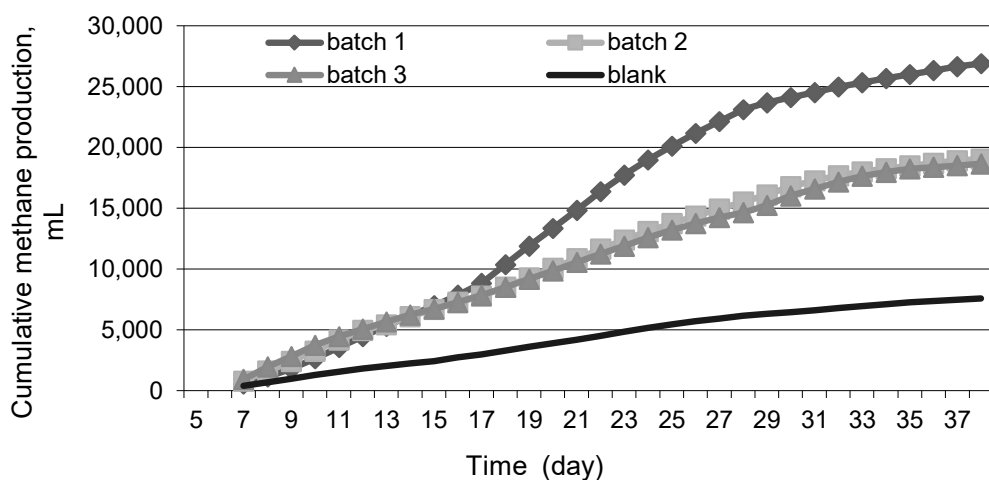
	DS/CS/GH/CM ratios			
	30/35/25/10	40/30/20/10	50/25/15/10	100% DS
Initial TS (% , FM basis)	8.00 $\pm$ 0.1	8.00 $\pm$ 0.1	8.00 $\pm$ 0.1	7.6 $\pm$ 0.1
Final TS (% , FM basis)	4.85 $\pm$ 0.4	5.08 $\pm$ 0.5	5.47 $\pm$ 0.4	6.55 $\pm$ 0.2
Initial TVS (% , FM basis)	6.87 $\pm$ 0.2	6.73 $\pm$ 0.2	6.59 $\pm$ 0.2	5.74 $\pm$ 0.2
Final TVS (% , FM basis)	3.68 $\pm$ 0.5	3.53 $\pm$ 0.6	4.06 $\pm$ 0.3	4.89 $\pm$ 0.1
Ash (% , FM basis)	1.87 $\pm$ 0.2	1.58 $\pm$ 0.1	1.23 $\pm$ 0.2	1.39 $\pm$ 0.2
Initial pH	6.79 $\pm$ 0.9	6.68 $\pm$ 0.2	6.94 $\pm$ 0.2	7.97 $\pm$ 0.0
Final pH	7.48 $\pm$ 0.1	7.47 $\pm$ 0.1	7.43 $\pm$ 0.1	7.55 $\pm$ 0.1
Cumulative biogas yield (L)	46.33 $\pm$ 7.7	32.18 $\pm$ 4.6	28.33 $\pm$ 1.5	16.18 $\pm$ 2.8
Volumetric biogas yield (L <sub>STP</sub> [biogas] L <sup>-1</sup> [wet weight])	46.33 $\pm$ 7.7	32.18 $\pm$ 4.6	28.33 $\pm$ 1.5	16.18 $\pm$ 2.8
Cumulative biogas yield (mL[biogas] L <sup>-1</sup> [wet weight] d <sup>-1</sup> )	1,029.56 $\pm$ 170.7	715.11 $\pm$ 101.8	629.56 $\pm$ 32.5	359.56 $\pm$ 62.0
Specific biogas yield (L <sub>STP</sub> [biogas] kg <sup>-1</sup> [TVS])	674.38 $\pm$ 126.5	478.16 $\pm$ 78.4	429.89 $\pm$ 31.9	281.88 $\pm$ 55.8
Cumulative methane yield (L)	26.91 $\pm$ 8.82	19.06 $\pm$ 6.54	18.66 $\pm$ 1.64	7.59 $\pm$ 2.49
Volumetric methane yield (L <sub>STP</sub> [methane] L <sup>-1</sup> [wet weight])	26.91 $\pm$ 8.82	19.06 $\pm$ 6.54	18.66 $\pm$ 1.64	7.59 $\pm$ 2.49
Cumulative methane yield (mL[methane] L <sup>-1</sup> [wet weight] d <sup>-1</sup> )	598 $\pm$ 196.0	423.55 $\pm$ 145.3	414.67 $\pm$ 36.4	186.67 $\pm$ 55.3
Specific methane yield (L <sub>STP</sub> [CH <sub>4</sub> ] kg <sup>-1</sup> [TVS])	336.34 $\pm$ 110.18	238.1 $\pm$ 81	233.23 $\pm$ 20.5	99.8 $\pm$ 32.49

