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Effect of alternative sources of input substrates on biogas production and its quality from anaerobic digestion by using wet fermentation

K. Krištof[1,*] and J. Gaduš[2]

[1]Slovak University of Agriculture in Nitra, Faculty of Engineering, Department of Machines and Production Biosystems, Tr.A. Hlinku 2, SK949 76 Nitra, Slovakia
[2]Slovak University of Agriculture in Nitra, Faculty of European Studies and Regional Development, Department of Regional Bioenergy, Tr.A. Hlinku 2, SK949 76 Nitra, Slovakia
[*]Correspondence: koloman.kristof@uniag.sk

Abstract. The aim of the study was to confirm the suitability of alternative input substrates for production of biogas in order to decrese the need of utilization of high quality maize silage. All of the experiments were conducted by employement of wet fermentation process in mesophilic conditions (temperature in fermentor 40 ± 1 °C) in experimental fermentor with volume 5 m^3. The experiments were realised in operating conditions of biogas station designed for utilization of agricultural biowaste. The experiments were divided into two alternatives (I and II cycle) and one controle input substates. In the first alternative (I cycle) was daily dosage formed by 33 kg of *Amaranth* and 250 L of controle manure mixture. In this cycle, more than 3–times greater specific production of biogas was observed with average methan content 63.9% in comparison with controle manure mixture (80 : 20%, liquid manure and manure). In the second alternative (II cycle) was daily dosage formed by 19.5 kg of sugar beer cuts, 3.3 kg of maize silage, 1.9 kg of oil-seed rape moldings, 2.5 kg of glycerine and 250 L of controle manure mixture. In this cycle, more than 5.9–times greater specific production of biogas was observed. The decrease in averare methan content 55.1% however also decrease in average content of hydrogen sulphide (128 ppm) was observed as well. An unquestionable advantage for both tested alternative mixed substrates was increase in biogas production and its quality in comparison with control substrate based on manure. At the basis of these findings can be concluded that both tested alternative input substrate mixtures are suitable as co–fermentation substances with great potential to increase the biogas production and its quality in case of wet fermentation processes.

Key words: biogas, co-fermentation, co-substrates, anaerobic digestion, wet fermentation.

INTRODUCTION

Increased energy security as well as efforts to mitigate climatic changes and impacts on the environment has become the main motivation for transformation of energy production from mainly used fosil fuels into the renewable energy sources. Biomass plays a key role in the considerations how to secure enough amount of energy for next generations while biomass is a source of energy which is available almost

everywhere in the world regardless of its form (Piszczalka & Jobbágy, 2011; Gaduš & Giertl, 2016). Furthermore, biosubstrate, which includes suprogenic green waste and bio-waste, represents more than 2/3 of total renewable energy sources which can be successfully transformed into energy supplies. Production of biogas from biomass offers the effective replacement for conventional sources of energy while it represents the source of energy with a great potential. The methan gase produced from biomass provides a lot of interesting ways of its utilization while among its greatest benefits is its storability (storage of energy). Mostly due to its possibility to post-treated and compressed. Therefore the produced energy carrier biogas would decrese our dependency on conventionaly used fosil fuels (Braun, 1982; Crabtree, 1995; Gerardi, 2003; Braun, 2013).

From an environmental point of view, the production of biogas containing methane is more efficient in terms of emissions than fossil fuel energy. Biogas is understood as a source of energy with zero carbon dioxide (CO_2) emissions into the atmosphere. Biogas is considered as CO_2 neutral. The utilization of biomass for energy purposes allows the consumption of air CO_2 during photosynthesis while its release back into the atmosphere is closed in a relatively short time. This fact distinguishes biogas from fossil fuels. Moreover, carbon dioxide produced by the controlled anaerobic digestion process can be again exploited by the plants which allow closing down the carbon cycle in nature (Gunaseelan, 1997; Ward et al., 2008). The emissions resulting from agricultural activities are estimated at between 10 and 12% of the total amount of greenhouse gases which is between 5.1 and 6.1 billion tonnes of carbon dioxide equivalent per year (Piszczalka & Jobbágy, 2011; Gaduš & Giertl, 2016).

The biogas is a mixture of gases that results from a complex of multi-stage process overall described as the biodegradation of organic substances under anaerobic conditions. The main component of the gaseous mixture of biogas is methane. Methan is a colorless, non-degassing gas which with air is forming a flammable mixture in the range 5.3-15% vol., respectively. The overall composition of the biogas is dependent on the input substrate composition and the digestion process. On the average, biogas mixtures contain from 65 to 75% of methane (CH_4), 25 to 35% of carbon dioxide (CO_2), 3 to 4% of water (H_2O) and 0.1 to 0.5% of hydrogen sulphide (H_2S). Among the other traceble elements are hydrogen (H), nitrogen (N), ammonia (NH_3) etc. The calorific value of biogas ranges from 17 to 25 MJ m^{-3} which represents in average of 2/3 of energy produced by natural gas as a source of energy. The energy contained in biogas should be used as efficiently as possible especially in connection with the development of high temperatures (Crabtree, 1995; Møller et al., 2004; Li et al., 2011; Vítěz et al., 2015).

At present, some modern factories, especially those that produce bio–waste, are currently undergoing the possibility of producing biogas directly in the plant within its current processing. The biogas is often produced mainly in production plants that have their own biological waste water treatment where the methane–rich biogas is produced as a byproduct of purification. If it is used further in the factories, for heating for example, it reduces the operating costs and replaces the other sources of energy which are commonly used for that purpose.

