ANAEROBIC DIGESTION OF ANIMAL MANURE AND MAIZE SILAGE IN PILOT PLANT FOR BIOGAS PRODUCTION

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Anaerobic digestion is a biochemical process of substrate decomposition under the action of several species of bacteria, being used on large scale for the treatment and recovery of biodegradable waste. This paper presents some aspects of anaerobic digestion of animal manure and maize silage used as feedstock for biogas production. Characteristics of the tested substrate were analyzed at different periods of the fermentation process: total soluble solids (TSS), content of sugars and soluble proteins, and also the quantity and composition of the generated biogas.

Keywords: anaerobic digestion, biogas, animal manure, maize silage

1. Introduction

Anaerobic digestion has proven to be an optimum process for the treatment of waste from agriculture and animal husbandry, offering multiple advantages, such as reduced pollution and emissions of greenhouse gases [1, 2].

This method of treatment complies with the legislative provisions of the European Union, which require the decrease and recovery of waste, and the promotion of clean technologies [3].

Biogas production by anaerobic digestion is a complex process involving four main stages, namely: hydrolysis, acidogenesis, acetogenesis and methanogenesis, stages that take place under the action of several species of bacteria [2, 4]. In the first stage, hydrolysis, fermentation bacteria convert the insoluble organic matter (cellulose) into sugars, amino acids and fatty acids.

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During this step are acting microorganisms of type *Clostridia, Micrococci, Bacteroides, Butyrivibrio, Fusobacterium, Selenomonas* and *Streptococcus* [5].

Acidogenesis is the fastest stage in the process of anaerobic conversion of complex organic matter, also known as acid fermentation stage. From this stage will result organic acids (acetic acid, propionic acid and butyric acid), fatty acids with short chain, alcohols, H_2, respectively CO_2 [6].

In the third stage, acetogenic bacteria convert fatty volatile acids and alcohols into hydrogen (H_2), carbon dioxide (CO_2) and acetic acid, which represents the substrate for the last stage of the process, methanogenesis [7].

In the last stage of anaerobic digestion, methanogenesis, are involved methanogenic bacteria which are very sensitive to changes of environmental factors, such as the pH and temperature. Under these conditions, the methanogenic bacteria are considered to be the limiting factor for the speed of the anaerobic digestion process [8]. During this stage, microorganisms convert the previously formed hydrogen and acetic acid into methane (CH_4) and carbon dioxide (CO_2).

The composition of biogas obtained in the process of anaerobic digestion varies depending on the used feedstock. Generally, the biogas has two major compounds, methane (CH_4) and carbon dioxide (CO_2), but it can also contains traces of impurities, such as hydrogen sulphide (H_2S), nitrogen (N_2) and ammonia (NH_3) [9].

The most used feedstock for biogas production is coming primarily from agriculture, and is composed of waste from livestock (animal manure and sludge) and also from plant residues (straws, leaves, fruits etc). Lately, new categories of feedstock were tested, currently being used in anaerobic digestion plants for biogas production [10, 11]. This is the case of energy crops, such as maize, sorghum, alfalfa, barley, miscanthus, energetic willow (willow *salix ssp*) and sunflower (sunflower *Helianthus annuus*) [12, 13].

Co-digestion or co-fermentation is an efficient process in terms of energy, that can improve the performance of anaerobic digestion by adding a secondary substrate which is rich in nutrients lacking from the initial feedstock [14]. According to *Mata-Alvarez et al*. [15], the digestion of various feedstock in the same digester can establish a positive sinergy, enabling the microbial growth.

H.M. El-Mashad and *R. Zhang* [16] tested the efficiency of co-digestion process for a substrate consisting of cow manure and food waste, compared to separate digestion of the two substrates. They reported that biogas production obtained from food waste was of 657 L/kgVS after 30 days of digestion, while from fine, coarse and non-sieved fraction of cow manure were obtained 436, 404, respectively 366 L/kgVS. In terms of co-digestion of the two substrates, two mixtures were formed with the following composition: 32% food waste and 68% cow manure, the first mixture, respectively 48% food waste and 52% cow manure for the second mixture. After a period of 30 days of digestion, biogas production was of 455 L/kgVS for the first mixture and 531 L/kgVS for the second mixture. It was found that cow manure in co-digestion with food waste favors the increase of biogas production.

