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Biogas Production from Steam-Exploded Maize Stover: Results from Continuous Anaerobic Tank Bioreactor Tests

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Abstract: Steam explosion pretreatment of lignocellulosic biomass presents a promising technology for agricultural residues before anaerobic degradation. This study aimed to assess biogas production in continuously stirred tank reactors using steam-exploded maize stover mono-digestion. The continuous digestion tests were carried out in four fermenters with a capacity of 150 L under mesophilic and thermophilic conditions. Maize stover was pretreated at 173 °C for 15 min. Four different organic loading rates (OLR) were tested, the biogas and methane production rate was monitored, and parameters such as dry matter (DM), volatile solids (VS), pH, and C:N were analyzed. The results of the tests showed that using steam-exploded maize stover in a continuous system over the range of an OLR from 1.0 to 3.5 kg VS m⁻³ d⁻¹ is feasible with nitrogen as an additive only. The maximum methane yield, 637 L_N m⁻³ d⁻¹, was measured under thermophilic conditions with an OLR of 3.5 kg VS m⁻³ d⁻¹. The trend of an increased gas production rate with an increasing OLR was observed over the range of the applied OLRs, although the average gas yield in the thermophilic mode was higher than it was in the mesophilic one.

Keywords: steam-explosion; maize stover; biogas; anaerobic digestion; CSTR

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1. Introduction

The development and implementation of clean and renewable energy technologies are crucial to reduce the utilization of fossil reserves and to reduce the emission of greenhouse gasses [1]. The sustainable use of bioenergy, in particular organic wastes and agricultural residues, whether competing with food/feed production or requiring any expansion of or conversion into cropland, is given high attention in the Net Zero Emissions Scenario [2]. For example, agricultural residues like straw or corn stover, rich in carbohydrates, are promising feedstocks for biogas production because of their abundant availability and low costs [3]. According to Croce, Wei, D'Imporzano, Dong and Adani [3], large quantities of straw are produced worldwide. About 1600 million Mg of straw comes from the three main crops rice (731 million Mg), wheat (584 million Mg) and maize (230 million Mg). However, a large amount of straw is currently not used to prevent soil erosion. According to Monteleone, et al. [4], no negative effects on soil quality were observed when straw was partially removed from fields. On the contrary, they claim that, concerning specific local conditions, a win-win situation can emerge by preserving soil quality on the one hand and producing renewable energy with remarkable savings in GHG emissions on the other hand. Therefore, the anaerobic digestion of straw could considerably contribute to the transition towards an environmentally friendly and socially accepted energy supply [3,5]. However, straw and corn stover contain high amounts of lignocellulosic compounds (lignin and hemicelluloses), resulting in reduced gas production [3,6,7]. Hence, due to the complex nature of lignocelluloses, the direct input of straw into a biogas reactor is limited [8,9]. Several pretreatment technologies have been introduced to improve the anaerobic digestion of lignocellulosic biomass. They can be classified into

chemical, biological, physical and physicochemical methods. [10]. Steam explosion (SE) is an effective, environmentally friendly and industrially scalable physicochemical pretreatment method for lignocellulosic biomass. Biomass is pretreated by a combination of high temperatures (in most cases, around 180–240 °C) and pressure (1–3.5 MPa), followed by explosive decompression to atmospheric pressure [1] after a retention time of 5 to 20 min [11]. The SE process makes substrates more easily fermentable by increasing surface area, porosity, and chemical composition [1,12].

As mentioned before, the key parameters affecting SE pretreatment are temperature and residence time. The degree of degradability of lignocellulosic biomass increases with an increase in severity [13,14]. At a certain point of pretreatment severity, inhibitors can be formed (e.g., weak acids, furan derivatives and phenolic compounds). The formation of inhibitors results in a decline in gas yields [6,15]. However, to achieve high degradation, the optimum pretreatment conditions depend on the type of biomass. According to Bauer, et al. [16], hay requires milder conditions to maximize gas yields than other biomasses require. They found the optimum gas performance of late harvested hay with the pretreatment conditions of 175 °C for 10 min and recorded an increase of 16% in methane compared to the untreated sample. According to the studies by Lizasoain, et al. [17] and Lizasoain, Trulea, Gittinger, Kral, Piringer, Schedl, Nilsen, Pothast, Gronauer and Bauer [6], an improvement of 89% in the methane yield of reed biomass has been reported after pretreating it at 200 °C for 15 min. The pretreatment of corn stover at 160 °C for 2 min improved methane yields by 22% during batch test investigations. Another set of experiments with steam-exploded rape straw, conducted by Vivekanand, Ryden, Horn, Tapp, Wellner, Eijsink and Waldron [14], found that after an 81-day trial, the cumulative methane yields of the pretreated samples were higher than those of the untreated sample. Furthermore, they showed that the mid-term yields at selected time points of the pretreated samples rose with pretreatment severity (increasing temperatures and time). Dererie, et al. [18] investigated the effectiveness of SE on the combined ethanol and methane yield of oat straw. They found the overall energy output of the pretreated biomass (190 °C/10 min), 9.5–9.8 MJ kg DM⁻¹, which was 28–34% higher than the yield of the untreated biomass digestion. Other studies which examined the effects of steam-exploded rice straw displayed increases in biogas yields of 51% (200 °C/120 min) [19] and even up to 147% (200 °C/120 min) compared to the untreated samples [20].

