

## Biogas generation from corn stalks and corn stalks bagasse resulted from ethanol production

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### Abstract:

The main purpose of the experiment was to compare the biogas yield from corn stalks differently pretreated with the biogas yield from corn stalks bagasse resulted from the process of lignocellulosic ethanol production. Cattle manure and liquid sludge from an on-farm biogas plant was used as co-substrate and inoculums. The laboratory scale experiment was carried out in batch system. Volume and composition of the produced biogas was measured by using BlueSens measuring equipment. By addition in fermentation batches of 8% DM corn stalks (milled to 10 mm particles), the gas yield is improved with 32.2 ml / 100 ml fermentation medium, while by addition of 8% DM corn stalks bagasse (mechanically pretreated before alcoholic fermentation) exhausted in the process of bioethanol production, the gas yield increased with 158.7 ml / 100 ml fermentation medium compared with control batch (cattle manure and inoculum).

In the case of physicochemical pretreated corn stalks bagasse exhausted in the process of bioethanol production, the gas yield decreased with 119.2 ml / 100 ml fermentation medium compared with control batch (this can be explained by accumulation in the physicochemical pretreatment of inhibitory compounds with negative effect on microbial activity). Mechanical pretreated corn stalks bagasse can be used as raw material for biogas production.

**Keywords:** agricultural wastes, corn stalks, biogas, bioethanol, anaerobic fermentation,

### Introduction

Biogas is a mixture composed mainly of CH<sub>4</sub> and CO<sub>2</sub>, during the degradation of organic substrate by anaerobic microorganisms. This biological process is called anaerobic digestion (AD). The degradation of organic substrate is carried out naturally in anaerobic environments such as the bottom of ponds, marshes (J. ROUSE & al. [1]), wetlands, the digestive tracts of ruminants and certain species of insects (J. RAPPORT & al. [2]).

References related to biogas production from organic substrate dating back to about 3000 years ago (U.S ENVIRONMENTAL PROTECTION AGENCY: WASTE PROGRAMS [3]). For example biogas was used to heat water bath in Assyria in the 10<sup>th</sup> century BC (P. D. LUSK [4]). In 1630 Van Helmont notices that the organic substrate is capable of producing an inflammable gas. For the first time, in 1808 Daviy recognized that methane was produced from decomposition of cattle manure (P. D. LUSK [4]).

A biological process like AD known from the past turns out to be a useful solution for the present and for the future in a situation where sources of alternative renewable energy are absolutely necessary to be found (D. ELANGO [5]).

The use of organic wastes as alternative energy sources is absolutely necessary because:

- year by year dramatically increase of organic wastes is recorded, as a consequence of global population growth (J. MOHAMMAD & al. [6]);
  - price of petroleum and fossil fuel is becoming more difficult to control. Energy security and reduced dependence on imported fossil fuels are very important for every country;
  - the wastes management by AD is proved as environmental friendly and decreases pollution;
- Using organic wastes as substrates for AD generates many benefits:
- Prevention of food and feed price increase: grains will be used only in human and animal nutrition (T. VINTILA & S. NEO [7]).
  - reduces emissions of greenhouse gases and decrease global warming (V.NIKOLIC & T. VINTILĂ [8]).
  - the energy stored in these wastes is not lost, but used to ensure human comfort.
  - contributes to social and economical development by creating jobs in areas with poor economies.

Many types of materials can be used as substrate for AD and biogas production: animal manure, agricultural waste, sewage sludge, municipal organic waste, industrial organic byproducts and residues (G. SHELEF, & al. [9]; I.R. ILABOYA, & al. [10]). Numerous potential studies indicate agricultural wastes as main source of renewable energy (T. VINTILA & S. NEO [7], T. VINTILA, & al. [11], SC PROJECT DEVELOPER [12]) at different levels (regional, national, global). Corn stalks represent important agricultural waste generated worldwide after corn grains harvesting and the quantity generated can be up to 200% from quantity of grain produced (T. VINTILA & S. NEO [7]). This agricultural residual biomass can be converted into lignocellulosic ethanol, the research in this field is very advanced (Z. CUI & al. [13], L. LUO & al. [14]), and technologies are already applied at pilot and industrial scale (<http://www.iogen.ca> [15], <http://ens-newswire.com> [16]). The corn stalks can be used as raw material for biogas production as well (V.NIKOLIC & T. VINTILĂ [8], X. LI & al. [17], S. NEO & al. [18]). However, it is important to study the possibility to use the exhausted bagasse resulted after pretreatment, hydrolysis and fermentation of corn stalks to produce lignocellulosic ethanol as feedstock for biogas production, as part of the cascade-processing of the biomass in biorefinery concept.

