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# Enhanced bio-methane production from co-digestion of different organic wastes

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This paper deals with an experimental study aimed at assessing the effect of mixing different organic wastes on the anaerobic digestion process. Livestock manure and organic solid wastes have been taken into account as substrates to verify if their mixing gives rise to higher methane production rates and lower risk of process failure. Bio-methane potential (BMP) tests have been conducted using the following substrates: buffalo manure (BM), poultry manure (PM), organic fraction of the municipal solid waste (OFMSW), greengrocery waste (GW) and two different mixtures composed of BM and OFMSW. Mixing BM with OFMSW resulted in 12% and 30% higher methane volumes after 30 and 15 days from the test start, respectively. Experimental data have been also used to calibrate and validate a mathematical model previously proposed by the authors, showing its capability to reproduce the synergistic effect on methane production promoted by co-digesting BM and OFSMW.

**Keywords:** co-digestion; methane production; anaerobic digestion; livestock manure; OFMSW

## 1. Introduction

Anaerobic digestion is widely used to treat organic wastes, representing a valid alternative to landfilling [1] as well as an attractive source of renewable energy [2,3]. The solid byproduct of the anaerobic process (digestate) could be used as agricultural fertilizer due to its nutrients and humus precursors content [4].

Anaerobic digestion is a self-inhibiting, multi-stage biological process that depends upon operational conditions [5], such as temperature, pH, carbon/nitrogen ratio (C/N) and the presence of inhibitors. The substrates most commonly used to feed the anaerobic digesters are livestock manure, organic fraction of the municipal solid waste (OFMSW), wastes generated from food factories and farming [6]. These substrates and their elements have specific characteristics of biodegradability that considerably affects the whole anaerobic digestion process. For instance, livestock manure and vegetable waste give rise to lower methane production rates, longer process reaction times and slower productions of volatile fatty acids (VFA) when compared with OFMSW and food factories wastes [7,8]. Furthermore, bio-methane produced from a mass unit of swine manure measured as volatile solids (VS) is different to that produced from the anaerobic digestion of an equal mass of poultry or cattle manure [9]. Over the last 15–20 years, these considerations have driven several authors to study the effects on the performance of the anaerobic digestion process produced by simultaneously treating several solid as well as liquid organic wastes or energy crops [10–15]. This method is commonly known as co-digestion and, if appropriately used, can give interesting results due to the synergistic effect shown by different organic substrates when digested simultaneously [16–18], e.g., mixing organic substrates can result in the production of a mixture with a C/N ratio included in the optimal range 20:1–30:1 [19]. Further benefits of the co-digestion process are: (1) dilution of the potential toxic compounds eventually present in any of the co-substrates involved; (2) adjustment of the moisture content and pH; (3) supply of the necessary buffer capacity to the mixture; (4) increase of the biodegradable material content; (5) widening the range of bacterial strains taking part in the process. All these benefits lead to an improved stability and performance of the anaerobic digestion process and higher biogas and energy production [20].

The main aim of this work is to investigate the effect on the anaerobic digestion process by mixing different organic substrates belonging to two particular categories of waste: livestock manure and municipal solid waste. In addition, the synergistic effect on methane production showed by co-digesting such wastes was successfully reproduced using the mathematical model recently proposed by the authors [21] to simulate the anaerobic co-digestion process.

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## 2. Materials and methods

### *Experimental design*

BMP tests were conducted using four different organic wastes whose main characteristics in terms of total solids (TS), VS, carbohydrates fraction ($F_{ch}$), proteins fraction ($F_{pr}$) and lipids fraction ($F_{li}$) are shown in Table 1. In particular, BMP tests were conducted on each pure substrate (identified by the test indexes $T_1$, $T_2$, $T_3$ and $T_4$) and on two mixtures containing BM and OFMSW in VS percentages of 50% and 50%, and 70% and 30%, respectively (identified by the test indexes $T_5$ and $T_6$). A further BMP test (identified by the test index $T_7$) was conducted on the inoculum to estimate the volume of methane produced by the fermentation of the organic solids in the anaerobic sludge. In total, seven BMP tests were conducted and each of them three times.