In recent years, numerous studies have shown a remarkable effect of the SE of different kinds of lignocellulosic material on biogas production. However, most experiments were conducted as lab-scale batch experiments only. Few works have been conducted on the pilot scale using a continuous stirred tank reactor (CSTR) with steam-exploded maize stover as an input material. A continuous stirred tank reactor (CSTR) is a common type of bioreactor for biogas production [21,22]. It is used efficiently on different scales, from a small scale, where it is applied to decentralized communities and remote areas, to large-scale biogas production plants [22].

The current study aimed to investigate the efficiency of steam-exploded maize stover in a CSTR under steady-state conditions. In detail, four different organic loading rates (OLR) (1, 1.5, 2.5, and 3.5 kg VS m⁻³ d⁻¹) and two different operating temperatures (mesophilic and thermophilic) were applied to examine the effects on the biogas and methane production rate as well as on process stability.

2. Materials and Methods

2.1. Inoculum

The inoculums for the fermentation were obtained from two full-scale biogas plants in Parndorf and Ziersdorf, Austria. At the time both inoculums were collected, the plant in Ziersdorf used maize silage and farm wastes, and the plant in Parndorf used steam-exploded maize stover as an input material.

2.2. Raw Material and SE Pretreatment

The maize stover used in this study was harvested in Burgenland (Austria). The straw was dried in the field, compressed in a round bale and sent to Parndorf, where it was pretreated with the steam explosion (SE) unit, developed and operated by the company “Biogas Systems GmbH” (Austria). The composition of untreated maize stover samples used in this study was determined by analyzing the following parameters: dry matter, volatile solids, raw ash, pH, crude protein and calorific value, and the specific biogas and methane yields (Table 1).

Table 1. Composition of untreated maize stover.

Parameter	Unit	Value
Dry matter	% FM	86.0
	SD	0.7
Volatile solid	% DM	94.3
	SD	0.1
Ash	% DM	5.7
	SD	0.1
pH-value		7.5
Crude protein	% DM	3.3
	min/max	0.2
Calorific value	kJ g^{-1}	17.9
	min/max	0.1
Biological biogas potential	$\text{LN kg}^{-1} \text{VS}$	501
	SD	10.2
Biological methane potential	$\text{LN kg}^{-1} \text{VS}$	305
	SD	2.9

The straw was dried in the field, compressed in a round bale and sent to Parndorf, where it was pretreated with the steam explosion (SE) unit, developed and operated by the company “Biogas Systems GmbH” (Austria). Figure 1 shows a schematic flow diagram of the SE unit in Parndorf.

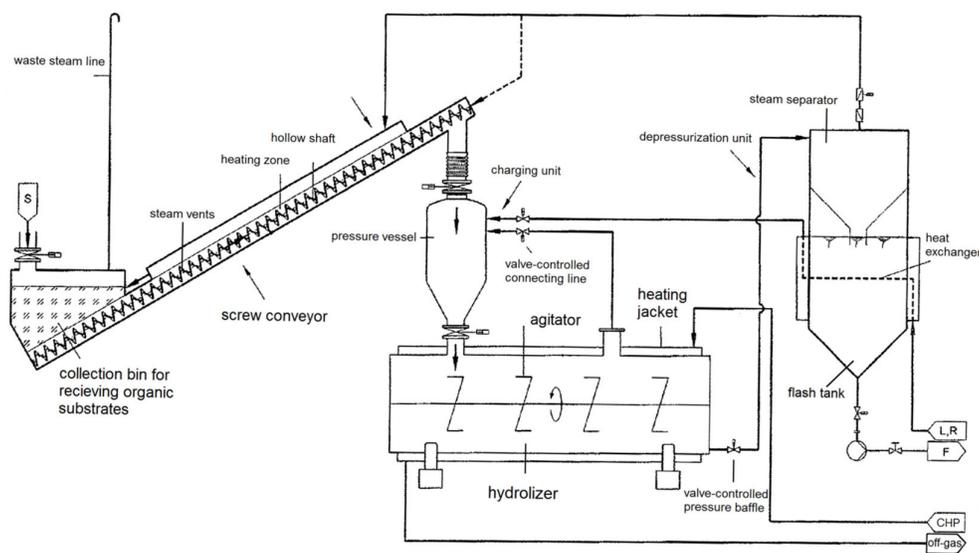


Figure 1. Schematic diagram of SE unit in Parndorf, Austria [23].