Another study of co-digestion was conducted by *Zhang T. et al.* [17], which followed the production of biogas from goat manure and several types of crop residues (wheat straws, corn stalks and rice straws) in different proportions of mixture. The results showed that the process of anaerobic co-digestion of goat manure with corn stalks or rice straws was effective, improving significantly the production of biogas. Thus, feedstock used in the following proportions, goat manure (GM)/corn stalks (CS) (30:70), GM/CS (70:30), GM/rice straw (RS) (30:70) and GM/RS (50:50) produced, after 55 days of digestion, 14840, 16023, 15608, respectively 15698 mL of biogas.

K. Bulkowska et al. [18], developed a study on the co-digestion process of energy crops of *Zea mays L.* and *Miscanthus sacchariflorus*, with pig manure in the following percentages: 0%, 7.5%, 12.5% and 25%. The results indicated that the highest production of biogas (1.41 L $L^{-1}d^{-1}$ with 0.80 L $L^{-1}d^{-1}$ methane) was obtained by adding 12.5% pig manure. The authors concluded that pig manure favors biogas production and the content of methane, unlike the anaerobic digestion of individual energy crops.

The main objective of this paper is the testing of substrate consisting of animal manure (pig and cow manure) and maize silage used in the process of anaerobic digestion, in order to obtain biogas. The control of anaerobic digestion process was achieved by monitoring the most important operating parameters, namely: total soluble solids (TSS %), pH, sugars amount and soluble proteins contained in the tested substrate. Also, there were monitored the quantity and composition of the resulted biogas.

2. Materials and methods

2.1. Collection and preparation of substrates

Animal manure and maize silage were obtained from an animal farm in Teleorman County, Romania, in May 2015.

Table 1

Composition of the tested substrate and its parameters

Substrate	Quantity (kg)	C/N ratio [19]	Dry matter (%) [19]	Moisture (%) [19]
Pig manure	18	13:1	13.5	86.5
Cow manure	10	25:1	14	86
Maize silage	4	40:1	30	70
Tap water	18	-	-	-

C/N ratio of the mixture of feedstock was calculated according to the method proposed by *T. Vintilă* and *V. Nicolik* [19], with the value 26:1. According to the literature, for a good functioning of anaerobic digestion, the C/N ratio of the mixture should range between 20:1 – 30:1 [20].

2.2 Biogas pilot plant design

Experiments were conducted using a small capacity biogas plant, from the laboratory of the National Research - Development Institute for Machines and Installations Designed to Agriculture and Food Industry – INMA Bucharest.

The anaerobic digester is made of stainless steel, has a capacity of 60 l, it is thermally insulated and the content is heated by an electric heater powered by photovoltaic panels. Also, the digester is equipped with a temperature sensor and a pH sensor, these parameters being set and monitored automatically by the control panel connected to the biogas plant. In order to homogenize the substrate, the digester is equipped with a paddle stirrer driven by an electric motor. The pressure inside the digester is measured by a low pressure transducer, with measuring range of 0 – 3 bar, type HONEYWELL – MLH 010BGC14B.

2.3 Experimental setup and operation

The experiment was carried out in the mesophilic range, and the temperature was gradually raised from 22^{o}C to 37 ± 1.5^{o}C, this value being kept constant throughout the experiment.

The initial pH of the substrate was of 6.4 units, being displayed on the control panel, the set value for self-maintaining in the digester being of 7 ± 0.5 pH units. pH adjustment was carried out using a solution of $CaCO_3$.

In this study, the entire process of anaerobic digestion lasted 23 days, until the production of biogas started to decrease.

In order to homogenize the mixture, the stirrer was set to start automatically at an interval of 30 minutes for 3 minutes.

2.4 Analytical methods

The pH of samples taken at certain intervals of time was determined using a Hanna pH-meter.

The total soluble solids (TSS) were determined on a thermobalance, after centrifugation of initial samples at 5000 rpm and then filtering through a membrane with pores of 0.45 µm. The TSS represents a decisive factor for the development and growth of microorganisms. Soluble proteins were measured using the Lowry method [21] and content of sugars were determined by the method with DNS [22]. All measurements were done using a T92+ UV VIS spectrophotometer.

