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Does the C/N ratio really affect the Bio-methane Yield? A three years investigation of Buffalo Manure Digestion

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In this paper we study the anaerobic digestion of water buffalo manure collected during a period of three years with the goal of investigating the effect of the substrate C/N ratio on the bio-methane production. The investigated samples show a C/N ratio between 9 and 50, this interval being much wider than that typically considered optimal in the literature for the waste digestion processes.
The experimental tests are performed in batch mode, at 37 °C and with a starting pH equal either to 6 or 7, uncontrolled during the digestion process.
We observe that, regardless of the C/N ratio of tested samples, the Gompertz productivity, expressed in terms of bio-methane volume fraction, is always larger than 54 %. A slightly larger productivity is recorded for substrates digested starting from an initial pH set to 7. We may then conclude that the C/N ratio of water buffalo manure does not really affect the bio-methane yield.

1. Introduction

Biomass includes a large amount of material as many different organic wastes like organic fraction of municipal solid waste, sewage sludge, food waste, animal manure (Li et al., 2009). The latter is a low cost substrate rich in carbohydrates, especially suitable to produce bio-fuel in anaerobic digesters.
In this work, we study the anaerobic digestion of buffalo manure. Buffalo farms are one of the most significant activities in southern Italy. One of the problems related to buffalo farms is the waste management. In fact, the nitrates directive [91/676/EEC] identifies the vulnerable zones to nitrates from agricultural sources, where spreading of animal manures is banned. A smart solution of this problem is the transformation of animal manure into bio-energy.
Anaerobic digestion consists of several interdependent, complex sequential and parallel biological reactions (Carillo et al., 2012, Merlin Christy et al., 2014), during which the products obtained from one group of microorganisms serve as the substrates for the next, resulting in transformation of organic matter mainly into a gas mixture of CH_4 and CO_2 with minor quantities of N_2, H_2, NH_3 and H_2S (Gujer and Zehnder, 1983).
In the literature attention has been paid to the individuation of the correct ratio C/N to maximize the bio-methane yield and Bardiya and Gaur (1997) suggested an optimal range from 20 to 30. Indeed, Al Juhaimi et al. (2014) use a C/N ratio equal to 30 for anaerobic digestion of palm wastes, Rao and Singh (2004) reported an optimum C/N ratio equal to 25 for municipal solid wastes, Yasin and Wasim (2011) individuated an optimal C/N ratio equal to 30 for biogas fermentation of buffalo dung. However, values slightly outside from this range have been found as in, e.g., Tewelde et al. (2012) who detected a C/N ratio equal to 17 to digest a brewery waste.
In a period long three years we observed a wide variability of the C/N ratio of the buffalo manures collected from the very same farm (di Cristofaro et al., 2014). This may be due to the differences in the animal food supply, the season of the year of the collection, and the hormonal phase of the cattle. We focus on the influence of the C/N ratio on the bio-methane yield measure in terms of CH_4 volume fraction in the produced

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biogas. We investigates a C/N ratio varying from about 9 to about 50, thus largely exceeding the optimal values range suggested in the literature. The water buffalo manure was digested in batch mode under mesophilic conditions, without additioning neither seed bacteria, nor external nutrients.

2. Materials and Methods

Buffalo dungs were taken from an Italian farm, located in Villa Literno (Caserta, Italy), and they were always collected in the morning, placed in plastic containers, transported to the laboratory and immediately placed in the fridge, at +4 °C. In this way, the manure is correctly stored, since any metabolism process is arrested or, at least, slowed down.
The manure samples are identified with a capital letter and are listed in Table 1 together with the amount of total solids (TS), volatile solids (VS), C, N, and C/N.

2.1 Total Solids and Volatile Solids

The calculation of the total solids (TS) (or dry matter) is specified by the European Standard [UNI EN 14346, (2007)]. This method applies to solid samples and samples which become solid during the drying process. It applies to samples containing more than 1 % of dry residue. The calculation of the volatile solids (VS) of samples is specified from the European Standard [UNI EN 15169, (2007)]. This procedure is applicable to all kinds of waste, sludge and sediments and it is often used to estimate the content of volatile organic matter.
TS varies from about 17 to 35 % (Table 1) and the mean value is 24.52 % ± 6.2 %. This large standard deviation is due not only to the unavoidable biological variability of the substrate, but also to the different collection conditions: for example, samples collected from stables without roof in a rainy period are usually wetter. VS varies from about 52 to 74 %.