The maize stover was chopped to a maximum particle size of 5 cm using a straw mill, mixed with water (target value DM content of straw being 25%), and inserted into a pre-conditioning tank. Here, the mixture was preheated with water vapor and was transported, under a further supply of water vapor, along a conveyor screw until the straw reached the feed tank. The feed tank was closed by automatic valves, and the biomass was heated up with water vapor until it obtained a temperature close to the final target. Subsequently, the biomass arrived at the hydrolysis tank, which was kept at the target pre-treatment temperature by using a heating jacket containing thermal oil, which was heated up with the exhaust gases gained from the CHP unit. Within the hydrolysis tank, homogeneous heat distribution in the biomass was ensured by an agitator, which also prevented the formation of coatings in the reactor. As a result, the pressure inside the reactor increased due to a rising temperature. After the specified residence time, the pressure was abruptly reduced to almost atmospheric pressure using a pressure diaphragm, whereby the water in the biomass immediately evaporated. This process supported the physical disruption of the biomass. The expansion took place in a tank, and the pretreated biomass was finally transported to a blow tank, from which the pretreated straw was removed. The pretreated material was subsequently filled into barrels and stored at 4 °C until further utilization. The pretreatment process was performed at a temperature of 173 °C and a residence time in the pressure vessel of 15 min.

2.3. CSTR Setup and Operation

The experiments were carried out in pilot-scale biogas fermenters. Four identical 200 L continuously stirred tank reactors with a 150 L active volume in the parallel mode were used. The fermenters used were vertical cylinders. The temperature was controlled by a heater mounted on the outside of the side walls. The reactors were continuously stirred by using a paddle stirrer. The stirrer was operated automatically and intercalated to 10 min of mixing with a 10 min break. Two reactors were operated under mesophilic (40 ± 0.5 °C) conditions and two were operated under thermophilic conditions (55 ± 0.5 °C). To ensure that the inoculum was suitable for mesophilic and thermophilic operation, batch tests were carried out in advance with maize straw to test the suitability of the inoculum for the experiments.

The SE stover was filled daily in the glass containers mounted above each fermenter. A vertical stirrer was installed in the glass containers to ensure material homogeneity during the feeding intervals. The feeding material was introduced into the fermenters through an electronically controlled peristaltic pump. The feeding intervals were adjusted to one hour. An overflow system was used to fix the level of the reactor content. Periodic sampling from the overflow stream was carried out.

The organic loading rates of 1.0, 1.5, 2.5 and 3.5 kg VS m⁻³ d⁻¹ were tested in the study. Urea was added to adjust the C:N ratio in the fermenters if needed. Gas production was measured continuously using a RITTER gas meter, TG5 (Bochum, Germany). The measured gas volumes were converted into standard conditions (273 K and 100 kPa) using a thermometer and an incorporated manometer, which measures the differential pressure at the inlet of a gas meter and the atmospheric pressure. The temperatures and pressures were read once per day. A portable gas analyzer, “Dräger X-am 7000” (Dräger, Lübeck, Germany), was used to determine the concentration of CH₄, CO₂, H₂, and H₂S in the biogas.

2.4. Biomethane Potential (BMP) Test

The biogas and methane production from untreated and steam-exploded maize stover were determined by randomized AD batch experiments carried out in triplicate, in accordance with VDI 4630 [24]. The inoculum used was a mixture of inoculums taken from a previous BMP test and inoculums from two biogas plants (located in Parndorf and Margarethen, Austria) that used lignocellulosic material and manure as input material at the time of the sampling. The inoculum was sieved and diluted to approximately 4% DM, the

pH value was controlled, and no nutritional additives were used. After two weeks, the fermenters were filled with 200 cm³ of inoculum. The substrates were added to the fermenter to achieve an substrate-to-inoculum ratio of 1 to 3 based on VS content. The fermenters were maintained in water baths under mesophilic conditions (37.5 °C) and continuously stirred during the 63-day process. The production of biogas was monitored daily. In addition, microcrystalline cellulose was used as a control. All gas volumes were reported under standard conditions (273.15 K and 1013 mbar) per kilogram of volatile solids (Lkg⁻¹ VS). The gas potential of the fermentation residues was determined using the same method, whereby only 200 mL of fermentation residue was used instead of the inoculum.

2.5. Physical and Chemical Characteristics

The dry matter content (DM) of the samples was determined by heating the samples at 105 °C in triplicate in a drying oven until a constant weight could be measured [25]. Ash content was determined after dry oxidation at 550 °C in triplicate in a muffle furnace, and volatile solid content (VS) was calculated by subtracting the raw ash content from the dry matter [26]. In addition, the contents of cellulose, hemicellulose and lignin of the pre-treated biomass were determined.

Nitrogen concentration in the food and content of the reactors was determined in duplicate according to the Kjeldahl method using SpeedDigester K-439 (Büchi, Flawil, Switzerland) as well as distillation unit type B-324 (Büchi, Switzerland). To determine the pH value of the solid or semi-solid samples, 10 g of fresh material was mixed with 100 mL of distilled water. The pH measurement was performed using a C933 multiparameter analyzer by Consort equipped with the standard pH cell ($\pm 0.1\%$). Cellulose and hemicellulose contents were determined using a standard procedure, the sulfuric acid hydrolysis procedure, provided by the National Renewable Energy Laboratory (NREL) [27]. The C and N content of the samples were measured in the Microanalytical Laboratory of the University of Vienna, Austria. The elemental analysis was performed according to standard procedures [28] using Element-Analyzer by Perkin Elmer (EA 1108 CHNS-O, Carlo Erba, Emmendingen, Germany).

