Biogas production from anaerobic co-digestion of cow manure and leaves of Miscanthus x giganteus

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Abstract

Lately, anaerobic digestion process has gained particular attention due to the environmental benefits on reducing greenhouse gases emissions and biogas generation, which is a promising source of renewable energy. The manure from livestock industry, mixed with energy plants or with agricultural residues, can be processed in an environmentally friendly manner through the process of anaerobic digestion, in order to obtain biogas for electrical or thermal energy production, and to use the digestate as soil fertilizer. In this paper was studied the production of biogas generated by the process of anaerobic digestion of cow manure mixed with Miscanthus x giganteus biomass, a C4 energy plant. Also, it has been characterized the substrate tested in various stages of anaerobic digestion. The recorded parameters were: pH, total soluble solids, content of soluble proteins and sugar content. The results showed that the maximum yield of biogas, after 15 days of anaerobic digestion, was of 0.420 Nm³/kg dry matter, and the biogas started to form on the second day of incubation. During the anaerobic digestion, it was observed that the values of TSS and pH were slightly decreasing due to the growth and multiplication of bacterial cell, the consumption of substrate and the accumulation of metabolites. Reducing sugars and soluble proteins were found in small concentrations at the beginning of anaerobic digestion process and at its end were undetectable.

Keywords: biogas composition, animal manure, renewable energy, soluble protein, sugar content

1. Introduction

The process of anaerobic digestion is considered to be a key technology for the sustainable use of biomass consisting of the organic fraction of municipal solid waste, animal manure, crop residues, and aquatic biomass and energy crops suitable for this process (1). The final product of anaerobic digestion is biogas, which is a mixture of methane (CH₄) and carbon dioxide (CO₂), along with traces of hydrogen, hydrogen sulphide and water vapours (2). Anaerobic digestion generating biogas is a complex and sensitive process because it involves several groups of microorganisms. The stages involved in the anaerobic digestion process are hydrolysis, acidogenesis, acetogenesis and methanogenesis (3). Each stage involves specific bacterial species responsible for the conversion of complex molecular structures into simpler substances, which in the end lead to the production of biogas (4).

The raw material used in the anaerobic digestion must ensure a favourable environment for the growth of microorganisms involved in the process, namely: C/N ratio should be between 15:1 and 25:1, the pH of the processed substrate must be neutral and should not contain substances to inhibit the process (5).
The most used substrate for the production of biogas by anaerobic digestion process is animal manure, which contains the necessary nutrients for the growth of anaerobic microorganisms. However, due to the low concentration of total solids of animal manure (usually <8%), its mixture with different crop residues or energy crops is often chosen (6). Lignocellulosic biomass is rich in carbon, therefore is very important that this material to be fermented with raw materials rich in nitrogen to obtain an optimal C/N ratio, a good yield and an adequate stability in the anaerobic digester (7).

The mixing process of two or more substrates of organic nature is known as co-digestion, offering a synergistic effect to the process of anaerobic digestion, leading to increased production of biogas (8).

In Germany, maize is the most commonly used co-substrate in the anaerobic digestion of animal manure, due to its low lignin content (9). However, this is not suitable for long-term use in biogas plants, as there is competition with food industry. For this reason, the interest in using agricultural residues as a substrate in biogas plants has increased; residues coming from the food industry, but also from perennial crops (*Miscanthus*, energy willow, sorghum etc.). Perennial crops need less fertilizer and pesticides, and they have less negative environmental impact compared with annual crops (10).

*Miscanthus x giganteus* is a perennial grass that can be mixed with other plant residues to produce heat and electricity, through the process of anaerobic digestion, by generating biogas. This grass can be grown without restrictions on soils polluted with heavy metals (11).

In the literature can be found a series of experiments conducted on the co-digestion process of animal manure with different energy crops. *M. Estevez & al.* (12) conducted a study in which they tested the anaerobic co-digestion process of cow manure and steam treated *Salix* willow for a good physical degradation of lignocellulosic structures. They found that the mixing with willow improved the percentage of methane in the biogas, compared to individual digestion of cow manure.

