# **Bio-Methane Potential Tests To Measure The Biogas Production From The Digestion and Co-Digestion of Complex Organic Substrates**

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Abstract: Bio-methane potential (BMP) tests are widely used in studies concerning the anaerobic digestion of organic solids. Although they are often criticized to be time consumer, with an average length longer than 30 days, such tests are doubtless easy to be conducted, relatively inexpensive and repeatable. Moreover, BMP tests give significant information about the bio-methanation of specific substrates and provide experimental results essential to calibrate and validate mathematical models. These last two aspects have been handled in this work where the following elements have been described in detail: i) the methods used to conduct the BMP tests; ii) the cumulative bio-methane curves obtained from three BMP tests, concerning respectively two pure organic substrates (swine manure-SM and greengrocery waste-GW) and an organic substrate obtained by mixing buffalo manure (BM) and maize silage (MS); iii) the procedure used to calibrate a mathematical model proposed by the authors to simulate the anaerobic digestion process; iv) the results of the calibration process. This paper shows that BMP tests are extremely helpful to determine the amount of bio-methane obtainable from different organic solids and under different operational conditions as well as the biodegradability of the investigated substrate, the relative specific rate of bio-methanation and the synergic effect of multiple co-digested substrates. Furthermore BMP tests represent an interesting tool for the technical and economical optimization of bio-methane producing plants.

**Keywords:** BMP, biodegradability, digestion, mathematical modelling, organic waste.

#### 1. INTRODUCTION

On the last decades the use of the anaerobic digestion as process to treat organic solid wastes became more and more frequent. The reason of this new tendency in treatments of solid wastes can be explained considering mainly three factors [1-3]: i) the need to apply a process to dispose of organic solid wastes more environmental friendly than landfills as requested by the latest rules concerning the environmental protection in many countries in the world; ii) the opportunity to obtain from this process a renewable fuel called biogas alternative to fossil ones; iii) the advantage of relatively low costs in starting up and managing this process.

Anaerobic digestion is a multi-steps biological process where the originally complex and big sized organic solid wastes are progressively transformed in simpler and smaller sized organic compounds by different bacteria strains up to have a final energetically worthwhile gaseous product, called biogas, and a semi-solid material, called digestate, rich in nutrients and thus suitable for its utilization in farming [4].

Despite the linearity according to which the anaerobic digestion of organic solid wastes evolves, this process is commonly prone to drops in performance due to the occurrence of dysfunctions or failures that make it strongly dependable on the choice of the substrates as well as on the environmental and operational conditions [5]. This last aspect can be reasonably considered the only drawback of this process in treating organic solid wastes.

Anaerobic digestion is easily performed in a biological reactor where mixers and heater exchangers could be the only technological and power consuming equipments needed. Moreover this process can gain money by disposing of organic solid wastes as well as selling the biogas or the power generated by its combustion and, when possible, the digestate as fertilizer in agriculture.

This process has therefore opened up interesting perspectives not only for the treatment of the organic solid wastes, but also for the production of a renewable source of power, that is cheap and easy to obtain.

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Nevertheless the anaerobic digestion is still affected by sceptical judges about its utilization as process to treat organic solid wastes because of not comforting past experiences mainly due to a lack of knowledge about this topic. From this point of view experimental tests represent the most powerful tools to remove all those doubts that still hamper the full establishment of the anaerobic digestion as the environmentally and economically most convenient and helpful process to treat organic solids. Among all available experimental methods, the bio-methane potential (BMP) tests are those that have been most successful, mainly thanks to their easy set up and conduction as well as the useful information obtainable from them.

The BMP tests are conducted in batch conditions and in bench scale, measuring the maximum amount of biogas or bio-methane produced per gram of volatile solids (VS) contained in the organics used as substrates in the anaerobic digestion process [6]. Furthermore, relevant elements coming from the conduction of such tests are mainly the environmental and operational condition that could lead the process to failure, the time needed to have a complete substrate degradation, the average rate of bio-methanation for each substrate investigated, the evaluation of the digestion kinetics by coupling the BMP tests results to a mathematical model simulating the anaerobic digestion.

The relevance of the BMP tests as useful tool to improve the knowledge on the anaerobic digestion process to treat organic wastes is claimed in this paper, where some BMP tests and the information obtainable from their results, such as the biodegradability of the substrate investigated, the relative specific rate of bio-methanation, the theoretical production of bio-methane and the disintegration process kinetics, are described as illustrative cases.

These tests were conducted using either pure substrates or a mixture of two substrates in order to investigate also the effect that the combination of different organic wastes has on the digestion process (co-digestion). Indeed according to recent studies [7-12], the concurrent presence in the same anaerobic reactor of different organic wastes can improve the performance of the digestion process.

