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Effect of Thermal and Mechanical pre-Treatments on the CH_4-H_2 Production from Water Buffalo Manure in Different Process Conditions

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Animal manure can be profitably used to produce energy in anaerobic fermenters increasing energy independence and reducing disposal costs and pollution. Animal manure is a low cost substrate rich in carbohydrates, especially suitable for biofuels production, such as hydrogen and methane.

We preliminary studied the effect of mechanical manure pre-treatments on the fermentation process focusing on the biogas yield and composition. In particular, we investigated both the H_2 and CH_4 oriented fermentation.

Our batch reactors consist in 280 ml serum glass bottles hermetically sealed with pierceable rubber cups. The products of fermentation were measured with a micro gas chromatograph, equipped with two capillary columns.

To individuate the most suitable conditions for H_2 production, a manure thermal pre-treatment, used to isolate the H_2-producing bacteria, was investigated.

Preliminary results show that the mechanical pre-treatments do not systematically alter the fermentation process. Concerning the thermal pre-treatment, we found that the CH_4 production is inhibited when the manure is heated at the 90 °C for several hours. Moreover, we identified the most favorable conditions to optimize the production of H_2: *i*) thermal pre-treatment on the substrate; *ii*) initial pH ~ 5.0; *iii*) process temperature of 55 °C. The favorable conditions to optimize the production of CH_4 are: *i*) initial pH ~ 7.0; *ii*) process temperature of 37 °C.

1. Introduction

One of the problems of the today's society regards the depletion of energy sources, their increase of costs, the intensification of the annual energy demand and, consequently, the climate changes induced by carbon dioxide and other greenhouse gases, such as those coming from the use of fossil fuels. The main options to limit the emission of carbon dioxide into the atmosphere are the de-carbonization of fossil fuels and the increase of renewable energy.

Among the most promising energy sources, the biomass is the oldest and includes the animal organic waste, sewage, energy crops, agricultural residues which may be used for biogas and biofuels production (Antonopoulou et al., 2007).

Since buffalo farms are largely diffused in Campania Region, in south Italy, and represent a sector in constant growth, we focused on the anaerobic digestion of buffalo manure. It is a low-cost substrate with plenty of carbohydrates, an optimal carbon/nitrogen ratio, and a rich microbial flora. For this reason it is considered a valuable source both of bio-methane and bio-hydrogen (Carillo et al., 2012).

The digestion process begins with bacterial hydrolysis of the input materials. Insoluble organic polymers, such as carbohydrates, are broken down to soluble derivatives that become available for other bacteria. Acidogenic bacteria then convert the sugars and amino acids into carbon dioxide, hydrogen, ammonia,

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and organic acids. Finally, methanogens transform these products to methane and carbon dioxide (Tabatabae et al., 2010). Hydrogen is an intermediate product of the fermentation; its yield can be optimized by inhibiting the final fermentation step in a batch process, or by optimizing the resident time in the first of two continuous bioreactors in series (Cooney et al., 2007). To study and optimize a continuous process at lab scale, the first problem to face is the substrate pumping. In fact, the manure must be diluted with water, homogenized and filtered in order to avoid feeding tubes clogging.

The purpose of this work is to analyze the fermentation anaerobic process in batch mode, with serum glass bottles, to investigate the effect of mechanical pre-treatments. In addition, the thermal pre-treatment is also analyzed; indeed, the methanogens colonies, responsible of hydrogen consumption during the final stage of the digestion process, are typically killed at high temperature, differently from hydrogen producing bacteria (such as *Bacillus* and *Clostridium* species) that form endospores or “structures of survival” in unfavorable environmental conditions (Carillo et al., 2014). When favorable environmental conditions are restored the spores germinate and become vegetative cells (Doyle, 1989).

2. Materials and Methods

Buffalo dungs were collected from an Italian farm, located in Villa Literno (South of Italy) and only from stables accommodating lactating heads. The manure was taken in March, April, May and June to follow the possible changes in the material composition. Once collected, the samples were stored in closed plastic bags, and no metallic tools were ever adopted to avoid any contamination of the samples. The majority of the manure was placed in a refrigerator, at a temperature of about 4 °C, to stop the likely fermentation reactions in progress.

As discussed in the Introduction, the manure is subjected to different thermal and mechanical pre-treatments and also different fermentation conditions were investigated. Some details of the sample preparation are discussed here.