In Table 2, the mass of BM, PM, GW, OFMSW, inoculum and $Na_2CO_3$ used to perform the seven BMP tests are indicated.

### *Substrates collection and preparation*

PM and BM were collected from a farm in Albanella near Salerno in southern Italy and stored in 10 l buckets at 4 °C. Granular anaerobic sludge, used as inoculum, was taken from an upflow anaerobic sludge blanket (UASB) reactor treating wastewater produced by a potato factory.

Representative samples of OFMSW and GW were collected according to the waste sampling methodology [22] from the household source-separated wastes and the fruit and vegetable market of Naples. Both samples of OFMSW and GW were ground and sieved to have a homogeneous material composed of particles with size ranging between 1 and 2 cm.

### *BMP tests set up and operation*

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 $Na_2CO_3$ powder, ranging from 0.10 to 0.60 g, were also added (Table 2) to prevent critical drops 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 ± 1 °C by 200 W 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 fitted on both ends with a needle, which could pierce the silicone disc.

### *Measurements*

TS, VS, chemincal oxygen demand (COD) and $F_{li}$ of each substrate were measured according to standard methods [23]. $F_{pr}$ was obtained by multiplying 6.25 by the

Table 1. Main characteristics of the organic solid wastes used in BMP tests.

| Parameter | Units | Organic solid wastes | | | | Inoculum |
|---|---|---|---|---|---|---|
| | | BM | PM | OFMSW | GW | |
| TS | g/kg, wet | 102.7 ± 0.8 | 867.1 ± 2.0 | 334.7 ± 0.9 | 151.8 ± 0.5 | 140.9 ± 0.8 |
| VS | g/kg, wet | 81.4 ± 0.6 | 365.2 ± 1.4 | 251.4 ± 0.7 | 112.8 ± 0.4 | 85.4 ± 0.5 |
| $F_{ch}$ | g/g, dry | 0.18 | 0.30 | 0.28 | 0.32 | |
| $F_{pr}$ | g/g, dry | 0.31 | 0.20 | 0.18 | 0.17 | |
| $F_{li}$ | g/g, dry | 0.017 | 0.015 | 0.25 | 0.02 | |

Note: *TS = total solids; VS = volatile solids; $F_{ch}$ = carbohydrates fraction; $F_{pr}$ = proteins fraction; $F_{li}$ = lipids fraction.*

Table 2. BMP tests.

| Test | BM Mass [g] | PM Mass [g] | OFMSW Mass [g] | GW Mass [g] | Inoculum Mass [g] | $Na_2CO_3$ Mass [g] |
|---|---|---|---|---|---|---|
| $T_1$ | 77.61 ± 0.64 | ------------- | ------------- | ------------- | 150.22 ± 0.62 | 0.35 ± 0.04 |
| $T_2$ | ------------- | 17.52 ± 0.51 | ------------- | ------------- | 150.15 ± 0.89 | 0.10 ± 0.02 |
| $T_3$ | ------------- | ------------- | 25.43 ± 0.38 | ------------- | 150.32 ± 0.65 | 0.60 ± 0.06 |
| $T_4$ | ------------- | ------------- | ------------- | 56.45 ± 0.72 | 150.27 ± 1.05 | 0.30 ± 0.04 |
| $T_5$ | 49.05 ± 0.51 | ------------- | 11.19 ± 0.22 | ------------- | 150.08 ± 0.93 | 0.35 ± 0.05 |
| $T_6$ | 68.68 ± 0.62 | ------------- | 6.71 ± 0.18 | ------------- | 150.24 ± 0.89 | 0.25 ± 0.02 |
| $T_7$ | ------------- | ------------- | ------------- | ------------- | 150.16 ± 0.77 | 0.10 ± 0.03 |

organic nitrogen content of each substrate (TKN minus $NH_4$-N) measured according to standard methods [23], whereas $F_{ch}$ was evaluated by subtracting the sum of proteins and lipids from VS content [24].