The co-digestion of different organic substrates has been studied during the last 10-15 years and the results have showed a synergic effect of the combined treatment as the biodegradability of the resulting mixture was higher than the biodegradability of the single substrates when investigated separately. In particular, the combination of different substrates with proper percentages of each fraction can result in the production of a mixture with a Carbon:Nitrogen (C:N) ratio included in the optimal range 20:1-30:1 [13]. Analogous results were obtained with regard to the Carbon:Phosphorous (C:P) ratio. Therefore the above-cited improvement of the biodegradability characteristics of the solid mixture is substantially due to the C:N:P ratio adjustment. Further benefits of the co-digestion are higher biogas and energy production [14] and the decrease of the amount of solid waste to be disposed due to the gasification of a higher percentage of the substrate.

This work is focused on the relevance of BMP tests as tool to measure the bio-methane potential of different substrates, both pure and mixed, as well as to determine their digestion bio-kinetic constants. A procedure to assess the kinetics of the limiting step of the anaerobic digestion process is presented, based on the use of a mathematical model proposed by the authors [15]. In particular, the kinetic constants for specific organic substrates are determined using the results of BMP tests carried out on such substrates to calibrate the above cited mathematical model.

#### 2. BMP TESTS METHODOLOGY

The BMP assay can be used as an index of the anaerobic biodegradation potential as it is the experimental value of the maximum quantity of methane produced per gram of VS. The BMP is measured with the BMP test, which consists in measuring the bio-methane or biogas produced by a known quantity of waste in batch and anaerobic conditions.

The approach of the BMP test is simple, an organic substrate is mixed with an anaerobic inoculum in defined operational conditions, and the gas evolved is quantified by a specific measurement method. In literature there are different attempts to define a standard protocol in order to gain comparable results but so far such standardization has not been reached.

One of the last attempts to define a common protocol for BMP testing with some basic guidelines for a common procedure was given in [16]. Some studies are also published in literature, aimed at collecting data and methods that are commonly used by different international laboratories [17]. The last collection of data, from 19 laboratories, was done in [18], with the aim of providing an extensive database for BMP results in terms of specific methane yield and degradation rates as a function of the experimental conditions selected.

Protocols for BMP tests should be provided for a clear setting of all those parameters that can affect significantly the experiments results, such as temperature, pH, stirring intensity, physical and chemical characteristics of substrates, substrate/inoculum (S/I) ratio.

Temperature affects the bio-methanation rate and usually higher temperatures imply greater methane yields in a shorter digestion time. Nevertheless sharp increases of temperature should be avoided because they can cause a decrease in bio-methane production due to the death of specific bacteria strains, particularly sensitive to temperature changes [19]. To keep constant the temperature during BMP tests it is needed to submerge the reactors in a water bath kept at the selected temperature [20] or to incubate them in a thermostatically controlled room [21].

BMP tests have to be carried out keeping the pH around the neutrality (values ranging between 7.0 to 7.8). pH values below 6.0-6.5 inhibit the methane bacteria activity. To avoid drops in pH chemicals are added to the organic substrate to supply a buffer capacity. Sodium bicarbonate, sodium hydroxide, sodium carbonate and sodium sulphide are the most used chemicals [22].

Table 1. Main Characteristics of the Organic Solids Used in BMP Tests

Parameter	Units	Organic Wastes				Inoculum
		SM	GW	ВМ	MS	moculum
TS	g/kg, wet	101.5±0.4*	125±1.0*	81.67±0.5*	278.82±0.3*	140.9±0.4*
VS	g/kg, wet	83.06±0.3*	113±0.7*	64.45±0.4*	264.07±0.2*	85.4±0.3*

<sup>(\*)</sup> Standard error.

Table 2. BMP Tests Design

Test index	SM	GW	BM	MS	Inoculum	Na <sub>2</sub> CO <sub>3</sub>
	Mass [g]	Mass [g]	Mass [g]	Mass [g]	Mass [g]	Mass [g]
A	76.87±0.37*				150.22±0.36*	0.35±0.02*
В		17.52±0.29*			150.15±0.51*	0.10±0.01*
С			68.68±0.22*	7.20±0.23*	150.32±0.38*	$0.35\pm0.03^*$
D					150.22±0.43*	0.10±0.01*

<sup>(\*)</sup> Standard error.

Stirring intensity guarantees an uniform moisture content and maximizes the contact between substrates and microorganisms. Mixing can be provided by several ways: turning manually up to down the reactors once a day [16], using stirring magnet bar, using an external agitation systems [23].

Substrate particles size affects significantly the BMP tests [15, 25-27] as it influences the ratio between surface and volume for each organic particle. This ratio is relevant since microorganisms can degrade only the substances present on the organic solids surface.

