

## Improvement of Methane Yield from Maize Silage by a Two-stage Anaerobic Process

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Growing interest in processes that involve the conversion of biomass to renewable energies, such as anaerobic digestion, has stimulated research in this field and a considerable number of research projects have been developed to assess ideal digestion conditions for different energy crops. Among these potential crops, great interest has been addressed to maize silage. Most of the research on maize silage has been carried out on 1 stage batch digestion tests which have shown a great variability of results depending on hybrid type, harvest time, silage time and particle size. In this research project, an experimental procedure for the assessment of a two stage anaerobic digestion process was developed: a bench-scale reactor system was assembled for the evaluation of methanogenic activity of maize silage submitted to an acidification step, which could reduce the variability issues previously mentioned. The system consisted of two pairs of 5 L batch-fed completely stirred reactors, of which one was employed for the hydrolytic step and one for the acetogenic-methanogenic step. The reactors were kept at mesophilic conditions (38°C). For the first stage reactors an inoculum, taken from an operating full scale acidogenic reactor, was diluted and used as starter for the hydrolysis of the maize. The acidification rate was evaluated by means of the carbon dioxide production, total COD and pH. Samples of the hydrolyzed substrate were then used as feed for the methanogenic reactors, in order to evaluate the methane yield of the produced biogas, which was 0.3 m<sup>3</sup> CH<sub>4</sub>/kg COD<sub>removed</sub>, the COD removal efficiency, that was over 80 % and methane productivity in terms of volatile solids (VS), which was 0.38 m<sup>3</sup> CH<sub>4</sub>/kg VS. The final total COD mass balances had a mean error lower than 5 %.

### 1. Introduction

In the present day energy has become a resource of primary importance. With the constant increase of fossil fuel prices and the growing evidence of environmental damage caused by their use, a lot of research has been focused on finding and developing new diversified sources of renewable energy. Until recent years maize has been used mainly for human and livestock nutrition, and to a smaller extent as raw material for industry. New utilization pathways have evolved with the promotion of climate protection policies and biomass is regarded to play a vital role in the development of sustainable energy systems such as biodiesel and anaerobic digestion (Fischer et al. 2010; Mata et al. 2012).

In particular the biogas production from energy crops is of growing importance. Maize, sunflower, grass and Sudan grass are the most commonly used energy crops (Karpenstein-Machan, 2005).

Maize is currently the most dominating crop for biogas production and there is a considerable amount of literature available on the use of maize and maize silage for anaerobic digestion. A recent literature review on the anaerobic digestion of maize silage, compiled by Hermann and Rath (2012), has highlighted that ideal digestion conditions have not been established yet, due to a high variability of the methane yield results depending on particle size, harvest time, ensiling time and crop variety. The authors suggest the need to take specific biomass quality requirements into consideration, that could be addressed by targeted breeding to optimize the nutritive values and therefore improve digestibility.

The microbiology of the anaerobic digestion process is a complex system that involves several groups of microorganisms. In a simplistic description the process includes four main steps. During the first step (hydrolysis), complex organic macromolecules are hydrolysed into simpler and soluble organic compounds. Acidogenesis and acetogenesis are the second and third steps respectively, during which the products of the hydrolysis get fermented into short chain organic acids (acidogenesis) and ultimately into acetic acid (acetogenesis), hydrogen is also an important product of these 2 steps. Finally the acetic acid and the hydrogen get used by the methanogenic population for the production of methane (Pavlostathis and Giraldo-Gomez, 1991).

The different growth rates and pH optima for acidogenic (between 5.5 and 6.5) and methanogenic microorganisms (pH around 7), which translate in different reactor condition requirements, have led to the development of the two-stage anaerobic digestion process, that was first promoted by Pohland and Ghosh, (1971). Ideally during the first step particulate organic matter is hydrolysed and then fermented to obtain short chain organic acids, alcohols, carbon dioxide and hydrogen, which then are converted into methane and carbon dioxide by the acetogenic and methanogenic bacteria in the second stage of the process (De La Rubia et al., 2009).