The main purpose of this experiment is to analyze what is the biogas generating capacity of corn stalks bagasse resulted from the process of lignocellulosic ethanol production and what is the total energy yield of corn stalks in the case of directly use as feedstock in biogas production compared with the case of previously passing of this biomass through the process of lignocellulosic ethanol production. As inoculums and additional fermentation substrate we used liquid sludge from a biogas plant and fresh cattle manure.

## **Materials and methods**

### ***1. Raw materials***

The raw materials used to construct the fermentation batches are: dried corn stalks resulted after harvesting corn grains in the stage of biological maturity; fresh dairy cows manure collected in the day of starting the experiment from didactic farm of University of Agricultural Science and Veterinary Medicine from Timișoara. The manure represents an important source of microorganisms for biogas fermentation. As main inoculums we used liquid sludge obtained from the pilot biogas plant belonging to the same farm. This plant is fed mainly with dairy cows manure for the production of biogas.

## 2. Pretreatment of the raw material and experimental pattern

A. Coarse milled biomass. In this type of pretreatment, we milled the biomass in a laboratory mill (Sample Mill SJ500, SWANTECH – France) to obtain coarse particles of raw material (up to 10 mm) to be directly used in biogas fermentation.

B. Mechanical pretreatment of the biomass. The corn stalks were mechanically pretreated in order to make available to cellulolytic enzymes the cellulose and hemicelluloses from the lignocellulosic complex. The raw material was milled in fine particles (under 2 mm), using a Cyclotec 1093 mill with 2.0 mm mesh (Foss Tecator, Sweden).

C. Physicochemical pretreatment. The corn stalks milled to coarse particles (10 mm - 20 mm) were pretreated by a combination of thermal and alkaline pretreatment (more correctly would be *mechanical-physical-chemical pretreatment*, but because the other types of biomass were milled as well, we have shorten to *physicochemical pretreatment*). The biomass soaked in 2% NaOH was autoclaved 30 minutes at 121°C. The biomass was afterwards washed with 10% H<sub>2</sub>SO<sub>4</sub> until pH 6.5 and with 12 equivalent volumes of water in order to remove the inhibitors resulted during pretreatments.

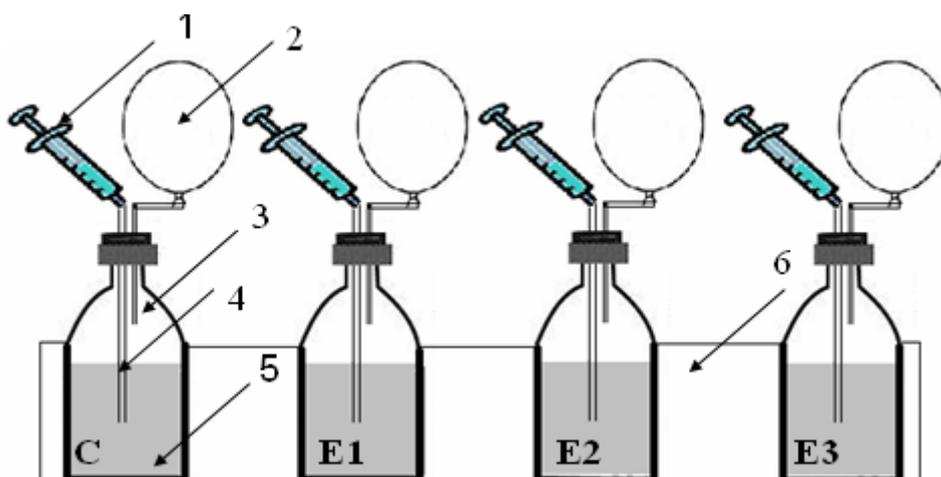
In a previous experiment the pretreated corn stalks were hydrolyzed using cellulolytic enzymes and the hydrolyzate was fermented with *Saccharomyces cerevisiae* to obtain ethanol. At the end of the fermentation, the solid fraction was separated of the liquid fraction by centrifugation. Ethanol was distilled from the liquid fraction. The solid fraction (containing mainly corn stalks bagasse and yeasts) was dried in an oven at 105°C and stored at the room temperature until was used as raw material for biogas fermentation in this experiment.