Also, experiments were conducted in order to optimize the process of anaerobic digestion by testing the substrate consisting of *Zea mays* and *Miscanthus sacchariflorus* in a ratio of 9:1, to which was added pig manure in different proportions (0%, 7.5%, 12.5% and 25%). The results showed that the addition of a percentage of 12.5% of pig manure was optimal for co-digestion with energy crops, resulting in a biogas production of 1.41 L L\(^{-1}\)d\(^{-1}\) (13).

Other experiments have shown that using *Miscanthus* crop is one of the best alternative to replace maize silage in the process of anaerobic digestion, recording a biogas production of 5.5 ± 1x 10\(^3\) m\(^3\) ha\(^{-1}\), compared to 5.3 ± 1x 10\(^3\) m\(^3\) ha\(^{-1}\) obtained in the case of maize silage (14).

In the present paper, it was studied the performance of anaerobic digestion process using a substrate composed of cow manure mixed with green leaves of *Miscanthus x giganteus* energy plant. There were evaluated specific parameters playing an important role in the process of anaerobic digestion, such as: pH, total soluble solids (TSS), content of soluble proteins and content of sugar. Also, the production of biogas and the percentage values of gases in biogas composition (methane, carbon dioxide and hydrogen sulphide) were monitored.
2. Materials and Methods

2.1. Preparation of feed material and experimental set-up

For this study were used fresh cow manure, obtained in June 2015 at a farm in Teleorman county, mixed with green leaves of Miscanthus x giganteus energy crop, a C4 perennial plant, steril hybrid, harvested during vegetation time from the culture of National Institute of Research – Development for Machines and Installations Designed to Agriculture and Food Industry, INMA Bucharest.

In the experiment, only the green leaves of the plant have been used, chopped to a size of up to 5 cm. From the literature it is known that besides biofuel production, Miscanthus x giganteus stems can be used both for ornamental purposes (15) and in the construction field (16).

The experiment took place in a digester made of stainless steel (figure 1), with a capacity of 60 l, thermally insulated, and the content was 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 stirred being set to start automatically at an interval of 30 minutes, with a time of mixing of 3 minutes. The pressure inside the digester is measured by a low pressure transducer, with measuring range of 0 – 3 bar, type Honeywell – MLH 010BGC14B. Experimental data obtained during the process of anaerobic digestion were recorded by the monitoring and control system fitted to the bioreactor.

Figure 1. Design of anaerobic digester used in the experiment

1 - actuator; 2 – lid with screws for sealing; 3 – paddle stirrer; 4 – temperature sensor; 5 - pH sensor; 6 – hot water circulation pipe; 7 – pressure transducer; 8 – pH correction pipe; 9 – sampling pipe
Cow manure was mixed with *Miscanthus x giganteus* green leaves and water, in the quantities presented in table 1.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Quantity (kg)</th>
<th>C/N ratio</th>
<th>Moisture content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miscanthus x giganteus (leaves)</td>
<td>2.5</td>
<td>26 [18]</td>
<td>85 [19]</td>
</tr>
<tr>
<td>Tap water</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

C/N ratio of the tested substrate was determined according to the method proposed by T. Vintilă and V. Nicolik (17), having a value of 25.4:1. According to data in the literature, for a good development of the anaerobic digestion process, the value of C/N ratio of the mixture must range between 20:1 and 30:1 (20).

The experiment was carried out in the mesophilic range, and the temperature was gradually raised (in approximately 4 hours) from 21°C to 37 ± 1.5°C, this value being kept constant throughout the experiment.

The initial pH of the substrate was of 8.2 units, being displayed on the control panel. pH adjustment was carried out automatically using a solution of CaCO₃.

Retention time of the substrate in the anaerobic digester was of 15 days, until the production of biogas almost stopped.

### 2.2. Analytical methods

Assessment of anaerobic digestion process was done by analysing and interpreting the following parameters: total soluble solids (TSS), content of soluble proteins and content of sugar. The analysis of samples was done by collecting samples of mixture from the bioreactor in different days of the anaerobic digestion process (days 1, 4, 7, 11 and 15).

The content of total soluble solids (TSS) was determined with a thermo-balance, after the centrifugation of initial samples at 5000 rpm followed by filtering through a membrane with pores of 0.45 µm. TSS is a decisive factor for the development and growth of microorganisms.