In order to achieve a good economically balanced biogas production plant it is necessary to evaluate the advantages of its situation prior to construction. The costs of building and purchasing of technology are considered as financially very demanding. Therefore, prior to construction of the biogas plant, it is necessary to ascertain the

amount of available raw material resources, to perform tests and determine the yield of biogas and its quality. The efficiency of the modern biogas plant operation can be increased by a suitable combination of input organic materials (co-fermentation). The term co-fermentation can be understood as the anaerobic treatment of partial substrates (within a fraction) alongside with the main component of the feed mixture (dominant organic materials). Co-fermentation is carried out due to the processing of several types of materials (if available) but also in order to increase of the methane content from the biogas mixture. In addition, by fermentation of suitable co–substrates with the primary types of substrate the total amount of gas produced can also be increased (Álvarez et al., 2010; Zhang et al., 2014).

By co–fermentation it is possible to refer to the anaerobic treatment of several materials. These are different types of biomass mixtures which are added together with the basic (major) substrate into joint fermentation. This process allows achieving higher production of biogas than in the fermentation of these substrates alone. Joint fermentation of materials can also be carried out due to the availability of these materials only in certain quantities whose total weight is sufficient to achieve the required power of the biogas plant. However, not all of organic materials can be fermented together with the production of a sufficient quantity of high–quality biogas. Determination of the suitability for co–fermentation and co–fermentation of individual biomass materials requires tests to be carried out prior to its utilization (Angelidaki & Ahring, 1994; Sosnowski et al., 2003).

As the main advantages of the co–fermentation can be considered the possibility of fermenting several types of biomass that are available directly in the vicinity of the biogas plant it self. In addition, the improvement of the economic efficiency of the biogas plant is therefore also achievable. Moreover, the possibility of increase in the production of biogas or production of biogas with higher methane content (higher energy value) is also considered as an undeniable advantage (Khalid et al., 2010).

The advantage of facilities capable of handling diverse types of organic substances is the rich availability of agricultural and food materials that otherwise would end up as waste. In the fermentation treatment of organic matter, in addition to the production of energy fuel, the carbon cycle is also closed. Therefore, it is considered as a highly rated biomass processing for energy purposes. With a well–established anaerobic decomposition process, co–fermentation can produce high volumes of biogas in comparison to fermentation of the input mono–substrate (Rajeshwari et al., 2000; Clemens et al., 2006; Lin et al., 2013).

The aim of the study was to verify the suitability of alternative substrates usable as input mixtures for anaerobic treatment in the biogas station with the result of production of energy fuel – biogas, under the operating conditions of a real biogas station. Another object was to design suitable alternative substrates with respect to their composition which would provide the required performance parameters while reducing operating costs as well as evaluating the advantages of their anaerobic treatment compared to processing the same volume of input substrate from some conventional fermentation mixtures such as cattle slurry, manure, maize silage, ect.

MATERIALS AND METHODS

Several methods can be used to determine the effect of substrate composition and its suitability for anaerobic fermentation. In addition, measurement of the biogas produced volume and the distribution of individual components volume, particularly methane, were also carried out. The measurements were carried out in order to determine the mixtures composition before the fermentation process itself and during the fermentation process. In these analyses it is possible to observe the process of anaerobic digestion with an increased or reduced content of substances, parameters indicating the state of the fermentation process. Therefore it is possible to describe the substrate distribution options. Since among the important indicators of the fermentation processes are the pH value during the process, the presence of ammonia and unsaturated fatty acids, etc. (Lehtomäki et al., 2007). When determining the suitability of substrates and combinations of substrate in the mixtures, one of the determining factors for the production of sufficient biogas is the presence of organic substances (Rajagopal et al., 2013).

Among one of the laboratories in which the properties and suitability of the substrates composition for anaerobic fermentation can be determined is set up at the workplace of the experimental biogas station VPP SPU in Kolíňany (48°21'47.3"N, 18°12'06.2"E). The laboratory is set up at the Slovak university of agriculture in Nitra, where a 5 m^3 experimental fermentor is installed with an automatic substrate dispenser and a homogenisation input tank. It is adapted and developed for testing substrates for anaerobic fermentation. Gas analyzer (HY-LiTE® 2 system, Merck Millipore, Prague, Czech Republic) and gas chromatograph (WTW PhotoLab S12, Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany) was used to measure gas composition in experimental biogas plant. The volumes of gas produced were measured by a gas meter (BK-G10, Mahrlo, MAHRLO ltd., Trenčín, Slovakia).

Sampling before fermentor

The samples taken before entering the fermentor suppose to be sufficiently homogeneous. Therefore, before sampling the substrate must be thoroughly mixed to bring the sample as close as possible to the entire composition of the substrate. The sampling temperature and pH should be measured during sampling. Between sampling and sample processing, a long time difference should be avoided due to possible chemical and biological changes in the sample over a longer period of time. After the sample has been transferred to the laboratory, these must be re-homogenized so that it can be further used for testing. Basic measurements of the sample before fermentation are the measurement of COD (chemical oxygen demand), total nitrogen and the presence of sulphates in the sample. The COD content is determined to observed the proportion of organic matter in the sample; the sulfate content predestined the content of hydrogen sulfide in the output biogas.

Chemical test of samples before fermentor (input substrate)

Methodology of determination COD (chemical oxygen demand)

The method is based on the procedure described in the MERCK Sulfate Cell Test Instructions – Cell Test. The chemical oxygen demand value is the amount of oxygen coming from the potassium dichromate that reacts with the oxidizable substances

contained in one litre (L) of water under the specified conditions. 1 mol of $K_2Cr_2O_7$ is equivalent to 1.5 mol of O_2. Thus, chemical oxygen demand indicates the amount of oxygen needed to oxidize organic matter with oxidizing agents, a method for expressing the amount of organic matter in the sample.