The biomass sorts resulted after processing as described above, mixed with cattle manure and liquid inoculums were introduced in batch bottles (capacity 2000 ml). The calculation of the quantity of each component introduced in the fermentation mixtures was made to obtain 8% dry matter (DM) in all batches.

**Table 1.** Experiment organization scheme

BATCH CODE	BATCH CONTENT	BIOMASS (g, DM)	INOCULUM (ml)*	FRESH MANURE (g)
C	Control (manure and inoculums)	-	1140	375
E1	Corn stalks (coarse milled), manure and inoculum	26.25	1150	375
E2	Mechanical pretreated corn stalks bagasse, manure and inoculum	26.25	1175	375
E3	Physicochemical pretreated corn stalks bagasse, manure and inoculum	26.25	1210	375

\*we used different quantities of inoculums to reach the same volume of fermentation medium in all batches.



**Figure 1** Experiment setup

1. Syringe for probes harvesting, 2. Gas collection balloon, 3. Tube connected to the gas collection balloon, 4. Tube immersed in the fermentation liquid, 5. Fermentable organic substrate, 6. Water bath.

Batch bottles are closed with caps provided with two rubber flexible tubes:

-one of the tubes has one head free in the interior of the fermentation flask and is not in contact with the substrate, and the external end of the tube was connected to a balloon for storing the produced biogas (S. NEO & al. [18]);

-the second tube, immersed into the fermentation liquid, has the external end connected to a syringe through for harvesting samples for pH control (S. NEO & al. [18]).

### **3. Parameters and conditions of fermentation**

At the start of the fermentation, the pH was adjusted in all batches to 6.8 in order to create the optimal conditions for the first phase of the biogas fermentation process. A Consort C932 pH meter has been used to determine the pH value during the experimental period. When required, the pH value was adjusted using 10%  $\text{NH}_4\text{OH}$  solution.

Regarding temperature, methane fermentations can be conducted in three temperature ranges: the psychrophilic (below  $20^\circ\text{C}$ ), the mesophilic (between  $20^\circ\text{C}$  and  $40^\circ\text{C}$ ) and the thermophilic (above  $40^\circ\text{C}$ ). Biogas yield increases proportionally with temperature. On the other hand, the increased temperature will also increase the concentration of free ammonia, the process will be inhibited and the methane production will be reduced (W. KOSSMANN [19]). Considering this, we chose the mesophilic range for our experiment and batches were incubated in a water bath (Memmert, Germany) at  $37^\circ\text{C}$ .

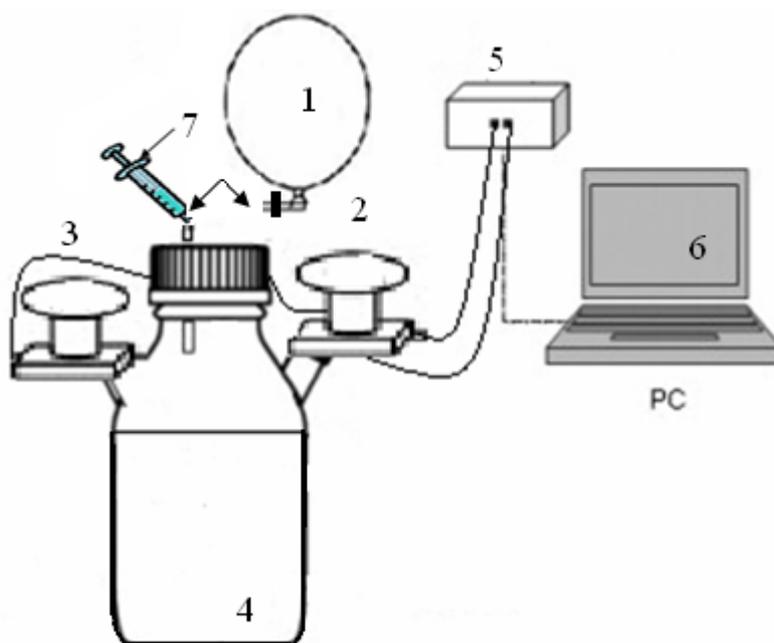
Stirring of the flasks content is very important for the dispersion of the biosolids for better contact with the methanogenic bacteria, reducing the scum build-up, diluting the level of inhibitors, retaining of the inorganic material in suspension and for reducing thermal stratification (R.H. ZHANG & al. [20]). Considering the aspects previously presented, during the 40 days of experiment the bottles contents were stirred for 5 minutes each 24 hours.