A) The material, if required, is thermally pretreated at 90 °C for 3 hours. The duration and temperature was chosen according to the literature (Khanal, 2008) because it was proved to be effective to inhibit, at least partially, the methanogenic bacteria (Carillo et al., 2012).
B) The material is then weighed and mixed with distilled water to achieve a ratio of manure and water equal to 30/70 in mass. Previous tests have shown that this ratio provides the best compromise between productivity potential and viscosity of the slurry.
C) The initial pH of the solution was acidified with a 1M solution of HCl.
D) The samples are finally mechanically treated in three possible ways:
    i. gently hand mixed: MM
    ii. first mixed by hand and then with an electrical homogenizer for 2 minutes: H
    iii. first mixed by hand, then with an electrical homogenizer for 2 minutes and finally filtered with a Büchner filter with the aid of a vacuum pump: F.

The different sample typologies that we have investigated are summarized in Table 1.

The anaerobiosis is obtained blowing nitrogen with a two-needles system inside the reactors, i.e. glass serum 280 ml bottles. The bottles are placed in a ventilated oven set at the desired temperature (Table 1). Each sample measurement is obtained using three replicates for statistical purposes.

The composition of the gas in the bottle headspace is analyzed with the MicroGC Agilent 3000 (S.R.A. Instruments, France) gas chromatograph equipped with two capillary columns: a *MolSieve 5 A* and a *Poraplot U*. The former is used to separate H_2, O_2, N_2 and CH_4; the injector and column temperature are respectively set at 90 °C and 110 °C with Ar as gas carrier. The latter is used for CO_2 measurements; the injector and column temperature are set at 90 °C and 85 °C, respectively, and He is the gas carrier. This column allows detecting also H_2S traces in the biogas. The gas sample is taken from the bottles using a needle inserted in their butyl rubber caps and directly connected, through a water vapor trap, to the gas chromatograph (Carotenuto et al., 2012).

Table 1: Prepared samples

Sample	Initial pH	T (°C)	Thermal pre-treatment	Mechanical pre-treatments
A	6.0	37	YES - NO	MM, H, F
B	6.0	37	NO	H, F
C	7.0	37	NO	F
D	5.0	55	YES - NO	F

3. Results and Discussion

Results are presented showing the measured values of gas volume fraction as a function of the time and they are interpreted by means of the modified Gompertz equation (Khanal et al., 2004):

$$H = P\,exp\left\{-exp\left[\frac{R_m \cdot e}{P}\,(\lambda - t) + 1\right]\right\} \tag{1}$$

where H is the cumulative production, R_m is the maximum production rate, e is Euler's number, λ the lag-phase time and t the incubation time.

Figure 1 shows CH_4 concentration as a function of time for samples A without thermal pre-treatment, and the values of Gompertz parameters P, R_m and λ, obtained from the best fit of the data with Eq(1), are reported in Table 2 together with the regression coefficient R^2.

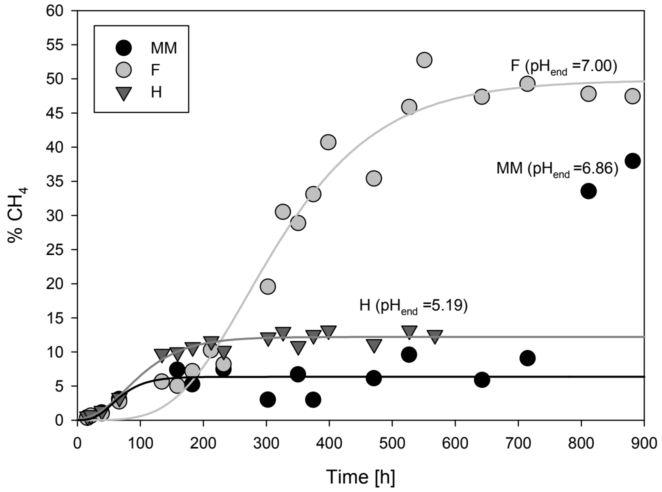

Figure 1: Evolution of bio-methane concentration during the fermentation process obtained with sample A, at initial pH 6.0 and 37 °C, for different mechanical pre-treatments and without thermal pre-treatment.