Methodology of determination slphur content

The method is based on the procedure described in the MERCK Sulfate Cell Test Instructions – Cell Test. The method of determining the sulphate content is based on reactions in which the sulfate ions react with barium ions of mildly soluble barium sulphate. The resulting concentration value is then determined photometrically.

Methodology of determination of nitrogen content

The method is based on the procedure outlined in the MERCK Nitrogen (Total) Cell Test Instructions – Cell Test. The method of determining the concentration of nitrogen is based on reactions in which nitrogen–containing organic and inorganic components are transformed into nitrates by the Koroleff method by the action of oxidizing agents in a thermo-reactor. In sulfuric and phosphoric acid, nitrates are further reacted with 2,6-dimethylphenol (DMP) to form 4-nitro-2,6-dimethylphenol, which is then determined photometrically. If COD is higher than 7,000 mg L it is necessary to dilute the sample with distilled water.

Sampling from fermentor – running process

Samples taken from the fermentor represent a fermentation mixture with the processes of acidogenesis is already started. The sample exhibits sufficient homogeneity as the fermentor is not sufficiently agitated. The sample exhibits a higher temperature as the fermentor is heated with thermophilic processes up to 40 °C. The sampling temperature and pH should be measured during sampling. Between sampling there should be a short time difference due to possible chemical and biological changes in the sample over a longer period of time. After the sample has been transferred to the laboratory, it must be re–homogenized so that it can be further used for testing. Among the basic measurements of the taken sample is determination of the ammonia, iron content, the determination of TS (total solids) as well as the presence of fatty acids.

A high amount of ammonia in the fermentation mixture may inhibit the anaerobic digestion process. The presence of fatty acids is conducted to determine the presence of acetic acid as the main constituent needed for methane formation. For a good fermentation process the pH should be in the slightly alkaline range of 7–8. The proportion of organic matter content is determined in dry matter, subsequently. The dry matter of the fermented mixture is lower than the dry matter of the feed material (Mata–Alvarez et al., 2014).

Chemical and physical tests of samples in process of fermentation (running process)

Determination of total solids (TS)

For measurements, TS is determined from the homogenized taken samples. The basic process for determination of dry matter was conducted by device KERN MLB 50-3 (Merck KGaA, Darmstadt, Germany). Ceramic plates are used to dry the sample in the apparatus, which are combusted in a muffle furnace at a temperature of 550 °C for 20 minutes prior to the drying process, then dried in a desiccator (device with an absorbent material, e.g. silicate gel).

Determination of fatty acids

Samples were analyzed immediately after collection. The samples were thoroughly homogenized. The method was performed by diluting the substrate sample with distilled water 1:50.

Determination of amonia (NH_4)

The method is based on the procedure described in the MERCK Sulfate Cell Test Instructions – Cell Test. Ammonium ions react at pH 12.6 with hypochlorite ions and salicylate ions in the presence of sodium nitrosophoric acid as a catalyst to give intense blue coloration (indophenol). The test was evaluated photometrically.

Determination of iron (Fe)

The method is based on the procedure described in the MERCK Sulfate Cell Test Instructions – Cell Test. Iron ions are the effects of ascorbic acid reduced to divalent iron ions. The thioglycolate–buffered solution is then reacted with the triazine derivative to give a red–colored complex which was photometrically evaluated.

Laboratory equipment

For sampling and homogenisation were used folloeing devices: Disperser for sample homogenization WTW Disper D8 (Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany) and Test reaction cuvettes for determination of COD content in water. For determination of COD and total nitrogen content in substrate samples and biogas was used Thermoreactor for heating of cuvettes WTW Cr4200 (Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany) and Photometer WTW PhotoLab S12 (Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany).

Laboratory equipment includes auxiliary glass banks, measuring cylinders, ceramic bowls, fungi, and pipetting piston dispensers for dosing exact quantities of samples or reagents. For determination of pH values was used device pH meter WTW 3310 (Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany).

For following analysis of biogas quality and other observed parameters were used distilation device (KjelFlex K-360, Donau Lab, Bratislava, Slovakia), laboratory scales with drying of samples (Kern MLS-D, KERN & SOHN GmbH, Balingen, Germany) and muffle furnace (Witeg FHP-3, Unimed ltd., Praque, Czech Republic).

RESULTS AND DISCUSSION

Experiments with anaerobic fermentation of biomass were conducted in two alternatives. In accordance with the stated objectives of the study there were carried out 2 cycles (Table 1). These cycles were conducted in the operating conditions of the biogas station with duration of 25 to 30 days. During these long–term experiments chemical analyses of the substrate were carried out according to the standard methodology (Donoso–Bravo et al., 2011). Methodology was briefly described in above

Table 1. Overview of realised experiments

Cycle	Time period (days)	Co–substrates	Amout of added co–substrates (kg)
I.	28	amaranth	33
II.	30	sugar beet slices	19.5
		maize silage	3.3
		rape moldings	1.9
		glycerine	2.5

chapters and sampling was conducted regularly (twice a day) along with the analysis of biogas quality.

As a comparative and starting biological material for fermentation the cattle manure was used along with pigs' slurry in a volume ratio of 20:80%. This mixture was mechanically pretreated in the main homogenization tank with a submersible propelled mixer. After coarse homogenization of the manure a daily dose of 250 L of substrate was pumped into an experimental fermentor with a volume of 5 m^3 thus maintaining a residence time of 20 days.

In the first (I) experimental cycle the cosubstrate – amaranth was added to a small homogenization tank in the prescribed quantity into a basic mixture consisting of 20% volume of cattle manure and 80% volume of pigs' slurry. In this tank the substrate was homogenized with a propelled mixer for 30 minutes before dosing. The daily dose was pumped from this tank through a slurry pump into a small fermentor.

