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# Temperature and pH Effect on Methane Production from Buffalo Manure Anaerobic Digestion

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## ABSTRACT

Anaerobic digestion is an established technology to treat different kinds of wastes and to simultaneously produce biogas, a mixture of methane and carbon dioxide, which is a useful and renewable energy source. In this work, the anaerobic digestion of buffalo manure is studied. The latter is a low cost substrate rich in carbohydrates, especially suitable to produce biogas in anaerobic digesters. The process is performed both under mesophilic (37 °C) and thermophilic (55 °C) conditions with pH spanning from 6.0 to 8.7. Many different samples, deriving both from lactating and non-lactating buffaloes, are used. Under mesophilic conditions, our results are essentially in line with literature, and the highest methane concentration in the biogas (even above 65 %) is obtained with pH = 7.0. Conversely, under thermophilic conditions, we observed that also with pH > 8.0, the anaerobic process runs efficiently. In particular, methane concentration in the biogas reaches values around 70 %, with production rates almost twice those obtained at 37 °C. This experimental observation is particularly interesting for those wastes showing a natural basic pH, as the buffalo manure at hand; in these cases, in fact, the digestion process can start without any manipulation of the pH.

**Keywords:** Anaerobic digestion, Bio-methane, Buffalo manure.

## 1. INTRODUCTION

Anaerobic digestion is widely used in the treatment of different organic wastes for production of biogas with whole range of benefits for their users that include: production of heat and electricity, transformation of organic waste into high-quality fertilizer, improvement of hygienic conditions through reduction of pathogens, reduction of work for firewood collection and cooking, and environmental advantages through protection of soil, water, air, and woody vegetation [1].

Anaerobic treatment comprises decomposition of organic material in the absence of free oxygen and production of methane, carbon dioxide, ammonia and traces of other gases and organic acids of low molecular weight [2]. It is a complex process, which can be divided into four main phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The degradation steps are carried out by different consortia of microorganisms [3], which partly stand in syntrophic interrelation and place different requirements on the environment [4]. The microorganisms producing methane are called methanogens and are usually archaea bacteria [5]. Microorganisms are classified according to their optimal pH range [6] and to maximize the $CH_4$ yield, pH typically varies from 6.5 to 8.2, with optimal values of 7.0 - 7.2 [7]. Apart from the pH, the production of methane from anaerobic digestion of livestock manures principally depends on the matter added to the digester, the solids loading, the hydraulic retention time and the temperature [8, 9]. Tewelde et al. [10] used a process temperature equal to 35 (±5) °C and a pH 7.5 in batch mode, obtaining a bio-methane concentration equal to 69 % by digestion of brewery wastes; Kalia [11] used 23 °C and pH 8.2 in batch mode, obtaining a bio-methane concentration equal to 60 % by digesting cattle dung, while Abubakar and Nasir [12] used a temperature equal to 53 °C and a pH equal to 7.0 obtaining methane concentration equal to 47 % for cow dung in a semi-continuous process.

In this study, the effect of temperature and pH on the bio-methane production from digestion of water buffalo manure is investigated. This is quite new since in the literature attention has not been paid to this kind of dung, moreover we will also treat separately manure coming from lactating and non-lactating buffaloes. The latters are fed differently from the formers also because of their different hormonal phase. This will allow highlighting whether these differences affect the digestion process. The amount of methane produced under different experimental conditions is compared to identify the optimal process parameters. The anaerobic digestion of water buffalo manure is performed at mesophilic (37 °C) and thermophilic (55 °C) conditions, with pH varying from acid (pH = 6.0) to basic (pH = 8.7) values.

## 2.1 MATERIAL AND PROCEDURE

### 2.1 Material

Manures from both lactating (LB) and non-lactating (N-LB) buffaloes are collected during a period of time covering more than three years, from the farm “La Valentina s.r.l.”, located in the municipality of Villa Literno, in Campania (South Italy). The manure samples are taken in the morning; they are placed in sterile plastic containers, transported to the laboratory and immediately stored in the fridge at +4 °C [13]. In this way, the bacteria metabolism slows down and the properties of the manure remain stable until the effective start of the digestion process.

The fresh manure samples studied in this work are characterized in terms total solids (TS) and volatile solids (VS) content, measured according to European Standard Methods [14, 15]. TS varied from about 17 to 35 % (Table 1) and the mean value is 26.8 % ± 6.3 %. This large standard deviation is probably due to differences of the animal food supply, the season of the year and the hormonal phase of the cattles [16]. VS varies from about 52 to 74 % and these values are similar to those observed in literature for manure and sewage sludge [17]. VS content indicates the organic matter content of the biomass and it is often used for the estimation of the effectively decomposable material fraction.