Daily methane production was monitored measuring the volume of alkaline solution displaced from the measure bottle and collected in a graduated cylinder. The $CO_2$ contained in the biogas did not affect the volumetric methane measurements because it was dissolved in the alkaline solution. Temperature and pH in each BMP bottle were also monitored at least once a day with a TFK 325 thermometer (WTW, Germany) and a pH meter (Carlo Erba, Italy).

### *Mass balance analysis and specific methane production calculation*

A COD mass balance was carried out for all $T_1 - T_7$ tests. In particular, the COD removal assessed as the difference between input COD and output COD was compared with the methane production expressed in terms of COD.

The specific methane production for tests $T_1 - T_4$ was calculated by the following procedure: the final methane production (tot $CH_4$) from each BMP test was reduced by 780 Nml (the final methane production from the BMP test $T_7$, i.e., test carried out considering inoculum as the only substrate) to give the net methane production (net $CH_4$). This value was divided by the VS content of the corresponding substrate to give the specific methane production.

### *Mathematical modelling*

A mathematical model that can predict the methane production has recently been proposed by the authors [21]. This model considers different organic substrates (e.g. sewage sludge and OFMSW) that are modelled with different disintegration kinetics. In this paper, a first-order kinetics according to the ADM1 [25] has been used to model the livestock manure and anaerobic sludge, whereas a surface-based kinetic expression [26,27] has been used to simulate the OFMSW and GW disintegration process, an essential step when the substrate to be disintegrated is highly complex.

Model calibration was performed by comparing model results with experimental measurements of methane production from tests $T_1 - T_4$ and adjusting the unknown parameter until the model results adequately fit the experimental observations.

After the calibration process was completed and the values of the disintegration constants determined, the model was validated using the results of the BMP tests $T_5$ and $T_6$ carried out on mixtures of two organic substrates i.e., OFMSW and BM. The $T_5$ and $T_6$ experimental measurements were compared to the corresponding model results obtained using the disintegration constants previously determined for BM (test $T_1$) and OFMSW (test $T_2$). Model calibration and validation were performed following the procedure detailed in [28], taking into account the modelling efficiency (*ME*) method, the index of agreement (*IoA*) method and the root mean square error (*RMSE*) method [29].

## 3. Results and discussion

### *Methane production from pure organic substrate*

Figure 1 shows the cumulative methane production from the BMP tests conducted using pure substrates. $T_2$ and $T_3$ gave the highest and lowest methane production, respectively and intermediate values were obtained from $T_1$ and $T_4$.

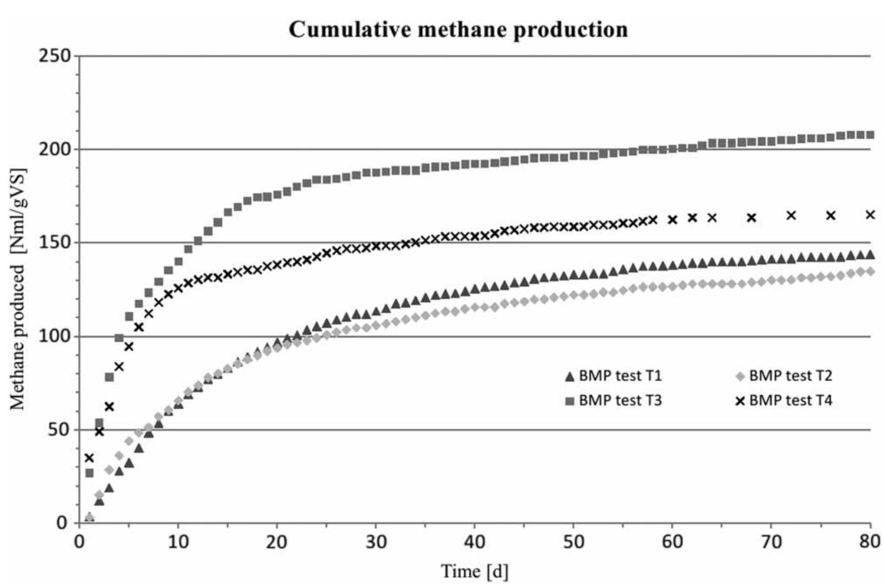


Figure 1. Cumulative methane production from BMP tests $T_1 - T_4$.