2.2 C, N content and C/N ratio

The determination of the content of C and N is obtained using the analyzer EA 1110 [Thermoquest, Italy]. The measurement method is based on complete and instantaneous oxidation (dynamic flash combustion) of the samples (Friis et al. 1998), with their conversion from organic substrates to gaseous products. The latter are separated with a gas chromatograph, equipped with a column Porapack PQS, and are quantified by a thermal conductivity detector. C/N ratio varies from about 9 to 50 (Table 1), largely outside the range 20-30 indicated as optimal in the literature (Bardiya and Gaur, 1997).

3. Experimental Protocol

The sample preparation follows a well-defined and tested experimental protocol (Guarino et al., 2014). The manure is mixed with distilled water to achieve a manure/water mass ratio equal to 30/70. Previous tests showed that this ratio is a good compromise between biogas productivity potential and viscosity of the slurry. The pH of the mixture is lowered with a 1M aqueous solution of HCl in order to achieve a value of either 6.0 or 7.0. In particular, we choose pH = 6 in four cases and pH = 7 in other four cases (Table 1). We set the initial pH of the process (pH_{in}), evolving spontaneously and monitoring it only when the digestion is stopped (pH_{fin}). Each sample is also subjected to a three steps mechanical pre-treatment: it is firstly hand mixed, then electrically homogenized for 2 min and finally filtered with a Büchner filter equipped with a vacuum pump.
The experimental tests are conducted in batch reactors consisting of borosilicate glass bottles [Wheaton, U.S.A] of 280 ml sealed with pierceable caps. The anaerobiosis is obtained blowing nitrogen inside the bottles with a two-needles system. The bottles are finally placed in a ventilated oven at 37 °C where the digestion process starts.

Table 1: Properties of buffalo manure samples

	TS [%]	VS [%]	C [%]	N [%]	C/N	pH_{in}
A	28.63	-	30.83	0.62	50.12	6.0
B	20.56	-	30.18	0.86	34.93	6.0
C	26.9	-	34.78	1.06	32.72	6.0
D	17.08	-	38.75	1.88	20.61	7.0
E	20.74	73.85	31.94	1.85	17.26	7.0
F	35.28	67.31	31.51	1.58	19.94	6.0,7.0
G	22.44	52.54	27.62	2.83	9.75	7.0

The anaerobic digestion of animal wastes usually does not require the addition of seed bacteria for biogas production (Carillo et al., 2014) and this feature distinguishes animal waste from other organic wastes (Yokoyama et al., 2007). Also in our study the manure was digested without any addition of nutrients or external microbial communities.

The gas composition of the bottle headspace is analysed with the MicroGC Agilent 3000 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 to 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 to 90 °C and 85 °C, respectively, and He is the gas carrier.

4. Results and Discussion

Results are presented in terms methane volume fraction in biogas as a function of the time; they are interpolated with the modified Gompertz equation (Khanal et al., 2004):

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

where *H* [%] is the cumulative production, *P* [%] is the productivity, i.e. the asymptotic value, R_m [h^{-1}] is the maximum production rate, *e* is Euler's number, *λ* [h] the lag-phase time and *t* [h] the incubation time.

Figure 1 shows CH_4 volume fraction as a function of time for samples A, B, C and F, i.e. the four samples digested with pH_{in} = 6. The corresponding 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 and the value of the pH_{fin} measured at the end of the digestion process.