### **4. Analysis**

Dry matter (DM %) of biomass and fermentation residues was determined by oven drying at  $105^\circ\text{C}$ .

Measurement of methane concentration and carbon dioxide concentration in biogas was possible by using BlueSens<sup>TM</sup> fermentation measuring system. This system contains infrared  $\text{CH}_4$  and  $\text{CO}_2$  sensors for gas concentration measurement and gas counters for biogas volume

measurement. The sensors (figure 2 marked with 2 and 3) have been attached to a glass flask filled with water for reducing the dead volume in the measuring chamber. The biogas produced in each batch was stored in collection balloons and periodically harvested with a syringe (see figure 2). The biogas collected with syringe was immediately injected in the glass flask equipped with sensors. Concentrations of CH<sub>4</sub> and CO<sub>2</sub> are measured during the gas flow and the injected gas is forwarded through a tube to the gas counter to measure the volume of the biogas collected from each batch bottle. The information obtained by gas sensors and gas counter are processed on-line in the function box (figure 2 marked with 5). Here a data file is generated and transferred into the computer where the measurements are processed by BACVis software.



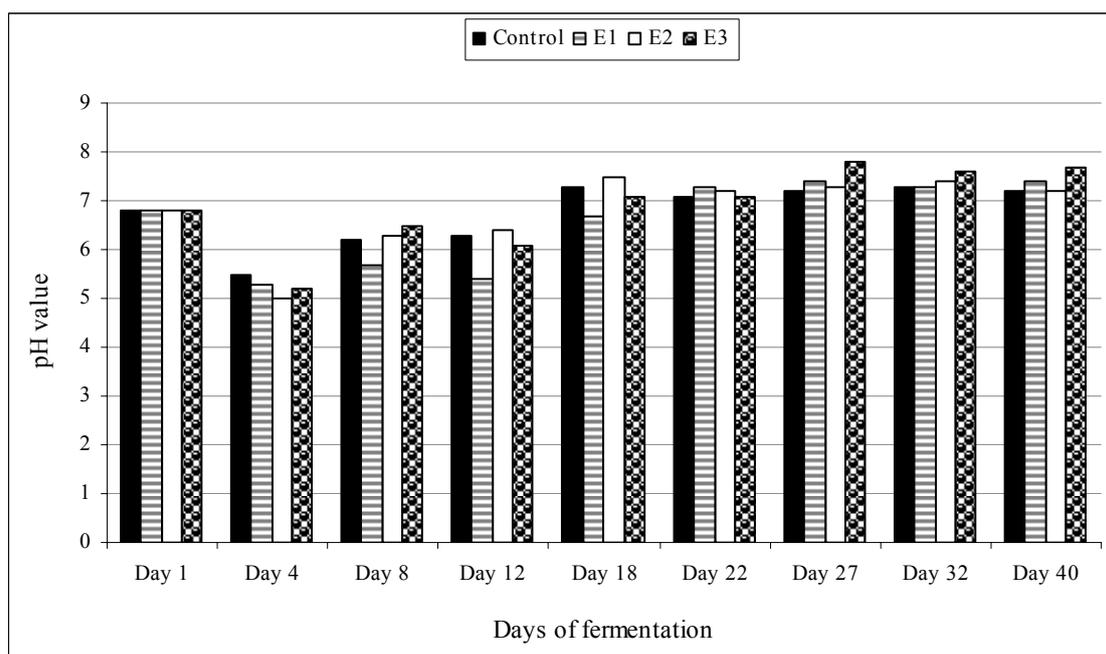
**Figure 2.** Data recording with BlueSens equipment.

1. Gas harvesting balloon; 2. CH<sub>4</sub> sensor; 3. CO<sub>2</sub> sensor; 4. Glass flask partially filled with water (empty space on the top is the measuring chamber); 5. Function box for data file generation; 6. Computer with BACVis software 7. Syringe for gas harvesting