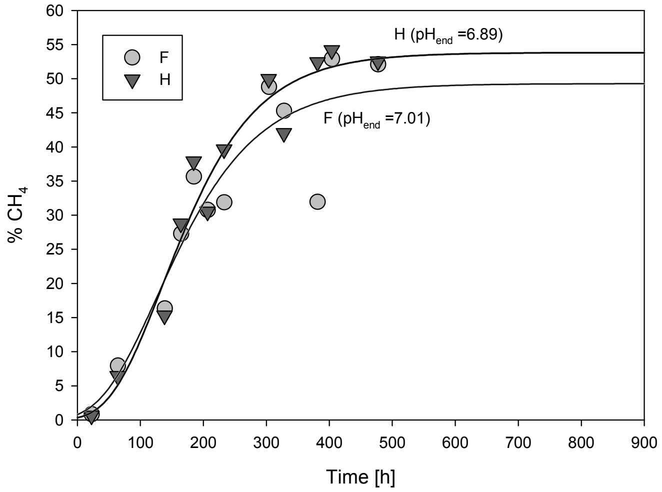

Figure 2: Evolution of bio-methane concentration during the fermentation process obtained with sample B, at initial pH 6.0 and 37 °C, for different mechanical pre-treatments and without thermal pre-treatment.

Table 2: Gompertz parameters for CH_4 Production (pH 6.0; T 37 °C)

Figure1: Sample A				Figure2: Sample B			Figure3: Sample C	
Parameters	MM	H	F	Parameters	H	F	Parameters	F
P [%]	6.37	12.20	49.84	P [%]	53.82	49.28	P [%]	68.28
λ [h]	26.80	26.63	155.80	λ [h]	52.12	37.14	λ [h]	99.78
R_m [%/h]	0.08	0.09	0.16	R_m [%/h]	0.24	0.20	R_m [%/h]	1.10
R^2	0.56	0.97	0.97	R^2	0.96	0.88	R^2	0.99

We observe from Figure 1 that the sample with the mechanical pre-treatment F behaves differently from the other two, and indeed it produces more methane. This result may have several explanations. It is reasonable to assume that the vacuum filtration removes all inert materials, such as straw and animal hair, initially present in the dung, which may obstacle the fermentation. Looking at the final pH values measured at the end of the entire process (see Figure 1), we notice that the samples with higher CH_4 yield (F and MM) show a final pH of about 7.0, while the H sample shows a final pH value of 5.19. The growth profiles of F and MM samples are, however, quite different, as also highlighted by the Gompertz parameters shown in Table 2, and we believe that the pH of sample MM increased only in the final stages of fermentation, in correspondence of the abrupt increase of CH_4 concentration (t = 700 hours). Probably, some uncontrollable factors influenced the pH evolution that brought about a different yield and we believe that this latter explanation is the real factor that induced the three different behaviors observed in Figure 1.

We repeated the comparison of H and F mechanical pre-treatments with sample B, *i.e*. the two that showed the larger differences in Figure 1. The results are shown in Figure 2, where the CH_4 evolution of H and F almost overlap and, indeed, the Gompertz coefficients are very similar (Table 2). So, we believe that the mechanical pre-treatments do not affect the CH_4 fermentation process. The final pH value, conversely, seems very sensitive to the process and, indeed, in this case the two final pH values are about 7.0.

Despite the indications found in the literature, where the typical pH for CH_4 production is 6.0 (Yasin et al., 2011), the results obtained suggest that buffalo manures produces a high CH_4 yield when the system (auto-)evolves towards a pH of 7.0. In Figure 3, we show the results obtained on sample C, fermented starting from a pH 7.0. The results are very promising and indeed the bio-methane reached a concentration of about 70 % (see Table 2). In any case, the methane concentration of 70 % is one of the highest reported in the literature for batch fermentation for fresh substrate. In fact, Zhang et al. (2007) attained a value of CH_4 concentration equal to 73 % by fermenting food waste for 28 days at 50 °C; Rao et al. (2004) achieved a value of CH_4 concentration of 72 %, at 25 °C, by fermenting for 100 days municipal garbage and adding cattle dung as process starter. It is worth noticing that both the yield and the lag-time of the fermentation can be improved if some digested manure is mixed with fresh one (Kalia et al., 2001; di Cristofaro et al., 2014).