Substrate/inoculum (S/I) ratio influences the performance of BMP tests. According to [28] a S/I ratio ranging between 0.5 and 2.3 gVS/gVS can prevent acidification phenomena. Instability in the anaerobic process, such as high COD content in the effluent and volatile fatty acids (VFAs) accumulation, occurs with S/I ratio lower than 0.5 [29]. Further studies [30] showed that the biogas yield is in an inverse proportion to the S/I ratio in the range 1.6-5.0.

In literature different methods to measure the biogas produced are used. The most common are the manometric and volumetric methods.

The manometric method measures the biogas production by the overpressure generated by biogas the reactor where volume and temperature are kept constant. The overpressure can be measured using a common differential manometer or a more sophisticated pressure transducer [31].

The volumetric method measures the biogas produced when pressure and temperature are kept constant.

One of simplest volumetric methods connects the reactor with a graduated piston [24]. Another equipment that uses the liquid displacement to measure the biogas produced is the Eudiometer described in detail in [32]. Systems similar to Eudiometer can be built using graduated reverse cylinder filled with a barrier solution [33]. Volumetric methods permit to know the biogas composition as percentages of CH<sub>4</sub> and CO<sub>2</sub> by using a gas chromatograph or measuring directly

the CH<sub>4</sub> flow after removing CO<sub>2</sub> from biogas by bubbling it through a NaOH 2N solution.

# 3. BMP TEST APPLICATIONS

#### 3.1. Experimental Design

In this paper some examples of BMP tests are illustrated that are concerned with four different organic wastes, whose main characteristics in terms of total solids (TS) and VS are shown in Table 1. In particular, BMP tests were conducted on two pure substrates, such as swine manure (SW) and greengrocery waste (GW) (identified by test indexes A and B) and a substrate obtained mixing buffalo manure (BM) with maize silage (MS) in percentages of 70% and 30%, respectively, in terms of VS, (identified by test index C). A further BMP test (identified by test index D) was conducted on the inoculum, to estimate the volume of methane resulting from the fermentation of the organic solids contained in the anaerobic sludge. In total, 4 BMP tests were conducted, each of them in triplicate (Table 2).

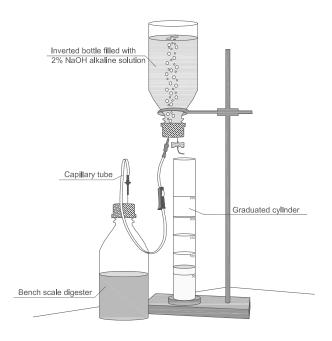
#### 3.2. Substrates Collection and Preparation

SW, BM and MS were collected from a farm in Albanella, near Salerno, in the southern Italy and stored in the fridge at 4 °C. Granular anaerobic sludge, used as inoculum, was taken from an Upflow Anaerobic Sludge Blanket (UASB) reactor treating the wastewater produced by a potatoes factory.

The representative sample of GW was collected from the fruit and vegetable market of Naples according to waste sampling methodology [34]. This sample was subsequently ground and sieved as far as to have a homogeneous material composed of particles with size ranging between 1 and 2 mm.

# 3.3. BMP Tests Setup and Operation

As there is not a standard protocol for BMP tests, the most common conditions used in the literature were applied.



**Fig. (1).** Experimental equipment used to measure the daily bio-methane production.

Each BMP test was performed under controlled and reproducible conditions in a 1000 mL glass bottle GL 45 (Schott Duran, Germany). Each bottle was partially filled with inoculum and a substrate, according to a ratio equal to 2 between their VS content; tap water was added up to a 500 mL total volume. Small amounts of Na<sub>2</sub>CO<sub>3</sub> powder, ranging from 0.10 to 0.60 g, were also added (Table 2) to prevent a critical drop in pH. Each bottle was sealed with a 5 mm thick silicone disc that was held tightly to the bottle head by a plastic screw cap punched in the middle (Schott Duran, Germany). All bottles were shaken for 30 min at 80 rpm speed by bottle shakers KL-2 (Edmund Bühler, Germany) and were immersed up to half of their height in hot water, kept at a constant temperature of 35  $\pm 1$  °C by 200W A-763 submersible heaters (Hagen, Germany). Once a day, each bottle was connected by a capillary tube to an inverted 1000 mL glass bottle containing an alkaline solution (2% NaOH) and sealed in the same way as done for the BMP bottle. To enable gas transfer through the two connected bottles, the capillary tube was equipped on both ends with a needle, sharp enough to pierce the silicone disc.

# 3.4. Analytical Measurements

TS and VS contents were measured according to Standard Methods [35]. Daily methane production was monitored measuring the volume of alkaline solution displaced from the measure bottle and collected in a graduated cylinder (Fig. 1). The  $\rm CO_2$  contained in the biogas did not affect the volumetric methane measurements as it was dissolved in the alkaline solution. Temperature and pH in each BMP bottle were also monitored for at least once a day with a TFK 325 thermometer (WTW, Germany) and a pH meter (Carlo Erba, Italy), respectively.