## Results and discussion

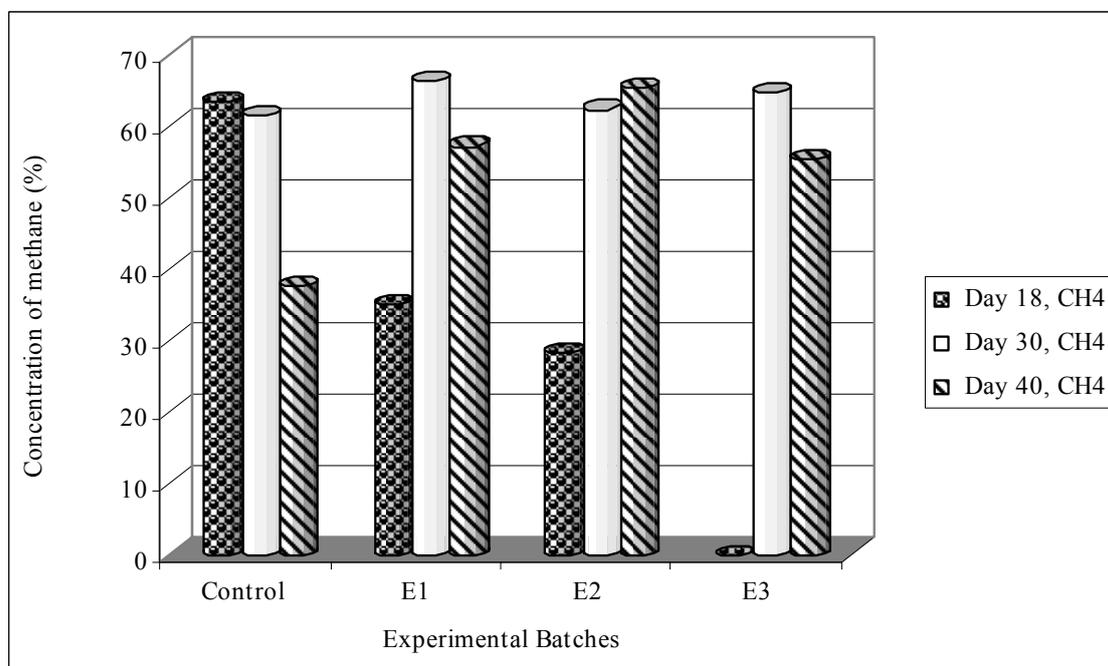
For pH monitoring and control during the 40 days of fermentation, samples were collected from each batch bottle every two days. When pH value decreases below 5.5, correction was achieved using 10% NH<sub>4</sub>OH solution. The pH decrease was substantial starting day 4 in all experimental batches. In the first 14 days of experiment the pH correction was required as presented in figure 4 (for lack of space not all pH determinations are shown). At each pH control, the pH value was adjusted to 7.0. In C batch bottles (cattle manure and inoculum), pH varied in the optimal limits of methanogenic bacteria (pH: 5.5–7.3). This can be explained by the fact that cattle manure acts as buffer protecting the fermentation process against the pH decrease resulted by accumulation of organic acids.

The pH correction was not necessary during the last 15 to 40 days of experiment, in any batches. In this period of time pH values varied between 6.6 and 7.8.



**Figure 2.** pH variation during anaerobic fermentation

During the experimental period, three harvestings of biogas accumulated in gas balloons were made: after 18 days, after 30 days and after 40 days of fermentation. The values presented in figure 3 represent the maximum concentration of CH<sub>4</sub> measured for each batch by BlueSens gas sensors.



**Figure 3** Concentration of CH<sub>4</sub> in the biogas harvested during the 40 days of anaerobic fermentation

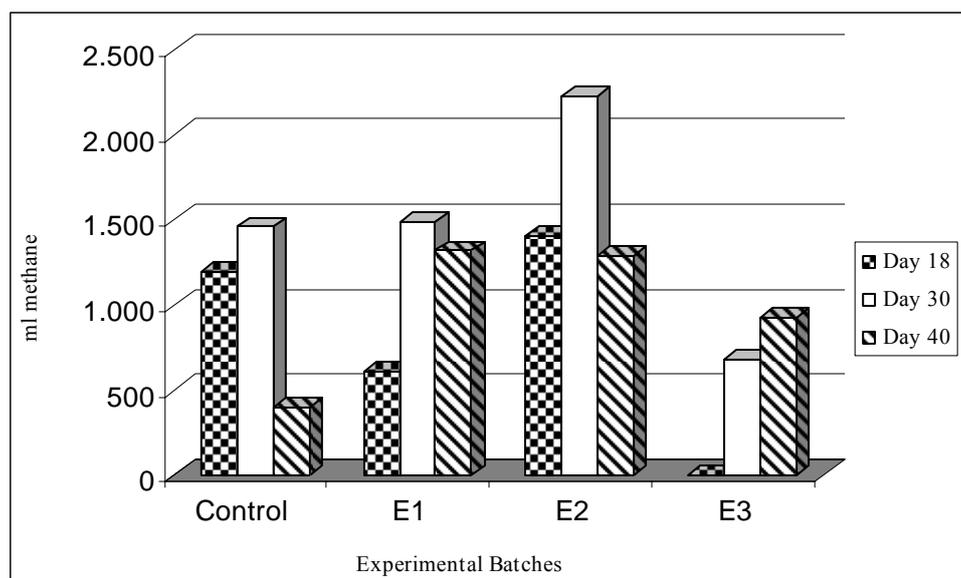
Concentration of CH<sub>4</sub> recorded at the first harvesting ranged between 28.25%, 34.97 % and 63.23% in case of E2, E1 and Control respectively. In the gas harvesting balloons of E3 no biogas has been accumulated. At the second harvest the CH<sub>4</sub> concentration ranged between