As established by Solera et al. (2002), two-stage systems have shown several advantages over conventional processes, mainly due to a high specialization of the microorganisms within each digester: there is an increase in the stability of the process caused by a better control of the acidification stage, which helps prevent overloading and the build-up of toxic materials, and ultimately this separation tends to increase the pH stability of the methanogenic population.

In the present work, a two stage digestion process was used for the anaerobic digestion of maize silage, based on the hypothesis that the variability in the methane yield depends greatly on its molecular composition (mainly complex molecules, such as cellulose and lignin, which show difficult digestibility). The separation of the first step allows for better hydrolysis and acidogenesis conditions; this could bring to an improved digestion of the substrate, in particular a higher degradation rate of the complex molecules that are part of the total composition of maize silage.

The improved digestion carried out in the first step could then translate into higher methane yields within the second stage reactor.

## 2. Materials and Methods

### 2.1 Maize silage and anaerobic seed cultures

The Maize silage was obtained from a silo after approximately 6 months of ensiling. Samples of the silage were collected for characterization, while the rest was stored in airproof bags at 4 °C. The results of the characterization of the substrate are presented in table 1.

*Table 1: Substrate characterization*

Sample	TS [%]	TVS [%]	COD <sub>tot</sub> [g/g TS]
Maize silage	28.10	96.57	1.99

The seeding cultures were obtained from a full scale 2 stage reactor in the Emilia Romagna region (Italy) and preserved at room temperature prior to use. After characterization (Table 2), 0.75 mL of the acidogenic sludge was used to seed the first stage reactors, and 1.5 L of methanogenic sludge to seed the second stage reactors; demineralized water was added to reach the final reactor volume of 4.5 L. The seeding was followed by a sludge stabilization process, to ensure, in the first stage reactors, a sufficiently high grade of digestion of the substrate: three consecutive loadings were performed in the course of three weeks. During this period, the second stage reactors were fed with mashed apples, in order to maintain the activity of the methanogenic microorganisms.

*Table 2: Sludge characterization*

Sample	TS [g/L]	TVS [g/L]	COD <sub>tot</sub> [g/L]
First stage sludge	64.55	56.18	74.26
Methanogenic sludge	37.38	29.00	38.84

## 2.2 Experimental set up

The bench scale plant used for the assays is composed of two pairs of independent reactors (four in total: reactors 1 and 2 were used for the first stage, reactors 3 and 4 were dedicated to the methanogenic step), each of which is connected to a gas-collecting unit for biogas accumulation and sampling.

Each reactor consists of a 5 L volume flask. The reactors are immersed in a 50 L Plexiglas vessel kept at 38 °C. Homogeneous conditions were maintained through magnetic stirring, and the system temperature was controlled within  $\pm 0.1$  °C using a heating immersion circulator (Julabo MB).

Each bioreactor was connected through stainless steel pipeline ( $\varnothing_{in} = 3$  mm) to a differential pressure transducer (RS Instr.) and to a gas collector. The fittings used to connect the glass vessels to the steel piping were made of Teflon in order to reduce gas diffusion and permeation through polymer materials.

The biogas produced was collected in a water displacement device consisting of 4 glass cylinders with 2 L of volume each, turned upside down and immersed in a Plexiglas vessel of 15 L filled with a NaCl barrier solution of 10% concentration by weight.

Temperature probes were inserted into the reactor vessel and the gas collector respectively. To monitor pH in the first stage, a pH probe (Hanna HI 62910) was inserted into each bioreactor used for the first stage of the process.

Temperature, pH and pressure data were sampled by a data logger with a temporal scan of 1 min and sent to a PC.

## 2.3 Analytical methods

Total solids (TS), total volatile solids (TVS), total suspended solids (TSS), total volatile suspended solids (TVSS) and total COD ( $COD_{tot}$ ) analyses were performed as described in standard methods (APHA, 2005).

The produced gas was analysed with a landfill gas analyser (GA2000 plus by Geotechnical Instruments).

## 3. Results and Discussion

At the end of the first 3 weeks of stabilization, the biogas produced by the acidogenic (first stage) reactors reached a steady composition of 23 %  $CO_2$  and 75 % of a mix of VFA (volatile fatty acids) and  $H_2$ , oxygen and methane were present in less than 1% each. The pH values within the first stage reactors were stable through the assays: in reactor 1 a minimum of 3.85 was measured at the beginning of the stabilization stage, after which the pH maintained a value around 4.1 ( $\pm 0.1$ ), while in reactor 2 pH values varied from a minimum of 3.35 at start-up to a stable value of 4.3 ( $\pm 0.1$ ).