#### 4. MODEL CALIBRATION

### 4.1. Mathematical Model

During the last years much research aimed at modelling the anaerobic digestion of complex organic substrates has been carried out [36]. The mathematical modelling of the digestion process allows to reproduce several empirical behaviours on a computer in a short time. The possibility to obtain several data from model simulations can reduce the number of BMP tests needed to evaluate the biodegradability of a specific organic substrate. However the possibility to use a mathematical model to predict the results of BMP tests relies on a proper calibration of the model itself. Once the model is properly calibrated it can be used also to improve the performance of full-scale digesters.

The BMP tests presented in this study have been used to calibrate a recently proposed mathematical model of the co-digestion process [15, 37, 38]. In this model the differential mass balance equations and the process kinetics and stoichiometry are modelled according to the Anaerobic Digestion Model no 1-ADM1 [39]. However, the model can consider different influent substrates, which are modelled with different disintegration kinetics. Classical first-order kinetics are used to model the disintegration of simple organic matter (e.g. sewage sludge, livestock manure), and a surface-based kinetic expression [15, 40] is used to simulate the disintegration of complex particulate matter.

This expression (equation 1) considers the dependence of the disintegration rate on the surface area (i.e. on the particles size distribution-PSD) of the solid waste to be disintegrated. However, the surface-based kinetic expression proposed in [26] cannot be used in its original form (equation 1) as the model structure needs the substrates to be expressed in terms of concentrations while equation (1) includes the organic particles in terms of mass:

$$\frac{dM}{dt} = -K_{sbk}A\tag{1}$$

where:

M = complex organic substrate mass [M];

 $K_{sbk}$  = disintegration apparent kinetic rate constant [M L<sup>-1</sup>];

 $A = disintegration surface area [L^2].$ 

Equation (1) has therefore been reformulated in terms of concentrations (equation 4) by including the following two parameters, a and a\*, which characterize the disintegration process:

$$a = \frac{A}{V_{liq}} \tag{2}$$

$$a^* = \frac{A}{M} \tag{3}$$

$$\frac{dC}{dt} = -K_{sbk} \cdot a * \cdot C \tag{4}$$

where:

Cconcentration of the complex organic substrate in the digester  $[M L^{-3}]$ ;

 $V_{lig}$ liquid working volume of the anaerobic digester

Assuming that all the organic solid particles have the same spherical shape and initial size and that they are progressively and uniformly degraded, equation (3) can be rewritten as follows:

$$a^* = \frac{\sum_{i=1}^{n} A_i}{\sum_{i=1}^{n} M_i} = \frac{nA_i}{nM_i} = \frac{n4\pi R^2}{n\delta \frac{4}{3}\pi R^3} = \frac{3}{\delta R}$$
 (5)

where:

disintegration surface area of the organic solid  $A_i$ particle  $i[L^2]$ ;

 $M_i$ mass of the organic solid particle i [M];

total number of organic solid particles [dimennsionless];

complex organic substrate density [M L<sup>-3</sup>]; δ

R organic solid particles radius [L], assumed to be time dependent according to the following expression proposed in [40]:

$$R = R_0 - K_{sbk} \frac{t}{\delta} \tag{6}$$

where:

 $R_0$ = initial organic solid particle radius [L], specified as the initial condition for model application.

Equation (4) therefore results in equation (7), which is used in the model:

$$\frac{dC}{dt} = -\left(\frac{3 \cdot K_{sbk}}{\delta}\right) \cdot \frac{C}{R} \tag{7}$$

Expressing C in equation (7) as the ratio between the mass of the organic solid particles and the digester volume results in the following quadratic dependence of the disintegration process rate on the particle radius:

$$\frac{dC}{dt} = -K_{sbk} \frac{n4\pi R^2(t)}{V_{lig}} \tag{8}$$

Because the radius of the organic solid particles varies according to a linear law (equation 6), equation (8) implies that the concentration of the complex organic substrate decreases during the disintegration process according to a cubic law.

If this model is compared with the ADM1 first-order disintegration kinetics, the main advantage of this model is that  $K_{sbk}$  is the same for any PSD and can thus be determined experimentally using organic waste samples of any PSD. If a typical first-order kinetic expression is applied and organic waste samples are used to determine experimentally the apparent kinetic rate constant, the latter can be used to simulate the anaerobic digestion of organic waste with only the same nature and PSD of the organic waste samples that are investigated.

Integration of the differential algebraic equations is performed using a multi-step solution algorithm based on the numerical differentiation formulas in the software tool MATLAB®.