TS: Total solid; TVS: Total volatile solids; FM: Fresh matter; DS: Digested sludge; CS: Corn silage; GH: grass haylage; CM: Cattle manure.



**Figure 3.** Cumulative biogas production of experimental batches.

Batch 1: 30% digested sludge (DS) + 35% corn silage (CS) + 25% grass haylage (GH) + 10% cattle manure CM; Batch 2: 40% DS + 30% CS + 20% GH + 10% CM; Batch 3: 50% DS + 25% CS + 15% GH + 10% CM; Batch 4: 100% DS. DS: Digested sludge; CS: Corn silage; GH: grass haylage; CM: Cattle manure.



**Figure 4.** Cumulative methane production of experimental batches.

Batch 1: 30% digested sludge (DS) + 35% corn silage (CS) + 25% grass haylage (GH) + 10% cattle manure CM; Batch 2: 40% DS + 30% CS + 20% GH + 10% CM; Batch 3: 50% DS + 25% CS + 15% GH + 10% CM; Batch 4: 100% DS; DS: Digested sludge; CS: Corn silage; GH: grass haylage; CM: Cattle manure.

**Table 4.** Biogas and methane yields obtained from the batches with distracted results of control sample portion in mixture (mean  $\pm$  standard deviation of 3 determinations)

	DS/CS/GH/CM ratios		
	0/35/25/10	40/30/20/10	50/25/15/10
Cumulative biogas yield (l)	9.25 $\pm$ 12.2	42.84 $\pm$ 9.5	40.48 $\pm$ 5.7
Volumetric biogas yield ( $L_{STP}[\text{biogas}] L^{-1}[\text{wet weight}]$ )	9.25 $\pm$ 12.2	42.84 $\pm$ 9.5	40.48 $\pm$ 5.7
Specific biogas yield ( $L_{STP}[\text{biogas}] \text{kg}^{-1}[\text{TVS}]$ )	42.594 $\pm$ 204.7	609.01 $\pm$ 167.9	577.9 $\pm$ 119.7
Cumulative methane yield (l)	2.7 $\pm$ 12.23	23.65 $\pm$ 9.3	24.2 $\pm$ 3.73
Volumetric methane yield ( $L_{STP}[\text{methane}] L^{-1}[\text{wet weight}]$ )	2.7 $\pm$ 12.23	23.65 $\pm$ 9.3	24.2 $\pm$ 3.73
Specific methane yield ( $L_{STP}[\text{CH}_4] \text{kg}^{-1}[\text{TVS}]$ )	07.32 $\pm$ 153.04	293.42 $\pm$ 126,4	300.04 $\pm$ 47.22

TVS: Total volatile solids; DS: Digested sludge; CS: Corn silage; GH: grass haylage; CM: Cattle manure.

Agricultural by-products, including those used in this study, cannot be digested by itself, thus extra methane-producing bacteria are needed to start the digestive process. (Yu et al., 2014). Inoculum is usually dosed/added in weight concentrations 10–20% of dry matter (i.e. 10% from 8% of dry matter). In the present experiment the concentrations of 30, 40 and 50% were applied due to the fact that previous attempts from the same biogas plant didn't succeed in batch anaerobic laboratory process properly going. The reason could be an application of antibiotics in cattle stables (10% of the experimental mixture are cattle manure). However, the objective of this research was to determine whether the mixture composition is appropriate for the AD in given biogas plant and the reduction of gas production is not caused by the substrate composition. The control sample – DS from biogas plant's reactor (batch 4) was subjected to laboratory fermentation in three repetitions as well. The results of the biogas production/yield of



this sample were then used to read out adequate biogas yield produced by inoculum from the mixed sample. Summarizing Table 4 presents average yields of biogas and methane of the mixed samples minus adequate production of inoculum, which reduces the overall production of the sample.