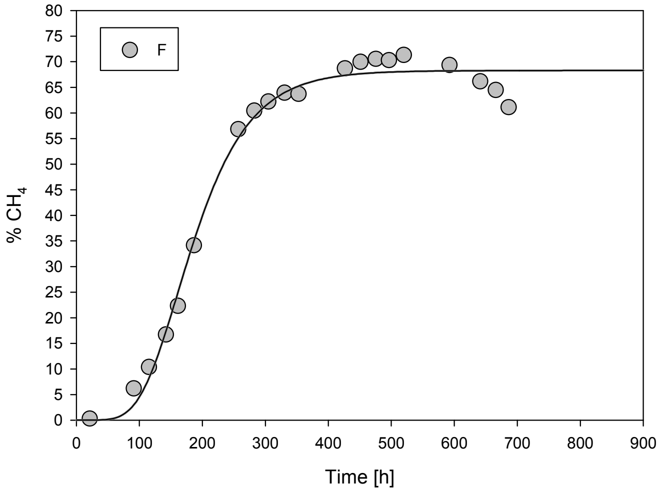

Figure 3: Evolution of bio-methane concentration during the fermentation process obtained with sample C, at initial pH 7.0 and 37 °C, for only mechanical pre-treatments F and without thermal pre-treatment.

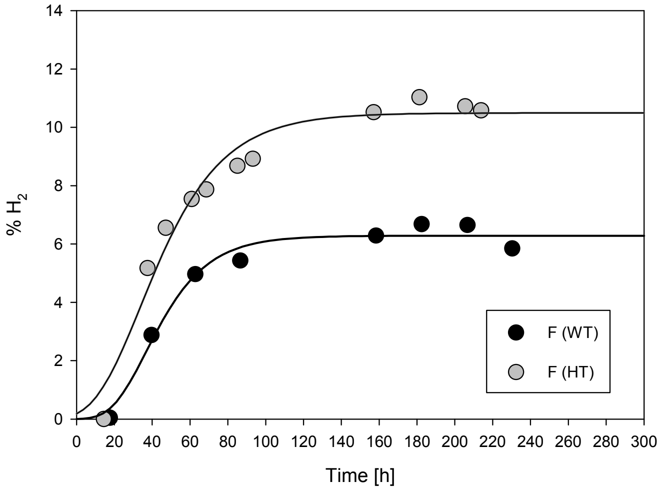

Figure 4: Evolution of bio-hydrogen concentration during the fermentation process for samples D at pH 5.0 and 55 °C, for only mechanical pre-treatments F, with (HT) and without (WT) thermal pre-treatment.

Table 3: Gompertz parameters for H_2 Production (pH 5.0; T 55 °C)

Figure 4: Sample D		
Parameters	F (WT)	F (HT)
P [%]	6.29	10.49
λ [h]	18.54	9.59
R_m [%/h]	0.34	0.43
R^2	0.98	0.97

Finally, sample A with the thermal pre-treatment was fermented at 37 °C and initial pH 6.0. No CH_4 was detected in the first 350 hours, and then it appeared reaching a maximum value of about 15 %. We do not show these results for the sake of simplicity, but they allow concluding that the thermal pre-treatment inhibits, at least partially, the methanogenic bacteria. We, then, investigated whether the thermal pre-treatment is also effective to select the correct bacteria colony to produce bio-hydrogen.

In Figure 4, we show the results obtained from the fermentation of sample D at 55 °C with (HT) and without (WT) thermal pre-treatment. In both cases we used the filtered manure (F). A rapid growth of H_2 concentration is observed for both typologies. However, the sample HT reaches the maximum asymptotic value of about 10 %, while sample WT of about 5 %. Both of them reach this maximum value in about 90 hours and then the concentration remains almost constant for more than 100 hours.

The Gompertz parameters, P, R_m and λ, obtained from the best fit of the data in Figure 4 are reported in Table 3 together with the relative regression coefficient R^2. It is worthwhile noticing that the lag time of HT sample is about 1/2 of that of WT sample. The thermal pre-treatment has clearly improved both the hydrogen production and the fermentation kinetics.

4. Conclusions

We have shown that buffalo manure can be used to produce both H_2 and CH_4 in anaerobic fermenters without addition of hexogen bacteria.

The mechanical pre-treatments, compulsories in a continuous process in the lab scale, do not affect the process. The optimal initial pH to be imposed to produce CH_4 resulted to be 7.0 and it does not require a control during the process, since the system auto-regulates. Conversely, if an initial pH of 6.0 is chosen, the system may evolve either towards a neutral pH, thus producing methane, or towards a slightly acid slurry to the detriment of CH_4 production.

Also the effect of a thermal pre-treatment to select the appropriate bacteria community is investigated and we showed that 3 hours at 90 °C are able to inhibit the methanogenic bacteria, thus promoting the eubacteria if the system is fermented at 55 °C with an initial pH equal to 5.0.

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