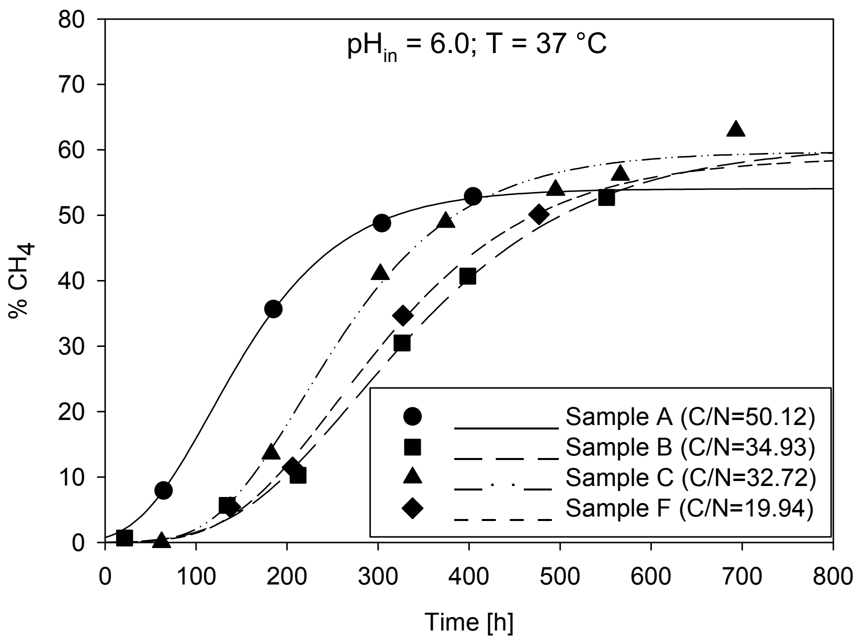

Figure 1: Evolution of bio-methane concentration with time at pH_{in} = 6, the fermentation temperature is 37 °C. Symbols are the experimental data, lines are the regression curves.

Table 2: Gompertz parameters for data of Fig. 1 (pH_{in} = 6.0; T = 37 °C)

	P [%]	*λ* [h]	R_m [h^{-1}]	R^2	pH_{fin}
Sample A	54.1	36.26	0.25	0.99	6.77
Sample B	60.9	140.74	0.16	0.99	7.01
Sample C	59.7	120.82	0.23	0.99	7.19
Sample F	58.9	137.83	0.21	0.99	6.86

Data in Figure 1 and Table 2 indicate that sample A has a behaviour slightly different from sample B, C and F. In particular, Sample A has the shortest lag-phase time (λ = 36.26 h) and the fastest production rate (R = 0.25 h^{-1}), while the value of P is the smallest one and equal to 54.1 %. Notice that the digestion process of sample A was stopped after about 400 h, while, in the other cases, we followed the process for at least than 500 h. This may lead to an underestimation of the productivity P of sample A. Samples B, C and F, have a similar lag-phase time (about 133 h) and a P equal to about 60 %, R varies from 1.16 to 0.23 h^{-1}. It is worth noticing that sample A has a C/N equal to 50.12, largely greater than the value suggested as optimal in the literature for wastes digestion, whereas the other C/N ratios fall close to the limit of the suggested interval [20-30]. We can also observe that pH_{fin} is auto-evolved, in all four cases, toward an almost neutral value (Table 2). Figure 2 shows CH_4 volume fraction as a function of time for samples D, E, F and G, i.e. the four samples digested with pH_{in} = 7. The corresponding values of Gompertz parameters P, R_m and λ, obtained from the best fit of the data with Eq(1), are reported in Table 3 together with the regression coefficient R^2 and the value of the pH_{fin} measured at the end of the digestion process. Data in Figure 2 and Table 3 show that all the four samples behave similarly. A slight difference can be observed for sample E (triangles), which has a slightly faster production rate equal to 0.39 h^{-1}. Let us emphasize that in this case the C/N ratio of sample G (represented with *stars*) is smaller than the values suggested as optimal, and no significant differences in the productivity P are found with respect to the other samples. It is interesting to remark that pH_{fin} is once again neutral and almost unchanged with respect to pH_{in}.