The quantity of generated biogas was measured using a Sacofgas Milano gas meter, fitted with a pulse counter, 1 pulse = 0.01 m^3. Maximum flow rate is 6 m^3/h and minimum flow rate is 0.04 m^3/h. The composition of the resulted biogas (methane (CH_4 %v/v), carbon dioxide (CO_2 %v/v) and hydrogen sulphide (H_2S% v/v) was determined using a COMB/ I-R gas analyser.

3. Results and discussion

Substrate characterization

The total soluble solids contain soluble sugars, soluble proteins, mineral salts, pigments and other water-soluble compounds that are used as nutritive substrate for different groups of microorganisms involved in anaerobic digestion and biogas production. During the anaerobic digestion process, TSS content becomes the main parameter governing the methane production. Analyzing the data, it is noted that the TSS value tends to increase on days 8 – 17, period in which the methanogenic bacteria are growing, as can be seen from the amount of recorded biogas. By the end of the co-digestion period (Table 2), the TSS content decreased because of the substrate consumption.

The pH value was kept in the optimal range for methanogenic bacteria growth (6.8 - 7.2) during the anaerobic digestion process.

Table 2 presents the characteristics of the substrate in various stages of anaerobic fermentation process.

Table 2

Substrate characteristics during the anaerobic digestion process

	Day 5	Day 8	Day 13	Day 15	Day 18	Day 23
TSS (%)	1.7	1.74	2.50	3.30	3.1	2.77
pH	6.78	7.16	7.09	7.15	6.94	6.87
Protein content (mg/ml)	0.73	0.76	0.79	0.85	0.89	0.90
Sugar concentration (mg/ml)	1.5	1.52	1.56	1.54	1.58	1.6

Biogas production

Biogas production was monitored every day, in Fig. 1 being shown the variation of biogas production during the 23 days of anaerobic digestion of animal manure and maize silage.

Variation of biogas production follows an exponential curve approximately Gaussian, as can be seen from Fig. 1. The regression curve of experimental data with the exponential law displayed on the figure has a relatively high cofficient of correlation (R^2 = 0.939), in the figure being displayed also the coefficients of the regression equation. The curve shows a certain asymmetry of the right so that the slope of the ascending zone is slower, while the slope of the descending zone is steeper. This shows that the gas is formed relatively slowly, while the ending process of biogas generation is faster.

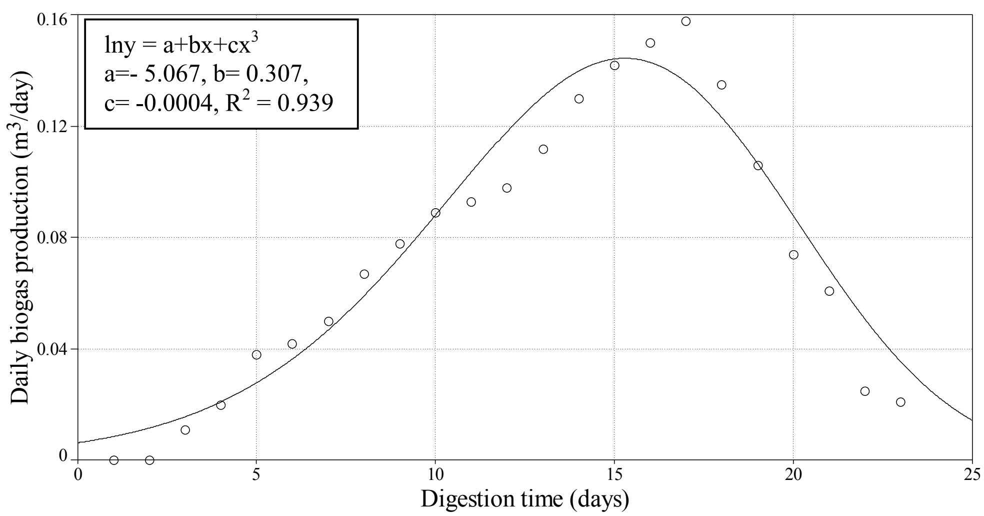


Fig. 1. Daily biogas production during the anaerobic digestion process

Biogas production accumulated throughout the anaerobic digestion process was 1.70 m^3/ batch.

Regarding biogas production curve (Fig. 1), we observed a delayed start, this phenomenon being due to the absence of a specific inoculum, and the duration of adapting the microorganisms present in tested substrate at the bioreactor conditions. The largest amount of biogas (0.158 m^3) and the highest concentration of methane (56 % v/v) were obtained on day 17. As we can see, the biogas production increased after first seven days.