The methanization process was faster when OFMSW ($T_3$) and GW ($T_4$) were used as a substrate rather than livestock manure, as shown by the initial slope of the cumulative curves (Figure 1).

After 80 days, the methane produced for all pure substrates was close to the maximum obtainable, indicated in Figure 1 by the achievement of the plateau. After 30 days, the methane obtained by $T_3$, $T_4$, $T_1$ and $T_2$ were 187.7, 148.3, 113.5 and 105.8 Nml $g^{-1}$VS, respectively, corresponding to 90%, 89%, 79% and 78% of the total methane produced after 80 days.

These differences in the amount of methane produced, as well as in the production rate between organic solid wastes and livestock manure, are due to the first substrates being more biodegradable than the second substrates because they were not initially passed through the digestive system of animals. Moreover, in the livestock manure a lower C/N ratio and a higher content of ammonia [30] due to the presence of urea makes the digestion process slower. On the other hand, a high biodegradable substrate, such as OFMSW, can cause problems in the progress of the digestion process because the production of VFA [31] can be faster than their conversion into methane. When this event happens a sharp drop in pH occurs and consequently the digestion process becomes slower or can even fail. The occurrence of these failures can be avoided if the organic substrate has a sufficient buffer capacity. From this point of view, the ammonia in livestock manure could turn from a cause of inhibition [32] into a positive element for the biological process, supplying the requested buffer capacity [33]. Livestock manure also contains enzymes and a high number of microorganisms that can make the biological process faster and also more efficient because enzymes help to consume the less biodegradable components of the municipal organic solid wastes such as cellulose.

On the basis of the previous considerations, it was decided to mix organic solid wastes and livestock manure and assess their synergistic effect by means of further BMP tests.

BMP tests were also used to evaluate the specific methane production (*spec $CH_4$*) obtained from all pure substrates investigated, according to the particular operational conditions chosen to perform the BMP tests. Each substrate showed a specific methane production in agreement with the values (Table 3) reported in literature [34–38]. It was not possible to have a direct comparison between the literature data and the data resulting from the BMP tests conducted in this study for the buffalo manure because this type of waste

Table 3. Methane productions from tests $T_1 - T_4$ and $T_7$

| | tot $CH_4$ [Nml] | net $CH_4$ [Nml] | substrate VS [g] | spec $CH_4$ [Nml/gVS] | | |
|---|---|---|---|---|---|---|
| | | | | This study | Literature | Ref. |
| $T_1$ | 2.763 ± 124 | 1.980 ± 189 | 6.32 ± 0.18 | 313.29 ± 38.83 | 220–350[(1)] | [34] |
| $T_2$ | 2.583 ± 145 | 1.803 ± 210 | 6.39 ± 0.24 | 282.16 ± 43.46 | 200–290 | [35] |
| $T_3$ | 4.032 ± 187 | 3.250 ± 252 | 6.39 ± 0.21 | 508.61 ± 56.15 | 298–573 | [36,37] |
| $T_4$ | 3.592 ± 118 | 2.810 ± 183 | 6.37 ± 0.11 | 441.13 ± 36.35 | 470–510 | [37,38] |
| $T_7$ | ---------- | 780 ± 65 | 12.83 ± 0.23 | 60.80 ± 6.16 | ---------- | ---- |

Note: [(1)] *Cow manure*.