3. Results and Discussions

3.1. Chemical Characteristics of the Steam-Exploded Substrate

The composition analysis of the substrate, treated in the SE unit in Parndorf, is presented in Table 2.

Table 2. Characterization of the feeding substrate.

Operating Stage	Unit	I	II	III	IV
Dry Mater	% FM	19.1	25.0	28.1	22.2
	SD	0.4	0.3	0.1	1.2
Volatile Solids	% DM	68.8	69.7	72.6	57.8
	SD	1.2	0.5	0.2	8.3
Ash	% DM	31.2	30.3	27.4	42.2
pH-value		5.2	5.2	5.6	4.6
TKN	% DM	1.2	1.5	1.8	1.5
	SD	0.2	0.4	0.2	0.2
C:N-ratio		n.m.	n.m.	23.0	36.0
Cellulose	% DM	24.9	24.0	20.2	24.8
	SD	1.0	0.2	1.0	0.9
Hemicellulose	% DM	3.3	3.2	2.4	2.9
	SD	0.3	0.2	0.3	0.1
Lignin	% DM	31.6	33.7	40.3	30.6

Biologic biogas potential	SD	0.7	0.4	0.4	0.4
	$L_N \text{ kg}^{-1} \text{ VS}$	348	356	325	364
Biological methane potential	SD	9.5	14.5	0.0	2.8
	$L_N \text{ kg}^{-1} \text{ VS}$	202	191	186	210
	SD	5.8	23.9	0.0	4.9

n.m.: not measured. Organic loading rate given in $\text{kg VS m}^{-3} \text{ d}^{-1}$.

The average DM content of the pretreated maize stover at different OLRs was 23.6%, while the native biomass had a DM content of 86.0% (Table 1). This increase in the water content of the pretreated material was due to the introduction of water and steam to the biomass in the SE unit. Water was added to the straw after milling to facilitate the mixing and transportation of biomass, and steam was added to increase the temperature and pressure in the pressure vessel. This result corresponds to the results of previous studies [6,13,16,17,29]. For example, Bauer, Lizasoain, Theuretzbacher, Agger, Rincón, Menardo, Saylor, Enguádanos, Nielsen, Potthast, Zweckmair, Gronauer and Horn [16], who examined the influence of steam-exploded hay on biogas production, found that the DM content of the native hay (87.1%) decreased in all pretreated samples between a range of 22.8% DM (220 °C for 15 min) and 40.6% DM (175 °C for 5 min), depending on the temperature and residence time in the SE unit. Lower DM content corresponded to a longer residence time and lower temperatures [16].

The average content of the organic matter in the solid fraction of the steam-exploded maize stover during the tests was 67.2%, which showed a significant decrease to 27.8% in comparison to that of the native one (94.3% VS of DM). This reduction could be related to the loss of organic material, such as volatiles, in the steam fraction of the pretreated biomass. Additionally, other studies have reported significant reductions in the VS content for different steam-exploded biomasses like wheat straw, hay, corn stover and reed [6,16,17,30,31]. Horn, Nguyen, Westereng, Nilsen and Eijsink [30] reported a reduction of 20% in the organic material of the wheat straw samples, which were pretreated at 210 °C for 10 min. The studies from Bauer, Lizasoain, Theuretzbacher, Agger, Rincón, Menardo, Saylor, Enguádanos, Nielsen, Potthast, Zweckmair, Gronauer and Horn [16] and Horn, Nguyen, Westereng, Nilsen and Eijsink [30] demonstrate that the loss of VS from steam-exploded biomass rises with the increase in the severity of the pretreatment.

Table 2 shows that the value of the pH of the steam-exploded maize stover was in the acidic range (4.6–5.6), while the pH of the native sample was relatively neutral (7.5). This trend of declining pH values agrees with the observations of the published reports on the SE of different types of lignocellulose biomasses [6,16,32]. The lower pH in the pretreated samples can be explained by the debranch reactions of the acetyl and uronyl groups which are linked comprehensively to the backbone of hemicellulosic components. Debranch reactions release acetic and uronic acids to the liquid fraction of the steam-exploded biomass [15,33,34].

The C:N ratio strongly influences the efficiency of anaerobic digestion. According to Deublein and Steinhauser [22], the optimum value of the C:N ratio for methane formation is between 20–30, while the C:N value of maize stover is 75 [35]. Thus, the nutritional value of straw should be adjusted before biological degradation to offset the N deficiency. The C:N ratio of the steam-exploded straw was 23 and 36 for the loading rates 2.5 and 3.5, respectively. However, the drop in the C:N ratio of the biomass occurred during the SE process. One possible reason for this effect could be that nitrogen-containing organic materials in the maize stover were more resistant to degradation within the SE process. The decrease in C:N has also been observed by Bauer, Lizasoain, Theuretzbacher, Agger, Rincón, Menardo, Saylor, Enguádanos, Nielsen, Potthast, Zweckmair, Gronauer and Horn [16] during the batch surveys on steam-exploded hay with different severities.