The pH of the manures, $pH_{sub}$, is also measured upon collection and resulted always basic; in particular, it varied from 7.1 to 8.8.

**Table 1.** Properties of buffalo manure samples

| n° | Typology | TS [%] | VS [%] | $pH_{sub}$ |
|---|---|---|---|---|
| **1** | LB | 28.6 | | 8.8 |
| **2** | N-LB | 29.8 | - | 8.7 |
| **3** | LB | 20.6 | - | 8.8 |
| **4** | LB | 26.9 | - | 7.6 |
| **5** | LB | 17.1 | - | 7.3 |
| **6** | LB | 20.7 | 73.9 | 7.6 |
| **7** | NL-B | 35.3 | 67.3 | 7.9 |
| **8** | N-LB | 22.4 | 52.5 | 7.1 |
| **9** | LB | 33.1 | 72.9 | 7.3 |
| **10** | N-LB | 33.8 | 73.9 | 7.7 |

### 2.2 Digester and operating conditions

Figure 1 shows the borosilicate glass bottle used as batch digester in this study. Each bottle has a total volume of about 280 ml; the effective working volume is maintained at 80 ml and 200 ml was left for the gas. Three replicates of each sample are prepared for statistical needs.

The manure is mixed with distilled water to achieve a manure/water mass ratio equal to 30/70. Preliminary tests showed that higher mass ratio increases the production of methane, at the cost of more viscous slurries, thus complicating their pumping. It is known, in fact, that a slurry viscosity increases by increasing the solid content [18, 19, 20, 21]. The pH of the samples is either corrected to reach two different values (6.0 and 7.0) by adding opportune amount of 1 M HCl water solution, or it is not modified. Manure/water slurries are opportunely mixed following a three steps procedure: they are firstly hand mixed, then electrically homogenized for 2 min and finally filtered with a Büchner filter equipped with a vacuum pump. Guarino et al. [22] showed that these mechanical three steps pre-treatments, compulsories in a continuous process in lab-scale reactors, do not alter the digestion process. The manure/water mixture is poured in the bottle and the anaerobiosis is obtained blowing nitrogen inside the closed reactors with a two-needles system. The bottles are manually shaken once a day to limit the sample sedimentation. The measurement of the produced gas composition is performed with the MicroGC Agilent 3000 equipped with two capillary columns: a MolSieve 5 A and a Poraplot U.

**Figure 1.** Batch digester

### 2.3 Data analysis

Results of each experimental campaign are here showed in terms methane volume fraction as a function of the digestion time. Data are interpolated using the modified Gompertz equation Eq(1) [23]:

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

where $H$ [%] is the cumulative production, $P$ [%] is the productivity, i.e. the volume fraction asymptotic value, $R_m$ [$h^{-1}$] is the maximum production rate, $\lambda$ [h] the lag-phase time, $e$ is Euler’s number and $t$ [h] the digestion time.

## 3. RESULTS AND DISCUSSION

Figure 2 and Figure 3 show $CH_4$ volume fraction as a function of time for digestion processes run with initial pH equal to 6.0 and 7.0, respectively, and temperature equal to 37 °C. In particular, Figure 2 shows the case study A where the samples 1, 2, 3, 4 and 7 of Table 1 are processed starting from an initial pH = 6.0 and Figure 3 shows the case study B where samples 5, 6, 7, 8 and 9 of Table 1 are digested starting from an initial pH = 7.0. In both cases, all data in the plot are interpolated with a single regression curve (Eq.1). The corresponding values of Gompertz parameters $P$, $R_m$ and $\lambda$, obtained from the best fit of experimental data, are listed in Table 2 together with the regression coefficient $R^2$ and the value of the $pH_{fin}$, measured at the end of the digestion process.

The case study A of Figure 2 (pH = 6.0, T = 37 °C) shows a regular sigmoidal trend with a methane productivity $P$ ~ 63 %, a lag-phase time of 108 h and a production rate $R_m$ = 0.18 $h^{-1}$. These samples typically have a final $pH_{fin}$ of about 6.9 ± 0.2

and this indicates that during the digestion of buffalo manures the system (auto)-evolved towards a neutral pH.

Case study B data in Figure 3 (pH = 7.0, T = 37 °C) and Table 2 show some small differences with respect to the samples digested starting from an initial $pH_{in}$ = 6.0: the value of $P$ is slightly higher and it is equal to 66 %, and the production rate is faster and equal to 0.25 $h^{-1}$; the lag-phase time is unchanged and results 108 h. It is interesting to remark that also in this case $pH_{fin}$ is neutral and this suggests that it remains almost constant during the entire digestion process.