In the second (II) experimental cycle a daily dose consit of: 19.5 kg of sugar beet slices, 3.3 kg of maize silage, 1.9 kg of rape moldings and 2.5 kg of glycerine. Used sugar beet slices (biowaste after production of sugar) was dosed in automatic mode via a newly designed and installed solid biomass dosing device after mixing with the rape moldings (biodiesel production waste) and after addition of a small amount of maize silage. Before the filling cycle it was programmed to mix the substrate in the tank with a rocking mixer for 5 minutes. The dosing itself took 4 minutes and the amount corresponding to a 1/4 daily dose was added to the fermentor. Glycerine (liquid waste from production of biodiesel) was added to fermentor by supply pump also in automatic mode directly in about 2/3 of the length of the lying fermentor.

The overall arrangement of the experimental device can be seen in Fig. 1 where the main parts and flow of the substrate, biomass, biogas and water for heating the fermentor are shown.

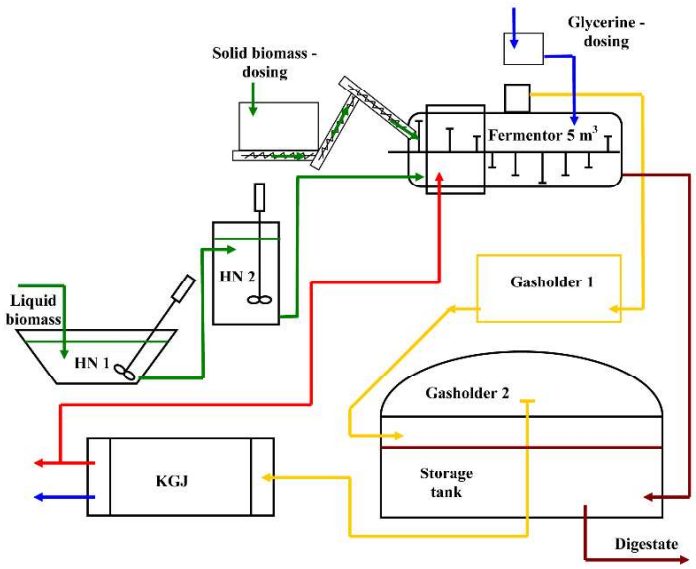

Figure 1. The overall arrangement of the experimental device for co-fermentation (HN1 – great homogenization tank 8 m^3, HN2 – small homogenization tank 1.6 m^3 and KGJ – co–generation unit).

The fermentor is the basic technological equipment for the biogas production. For testing purposes, an experimental (horizontal) 5 m^3 fermentor was used which operates under mesophilic conditions at 40 °C±1 °C. The content of the fermentor was automatically, at regular intervals, driven by the blades of the slow stirrer. Bacteria formed by the raw biogas from the fermentor were accumulated in a small gas jug. From there biogas was pumped through the gas volume meter into a large gas tank located above the final storage tank (Fig. 1).

The main observed parameters were the amount of biogas produced (BP) in terms of its specific production (m^3 m^{-3} d^{-1}) per unit of fermentor volume, methane content (CH_4) and other biogas components (CO_2, O_2 and H_2S). The analysis was carried out on a regular basis, twice a week, as well as chemical analysis of the substrate, with total solids (TS) content, organic total solids (OTS), and annealing loss (VSS), ammonium ions (NH^{4+}). For assessing the proper course of the fermentation process the pH and temperature of the substrate in the fermentor were also monitored (Alatriste–Mondragón et al., 2007).

The share of individual components (CH_4, CO_2, O_2 and H_2S) in the raw biogas was detected by the Schmack SSM 6000 gas analyzer (SCHMACK BIOGAS AG, Schwandorf, Germany). The measurement was performed automatically twice a day.

I. cycle of experiments

The experimental measurements were performed for 28 days. The amount of added amaranth to the base substrate was 33 kg. In experiments the same residence time was chosen as the reference biological material (pigs slurry and cattle manure) at least 20 days. The daily dose was maintained as for the base substrate, ie 250 L.

The content of the original biomass in the fermentor was gradually reduced by adding the daily dose of the monitored substrate (250 L). It can be seen from the graph (Fig. 2) that the temperature in the fermentor was on average 38.9 °C and was very stable. Throughout the experiment the methane content was averaged to 63.9%. The content of dry matter in the substrate (5.58%), thanks to the daily addition of 33 kg of amaranth was higher which also ensured a higher average specific production of biogas (0.542 Nm^3 m^{-3} d^{-1}) compared to the reference cycle.

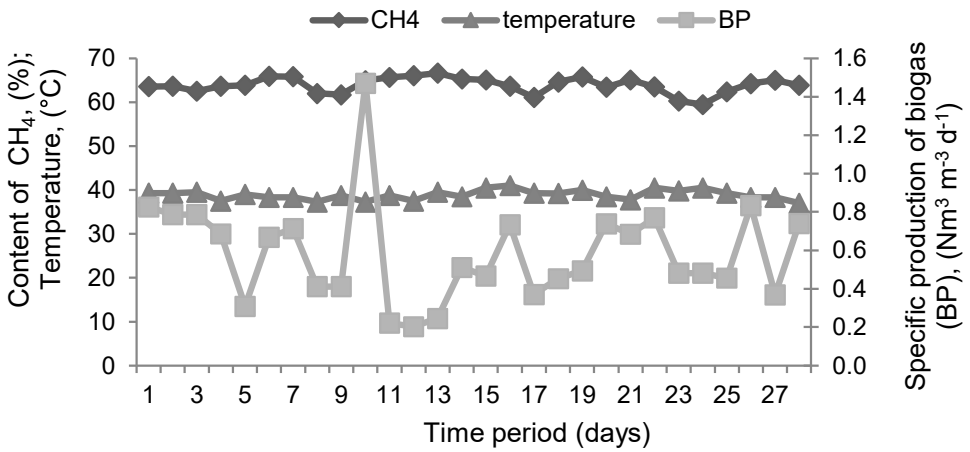

Figure 2. The course of monitored parameters for substrate fermentation **– I. cycle**.