61.47%, 62.23%, 64.52% and 66.29% in case of Control, E2, E3 and E1 respectively. At the last harvesting of biogas the concentration of CH<sub>4</sub> was 37.66%, 55.35%, 57.08% and 65.25% for Control, E3, E1 and E2 respectively.

Analyzing the data regarding the concentration of CH<sub>4</sub> produced in these anaerobic digestion batches, we conclude that our results are comparable with those reported by other researches. Pellerin et al. (1987) indicate CH<sub>4</sub> yields between 50-60% in fermentation of dairy manure wastes. K. L. Bothi (2007) observed that concentration of CH<sub>4</sub> is 70% when is used food manure mixtures compared with 50-60% when manure alone is used (R.A. PELERIN & al. [21], K.L. BOTHI [22]).

In figure 4 the biogas yields are presented. The experiment was stopped after 40 days because the production of biogas with all batches became insignificant. The results regarding biogas production show that the volume of biogas generated is higher in most cases after 30 days of fermentation. In the case of E3 batch, the dynamics is atypical and the biogas volume is lower than in control.

At the first harvest (after 18 days of anaerobic fermentation) the highest amount of biogas was obtained in E2 (1405 ml), while in the gas harvesting balloons of E3 no biogas has been accumulated.



**Figure 4** Biogas production rate in the experimental batches

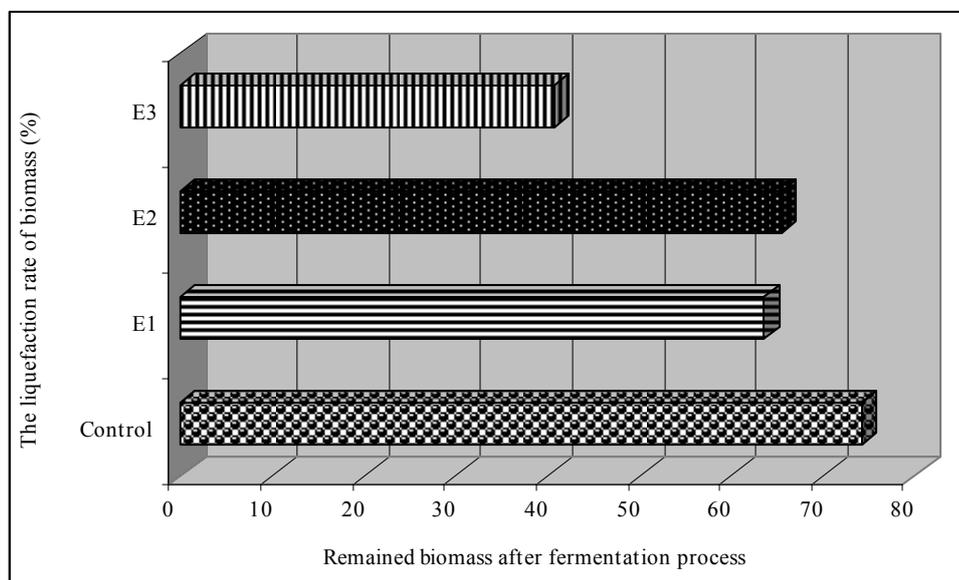
At the second harvest (after 30 days of experiment) the highest amount of biogas was obtained again in E2 (2225 ml) and the lowest quantity was recorded in E3 (683 ml). In case of C and E1 the amount of produced biogas was relatively equal 1460 ml and 1490 ml respectively.