Table 2 shows that the cellulose concentration in the steam-exploded maize stover ranged between 20.2 and 24.9% DM, that from the hemicelluloses ranged between 2.4 and 3.3% DM and that from lignin ranged between 30.6 to 40.3% DM. For native maize stover, these values have been reported to be 34.0% DM for cellulose, 37.5% DM for hemicellulose and 22.0% DM for lignin [35]. Different studies regarding the pretreatment of steam-exploded lignocelluloses showed that the cellulose and hemicellulose contents increase while the lignin content decreases in a pretreated sample. Similar trends regarding changes in the content of hemicellulose and lignin have also been reported in other studies [7,16,36,37], but no clear tendency could be found in the literature regarding the changes in cellulose content after SE. A reason for the different responses to pretreatment could be that the cellulose fraction of diverse biomass types can vary [17]. Thus, some authors reported relatively constant concentrations of cellulose for miscanthus, hay, barley, oat, reed, spring and winter wheat [7,16,38], while others describe a slight increase for wheat [30]. Additionally, a reduction in cellulose content for reed and corn stover under harsh pretreatment conditions was observed [6,17]. The reduction can be explained by the degradation of a part of the cellulosic and hemicellulosic material to monosaccharides and other degradation compounds during the SE, which are partly removed by the steam fraction. At the same time, lignin remains almost intact [14]. Moreover, the lignin fraction of steam-exploded biomass can be increased by pseudo-lignin, formed by reactions of released sugars from the hydrolyzed hemicellulose fraction [16]. Vivekanand, Ryden, Horn, Tapp, Wellner, Eijsink and Waldron [14] investigated the changes in rape straw composition after a SE process using mid-infrared spectroscopy. Whereas hemicellulose was solubilized through an auto-hydrolysis reaction caused by acetic acid released during the process, the SE process did not change the lignin content significantly. Therefore, methane potential from the anaerobic digestion of steam-exploded rape straw could be related to the degradation of cellulose and hemicelluloses, respectively [14,33].

The decrease in cellulose and hemicellulose and the increase in the content of lignin in the steam-exploded straw resulted in a drop in the BMP compared to the untreated sample, as illustrated by the data in Table 2. The specific biogas and methane yields of the steam-exploded maize stover decreased to 30.5% and 35.4% of the native sample.

Further, the ash content of the pretreated material was raised compared to that of the native material, from 5.7% DM (native) to an average of 32.8% DM (pretreated), which underlies the loss of volatile solids as a result of the SE process. These results are in accordance with previous studies, which also reported an increase in the ash content in steam-exploded biomass [7,16,17,30].

3.2. Gas Production

Table 3 shows the summary of the operating parameters of the reactors during the test period. The calculations were based on the last six measurements during steady states. The presented values correspond to mean values ± standard deviation.

Table 3. Summary of the operating conditions of the reactors over the runtime.

Operating Stage	I		II		III		IV	
Period [d]	1–54		55–97		98–152		153–207	
Mode of Operation	Meso-philic	Thermo-philic	Meso-philic	Thermo-philic	Meso-philic	Thermo-philic	Meso-philic	Thermo-philic
T [°C]	39.99	54.08	39.77	53.13	41.06	54.33	37.74	54.80
pH	7.5	7.7	7.3	7.5	7.4	7.4	7.5	7.4
OLR [kg VS m ⁻³ d ⁻¹]	1.0	1.0	1.5	1.5	2.5	2.5	3.5	3.5
DM [% FM]	6.8 ± 0.32	6.3 ± 0.50	6.8 ± 0.50	7.1 ± 0.45	7.6 ± 0.5	8.8 ± 0.51	15.1 ± 0.36	16.2 ± 0.88
VS [% DM]	69.7 ± 0.74	70.2 ± 0.89	71.8 ± 1.68	71.7 ± 1.55	71.3 ± 2.86	71.3 ± 3.31	57.3 ± 0.72	59.6 ± 1.19
TKN [g kg ⁻¹]	3.6 ± 0.57	3.6 ± 0.78	3.5 ± 0.23	3.4 ± 0.24	3.8 ± 0.31	3.6 ± 0.79	2.9 ± 0.48	2.8 ± 0.45
NH ₄ -N [g kg ⁻¹]	1.4 ± 0.40	1.2 ± 0.32	1.9 ± 0.21	1.8 ± 0.23	1.8 ± 0.16	1.6 ± 0.18	1.3 ± 0.23	1.2 ± 0.23

C [% DM]	55 ± 0.37	56 ± 0.14	57 ± 0.10	57 ± 0.88	62 ± 0.72	61 ± 0.10	59 ± 0.60	57 ± 0.75
N [% DM]	4.0 ± 0.02	4 ± 0.03	3.0 ± 0.04	3 ± 0.01	3 ± 0.00	3 ± 0.12	4 ± 0.03	3 ± 0.08
C:N	14	14	18	20	20	20	17	18
HRT [d]	68 ± 1	68 ± 1	59 ± 0	59 ± 0	35 ± 1	35 ± 1	41 ± 0	41 ± 0
CH ₄ in biogas [%]	61	56	56	55	55	54	51	52

The results of the measurements of biogas and methane production at the four different loading rates (1.0, 1.5, 2.5 and 3.5 kg VS m⁻³ d⁻¹) are summarized in Table 4 as well as in Figure 2.