The average values of the monitored parameters are shown in the Table 2 and Table 3. From Table 2 it is possible to extract information that the input substrate (slurry+manure+ amaranth) in fermentor has shown the higher value of organically dispersible organic total solids (OTS = 74.11% TS). In addition, samples from the homogenisation tank (before entering the fermentor) showed an average chemical oxygen demand (COD) of 49,100 mg L^{-1}.

Table 2. Average values of monitored parameters and chemical composition of substrates (slurry+manure+amaranth) **– I. cycle**

Parameter	Unit	Substrate samples	
		MHN	Fermentor
pH	-	5.90	7.33
temperature	°C	20.00	38.9
TS	%	4.90	5.58
VSS	%	-	3.72
OTS	% TS	-	74.11
CHSK	mg L^{-1}	49,100	-
N_{tot}	mg L^{-1}	153	-
NH_4^+	mg L^{-1}	-	1,040
SO_4^{2-}	mg L^{-1}	163	-
Fe	mg L^{-1}	-	8.53

Where TS – dry matter content; VSS – loss on ignition; CHSK – chemical oxygen demand; N_{tot} – total nitrogen; NH_4^+ – amonia ions; SO_4^{2-} – sulphur anions; Fe – iron content.

Table 3. Average values of monitored parameters and chemical composition of substrates (slurry + manure + cosubstrates: sugar beet slices, maize silage, rape moldings and glycerine) **– II. cycle**

Parameter	Units	Substrate samples	
		MHN	Fermentor
pH	-	6.25	7.33
temperature	°C	20.00	39.3
TS	%	4.90	2.92
VSS	%	-	2.18
OTS	% TS	-	78.43
CHSK	mg L^{-1}	93,200	-
N_{tot}	mg L^{-1}	1,530	-
NH_4^+	mg L^{-1}	-	298
SO_4^{2-}	mg L^{-1}	263	-
Fe	mg L^{-1}	-	-

Where TS – dry matter content; VSS – loss on ingnition; CHSK – chemical oxygen demand; N_{tot} – total nitrogen; NH_4^+ – amonia ions; SO_4^{2-} – sulphur anions; Fe – iron content.

II. cycle of experiments

The experiment was performed for 30 days. Glycerin was added for up to 10 days. The content of the original biomass (slurry+manure) in the fermentor was gradually reduced by the addition of the daily dose of the monitored substrate. As shown in Fig. 3, an increase in biogas production and slightly methane content (the first 13 days) was recorded immediately. The production of biogas grew gradually and at 14 days (after the start of glycerine dosing) the specific production of biogas was 1.289 $Nm^3 m^{-3} d^{-1}$ which was almost the highest value recorded. Both biogas and methane production were very stable throughout the 30–day trial. Moreover, the temperature in the fermentor was also maintained at a very stable value at average of 39.90 °C. However, very low content of dry matter in the substrate was recorded at average of 3.92%. It can be concluded that the daily dose of the substrate under investigation could be increased to four times to achieve a more efficient use of the fermentor (Raposo et al., 2012; Ariunbaatar et al., 2014; Lee et al., 2014). Moreover, lower dry matter content (5.69%) was recorded during the whole experiment in comparison with the reference substrate and almost the ideal average pH in the fermentor (7.33) was observed as well.

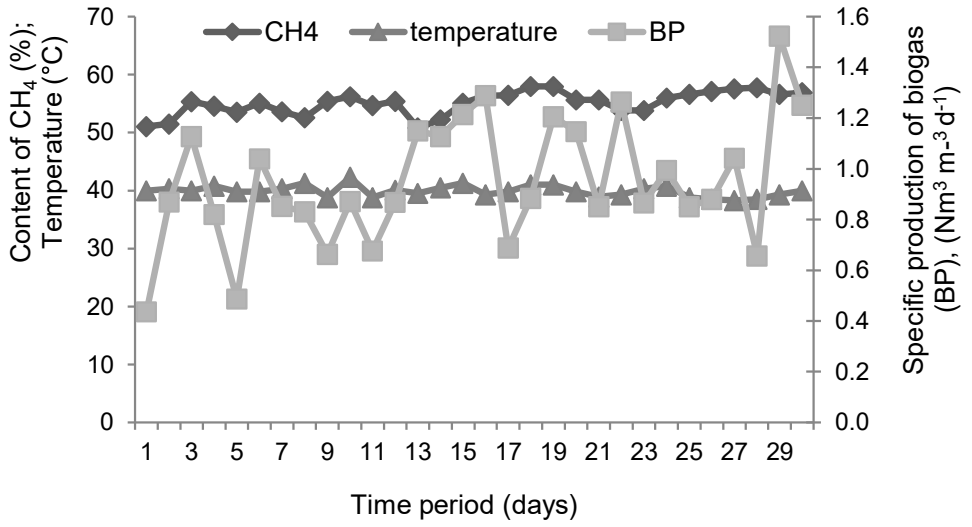

Figure 3. The course of monitored parameters for substrate fermentation **– II. Cycle**.

The average values of the monitored parameters are shown in Table 3 and Table 4. From the values given in Table 3 it can be concluded that the input substrate (slurry + manure + cosubstrates: sugar beet slices, rape moldings, maize silage and glycerine) showed a good value of organic degradable dry matter (OTS = 78.43% TS) in the fermentor. In addition, samples from the homogenisation tank (before entering the fermentor) showed an average chemical oxygen demand (COD) of 93,200 mg L^{-1}.