At the last harvest (after 40 days of experiment) the highest biogas production was observed in E1 (1380 ml), followed by E2 (1320 ml) and E3 (930 ml). The lowest biogas production was obtained in C (400 ml).

In C, E1 and E2 gas generation increased in the interval day 19-30. The decrease of gas production in the period 30-40 days of experiment can be explained by the fact that at the end of the experimental period the microorganism responsible for biogas production has consumed most of the organic substrate and their metabolic activity has slowed down. The increase of gas production in the interval 19-30 days can be explained by the fact that in this period the pH of the fermentation media is stable in the ranged between 6.6 and 7.8 – the optimal pH range for methanogenic microorganisms.

In E3 (physicochemical pretreated corn stalks exhausted in the process of bioethanol production) the production of biogas was low compared with the other batches. One explanation for this could be that the physicochemical pretreatment applied to the corn stalks generated inhibitory compounds that inhibited the fermentation process.

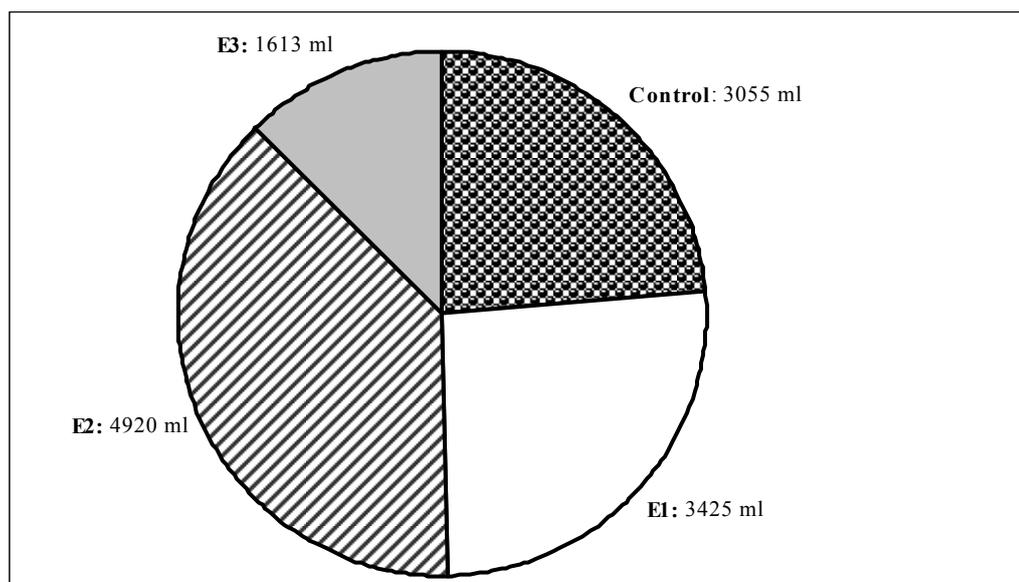
In figure 5 we represented data regarding the liquefaction rate of biomass in the experimental bathes.



**Figure 5.** The hydrolysis and liquefaction rate of biomass (%)

In each of these batches: E1, E2, E3 the initial concentration of biomass was 26.25 g (DM), as in C no biomass was introduced. During fermentation the biomass was decomposed and at the end of the experiment the following quantities of total solids were recovered (DM): 98 g (C), 130 g (E1), 134 g (E2), 200 g (E3). This indicates different values of biomass hydrolysis and liquefaction rates (see figure 4). There is a visible correlation between the biogas production and hydrolysis rates in the experimental batches. These results demonstrate that first period of hydrolysis in biogas fermentation, when mainly CO<sub>2</sub> is generated, is the key for a high production of biogas and later methanogenic activity.

Summing up the volumes of biogas from the three harvestings, we obtain the total biogas yields for each experimental batch during the 40 days of experiment. The data are shown in figure 6 and are comparable with those reported by Maramba (1978), Amon et al (2004), and Bond & Templeton (2011) in crop residues like straw, corn stalks and cobs, bagasse, peanut shell and found biogas yields between 200 and 400 m<sup>3</sup> per ton DM (F. MARAMBA [23], T. AMON et al [24], T. BOND and M. R. TEMPLETON [25]), Nikolic (2009) was reporting up to 200 l biogas/kg DM in corn stalks (V.NIKOLIC & T. VINTILĂ [8]). This yields are comparable to the biogas yields obtained from animal manure and animal slurry, which ranges from 370 m<sup>3</sup> per ton DM pig manure to 450 m<sup>3</sup> per ton DM cattle manure (LINKE & al [26]).