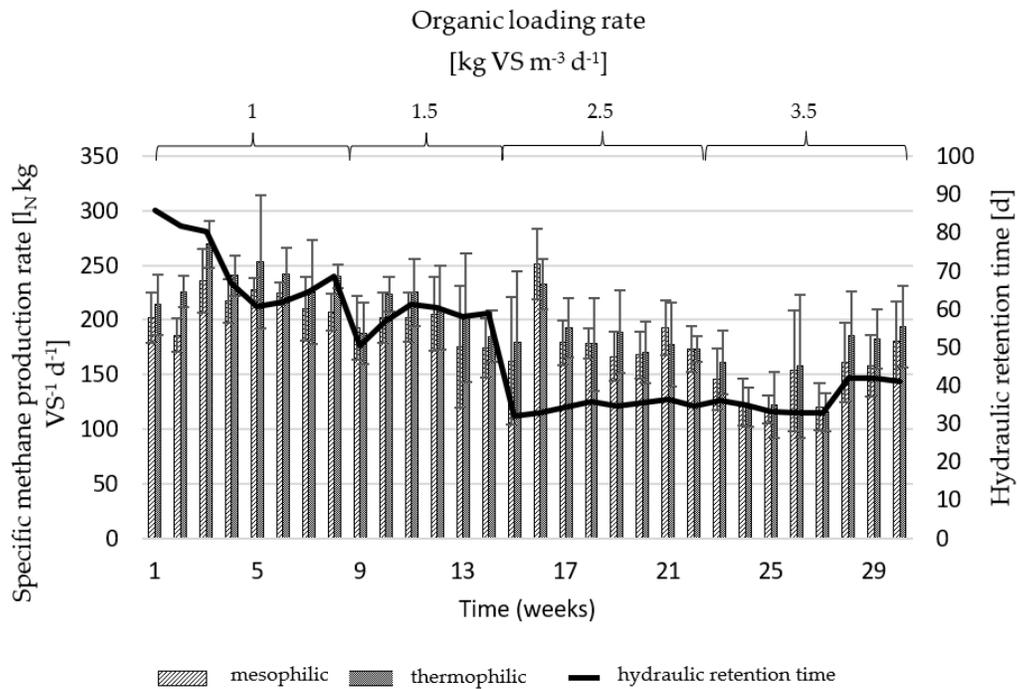


Figure 2. Weekly variations in the specific yield of the reactors for methane production over the operating period.

Table 4. The values of the volumetric gas and specific gas production rates under the steady state conditions of the reactors.

	Organic Loading Rate			Mesophilic		Thermophilic	
	[kg VS m ⁻³ d ⁻¹]	n	Average	SD	n	Average	SD
biogas production [LN m ⁻³ d ⁻¹]	1.0	10	341	31	10	432	23
	1.5	10	455	77	10	510	73
	2.5	14	847	66	14	833	67
	3.5	14	1166	233	14	1269	245
methane production [LN m ⁻³ d ⁻¹]	1.0	10	207	17	10	240	11
	1.5	10	254	38	10	278	38
	2.5	14	464	43	14	451	42
	3.5	14	600	121	14	637	128
specific biogas produc- tion [LN kg ⁻¹ VS d ⁻¹]	1.0	10	341	31	10	432	23
	1.5	10	303	52	10	340	49
	2.5	14	339	26	14	333	27
	3.5	14	333	67	14	363	70

specific methane production [L _N kg ⁻¹ VS ⁻¹ d ⁻¹]	1.0	10	207	17	10	240	11
	1.5	10	169	26	10	185	26
	2.5	14	186	17	14	180	17
	3.5	14	171	34	14	182	36

The values shown represent the mean values of gas and specific gas production rates based on the measurements of the last week of each testing regime before changing the OLR. In this phase, the average gas production value changes were less than 5% compared to the previous weeks. The amount of gas produced in this phase of each operation period can be considered as the gas yield under the steady state condition.

3.2.1. Volumetric Gas Production Rates

The maximum biogas and methane yields under the steady state condition, 1269 and 637 L_N m⁻³ d⁻¹, were obtained under the thermophilic condition with the OLR of 3.5 kg VS m⁻³ d⁻¹ (Table 4). Research work on Laboratory CSTR reactors conducted by Risberg, et al. [39] used steam-exploded wheat straw for co-digestion with cattle manure at an OLR of 2.6 kg VS m⁻³ d⁻¹ and showed a biogas yield between 0.88 and 0.92 m³_N m⁻³ d⁻¹ under mesophilic conditions (manure/straw ratio on VS basis was 26/74). In accordance with that, the biogas yield in the current study at the OLR of 2.5 kg VS m⁻³ d⁻¹ was similar and amounted to 847 L_N m⁻³ d⁻¹, also under mesophilic conditions.