After stabilization, the methanogenic reactors (3 and 4) were fed with a volume of effluent and COD withdrawn from the first stage reactors, which was replaced with demineralized water and fresh substrate. A run would be considered over, when the biogas production rate would be less than 15 mL/h. At this point the reactors would be fed with more substrate, as a result run duration was variable. Nevertheless, as can be observed from the production curves (Figures 1 and 2), the biogas production rate presents a high overlapping on the second and third run, which can be considered as an indicator of a stable digestion rate.

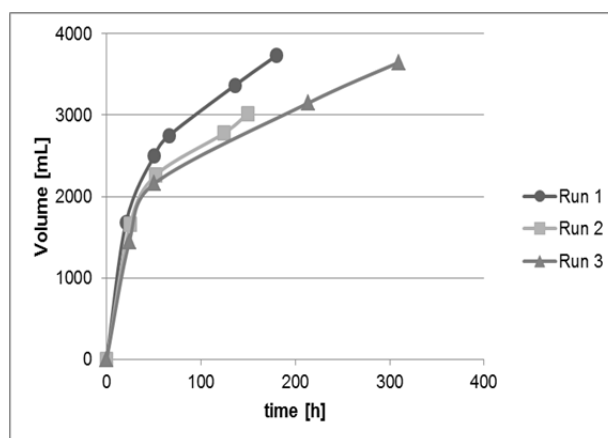


Figure 1: Biogas production for reactor 3 during loadings with samples taken from reactor 1.

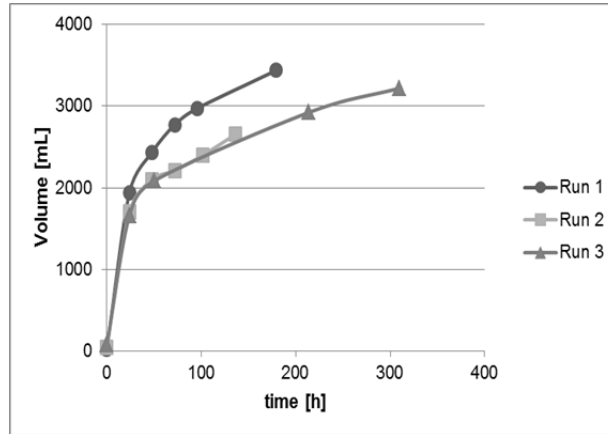


Figure 2: Biogas production for reactor 4 during loadings with samples taken from reactor 2.

Although the first run had the highest biogas production rate, the quality of the gas content in terms of methane was higher in the last run (Figures 3 and 4) with an average methane content of 50%. The curves in figure 4 represent this improvement very clearly.

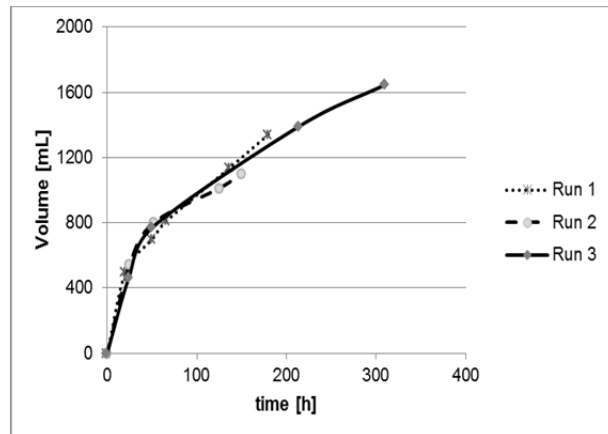


Figure 3: Methane production for reactor 3 during loadings with samples taken from reactor 1.