**Figure 6.** Total amount of biogas produced during the 40 days of anaerobic fermentation

Data referring to the total amount of biogas produced indicates that E2 (mechanical pretreated corn stalks bagasse exhausted in the process of bioethanol production combined with cattle manure) produced the highest amount of biogas: 4920 ml. The next follows E1 (corn stalks combined with cattle manure), with a total production of 3425 ml respectively. In the case of E3 (physicochemical pretreated corn stalks bagasse exhausted in the process of bioethanol production combined with cattle manure) the potential of producing biogas is lower than in control (1613 ml compared with 3055 ml).

These results indicates that by addition of coarse milled corn stalks, the gas yield is improved with 32.2 ml / 100 ml fermentation medium, while by addition of corn stalks bagasse exhausted in the process of bioethanol production (mechanical pretreated before alcoholic fermentation), the gas yield increased with 158.7 ml / 100 ml fermentation medium compared with control batch (cattle manure and inoculums). By contrary, in the case of physicochemical pretreated corn stalks bagasse exhausted in the process of bioethanol production, the gas yield decreased with 119.2 ml / 100 ml fermentation medium compared with control batch.

We consider these important findings, as in case of E2 we must take into account the fact that the corn stalks bagasse is generated after production of ethanol, which means that even more energy is produced if the corn stalks are processed by the combination of the two technologies: lignocellulosic ethanol production and biogas production of the resulted corn stalks bagasse. In ethanol fermentation of mechanical pretreated corn stalks, the concentration of ethanol in fermentation medium was 3.10%, as in the case of physicochemical pretreated corn stalks, the concentration of ethanol in fermentation medium was 2.80% (unpublished data). These results indicate once more that the physicochemical pretreatment leads to accumulation of inhibitory compounds with visible negative effect on microbial activity. Calculating the yields of ethanol reported to corn stalks biomass (DM), the calculations generated the following results: 0.206 g g<sup>-1</sup> DM mechanical pretreated corn stalks and 0.186 g g<sup>-1</sup> DM for physicochemical pretreated corn stalks (unpublished data).

**Table 2.** Total energy produced

	Ethanol produced, $\text{g g}^{-1}$	Energy from ethanol*, $\text{J g}^{-1}$	Biogas produced $\text{ml g}^{-1}$	Energy from biogas**, $\text{J g}^{-1}$	Total energy produced, $\text{J g}^{-1}$
Direct fermentation of corn stalks to produce biogas	0	0	131	2829	2829
Cascade processing by of corn stalks	0.206	5500	189	4082.4	9582

\*calculated for an energy content of 26700 J/g ethanol [27]

\*\*calculated for an energy content of 21.6 J/ml biogas containing 60% methane [27]

If we quantify the energy produced in the two cases (the case of direct fermentation of corn stalks to produce biogas, and the case of cascade processing by of corn stalks by hydrolysis, ethanol fermentation and biogas fermentation), we find that the total energy yield is much higher in the case of cascade processing then in the case of direct biogas fermentation (table 2). But, the most important finding in this work is that corn stalks bagasse produce higher yields of biogas than whole corn stalks. This can be explained by the pretreatment and enzymatic hydrolysis of the corn stalks before ethanol fermentation, which make the biomass more accessible to microorganisms in the biogas fermentation. Also, the addition and growth of yeasts during ethanol fermentation increases the organic load of the biomass and consequently the biogas production.

## Conclusions

Data generated in this work demonstrates that corn stalks bagasse resulted in the process of lignocellulosic ethanol production can be used as raw material for biogas production.

Corn stalks bagasse produces higher yields of biogas than mechanical pretreated (coarse milled) corn stalks.

Highest amount of biogas is produced from corn stalks bagasse resulted after the application of mechanical pretreatment, enzymatic hydrolysis and ethanol fermentation.

By cascade processing of the corn stalks through lignocellulosic ethanol production process and biogas production process, the highest energy yield is obtained.

The corn stalks bagasse resulted after the application of physicochemical pretreatment, enzymatic hydrolysis and ethanol fermentation produced lower amount of biogas. This can be explained by the fact that during the physicochemical pretreatment of corn stalks, inhibitory compounds are generated and accumulated in the biomass, with negative effect on the microorganisms involved in fermentation process.

## Acknowledgements

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