The data in Table 4 shows that the biogas and methane production rates in the steady state increased when the OLR was raised from 1.0 to 3.5 kg VS m⁻³ d⁻¹. The statistical analysis results showed that the increases in the averages of gas production between the different OLRs were significant, except for the difference between the OLRs of 1.0 and 1.5 kg VS m⁻³ d⁻¹. This effect was observed for both operating temperature modes. The same result can be seen for the volumetric methane yields. Other researchers have detected a similar trend of increasing gas production when raising the OLRs during the continuous digestion of different feedstocks [40,41]. A linear increase in methane production with increased OLRs over the range of 1.5 to 9.2 g COD L⁻¹ d⁻¹ was observed in a continuous bioreactor fed with an olive mill solid residue. In this study, the maximum methane yield obtained was 1.7 L STP L⁻¹ d⁻¹, at an OLR of 9.2 g COD L⁻¹ d⁻¹ [40].

Additionally, a fluctuation in the gas production rate in the third and fourth stages of the tests was noticeable, starting immediately after the new load of steam-exploded maize stover was applied. The fluctuation in methane production over the OLRs of 1.0 and 1.5 kg m⁻³ d⁻¹ was low. In the third stage, when the OLR was increased to 2.5 kg m⁻³ d⁻¹, the methane yield showed a rapid increase followed by a strong decrease. However, the average weekly production in this period was higher than that in the previous stages. This pattern of fluctuations was repeated with greater intensity in the fourth stage when the OLR was increased to 3.5 kg m⁻³ d⁻¹. After an initial increase, the average methane production decreased to 392 and 400 L_N m⁻³ d⁻¹ for the mesophilic and thermophilic reactors, respectively, which were even less than those of the minimum methane production in the operating period with the OLR of 2.5 kg m⁻³ d⁻¹. A similar trend of fluctuation was observed by the researchers who studied the anaerobic digestion of pulp and paper sludge in a CSTR reactor at 37 °C when OLR was increased from 1.5 to 5 kg VS m⁻³ d⁻¹ [42].

Despite the increasing fluctuations in gas production, after 4 to 5 weeks, the gas production became stable. The pH values of the reactors, as an index for reactor stability, in both temperature modes, were in the neutral range, which could provide appropriate conditions for methanogenesis. The optimum pH of the reactor for methane formation organisms lies in the narrow range of 6.7–7.5 [22]. Increasing the OLR may cause an increase in the pH of the reactor and the deterioration in gas production, consequently. In this case, the OLR of the reactors is limited. Typical volume loads in agricultural bioreactors do not exceed 4.0 kg VS m⁻³ d⁻¹, although higher values have been reported [21,22,43]. In a set of experiments, an accumulation of volatile fatty acids (VFA), accompanied by a gradual

deterioration in pH, was observed when the OLR of the anaerobic digester was raised to $7 \text{ kg VS m}^{-3} \text{ d}^{-1}$ with grass silage under thermophilic conditions. At the same time, reaction failure did not occur yet [43]. Additionally, the results obtained from a study on the anaerobic digestion of pulp and paper sludge demonstrated that an OLR of $5.0 \text{ kg VS m}^{-3} \text{ d}^{-1}$ led to a decrease in the pH and total volatile fatty acids, causing the destabilization of the reactor and process failure [42]. In the current study, no significant drop in pH and reactor failure was observed when digesting the steam-exploded maize stover with OLRs of up to $3.5 \text{ kg VS m}^{-3} \text{ d}^{-1}$, which is a value close to that of typical agricultural digesters.

3.2.2. Specific Gas Production Rates

The data in Table 4 shows the variation in the specific biogas and methane production of each operating regime under the steady state condition. The specific yield describes the daily volume of the gas produced per unit mass of the volatile solids inserted into the reactor. Contrary to the volumetric biogas and methane production rate, the highest specific biogas and methane production rate was related to the operation regime with the OLR of $1.0 \text{ kg VS m}^{-3} \text{ d}^{-1}$, amounting to 432 ± 23 and $240 \pm 11 \text{ L}_N \text{ kg}^{-1} \text{ VS d}^{-1}$ under thermophilic conditions, respectively. This observation is in accordance with those of other studies on continuous anaerobic reactors digesting protein-rich substrate [44], cow slurry [45], maize silage, whole-crop rye silage and fodder beet silage [46].

The decrease in the specific methane yield can be related to the reduction in the HRT. The operation of reactors with low retention times decreases degradation efficiency, mainly when the substrate contains organic material with a resistant structure [21]. Therefore, a decline in the specific yield may lead to an increase in the residual methane potential of the effluent. During a study carried out to investigate the increase in the organic loading rate from 2.1 to $4.3 \text{ kg VS m}^{-3} \text{ d}^{-1}$ in a two-stage agricultural biogas plant, biogas productivity almost doubled, while the residual methane potential of the effluent was multiplied by the factor 10 [47].