The methane yield obtained from anaerobic digestion process of animal manure and corn silage during the 23 days is presented in Fig. 2.

Fig. 3 shows the presence of the two biogas components, hydrogen sulphide, H_2S (% v/v) and carbon dioxide, CO_2 (% v/v). It can be seen that the maximum hydrogen sulphide percentage was about 0.47% on day 7, this aspect indicating the need of biogas purification in order to obtain energy in a productive and profitable manner. Thus, removing hydrogen sulphide from biogas is particularly important because it can cause corrosion, which can seriously affect the co-generation energy equipment or other installations. Moreover, water and carbon dioxide must be removed, they causing condensation in the pipeline or decreasing the energetic potential of the biogas [23].

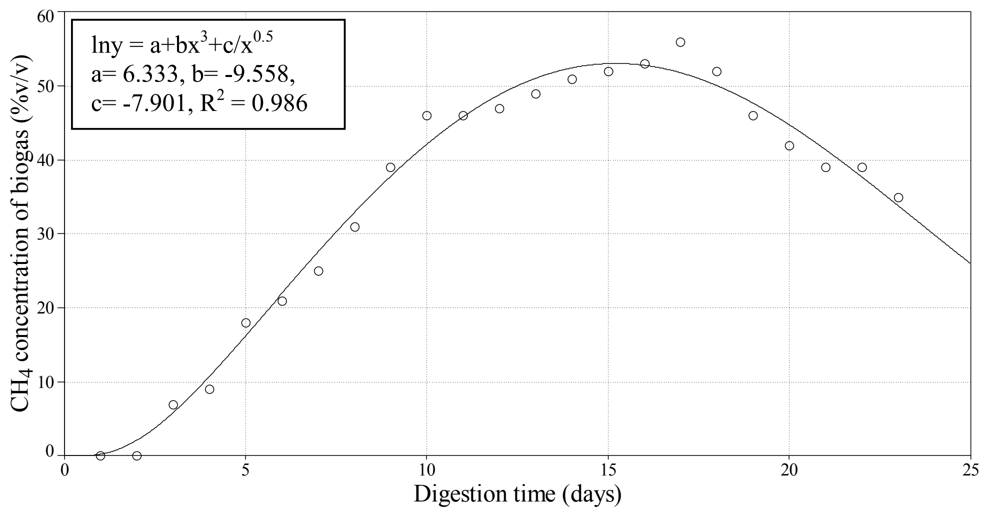
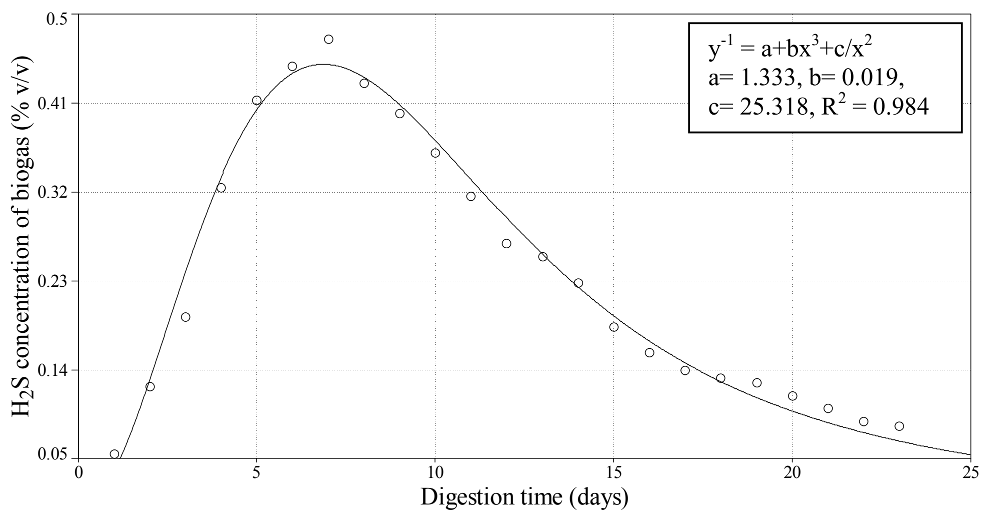