# 4.2. Calibration Procedure

The mathematical model can be used to estimate several apparent kinetic rate constants. In this work model calibration was used to estimate  $K_{sbk}$ , [M L<sup>-2</sup> T<sup>-1</sup>], i.e. the apparent kinetic rate constant of the surface-based disintegration process and  $K_{dis}$  [T<sup>-1</sup>], i.e. the first-order apparent kinetic rate constant of the disintegration process. Calibration was performed by comparing model results with experimental measurements of methane production and adjusting the unknown parameter until the model results adequately fit the experimental observations. Input, operational and output data from experiment A, B and C. (Table 2) were used, and a specific procedure was developed.

In this text the calibration procedure is referred to  $K_{sbk}$ , even if it can be applied for any apparent kinetic rate constant.

The calibration procedure is structured in four steps as follows.

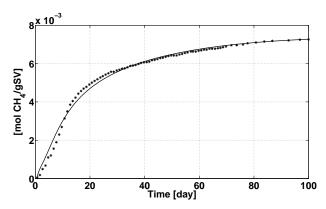
- 1. Step 1 determines a variation range for  $K_{sbk}$ ;
- Step 2 generates as many different values of  $K_{sbk}$  as the estimation accuracy requires. This calculation was performed taking n+1 constant step values of  $K_{sbk}$ , between the two bounds of the variation range, according to the following expression:

$$K_{sbk}^{j} = K_{sbk}^{j-1} + \Delta_{K_{sbk}}$$
, with j=1...n (9)

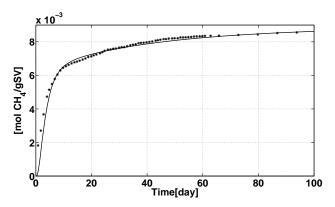
- $K_{sbk}^0 = 0$  and  $K_{sbk}^n = 1$  are the lower and upper bounds of the variation range, respectively, and  $\Delta_{K_{shk}}$  is the ratio between the width of the range and n. To set the accuracy of the results at two significant digits, n was fixed to be equal to 100;
  - Step 3 is performed by plotting a simulated curve for each value of  $K_{sbk}$  from the development of step 4 and by comparing simulated results with observed data. A comparison is performed by applying the Root Mean Square Error (RMSE) method that is commonly used for the model calibration process [38, 41];
  - Step 4 determines the value of  $K_{sbk}$  that best fits the observed data using the RMSE method [38].

#### 5. RESULTS AND DISCUSSION

The main results of the BMP tests are the cumulative biomethane curves (Figs. 2-4) where on the X-axis is displayed



**Fig. (2).** Cumulative bio-methane production from BMP test A: simulated data (line); experimental data (points).



**Fig. (3).** Cumulative bio-methane production from BMP test B: simulated data (line); experimental data (points).

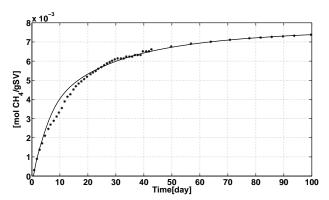
the observation time whereas on the Y-axis the corresponding cumulative bio-methane production per gram of VS. Usually these curves are either reverse L shaped or S shaped curves, both tending to a horizontal asymptote that represents the maximum theoretical bio-methane production per gram of VS obtainable from the substrate considered. The slope of the tangent line to the curve at any point indicates the bio-methanation rate at that specific time. Both reverse L shaped and S shaped graphs are divided in 3 main zones:

- initial phase;
- · intermediate phase;
- final phase.

In the reverse L shaped graphs the initial phase is distinguished by the higher bio-methanation rate that progressively decreases during the intermediate phase up to tend to zero at the end of the final phase. In the S shaped graphs, the initial phase is distinguished by a pretty high biomethanation rate, but lower than that one characterizing the intermediate phase, whereas during the final phase the biomethanation rate tends to zero, as well as already claimed about the reverse L-shaped graphs.

The bio-methanation rate depends on several factors:

1. Total amount of organic solids left. The biomethanation rate is in direct ratio to the current amount of organic solids present in the system. Since



**Fig. (4).** Cumulative bio-methane production from BMP test C: simulated data (line); experimental data (points).

BMP tests are batch tests, as time goes by, the amount of solids left decreases as well as the bio-methanation rate. This behaviour is well described by the reverse L shaped curves (Figs. 2 and 3);