Table 4. Comparison of the average parameters of biogas production from 2 cycles of experiment with reference biomass (slurry and manure)

Material	CH_4	H_2S	CO_2	Biogas production	Specificproduction of biogas
	obj%	ppm	obj%	$Nm^3\ h^{-1}$	$Nm^3\ m^{-3}\ d^{-1}$
Cosubstrate – 33 kg of amaranth	63.9	998	28.9	0.113	0.542
Cosubstrate – 19.5 kg of sugar beet slices + 3.3 kg of maize silage + 1.9 kg of rape moldings + 2.5 kg of glycerine	55.1	128.3	34.8	0.199	0.955
Slurry:manure (80:20) obj%	60.8	1,343	31.2	0.032	0.160

Where CH_4 – methane; H_2S – hydrogen sulfide; CO_2 – carbon dioxyde; Nm^3 – standardised cubic meter.

Throughout the whole test period, the biogas composition was also monitored by the gas analyzer. The overview of the average values achieved by percentage representation is shown in the Table 4. Moreover, the average content of hydrogen sulphide which is an undesirable component in the biogas reached very acceptable values (on average 128.3 ppm), which is only 9.5% value than in the reference substrate. Therefore, based on the experience of co–generation units and the results obtained by

other researchers it can be concluded that no expensive desulphurisation equipment is necessary (Hobson et al., 1974; Yadvika et al., 2004; Nielsen et al., 2007; Cantrell et al., 2008; Karafiát et al., 2012; Menon & Rao, 2012).

Table 4 lists the average parameters describing biogas production compared to the reference substrate parameters (pigs slurry and cattle manure in ration 80 and 20% volume).

In the co–fermentation of the amaranth an increase in biogas production was observed. Moreover, very stable and high methane content in the biogas was achieved. A significant reduction in the production of hydrogen sulphide content, which is an undesirable component due to highly corrosive effects, was also observed. It is important especially for the further processing of biogas in cogeneration units while its removal with the regular biogas cleaning represents the most costly operation (Gelegenis et al., 2007; Parawira et al., 2008; Fodora et al., 2013; Mao et al., 2015; Gaduš & Giertl, 2016).

In comparison with the reference substrate in the I–cycle of experiments (dosing of 33 kg of amaranth) it was observed an increase of specific biogas production in almost 3.39 times higher. Moreover, average methane content of 63.9% was observed as well. This represents very significant results. These results have demonstrated the suitability of using the amaranth as a co–substrate in biogas stations in our study as well as it was concluded by other authors (Gunaseelan, 1997; Seghezzo et al., 1998; Lehtomäki et al., 2007; Khalid et al., 2011).

Based on the results obtained from II. cycle of the experiments it can be concluded that the tested biomass showed very good results at suitable dosages and can therefore be considered as useful for the production of biogas by anaerobic fermentation. Similar conclusions were also indicated in other studies, e.g. Fatih Demirbas et al. (2011), Lee et al. (2014), Mao et al. (2015). The high energy value of both sugar and biodiesel waste, as demonstrated by the results of the experiments, ensures a high biogas production of more than 5.9 times than in case of the reference material. Moerver, there are still reserves of increase in potential increase of the daily dose, which would also provide a multiple times increase in biogas production (Alatriste–Mondragón et al., 2006; Nielsen et al., 2007; Parawira et al., 2008; Wang et al., 2012; Baeyens et al., 2015).

For methane production the most preferable pH is from 7.2 to 7.8 as can be seen from the tables above. In the first substrate measurement cycle the pH was lower than 6 and in the second cycle was less than 7. An unquestionable advantage for both experimental substrates was that the biogas production and specific biogas production were significantly higher than in case of utilization of slurry and manure combination. The differences in the amount of biogas produced are significant despite the lower value of methane from the fermented mixture of sugar beet slices, maize silage, rape moldings and glycerine. However, it should be added that the methane content in biogas of less than 50% may be limiting for its combustion in the cogeneration unit. This criterion is critical in terms of reliable operation while biogas has a lower calorific value (Hansen et al., 1998; Al Seadi et al., 2008; Gelegenis et al., 2007; Demirbas, 2011; Losak et al., 2014; Vítěz et al., 2015; Laštůvka et al., 2016). The methane content in biogas produced by fermentation of amaranth with slurry and manure mixture reached even higher levels of methane till up to 64%.

Based on the above results it is possible to conclude that the tested co–substates like amaranthus, as well as the addition of a mixture of biomass consisting of sugar beet slices, rape moldings, maize silage and glycerin are suitable biomass for the production of biogas by anaerobic fermentation. In addition, experience from biogas station situated in Kolíňany confirms that it is appropriate to look up for and verify the use of other biomass. These findings are more important while it may lead to reduction of utilization of high quality of maize silage for biogas production which is currently used in Slovakia for more than 85% and it is considered as very high demanding from the economic point of view.

CONCLUSIONS

The aim of the study was to verify the suitability of alternative substrates usable as input mixtures for anaerobic treatment in the biogas station with the result of production of energy fuel – biogas, under the operating conditions of a real biogas station. The experiments were divided into two alternatives (I and II cycle) and one controle input substates. In the first alternative (I cycle) was daily dosage formed by 33 kg of Amaranth and 250 L of controle manure mixture. In this cycle, more than 3–times greater specific production of biogas was observed with average methan content 63.9% in comparison with controle manure mixture (80 : 20%, liquid manure and manure). In the second alternative (II cycle) was daily dosage formed by 19.5 kg of sugar beer cuts, 3.3 kg of maize silage, 1.9 kg of oil-seed rape moldings, 2.5 kg of glycerine and 250 L of controle manure mixture. In this cycle, more than 5.9–times greater specific production of biogas was observed. The decrease in averare methan content 55.1% however also decrease in average content of hydrogen sulphide (128 ppm) was observed as well. An unquestionable advantage for both tested alternative mixed substrates was increase in biogas production and its quality in comparison with control substrate based on manure. These findings are more important while it may lead to reduction of utilization of high quality of maize silage for biogas production which is currently used in Slovakia for more than 85% and it is considered as very high demanding from the economic point of view.