Fig. 2. Daily methane content of the produced biogas



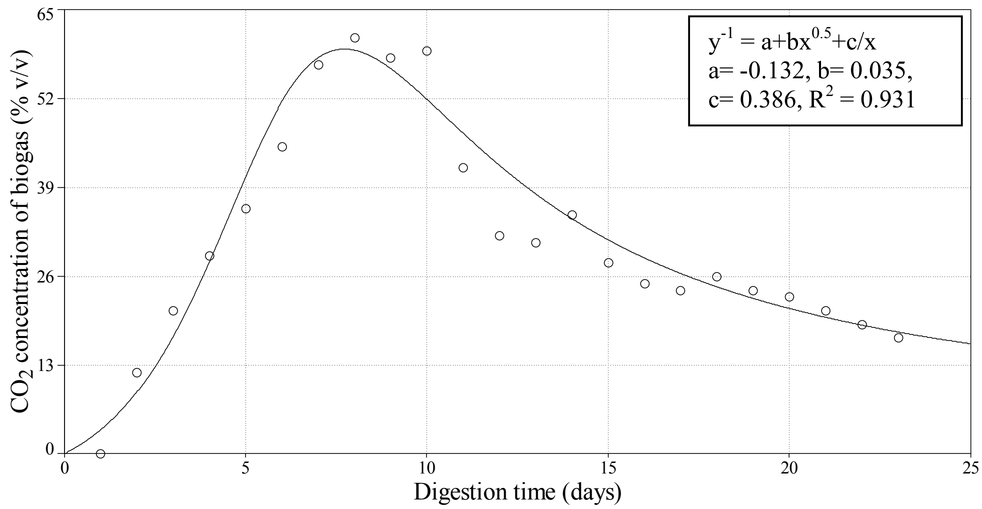


Fig. 3. Daily H_2S (% v/v) and CO_2 (% v/v) content of the produced biogas

From Fig. 2 and 3 can be observed that the trend for the three components of biogas presents approximately the same variation, the correlation coefficients of curves with the experimental points showing high values ($R^2 \geq 0.931$). Analysis of the curves show that H_2S is formed more quickly than the other components, followed by the CO_2 and then by the CH_4.

4. Conclusions

In this paper, the experimental part was carried out using as a substrate for anaerobic digestion animal manure and maize silage, with a retention time in the digester of 23 days.

It has been proved that maize silage, animal manure and their mixture, are adequate substrates for the process of anaerobic digestion in order to obtain biogas. The obtained results are in accordance with the ones from the literature. In the literature, the results obtained from the use of maize as substrate in the co-fermentation process show better results compared to other substrates.

For example, Liew N. and co-workers [24] reported a total methane production of 81.2 L kg^{-1} VS during anaerobic digestion of maize, followed by wheat straw (66.9 L kg^{-1} VS), yard waste (40.8 L kg^{-1} VS) and leaves (55.4 L kg^{-1} VS). Also, Zheng *et. al* reported a methane production between 125 – 160 L kg^{-1} VS during liquid anaerobic digestion of maize [25].

In the same context, the use of maize in the co-digestion process shows a positive effect on the concentration of resulted methane.

Thus, Adebayo *et. al* [26] determined the biogas production and methane concentration for cow slurry and maize stalk at mesophilic temperature (37^{o}C).

They reported that the higher biogas (0.426 m^3/kg organic dry matter) and methane (0.297 m^3/CH_4/kg organic dry matter) yields were obtained at ratio 3:1 (75% VS of cow slurry co-digested with 25% VS of maize stalk).

It has been found that the TSS parameter has an uneven evolution, which can be attributed to regular stirring of the feedstock. Its values ranged between 1.7 and 3.3%, corresponding to the degree of extraction of soluble compounds present in the water.

The concentration of soluble protein was in a relatively stable range, between 0.7 and 0.9 mg/ml, a phenomenon explained by the relatively low consumption and by the formation of protein specific to bacterial cells. Also, the concentration of sugars was maintained between 1.5 and 1.6 mg/ml. These values demonstrate that the phenomenon of growth, cell multiplication and biogas generation is a complex one.

It is important to mention that the maximum amount of biogas obtained in the experiment was recorded after the 17^{th} day, and then it had a downward evolution with a steeper slope than that in the first part of digestion. The same phenomenon is recorded for the two components of biogas (H_2S and CO_2) that present a maximum much earlier than the maximum of biogas production curve.

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