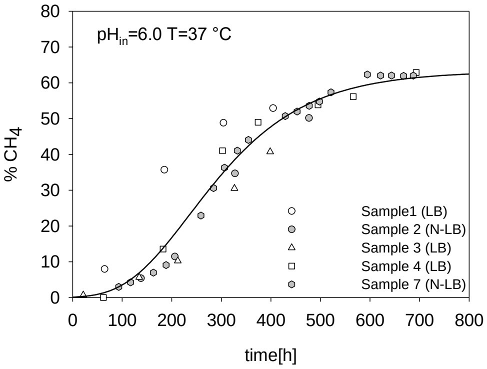


**Figure 2.** Case study A: Evolution of $CH_4$ concentration with time at $pH_{in}$ = 6.0 and T = 37 °C. Symbols are the experimental data, line is the global fit curve

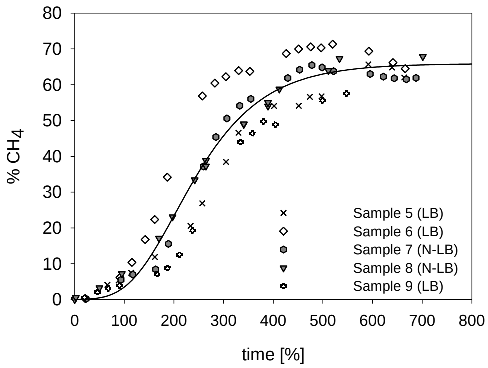


**Figure 3**. Case study B: Evolution of bio-methane concentration with time at $pH_{in}$ = 7.0 and temperature 37 °C. Symbols are the experimental data, line is the global fit curve

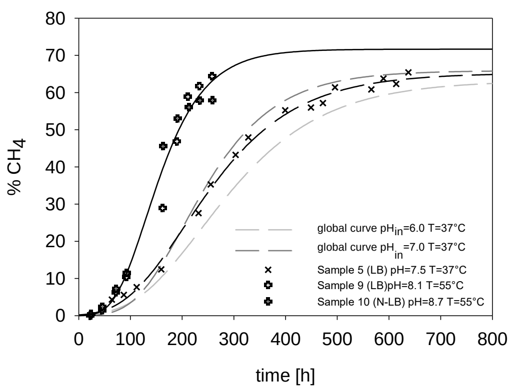


**Figure 4.** Evolution of bio-methane concentration with different process conditions. Symbols are the experimental data, lines are the regression curves

**Table 2.** Gompertz parameters

| Case study | $pH_{in}$ | T [°C] | $P$ [%] | $\lambda$ [h] | $R_m$ [$h^{-1}$] | $R^2$ | $pH_{fin}$ |
|---|---|---|---|---|---|---|---|
| **A** | 6.0 | 37 | 63.2 | 108 | 0.18 | 0.94 | 6.9 |
| **B** | 7.0 | 37 | 65.9 | 108 | 0.25 | 0.93 | 7.0 |
| **C** | 7.5 | 37 | 65.2 | 88.6 | 0.21 | 0.99 | 7.0 |
| **D** | 8.1-8.7 | 55 | 71.6 | 68.5 | 0.43 | 0.98 | 7.7 |

The results of the case studies A and B (Table 2) suggest that all samples auto-adjust their pH during the digestion process towards neutrality. Thus, it was then decided to investigate the digestion process without setting the pH of the sample to an initial desired value. This new process condition is imposed to the digestion of sample 5 of Table 1, whose initial pH, i.e. which obtained after mixing the manure with bi-distilled water, resulted to be 7.5, only slightly different from its $pH_{sub}$ (Table 1). The bio-methane evolution in time of this case study (C) is shown in Figure 4. The regression curves of case studies A and B, already shown in Figure 2 and 3, respectively, are also reported for comparison. Data in Figure 4 and Table 2 indicate that the digestion sample 5 starting from a $pH_{in}$ = 7.5 show a methane growth very similar to that of case A ($pH_{in}$ = 6.0) and case B ($pH_{in}$ = 7.0), but a shorter lag-phase time. In particular, it has a lag-phase time $\lambda$ = 88.6 h, a production rate $R_m$ = 0.21 $h^{-1}$, and a productivity $P$ equal to 65.2 %. It is interesting to remark that also in this case $pH_{fin}$ is neutral.