- 2. Biodegradability of substrate. The higher the biodegradability of substrate is, the higher the biomethanation rate is, keeping equal all operational conditions. This aspect affects the distance of the cumulative bio-methane curve from the Y-axis during the initial phase: the higher the biodegradability is, the closer to the Y-axis the curve is (Fig. 3);
- 3. Presence of inhibitors. The presence of inhibitors is one of the main causes of a lower bio-methanation rate than expected or even of a bio-methanation rate equal to zero. This aspect is responsible for a curve any time lower in value as well as longer in time than expected or even for a curve that reaches an asymptote earlier than all organic solids are degraded;
- 4. Drops and jumps in pH. The bacteria strains that take part in the anaerobic digestion process are particularly sensitive to the pH. For this reason when the pH in the system is far from the pH interval [6-8] the biomethanation rate is lower than expected. When a drop as well as a jump in pH occurs the cumulative biomethane curve shows an asymptotic value lower than the theoretical one and earlier than expected;

Substrate particles size and its complexity. The bigger or more complex the organic solid particles are, the lower the bio-methanation rate is during the initial phase since the first step of the anaerobic digestion, i.e. disintegration and hydrolysis, takes a long time to be complete, revealing this step as the limiting step of the whole process. This aspect results either in a big distance of the cumulative bio-methane curve from the Y-axis during the initial phase, or in the shape of the curve closer to S shaped than reverse L (Fig. 4).

Figs. (2-4) show the cumulative curves of bio-methane produced from the BMP tests A, B and C, respectively: B gave the highest methane production and A the lowest one. The bio-methanization process was faster for the test B.

For all tests, after 100 days the bio-methane produced was close to the maximum obtainable, as graphically shown by the achievement of the plateau. The differences in the amount of methane produced as well as in the production

**Table 3. Calibration Process Results** 

BMP Test	Estimated Parameters		Parameter	Estimator Value	
	K <sub>dis</sub>	$K_{sbk}$	a*	RMSE	
	[s <sup>-1</sup> ]	[kg m <sup>-2</sup> s <sup>-1</sup> ]	[m² kg-¹]		
A	0.40			0.0057	
В		0.30	4.545	0.0054	
С		0.28	0.300	0.0046	

rate among the BMP tests considered in this study are explainable taking into account that the substrate used in test B is more biodegradable than the ones used in tests A and C, since it was not preliminary passed through the digestive system of animals. Moreover, in SM (test A) a higher content in ammonia due to the presence of urea is one of the reasons that makes slower the digestion process. Nevertheless, livestock manure, such as BM, contains enzymes and a high number of microorganisms that can make faster the biological process when used in the co-digestion process with substrate rich in cellulose such as MS, as shown by the bio-methane curve coming from the BMP test C. Furthermore, in the co-digestion process with high biodegradable substrates the ammonia contained in livestock manure could turn from a cause of inhibition [42] into a positive element for the biological process since the ammonia can supply the requested buffer capacity [43]. The results obtained from the BMP tests were used to calibrate the mathematical model proposed by the authors [15]. For this aim, the procedure detailed in sub-paragraph 4.2 and relating to  $K_{sbk}$  has been applied. The same procedure has been also used to estimate the disintegration apparent kinetic rate constant  $(K_{dis})$  of the digestion process fed with SM as substrate (BMP test A).

The calibration process led to obtain the apparent kinetic rate constants of the disintegration process for each considered organic substrate (Table 3). In Figs. (2-4) a good fitting between the experimental and modelled data is evident for all of them.

# **CONCLUSIONS**

The BMP tests are a powerful tool to assess the methane yield from the digestion of organic solids. However the lack of a standard protocol to carry out these experiments is an important problem for the interpretation of the data, which results in a serious drawback of the application of such tests. Therefore efforts are needed to achieve a unique recognized and accredited method to conduct the BMP tests, which will give to these tests the reliability that is still missing.

The results of the experiments described in this paper show that the BMP tests can be used to assess not only the maximum methane production from an organic substrate, but also the biodegradability of the investigated substrate, the relative specific rate of bio-methanation and the synergic effect of multiple co-digested substrates.

Furthermore the BMP tests can be used for the calibration of mathematical models suitable to simulate the digestion process and predict the performances of full-scale anaerobic digesters.