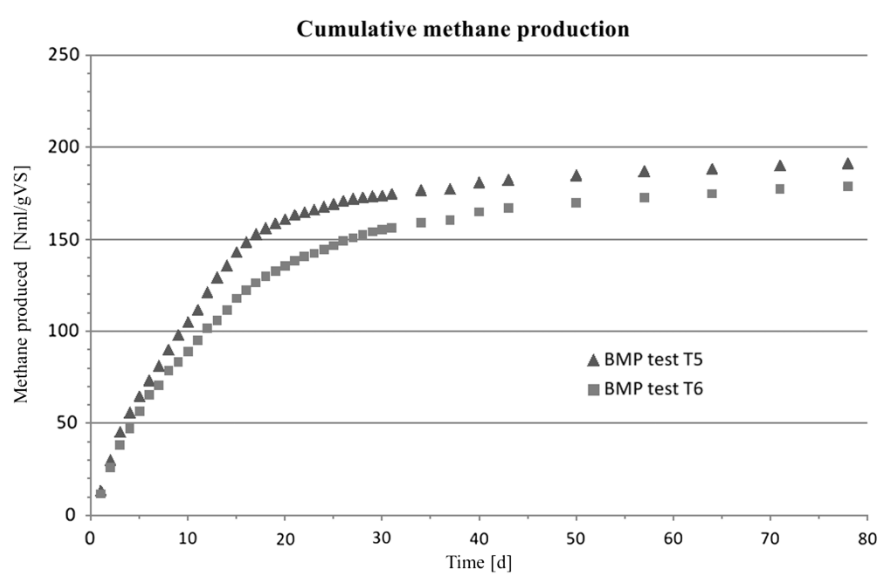


Figure 2. Cumulative methane production from BMP tests $T_5$ and $T_6$.

has not been sufficiently studied or, at least, not as much as cow manure.

### *Methane production from mixtures of organic substrates*

Tests $T_5$ and $T_6$ were conducted on mixtures composed of the two pure substrates chosen from livestock manure and organic solid wastes which had the highest production of methane i.e., BM and OFMSW. These tests focused on the effect of mixing different substrates on methane production (Figure 2). The highest methane production was achieved in test $T_5$, corresponding to the mixture characterized by the higher percentage of OFMSW.

It is interesting to note that the co-digestion of two substrates was completed in almost 60 days, which is faster when compared to the digestion of the pure substrates, completed in more than 80 days. This is due to the following main characteristics of the mixtures of BM and OFMSW: (1) higher buffer capacity if compared with pure OFMSW; (2) lower effect of inhibiting factors, like ammonia, when compared with pure BM; and (3) better balance in carbon and nutrient content.

Figure 3 shows that the methane volume obtained at any day from the mixture of organic wastes is higher than the sum of the amounts produced when the same organic wastes were digested separately. For instance, after 15 and