Since the digesters of waste are typically installed within a cogeneration plant, where heat is abundantly available, we considered appropriate to investigate the manure digestion process in thermophilic conditions, e.g. 55 °C. In the literature there are few papers [12, 24] indicating that bio-methane can be produced also at 53 - 55 °C. In Figure 4, experimental results obtained digesting the manure at 55 °C (samples 9 and 10) without adjusting the initial pH are shown and compared with all the results obtained at 37 °C. The initial process pH of Samples 9 and 10 resulted equal to 8.1 and 8.7, respectively. Also in this case $pH_{in}$ is slightly more basic than $pH_{sub}$.

Case study D, where the manures are digested at 55 °C without setting the initial pH to a preset value, presents the best bio-methane production (Figure 4 and Table 2) with an estimated productivity of 71.6 %; the shortest lag time $\lambda$ = 68.5 h and a production rate of 0.43 $h^{-1}$, which is almost the double of the cases at 37 °C. The estimated plateau value of $CH_4$ volume fraction ($P$ = 71.6 %) is reached in only 400 h that is a much shorter time than that required at 37 °C, equal to about 700 h. Notice that in this case the real plateau of concentration was not reached, thus indicating that the digestion process was still on go and consequently the productivity P is probably underestimated. These last samples showed a final $pH_{fin}$ = 7.7, which is slighter less basic than the initial value. This suggests that in any case the system tends to auto-evolve towards neutrality that is reached when the manure is digested at 37 °C, while it is only approached in thermophilic conditions where a slightly basic environment persists during all the process. Once more, in this case the system was still evolving and possibly with it its pH that might also tend to a true neutrality.

The measured high productivity may be due to the higher initial pH that may promote the hydrogenotrophic methanogenesis during which the $CO_2$ and $H_2$ are converted into $CH_4$ and $H_20$ [25]. Also the pH decrease in time suggests that the acidogenic phase is well operated during which fatty acids are released, due to the degradation of cellulosic material of the manure, and they balance the system pH.

The results obtained at 55 °C and $pH_{in}$ = 8.1 and 8.7 (sample 9 and 10) sounds very promising, but the generalization of this experimental observations requires further studies with also other manure samples. In fact, samples 9 and 10 are peculiar substrate, probably particularly rich in methanogenic bacteria. In fact, these samples were able to produce methane also after a thermal pretreatment at 90 °C for 6 h, specifically carried out to suppress all methanogenic bacteria [26, 27] and by using a $pH_{in}$ = 5.5, typically unfavorable to bio-methane production.

## 4. CONCLUSIONS

In this work, we compared the bio-methane produced by digesting water buffalo manure, in batch mode, under different process conditions. In particular, we modified two of the most important parameters affecting the process [28]: the pH and the temperature. The pH is not controlled during the digestion process, but only at its beginning and it is set at five different values varying from 6.0 to 8.7. Since the natural pH of the manure is slightly basic (Table 1), to obtain the value of pH = 6.0 and 7.0, we acidified the system with 1 M HCl water solution, while values of $pH_{in}$ = 7.5, 8.1 and 8.7 were obtained without any pH correction. The pH is always measured at the end of digestion process. The temperature was set to 37 and 55 °C.

We observed that all the samples digested at 37 °C tend to a final neutral pH value, both when the initial one was acid or basic. A starting basic pH favored the production of bio-methane, since it reduced the lag-time while increasing the production rate (Table 2). This observation suggested that the digestion process of naturally basic substrate, as the manure at hand, can start without any manipulation of the substrate pH. Experimental results also showed that at 55 °C the production of bio-methane is favored with respect to that obtained at 37 °C and in this case though the system tends to auto-evolve towards neutrality, as for the cases at 37 °C. This may indicate that the methanogenic bacteria community has an optimal operative pH interval at 55 °C different from that at 37 °C, or that the bacteria community that prevails at 55 °C is different from that prevailing at 37 °C. To elucidate this point further investigations are deserved, however, the use of high temperatures represents a cost of the process and its effective convenience must also be carefully evaluated.

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## NOMENCLATURE

| | |
|---|---|
| LB | lactating buffalo manure. |
| NL-B | no lactating buffalo manure. |
| TS | total solids. % |
| VS | volatile solids. % |
| $pH_{sub}$ | original substrate pH. |
| $pH_{fin}$ | pH at the end of the digestion process. |
| $H$ | cumulative production. % |
| $P$ | Productivity. % |
| $R_m$ | maximum production rate. $h^{-1}$ |
| $\lambda$ | lag-phase time. h |
| $e$ | Euler's number. |
| $t$ | digestion time. h |