The content of soluble proteins was determined according to the Lowry method (21) the protein used to make the standard curve being bovine serum albumin (Sigma Co.). The absorbance was measured at 660 nm using a T92+ UV VIS spectrophotometer, PG Instruments. The Lowry method is based on the biuret reaction, in which the peptide bonds of the protein react with copper under basic conditions to produce Cu²⁺, which in turn reacts with the Folin reactive.

In order to estimate the concentration of sugars in samples, DNS method was used (22). DNS solution is yellow, but after reaction with reducing sugars, the reactive is converted into 3-amino-5-nitrosalicylic acid, which is dark red. In this case, the absorbance was measured at 540 nm using the same T92+ UV VIS spectrophotometer, PG Instruments.

The quantity of generated biogas was measured daily using a Sacofgas Milano gas meter, fitted with a pulse counter, 1 pulse = 0.01 m³. Maximum flow rate is 6 m³/h and minimum flow rate is 0.04 m³/h. The composition of the resulted biogas, (methane (CH₄ % v/v), carbon dioxide (CO₂ % v/v) and hydrogen sulphide (H₂S % v/v) was measured using
a portable Mentor/COMB/IR Series gas analyser, fitted with sensors for methane, carbon
dioxide and hydrogen sulphide.

3. Results and discussion

Determination of the content of soluble proteins in the tested substrate can provide
important information regarding the decomposition of raw materials and the production of
generated biogas.

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 1                       | Day 4                       | Day 7                       | Day 11                      | Day 15                      |
| TSS (%)                     | 1.20                        | 0.72                        | 0.65                        | 0.34                        | 0.21                        |
| pH                          | 8.21                        | 7.23                        | 7.19                        | 7.32                        | 7.27                        |

Regarding the pH of the substrate subjected to the anaerobic digestion process, it
presents a slightly decreasing trend, but presenting optimum values for the growth of
microorganisms in the anaerobic digester (between 8.21 units and 7.27 units).

During the process of anaerobic digestion, the content of TSS (%) shows a downward
trend, indicating the consumption of substrate for cell growth and maintenance. Thus, it
can be observed that during anaerobic digestion, the values of TSS decreased from 1.20%,
value recorded in the first day of the process, to 0.21% in the last day of substrate
digestion.

Regarding the concentration of the soluble proteins and reducing sugars, these
recorded small concentrations at the beginning of anaerobic digestion process of about 0.6
mg/ml, respectively 3.8 mg/ml, and at the end of fermentation process were undetectable.

However, T. Vintila & al. (23) reported that Miscanthus can provide more fermentable
sugars and higher glucose from total reducing sugars than other types of agricultural
biomass (corn stover and wheat straw).

The recorded experimental data for the daily production of biogas and for the gases in
its composition (CH₄, CO₂ and H₂S), were fitted using Table Curve 2D program, which
gave the analytical equations with coefficients values and the Pearson correlation
coefficient (R²).

From figure 2 it can be observed that in the first 24 hours the production of biogas is
almost zero, and this aspect is probably due to the small number of microorganisms
initially found in the feedstock and to the necessary time of adapting of bacterial cells to
the new conditions of digestion. After this period, the accumulation of biogas increases
quite rapidly, following the equation \( y = a + bx^{1.5} + cx^3 \). The curve of experimental data
with the equation displayed on the graphic shows a high correlation coefficient, \( R^2 = 0.998 \).

A value of biogas production of 0.003 Nm³/kg dry matter was observed after the
second day. This fact may be attributed to the presence of easily biodegradable compounds
into the soluble fraction, which were continuous metabolized into fermentative products.

The maximum value of total biogas production during the 15 days of anaerobic
digestion of animal manure and Miscanthus x giganteus was about 0.420 Nm³/kg dry
matter.
Specific methane percent from animal manure and leaves of *Miscanthus x giganteus* after 15 days of fermentation at mesophilic conditions was determined, and the registered values are plotted in figure 3.