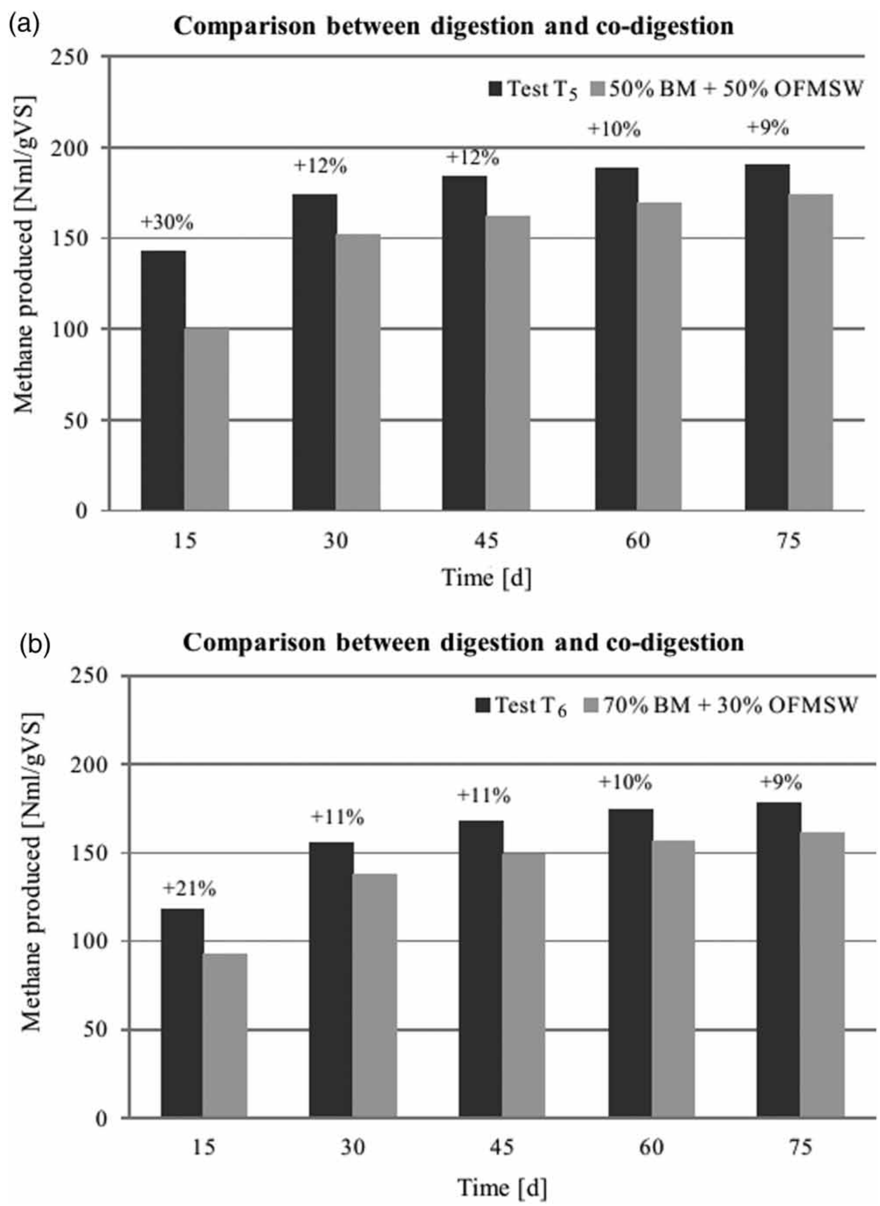


Figure 3. Comparison between digestion and co-digestion: (a) mixture with 50% BM and 50% OFMSW; (b) mixture with 70% BM and 30% OFMSW.

30 days the mixture of test $T_5$ gave a 30% and 12% higher methane production, respectively (Figure 3a), whereas the same volume of methane produced in test $T_6$ at day 30 is obtainable by adding the amounts produced individually by the single pure substrates after almost 75 days (Figure 3b).

This means that the co-digestion of different substrates reduces the time needed to complete the digestion process, making it possible for smaller size digesters.

### *Mass balance results and pH measurement*

A COD mass balance analysis was performed to compare the COD removal during the overall digestion process with the $CH_4$ produced. All data calculated according with the procedure illustrated in sub-section 2 are reported in Table 4. For all tests $T_1 - T_7$ the deviation between the COD removed from the liquid phase and the $CH_4$ produced is lower than the standard deviation of the COD and $CH_4$ measures, confirming that all the removed COD is converted in $CH_4$. The $CH_4$ and sulfide dissolved in the alkaline solutions were not taken into account in the mass balance because they are lower than the standard deviations of the other measures and thus negligible.