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REFERENCES

Alatriste–Mondragón, F., Samar, P., Cox, H.H.J., Ahring, B.K. & Iranpour, R. 2006. Anaerobic codigestion of municipal, farm, and industrial organic wastes: A survey of recent literature. *Water Environment Research* **78**(6), 607–636.

Al Seadi, T., Rutz, D., Prassl, H., Köttner, M., Finsterwalder, T., Volk, S. & Janssen, R. 2008. *Biogas handbook.* Esbjerg: University of Southern Denmark Esbjerg, Niels Bohrs Vej 9–10, DK-6700 Esbjerg, Denmark, 2008. ISBN 978–87–992962–0–0.

Álvarez, J.A., Otero, L. & Lema, J.M. 2010. A methodology for optimising feed composition for anaerobic co–digestion of agro–industrial wastes. *Bioresource Technology* **101**(4), 1153–1158.

Angelidaki, I. & Ahring, B.K. 1994. Anaerobic thermophilic digestion of manure at different ammonia loads: Effect of temperature. *Water Research* **28**(3), 727–731.

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F. & Lens, P.N.L. 2014. Pretreatment methods to enhance anaerobic digestion of organic solid waste. *Applied Energy* **123**, 143–156.

Baeyens, J., Kang, Q., Appels, L., Dewil, R., Lv, Y. & Tan, T. 2015. Challenges and opportunities in improving the production of bio–ethanol. *Progress in Energy and Combustion Science* **47**, 60–88.

Braun, R. 1982. *Biogas – Methane treatment of organic waste.* Monograph. Wien–New York: Springer Verlag, 1982 (In German).

Braun, R. 2013. *Biogas – Methassification of organic waste materials Basics and application examples.* Wien: Springer, 2013 (in German).

Cantrell, K.B., Ducey, T., Ro, K.S. & Hunt, P.G. 2008. Livestock waste–to–bioenergy generation opportunities. *Bioresource Technology* **99**(17), 7941–7953.

Carlsson, M., Lagerkvist, A. & Morgan–Sagastume, F. 2012. The effects of substrate pre–treatment on anaerobic digestion systems: A review. *Waste Management* **32**(9), 1634–1650.

Clemens, J., Trimborn, M., Weiland, P. & Amon, B. 2006. Mitigation of greenhouse gas emissions by anaerobic digestion of cattle slurry. *Agriculture, Ecosystems and Environment* **112**(2–3), 171–177.

Crabtree, R.H. 1995. Aspects of Methane Chemistry. *Chemical Reviews* **95**(4), 987–1007.

Gerardi, M.H. 2003. *The Microbiology of Anaerobic digesters.* New Jersey: John Wiley & Sons, Inc., Hoboken, 2003. ISBN 0–471–20693–8.

Demirbas, A. 2011. Waste management, waste resource facilities and waste conversion processes. *Energy Conversion and Management* **52**(2), 1280–1287.

Donoso–Bravo, A., Mailier, J., Martin, C., Rodríguez, J., Aceves–Lara, C.A. & Wouwer, A.V. 2011. Model selection, identification and validation in anaerobic digestion: A review. *Water Research* **45**(17), 5347–5364.

Fatih Demirbas, M., Balat, M. & Balat, H. 2011. Biowastes–to–biofuels. *Energy Conversion and Management* **52**(4), 1815–1828.

Fodora, M., Gaduš, J., Mareček, J., Vítěz, T. & Kažimírová, V. 2013. Design of laboratory cyclone separator for biogas purification. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* **61**(3), 631–635.

Gaduš, J. & Giertl, T. 2016. Technology for low–temperature thermochemical conversion of biomass. *MM Science Journal* **2016** (DECEMBER), 1545–1548.

Gelegenis, J., Georgakakis, D., Angelidaki, I. & Mavris, V. 2007. Optimization of biogas production by co–digesting whey with diluted poultry manure. *Renewable Energy* **32**(13), 2147–2160.

Gunaseelan, V.N. 1997. Anaerobic digestion of biomass for methane production: A review. *Biomass and Bioenergy* **13**(1–2), 83–114.

Hansen, K.H., Angelidaki, I. & Ahring, B.K. 1998. Anaerobic digestion of swine manure: Inhibition by ammonia. *Water Research* **32**(1), 5–12.

Hobson, P.N., Bousfield, S., Summers, R. & Kirsch, E.J. 1974. Anaerobic digestion of organic matter. *C R C Critical Reviews in Environmental Control* **4**(1–4), 131–191.

Karafiát, Z., Vítěz, T., Somerlíková, K., Gaduš, J., Haitl, M. & Koutný, T. 2012. Employment of maize silage in non–liquid fermentation for biogas production. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* **60**(6), 153–160.

Khalid, A., Arshad, M., Anjum, M., Mahmood, T. & Dawson, L. 2011. The anaerobic digestion of solid organic waste. *Waste Management* **31**(8), 1737–1744.

Laštůvka, I., Vítěz, T., Chovanec, J. & Mareček, J. 2016. Zero waste; energy recovery from non–recyclable mixed municipal waste. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* **64**(1), 99–107.