In this plant and for the mentioned substrate, the amount of CH₄ (% v/v) had values comparable to those in the literature, which are found in the range of 55 - 80% vol. The maximum value of 56.8% v/v was reached on day 15, this representing 0.023 Nm³CH₄/kg dry matter.
The production of hydrogen sulphide in the process of anaerobic digestion is due to sulphate-reducing bacteria, which use as substrate acetate, fatty acids, propionate and hydrogen, formed in the stages of substrate decomposition. Propionate decomposition by sulphate-reducing bacteria produces carbon dioxide, sulphide and acetate. Equations 1 and 2 describe the formation of hydrogen sulphide during the process of anaerobic digestion (24).

\[
CH_3COOH + SO_4^{2-} \rightarrow 2CO_2 + 2H_2O + H_2S \\
4H_2 + SO_4^{2-} + 2H^+ \rightarrow H_2S + 4H_2O
\]

(1)

(2)

As it can be seen from figure 4, the values of hydrogen sulphide concentration recorded a rapid growth in the first 5 days of digestion due to substrate decomposition, followed by the action of sulphate-reducing bacteria to the forming of CO2 and H2S.

After 4 days is recorded a peak of 3.696 %, and then the sulphate-reducing bacteria seemed to slow their activity, the hydrogen sulphide reaching to a value of 0.83% at the end of digestion period.

Figure 5 shows the CO2 content (% v/v) of the produced biogas. Carbon dioxide resulted from various digestion processes (3) or from reaction (1) that takes place in the first stage of digestion, recorded an increase of 10% in the first 5 hours, reaches a maximum of 53% after 5 days and then decreases to 19% at the end of digestion. Equations (3) – (5) describe the formation of carbon dioxide and methane in the process of anaerobic digestion (25, 26).

\[
C_6H_{12}O_6 \leftrightarrow 2CH_3CH_2OH + 2CO_2 \\
CH_3COOH \leftrightarrow CH_4 + CO_2 \\
CO_2 + 4H_2 \leftrightarrow CH_4 + 2H_2O
\]

(3)

(4)

(5)
4. Conclusion

In this paper were evaluated the biogas production, methane yield, carbon dioxide and hydrogen sulphide concentrations, as well as the time variation of substrate characteristics consisting of animal manure mixed with green leaves of *Miscanthus x giganteus* energy crop. Experiments were conducted by using a small capacity biogas plant at a temperature of 35 (±1) °C, neutral pH and intermittent mixing process. The plant operated for 15 days, the required time for complete anaerobic digestion of the used substrate.

The maximum yield of biogas, after 15 days of anaerobic digestion of animal manure and *Miscanthus x giganteus* was about 0.420 Nm³/kg dry matter.

It has been proved that *Miscanthus x giganteus*, 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 *Miscanthus x giganteus* as substrate in the co-fermentation process show better results compared to other substrates.

Thus, A.O. Adebayo & al. (27) determined the biogas production and methane concentration for cow slurry and maize stalk at mesophilic temperature (37°C). They reported that the higher biogas (0.426 m³/kg organic dry matter) and methane (0.297 m³CH₄/kg organic dry matter) yields were obtained at ratio 3:1 (75% VS of cow slurry co-digested with 25% VS of maize stalk).

R. Wahid & al. (28) evaluated the methane yields from stems and leaves of *Miscanthus x giganteus* and *Miscanthus sinensis* harvested green. They reported that after 90 days of anaerobic digestion, the methane production for *Miscanthus x giganteus* varied for stems from 285 to 333 NL/kg VS and for leaves from 286 to 314 NL/kg VS and for *Miscanthus sinensis* from 291 to 312 NL/kg VS for stem and from 298 to 320 NL/kg VS for leaves.

During anaerobic digestion process, it was observed that TSS values decreased from 1.20% to 0.21% and pH had a decreasing trend from 8.21 units to 7.27 units.

The results recorded for daily biogas production and for its component gases, were fitted using Table Curve 2D software, the curves of experimental data with the displayed equations showing a very good correlation coefficient, $R^2 > 0.970$. 

![Figure 5. Daily CO₂ content (% v/v) of the produced biogas](image-url)
Assessing the potential for biogas production of *Miscanthus x giganteus* energy crop is very important, because lately it increased the interest in finding effective crops of biomass with a high energy yield, low production cost and minimal effects on the environment.

5. Acknowledgements

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