From the obtained research data is visible that the best results had shown batch 1, wherein 30% of inoculum dry matter may be still considered as a standard. Biogas yield of mixed sample with inoculum ( $674.38 \pm 126.54 \text{ L}_{\text{STP}}[\text{CH}_4] \text{ kg}^{-1}[\text{TVS}]$ ) and without inoculum ( $842.594 \pm 204.69 \text{ L}_{\text{STP}}[\text{CH}_4] \text{ kg}^{-1}[\text{TVS}]$ ) was very high. Batches 2 and 3 only proved that the fermentation of substrate with the stated composition of green matter, i.e. corn silage and grass haylage in the ratio 1.4 to 1.6 with an addition of cattle manure is selected properly and is suitable for usage at the biogas plant.

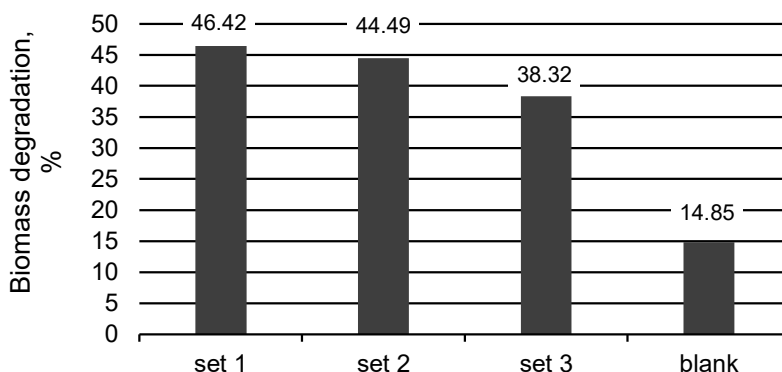
### ***Substrates and effluents characteristics***

Table 2 shows that at the beginning of experiment higher TS and TVS contents were detected in CS and GH than in the DS and CM. But DS and CM both presented a greater ash content comparing to the other two co-substrates. Similar analysis results of that matters and analogous investigation in terms of AcoD can be found in the articles of Cavinato et al. (2010), Kalamaras & Kotsopoulos (2014) and Pokoj et al. (2015).

Cattle manure presents an insignificant C:N ratio (less than 15) (Cestonaro et al., 2015). Crop materials can improve the C:N ratio evading inhibition of ammonia (Xavier et al., 2015). It can be considered that addition of corn silage and grass haylage developed C:N ratio which afterwards enhanced final biogas production.

The final pH levels were upper up to 10% in all batches except of the blank sample where pH level was less than 5.7%. From the Fig. 5 it is possible to assess the degradation of TVS in various variants (batches). The best results again showed batch 1, which presented more advanced TVS degradation than other samples. It confirms the results of the Table 3 and 4.

The control sample of DS after processing produced a limited amount of biogas and it is evidenced by a small percentage of organic matter degraded (Fig. 5).



**Figure 5.** Degraded organic matter in the experimental batches in %.

Batch 1: 30% digested sludge (DS) + 35% corn silage (CS) + 25% grass haylage (GH) + 10% cattle manure CM; Batch 2: 40% DS + 30% CS + 20% GH + 10% CM; Batch 3: 50% DS + 25% CS + 15% GH + 10% CM; Batch 4 (blank): 100% DS; DS: Digested sludge; CS: Corn silage; GH: grass haylage; CM: Cattle manure.

## CONCLUSIONS

Corn silage, grass haylage as co-substrates for anaerobic co-digestion of cattle manure improved total solids and total volatile solid content of the working mixture. This improvement in terms of substrate composition significantly increased volumetric and specific biogas production as well as methane yield. The best results showed batch 1 (30/35/25/10 for digested sludge/corn silage/grass haylage/cattle manure), wherein 30% of the inoculum dry matter can be still considered as a standard. Summarizing the results which were obtained from batch 2 (40/30/20/10 for digested sludge/corn silage/grass haylage/cattle manure) and batch 3 (50/25/15/10 for digested sludge/corn silage/grass haylage/cattle manure) it is concluded that the composition proportions of materials, i.e. vegetable biomass to animal manure were selected correct, thus, they can be used in biogas plants as working substrate mixtures to produce biogas.

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