Table 4. COD mass balance for tests $T_1 - T_7$.

| | $COD_{INPUT}$ [gCOD] | $COD_{OUTPUT}$ [gCOD] | $COD_{REMOVAL}$ [gCOD] | $CH_4$ [gCOD] |
|---|---|---|---|---|
| $T_1$ | 27.4 ± 0.4 | 19.5 ± 0.3 | 7.9 ± 0.7 | 7.7 ± 0.4 |
| $T_2$ | 28.8 ± 0.3 | 21.4 ± 0.3 | 7.4 ± 0.6 | 7.3 ± 0.4 |
| $T_3$ | 30.7 ± 0.8 | 19.5 ± 0.2 | 11.2 ± 1.0 | 10.9 ± 0.5 |
| $T_4$ | 29.4 ± 0.8 | 19.2 ± 0.4 | 10.2 ± 1.2 | 10.1 ± 0.3 |
| $T_5$ | 29.6 ± 0.7 | 19.5 ± 0.4 | 10.1 ± 1.1 | 10.0 ± 0.4 |
| $T_6$ | 29.7 ± 0.6 | 20.2 ± 0.4 | 9.5 ± 1.0 | 9.4 ± 0.3 |
| $T_7$ | 18.0 ± 0.3 | 15.9 ± 0.3 | 2.1 ± 0.6 | 2.0 ± 0.2 |

No significant drop in pH was noticed during the daily monitoring in any BMP bottle. The pH slightly fluctuated between 7.9 and 8.2 during each experiment.

### *Model results and application*

The results obtained from the BMP tests were used to calibrate and validate a mathematical model proposed by the authors [21].

Model calibration was used to estimate the first order disintegration kinetic constant for the livestock manure and the surface-based disintegration kinetic constant for the OFMSW and GW.

The $F_{ch}$, $F_{pr}$ and $F_{li}$ parameters are shown in Table 1, whereas all other model parameters considered in the simulations are in agreement with [25,39].

The calibration process was performed using the results of the BMP tests $T_1 - T_4$ to obtain the kinetic constant of the disintegration process for each considered pure substrate. Such kinetic constants and the corresponding values of the estimators (i.e., *ME*, *IoA* and *RMSE*) are shown in Table 5.

Table 5. Calibration process results.

| | Evaluated parameters | | Estimators value | | |
|---|---|---|---|---|---|
| BMP Test | $K_{dis}$ [$s^{-1}$] | $K_{sbk}$ [kg $m^{-2}s^{-1}$] | RMSE | IoA | ME |
| $T_1$ | 0.22 | | 0.0009 | 0.999 | 0.9989 |
| $T_2$ | 0.35 | | 0.0013 | 0.9999 | 0.9972 |
| $T_3$ | | 1.63 | 0.0040 | 0.999 | 0.9999 |
| $T_4$ | | 1.23 | 0.0020 | 0.9999 | 0.9926 |

Note: $K_{dis}$ = *First order disintegration kinetic constant*; $K_{sbk}$ = *Surface − based disintegration kinetic constant*; *RMSE* = *Root mean square error*; *IoA* = *Index of agreement*; *ME* = *Modelling efficiency*.