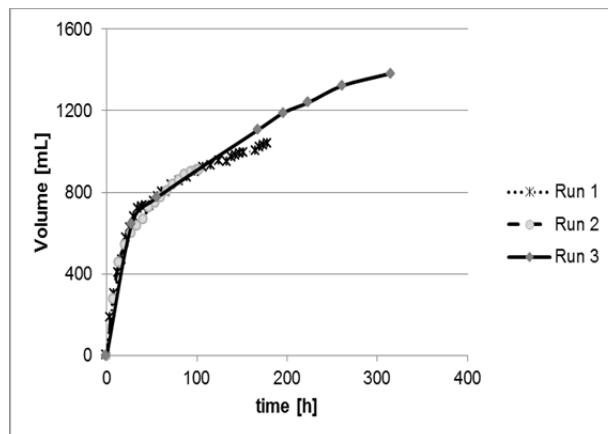


Figure 4: Methane production curves for reactor 4 during loadings with samples taken from reactor 2.

The formula used to calculate the mass balances is depicted in equation (1), where the final COD ( $COD_f$ ) is given by the base total COD ( $COD_b$ ) within the reactors summed to the total COD loaded ( $COD_l$ ) from the first stage reactors, minus the amount of COD taken ( $COD_d$ ) during control tests and minus the amount of COD equivalent to the total final volume of  $CH_4$  ( $COD_g$ ). The mass balances for both reactors confirm the steady state with an error range of 0.4 - 1.4 % (see Table 3).

$$COD_f = COD_b + COD_l - COD_d - COD_g \quad (1)$$

Table 3: Mass balance results

Reactor	Final calculated $COD_f$ [g]	Final measured $COD_m$ [g]	$COD_l - COD_m$ [g]	Error [%]
1 <sup>st</sup> Methanogenic reactor	57.85	57.61	0.24	0.41
2 <sup>nd</sup> Methanogenic reactor	57.41	56.63	0.78	1.37

The specific methane yield (calculated as  $m^3 CH_{4\text{produced}}/kg COD_{\text{fed}}$ ) was an average  $0.30 m^3 CH_4/kg COD$  on the first methane producing reactor, with a peak of  $0.38 m^3/kg COD$  during the last assay. The methane yield in the second methanogenic reactor had an average value of  $0.27 m^3 CH_4/kg COD$ , with a peak of  $0.34 m^3 CH_4/kg COD$  in the last run.

As reported by Giuliano et al. (2013), the SGP (Specific Gas Production) for maize silage in a single stage process can have relatively average values in both mesophilic and thermophilic conditions ( $0.73$  and  $0.82 m^3$  biogas/ $kg TVS_{\text{fed}}$ ).

The SGP obtained from the 2 stage digestion process reactors was  $1.56 m^3$  biogas/ $kg TVS$  and  $1.7 m^3$  biogas/ $kg TVS$ , for the first and second reactor respectively, which is almost twice the value obtained by the single stage digestion process. This means that a load taken from the first reactor in the 2 stage process, with a TVS content equivalent to the amount of TVS contained in the substrate fed in a single stage reactor process, produces almost twice as much biogas as the single stage process. Considering the methane yields of the process, this is a significant increase in the overall amount of methane obtained at the end of the process.

The COD removal efficiency was 84.35 % on the first methanogenic reactor and 85.50 % on the second methanogenic reactor.

Although both methanogenic reactors were maintained at the same working conditions, seeded with the same sludge, and fed with the same loading rates, the results obtained are different, but this difference is no greater than 10 %; this is probably due to the inherent nature of the populations of microorganisms, and the differentiation that occurred since the start of the experiments.

#### 4. Conclusions

In this study, a 2 stage process was applied with maize silage, a substrate, which shows difficult digestibility. The results obtained from this study have shown that a 2 stage digestion process can greatly increase the biogas production rate by improving the digestion efficiency of the substrate and therefore increasing the methane yield. The process reached stability in a relatively short time, and the methane production improved over time. The first stage reactors showed good stability with little pH variations after the stabilization period. Although the pH remained stable, it was still lower than the pH values of 5-6, which are recommended for optimal microbial growth (Verrier et al., 1987; Mtz.-Vitturia et al., 1989; Bouallagui et al., 2004; Demirer and Chen, 2004). Further study of the effects of pH influence, maintenance and optimization are needed to verify if this parameter influences the methane yield.

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