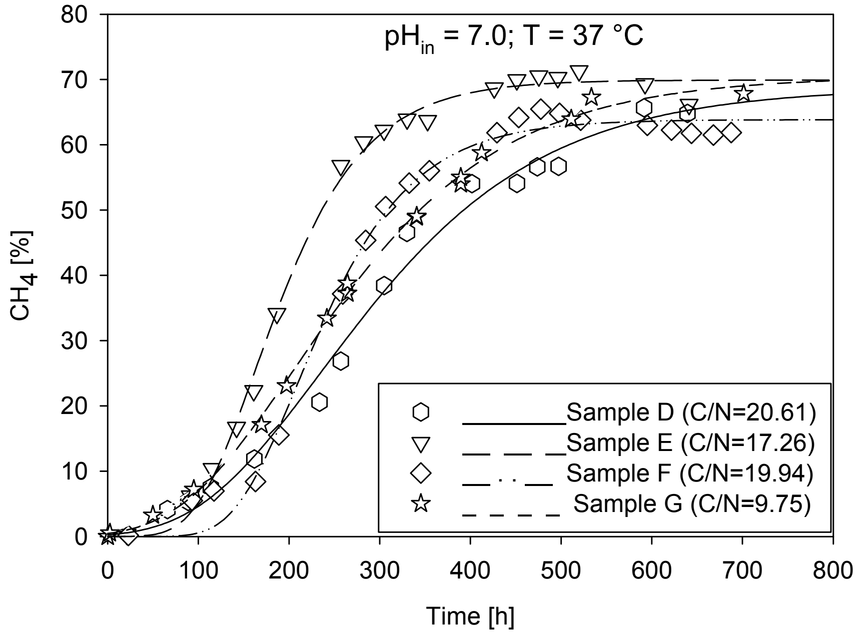

Figure 2: Evolution of bio-methane concentration with time at pH_{in} = 7, the fermentation temperature is 37 °C. Symbols are the experimental data, lines are the regression curves.

Table 3: Gompertz parameters for data of Fig 2 (pH_{in} = 7.0; T = 37 °C)

	P [%]	λ [h]	R_m [h^{-1}]	R^2	pH_{fin}
Sample D	68.81	99.07	0.18	0.99	7.13
Sample E	69.93	97.19	0.39	1.00	6.95
Sample F	63.86	139.67	0.33	0.99	7.05
Sample G	70.51	79.25	0.20	1.00	-

5. Conclusions

Aim of this work is to understand whether the value of C/N ratio really affects the anaerobic digestion process of water buffalo manure in mesophilic conditions. Despite the values considered optimal in the literature varying from 20 to 30, the samples we tested show a high bio-methane productivity in a wider C/N interval from 9 to 50. Figure 3 summarizes all our experimental results reported in terms of productivity *P* as a function of the C/N ratio. By chance, only one point (sample D) falls really within the optimal interval, delimited in figure with dashed lines, and two points (samples F) are on the lower limit, all the others are outside. The productivity of all the samples varies between 54.1 % and 70 %. An ever decreasing trend could be recognised in Fig. 3, however it is important to note that: i) the productivity of sample A can be underestimated due to the short incubation time; ii) in Fig. 3 the obtained productivities refer to samples digested starting from two different pH_{in}'s. More critical appears the observation that, on average, the productivity of samples with pH_{in} = 7 (*hole symbols*) is slightly larger than that of samples with pH_{in} = 6 (*filled symbols*). The process seems thus promoted by a pH = 7 and indeed also the samples that started with an initial pH = 6 evolved spontaneously towards a neutral pH.

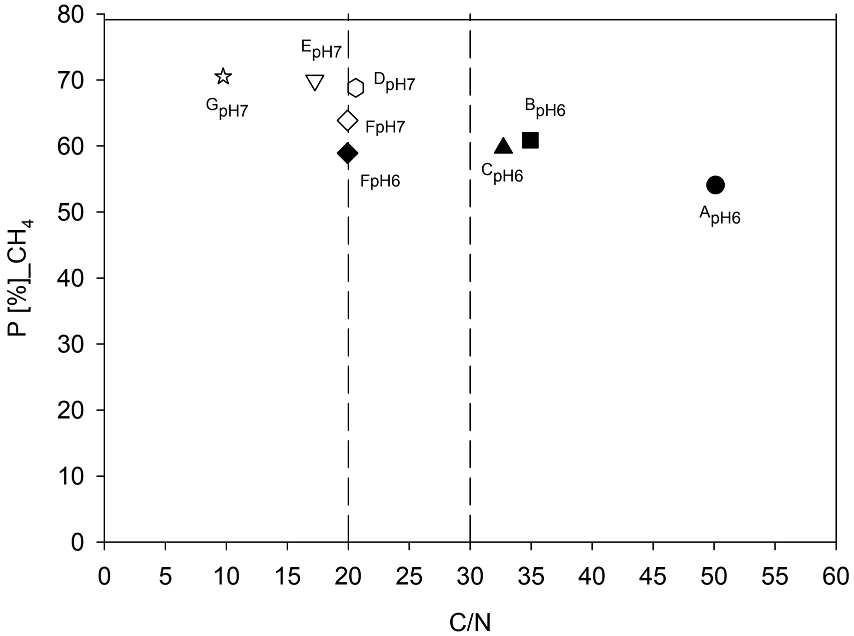

Figure 3: CH_4 productivity Vs. C/N ratio. Dashed lines delimit the literature optimal C/N ratio interval.

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