The results of the BMP test of the reactor residues under steady-state conditions are presented in Figure 3. The figure shows an increase in the BMP of the reactor residues up to an OLR of $2.5 \text{ kg VS m}^{-3} \text{ d}^{-1}$, which dropped at an OLR of $3.5 \text{ kg VS m}^{-3} \text{ d}^{-1}$. The BMPs of the residuals were higher under mesophilic conditions than under thermophilic conditions. This effect could be explained by the faster degradation of organic matter under the thermophilic condition [21]. Therefore, less undigested organic matter was released from the reactor outlet.

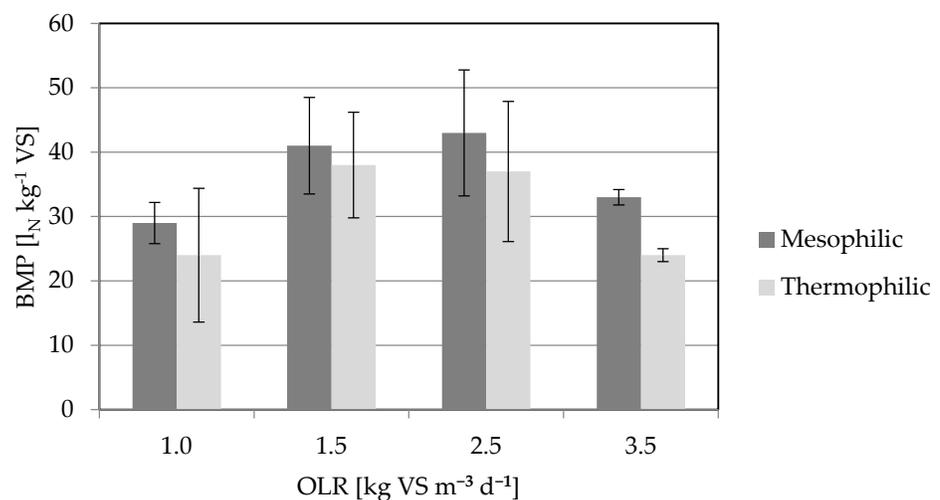


Figure 3. BMP of the residuals of the reactors.

3.3. The Effect of the Operating Temperature

As shown in Table 4, more biogas and methane were produced in the thermophilic mode of operation compared to the mesophilic one, except for the OLR of 2.5 kg VS m⁻³ d⁻¹. Under thermophilic conditions (45–55 °C), biological reactions typically proceed faster than they do under mesophilic conditions (25–40 °C), allowing higher OLRs [20,43,47] [21,48]. However, the statistical analysis of the current study solely showed a significant difference between the mean values at an OLR of 1.0 kg VS m⁻³ d⁻¹. At the OLR of 1.0 kg VS m⁻³ d⁻¹, the amount of biogas produced under mesophilic conditions was 341 ± 31, 4 L_N m⁻³ d⁻¹. Under thermophilic conditions, 432 ± 22.7 L_N m⁻³ d⁻¹ of biogas was produced, which corresponds to a significant increase of 11% at higher temperatures ($t(18) = 7.411$; $p < 0.001$). At higher OLRs, the biogas production rate does not follow the same trend. No significant difference was observed between the mesophilic and thermophilic conditions. The statistical analysis showed that the methane yields under mesophilic and thermophilic conditions were not significantly different at any OLR. This conclusion is in accordance with that of Sun, et al. [49], who analyzed steam-exploded wheat straw in a continuously stirred tank reactor (OLR 2.8 kg m⁻³ d⁻¹, HRT 25 days). Despite the changes in the cellulose-degrading community structure, there was no difference in the biogas yield of the digesters when the operating temperature rose from 37 to 52 °C. Additionally, some reports on the anaerobic co-digestion of energy crops [50] and steam-exploded wheat straw with manure [39] showed no significant improvement in the methane yield when the operating temperature was increased from 37 to 55 °C.

4. Conclusions

The results obtained from this study on the performance of the anaerobic digestion of steam-exploded maize stover in continuous stirred tank reactors are promising. The maximum biogas and methane yields under the steady state condition, 1269 and 637 L_N m⁻³ d⁻¹, were obtained under the thermophilic condition with the OLR of 3.5 kg VS m⁻³ d⁻¹. The biogas production process was stable over the OLR range of 1.0 to 3.5 kg VS m⁻³ d⁻¹, which corresponds to the most common range of OLR among industrial digesters, though higher organic loads could be applied to the reactor without negative effects on the reactor stability. However, identifying the upper limit of allowable OLR and the maximum available biogas yield needs further investigation. Optimizing the integrated system, including the SE of maize stover followed by anaerobic digestion, regarding the severity of SE, may also improve the energy yield of the whole system. Co-digestion of steam-exploded straw with nitrogen-rich wastes could be considered for further investigation to utilize cheap input materials and enhance environmental sustainability.

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