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# REFERENCES

- G. Plaza, P. Robredo, O. Pacheco and A. Saravia Toledo, "Anaerobic treatment of municipal solid waste", Water Sci. Technol., vol. 33, pp. 169-175, 1996.
- A. Karagiannidis and G. Perkoulidis, "A multi-criteria ranking of [2] different technologies for the anaerobic digestion for energy recovery of the organic fraction of municipal solid wastes", Bioresour. Technol., vol. 100, pp. 2355-2360, 2009.
- M. Pöschl, S. Ward and P. Owende, "Evaluation of energy effi-[3] ciency of various biogas production and utilization pathways", Appl. Energy, vol. 87, pp. 3305-3321, 2010.
- F. Tambone, P. Genevini, G. D'Imporzano and F. Adani, "Assess-[4] ing amendment properties of digestate by studying the organic matter composition and the degree of biological stability during the anaerobic digestion of the organic fraction of MSW", Bioresour. Technol., vol. 100, pp. 3140-3142, 2009.
- J. Mata-Alvarez, S. Macé and P. Llabrés, "Anaerobic digestion of [5] organic solid wastes: an overview of research achievements and perspectives", Bioresour Technol., vol. 74, no. 1, pp. 3-16, 2000.
- [6] T. L. Hansen, J. E. Schmidt, I. Angelidaki, E. Marca, J. C. Jansen, H. Mosbæk and T. H. Christensen, "Measurement of methane potentials of solid organic waste", Waste Manage., vol. 24, no. 4, pp. 393-400, 2004.
- H. Carrère, Y. Rafrafi, A. Battimelli, M. Torrijos, J. P. Delgenès [7] and G. Ruysschaert,"Methane potential of waste activated sludge and fatty residues: Impact of codigestion and alkaline pretreatments", Open Environ. Eng. J., vol. 3, pp. 71-76, 2010.
- [8] J. Rantala and B. Ahring, "A two-stage thermophilic anaerobic process for the treatment of source sorted household solid waste", Biotechnol. Lett., vol. 16, pp. 1097-1102, 1994.

- [9] F. G. Pohland, "Landfill bioreactors: fundamentals and practice", Water Qual. Int., vol. 9/10, pp. 18-22, 1996.
- [10] D. Reinhart and T. Townsend, Landfill Bioreactor Design and Operation. Lewis Publishers, Boca Raton, FL, 1998.
- [11] N. Hamzawi, K. J. Kennedy and D. D. McLean. "Technical feasibility of anaerobic codigestion of sewage sludge and municipal solid waste", *Environ. Technol.*, vol. 19, pp. 993-1003, 1998.
- [12] P. Sosnowski, A. Wieczorek and S. Ledakowicz, "Anaerobic codigestion of sewage sludge and organic fraction of municipal solid wastes", Adv. Environ. Res., vol. 7, pp. 609-616, 2003.
- [13] D. L. Hawkes, "Factors affecting net energy production from mesophilic anaerobic digestion", in *Anaerobic Digestion*, D. A. Stratford, B. I. Wheatley and D. E. Hughes, 1980, pp. 131-150.
- [14] G. Tchobanoglous, H. Theisen and S. Vigil, *Integrated Solid Waste Management*. McGraw-Hill Inc, New York, 1993.
- [15] G. Esposito, L. Frunzo, A. Panico and F. Pirozzi, "Modelling the effect of the OLR and OFMSW particle size on the performances of an anaerobic co-digestion reactor", *Process Biochem.*, vol. 46, no.2, pp. 557-565, 2011.
- [16] Angelidaki, M. Alves, D. Bolzonella, L. Borzacconi, J. L. Campos, A. J. Guwi S. Kalyuzhnyi, P. Jenicek and J. B van Lier, "Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays", Water. Sci. Technol., vol. 59, pp. 927-934, 2009.
- [17] U. Pagga and D. B. Beimborn, "Anaerobic biodegradation test for organic compounds", *Chemosphere*, vol. 27, pp. 1499-1509, 1993.
- [18] F. Raposo, V. Fernandez-Cegri, M. A. De la Rubia, R. Borja, F. Beline, C. Cavinato, G. Demirer, B. Fernandez, M. Fdz-Polanco, J. C. Frigon, R. Ganesh, P. Kaparaju, J. Koubova, R. Mendez, G. Menin, A. Peene, P. Scherer, M. Torrijos, H. Uellendahl, I. Wierinckm and V. de Wilde, "Biochemical methane potential (BMP) of solid organicsubstrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study", J. Chem. Technol. Biot., vol. 86, pp. 1088-1098, 2011.
- [19] J. Chae, A. Jang, S. K. Yim and S. Kim. "The effects of digestion temperature and temperature shock on the biogas yields from the mesophilic anaerobic digestion of swine manure", *Bioresour. Tech*nol., vol. 99, pp. 1-6, 2008.
- [20] A. Del Borghi, A. Converti, E. Palazzi and M. Del Borghi, "Hydrolysis and thermophilic anaerobic digestion of sewage sludge and organic fraction of municipal solid waste", *Bioprocess Eng.*, vol. 20, pp. 553-560, 1999.
- [21] G. Gungor-Demirci and G. N. Demirer, "Effect of initial COD concentration, nutrient addition, temperature and microbial acclimation on anaerobic treatability of broiler and cattle manure", *Bioresour. Technol.*, vol. 93, pp. 109-117, 2004.
- [22] W. F. Owen, D. C. Stuckey, J. B. Healy Jr., L. Y. Young and P. L. McCarty, "Bioassay for monitoring biochemical methane potential and anaerobic toxicity", Water Resour., vol. 13, pp. 485-492, 1979.
- [23] T. Forster-Carneiro, M. Pérez and L. I. Romero, "Influence of total solid and inoculum contents on performance of anaerobic reactors treating food waste", *Bioresour. Technol.*, vol. 99, pp. 6994-7002, 2008.
- [24] N. Hamzawi, K. J. Kennedy and D. D. Mclean, "Anaerobic digestion of co-mingled municipal solid-waste and sewage-sludge", Water Sci. Technol., vol. 38, no. 2, pp. 127-132, 1998
- [25] M. A. Barlaz, "Methane production from municipal refuse: a review of enhancement techniques and microbial dynamics", Crit. Rev. Env. Control, vol. 19, pp. 557-584, 1990.