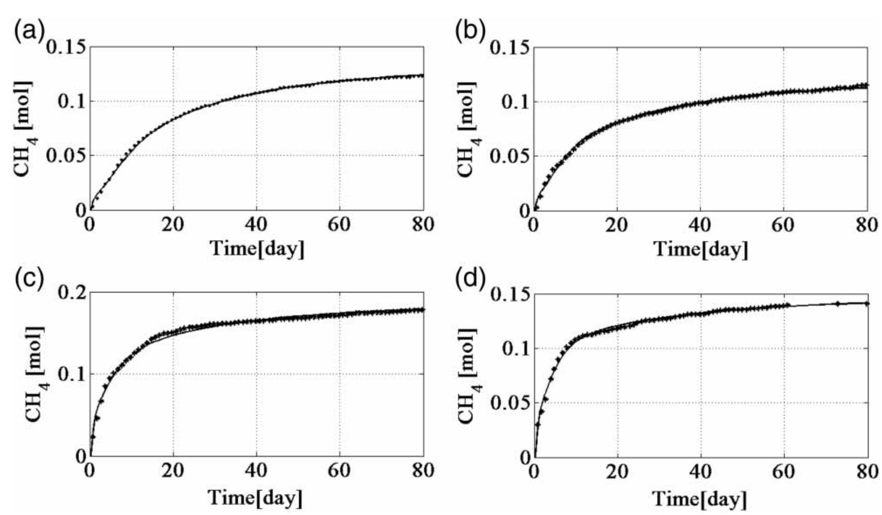


Figure 4. Comparison between experimental (points) and simulated (lines) methane production from BMP tests $T_1$ (a), $T_2$ (b), $T_3$ (c) and $T_4$ (d).

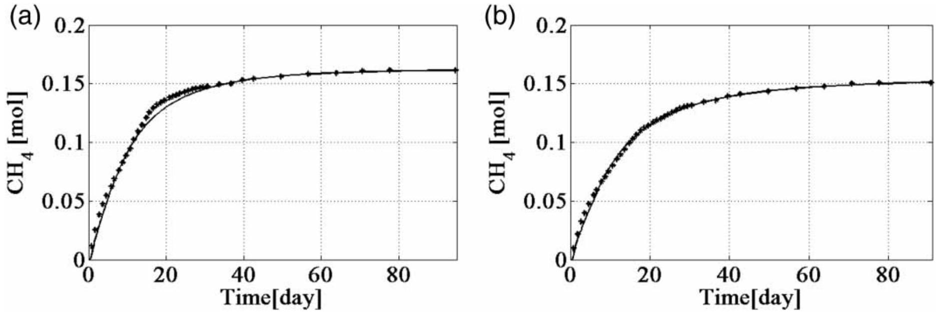


Figure 5. Comparison between the experimental (points) and simulated (line) methane production from BMP tests $T_5$ (a), $T_6$ (b).



Table 6. Validation process results.

| | Estimators value | | |
|---|---|---|---|
| BMP Test | RMSE | IoA | ME |
| $T_5$ | 0.0024 | 0.9999 | 0.9959 |
| $T_6$ | 0.0046 | 0.9999 | 0.9873 |

Note: *RMSE = Root mean square error; IoA = Index of agreement; ME = Modelling efficiency*.

Figure 4 shows a good fitting between experimental and modelled data for all curves related to each substrate.

The good fitting between simulated and experimental curves of cumulative methane production shown in Figure 5 validates the model; the optimal values obtained for *ME*, *IoA* and *RMSE* parameters for such curves (Table 6) confirm the model validity and indicate that, once the disintegration kinetic constants of different substrates have been evaluated by the calibration process, the mathematical model can be used to predict the bio-methane produced from a co-digestion process fed with two substrates of any percentage, among all those considered during the calibration process. This completes the model validation carried out in [28]. In [28] the model was validated with substrates of particle size different from the particle size of the substrate used for model calibration. The optimal values obtained for *ME*, *IoA* and *RMSE* demonstrated the main model peculiarity when compared to other ADM1 based models i.e., the independence of the disintegration kinetic constant on the size of the organic particles. However the model validation performed in [28] was limited by the use of similar substrates for calibration and validation and only synthetic substrates.

## 4. Conclusions

The experimental tests described in this paper demonstrate the advantages for the anaerobic digestion process resulting from mixing different organic wastes. The first benefit is related to the possibility to make the digestion process faster, producing a significant amount of methane even in digesters with a low hydraulic retention time (HRT). The second benefit is related to the possibility to decrease the risk of failures for the biological process when the digester is fed with a highly degradable organic substrate, such as OFMSW. Mixing OFMSW with a lower degradable but ammonia-rich substrate, such as livestock manure, suggests that the system is protected from a sharp drop in pH, which is the main cause of the digestion process failure.

Finally, once the characteristics of the pure substrates are known, the proposed mathematical model evaluates *a priori* which substrates and their percentages should be mixed to optimize the performance of the process. Therefore, this mathematical model can be reasonably applied for the co-digestion systems design.

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