Lee, W.S., Chua, A.S.M., Yeoh, H.K. & Ngoh, G.C. 2014. A review of the production and applications of waste–derived volatile fatty acids. *Chemical Engineering Journal* **235**, 83–99.

Lehtomäki, A., Huttunen, S. & Rintala, J.A. 2007. Laboratory investigations on co-digestion of energy crops and crop residues with cow manure for methane production: Effect of crop to manure ratio. *Resources, Conservation and Recycling* **51**(3), 591–609.

Li, Y., Park, S.Y. & Zhu, J. 2011. Solid–state anaerobic digestion for methane production from organic waste. *Renewable and Sustainable Energy Reviews* **15**(1), 821–826.

Lin, C.S.K., Pfaltzgraff, L.A., Herrero-Davila, L., Mubofu, E.B., Abderrahim, S., Clark, J.H., Koutinas, A.A., Kopsahelis, N., Stamatelatou, K., Dickson, F., Thankappan, S., Mohamed, Z., Brocklesby, R. & Luque, R. 2013. Food waste as a valuable resource for the production of chemicals, materials and fuels. Current situation and global perspective. *Energy and Environmental Science* **6**(2), 426–464.

Losak, T., Hlusek, J., Zatloukalova, A., Musilova, L., Vitezova, M., Skarpa, P., Zlamalova, T., Fryc, J., Vitez, T., Marecek, J. & Martensson, A. 2014. Digestate from biogas plants is an attractive alternative to mineral fertilisation of Kohlrabi. *Journal of Sustainable Development of Energy, Water and Environment Systems* **2**(4), 309–318.

Mao, C., Feng, Y., Wang, X. & Ren, G. 2015. Review on research achievements of biogas from anaerobic digestion. *Renewable and Sustainable Energy Reviews* **45**, 540–555.

Mata–Alvarez, J., Dosta, J., Romero-Güiza, M.S., Fonoll, X., Peces, M. & Astals, S. 2014. A critical review on anaerobic co–digestion achievements between 2010 and 2013. *Renewable and Sustainable Energy Reviews* **36**, 412–427.

Menon, V. & Rao, M. 2012. Trends in bioconversion of lignocellulose: Biofuels, platform chemicals & biorefinery concept. *Progress in Energy and Combustion Science* **38**(4), 522–550.

Møller, H.B., Sommer, S.G. & Ahring, B.K. 2004. Methane productivity of manure, straw and solid fractions of manure. *Biomass and Bioenergy* **26**(5), 485–495.

Nielsen, H.B., Uellendahl, H. & Ahring, B.K. 2007. Regulation and optimization of the biogas process: Propionate as a key parameter. *Biomass and Bioenergy* **31**(11–12), 820–830.

Parawira, W., Read, J.S., Mattiasson, B. & Björnsson, L. 2008. Energy production from agricultural residues: High methane yields in pilot–scale two–stage anaerobic digestion. *Biomass and Bioenergy* **32**(1), 44–50.

Piszczalka, J. & Jobbágy, J. 2011. Bioenergy: *Green Energy*. 1st edition. Slovak university of agriculture in Nitra, Nitra, 2011. 143 pp.

Piszczalka, J. & Jobbágy, J. 2012. Bioenergy: *Green energy*. 1st edition. Slovak university of agriculture in Nitra, Nitra, 2012. 149 pp.

Rajagopal, R., Massé, D.I. & Singh, G. 2013. A critical review on inhibition of anaerobic digestion process by excess ammonia. *Bioresource Technology* **143**, 632–641.

Rajeshwari, K.V., Balakrishnan, M., Kansal, A., Lata, K. & Kishore, V.V.N. 2000. State–of–the–art of anaerobic digestion technology for industrial wastewater treatment. *Renewable & sustainable energy reviews* **4**(2), 135–156.

Raposo, F., De La Rubia, M.A., Fernández–Cegrí, V. & Borja, R. 2012. Anaerobic digestion of solid organic substrates in batch mode: An overview relating to methane yields and experimental procedures. *Renewable and Sustainable Energy Reviews* **16**(1), 861–877.

Seghezzo, L., Zeeman, G., Van Lier, J.B., Hamelers, H.V.M. & Lettinga, G. 1998. A review: The anaerobic treatment of sewage in UASB and EGSB reactors. *Bioresource Technology* **65**(3), 175–190.

Sosnowski, P., Wieczorek, A. & Ledakowicz, S. 2003. Anaerobic co–digestion of sewage sludge and organic fraction of municipal solid wastes. *Advances in Environmental Research* **7**(3), 609–616.

Vítěz, T., Koutný, T., Geršl, M., Kudělka, J., Nitayapat, N., Ryant, P., Hejduk, S., Lošák, T., Vítězová, M. & Mareček, J. 2015. Biogas and methane yield from rye grass. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* **63**(1), 143–146.

Wang, X., Yang, G., Feng, Y., Ren, G. & Han, X. 2012. Optimizing feeding composition and carbon-nitrogen ratios for improved methane yield during anaerobic co–digestion of dairy, chicken manure and wheat straw. *Bioresource Technology* **120**, 78-83.

Ward, A.J., Hobbs, P.J., Holliman, P.J. & Jones, D.L. 2008. Optimisation of the anaerobic digestion of agricultural resources. *Bioresource Technology* **99**(17), 7928–7940.

Yadvika, Santosh, Sreekrishnan, T.R., Kohli, S. & Rana, V. 2004. Enhancement of biogas production from solid substrates using different techniques - A review. *Bioresource Technology* **95**(1), 1–10.

Zhang, C., Su, H., Baeyens, J. & Tan, T. 2014. Reviewing the anaerobic digestion of food waste for biogas production. *Renewable and Sustainable Energy Reviews* **38**, 383–392.