- [26] W. T. M. Sanders, "Anaerobic hydrolysis during digestion of complex substrates", Ph.D. Thesis, Wageningen Universiteit, Wageningen, The Netherlands, 2001.
- [27] V. A. Vavilin and I. Angelidaki, "Anaerobic degradation of solid material: Importance of initiation centers for methanogenesis, mixing intensity and 2D distributed model", *Biotechnol. Bioeng.*, vol. 89, no.1, pp. 113-122, 2005.
- [28] Neves, R. Oliveira and M. M. Alves, "Influence of inoculum activity on the bio-methanization of a kitchen waste under different waste/inoculum ratios", *Process Biochem.*, vol. 39, pp. 2019-2024, 2004.
- [29] F. Raposo, M. A. de la Rubia, R. Borja, M. Alaiz, J. Beltran and C. Cavinato, "An interlaboratory study as a useful tool for proficiency testing of chemical oxygen demand measurements using solid substrates and liquid samples with high suspended solid content", *Talanta*, vol. 80, pp. 329-337, 2009.
- [30] G. Liu, R. Zhang, H. M. El-Mashad and R. Dong, "Effect of feed to inoculum ratios on biogas yields of food and green wastes", *Biore-sour. Technol.*, vol. 100, no. 21, pp. 5103-5108, 2009.
- [31] F. Concannon, P. J. Reynolds, A. Hennigan and E. Colleran, "Automated measurement of the specific methanogenic activity of anaerobic digestion biomass", *Biochem. Soc. Trans.*, vol. 17, p. 425, 1988.
- [32] ISO/DIS 14853, "Determination of the ultimate anaerobic biodegradability of plastic materials in an aqueous system Method by measurement of biogas production", 1999.
- [33] D. Valcke and W. Verstraete, "A practical method to estimate the acetoclastic methanogenic biomass in anaerobic sludge", J. Water Pollut Control Federation, vol. 55, pp. 1191-1195, 1983.
- [34] ASTM International. "Annual book of ASTM standards: Waste Management", vol. 04.11, 2011.
- [35] APHA/AWWA/WEF, Standards Methods for the Examination of Water and Wastewater, 20th ed., United Book Press, Inc., Baltimore, Maryland, 1998.
- [36] J. Mata-Alvarez, J. Dosta, S. Macè and S. Astals. "Codigestion of solid wastes: A review of its uses and perspectives including modeling", Crit. Rev. Biotechnol., vol. 31, pp. 99-111, 2011.
- [37] G. Esposito, L. Frunzo, A. Panico and G. d'Antonio, "Mathematical modelling of disintegration-limited co-digestion of OFMSW and sewage sludge", *Water Sci. Technol.*, vol. 58, pp. 1513-1519,
- [38] G. Esposito, L. Frunzo, A. Panico and F. Pirozzi, "Model calibration and validation for OFMSW and sewage sludge co-digestion reactors", Waste Manage., vol.31, pp. 2527-2535, 2011.
- [39] D. J. Batstone, J. Keller, I. Angelidaki, S. V. Kalyuzhnyi, S. V. Pavlostathis, A. Rozzi, W. T. M. Sanders, H. Siegrist and V. A. Vavilin, *Anaerobic digestion model no.1, Rep. No.* 13, ed. IWA Publishing, London, 2002.
- [40] W. T. M. Sanders, M. Geerink, G. Zeeman and G. Lettinga, "An-aerobic hydrolysis kinetics of particulate substrates", Water Sci. Technol., vol. 41, pp. 17-24, 2000.
- [41] P. H. M. Janssen and P. S. C. Heuberger, "Calibration of processoriented models", *Ecol. Model.*, vol. 83, pp. 55-66, 1993.
- [42] Y. Chen, J. J Cheng, and K. S. Creamer, "Inhibition of anaerobic digestion process: A review", *Bioresour. Technol.*, vol. 99, pp. 4044-4064, 2008.
- [43] I. Angelidaki and B. K. Ahring, "Thermophilic anaerobic digestion of livestock waste: the effect of ammonia", Appl. Microbiol. Biot., vol. 38, pp. 560-564, 1993.

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