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ORIGINAL PAPER

Exploring the Possibility to Shorten the Duration and Reduce the Number of Replicates in Biomethane Potential Tests (BMP)

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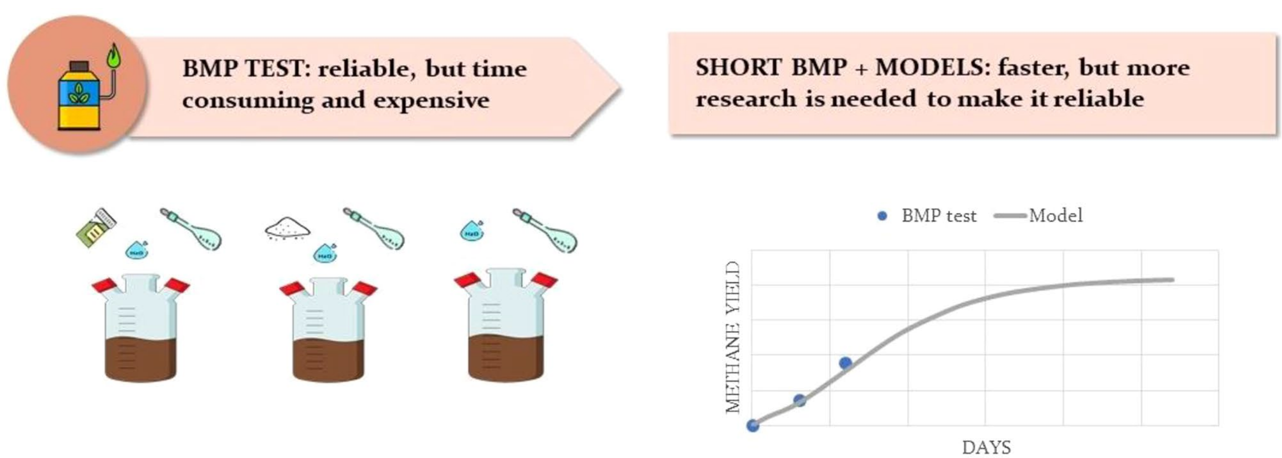
Abstract

Biochemical methane potential (BMP) tests are the most reliable method for the direct evaluation of the methane yield from a specific feedstock in anaerobic digestion. However, these tests are time-consuming (about 1 month) and quite expensive (need of no less than two or three replicates). This study evaluates the accuracy of the "first-order kinetic", "logistic" and "Gompertz" models in predicting the BMP values, calibrating the models' parameters with the data collected in shorter BMP tests (i.e., 5, 7, 10, 14 and 21 days) than usually (28 days or more). Moreover, the influence of the number of replicates (i.e., two or three) on the model prediction accuracy was also evaluated. A database from 32 BMP tests, previously carried out on different substrates, was adopted for these evaluations. The test duration significantly influences the prediction accuracy for two models (Gompertz and first-order kinetic), while the number of replicates is less influencing. The ultimate methane production is not accurate if the models use parameters from short (less than 10 days) BMP tests. The increase in test duration to 21 days gives BMP predictions with errors below 10% for Gompertz and logistic models.

Graphical Abstract



Keywords Anaerobic digestion · BMP tests · First-order kinetic model · Gompertz model · Logistic model · Replicates

Statement of Novelty

Prediction models are useful tools to estimate the ultimate biochemical methane potential (BMP) of a feedstock in anaerobic digestion (AD) under optimal conditions. However, in order to calibrate their parameters, these models require preliminary BMP tests, which are expensive (since

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require replications) and time-consuming (up to 3 months, but usually about 1 month is sufficient). To reduce the test time and save money, it is important to evaluate whether it is possible to reduce the test duration and the number of replicates. This study proposes a novel "hybrid" approach, where the "first order kinetic", "logistic" and "Gompertz" models are applied in predicting the BMP values, using the data collected in short BMP tests (between 5 and 21 days). Moreover, the model accuracy is also evaluated under the hypothesis of reducing the number of replicates (commonly three) to only two. To the authors' best knowledge, no relevant evaluations are available in the literature. This is an important approach, since a fast and reliable method for BMP prediction is strictly linked to the management of full-scale AD plants.

Introduction

Anaerobic digestion (AD) is a biochemical process that degrades organic substances thanks to anaerobic microorganisms. The final product of AD is the biogas, a mixture of methane (60–70%), carbon dioxide (30–40%) and traces of other gases (such as nitrogen, hydrogen, ammonia and hydrogen sulphide). The methane produced by the AD (the so-called "biomethane") is a valid alternative to fossil fuels, as renewable resource. In addition and, more importantly, the biomethane can be produced from biodegradable waste, and this enhances the sustainable management of the organic fraction of municipal and agro-industrial waste and wastewater [1]. As well-known, the methane produced from a specific substrate by AD under optimised environmental conditions is the biochemical methane potential (BMP), generally expressed as mL or L of CH_4 per gram of volatile solids (VS) of biodegradable substrate. The BMP depends on the physico-chemical characteristics of the substrate as well as on the environmental conditions of the AD process (such as the temperature, hydraulic retention time, inoculum to substrate ratio, possible presence of inhibitory compounds). The BMP evaluation is an essential step to optimise the AD [2], as it measures, under optimal conditions, the potential biodegradation of the substrate and therefore its methane yield. In other words, the BMP determination measures the substrate biodegradability, considering that the methane production increases with the anaerobic biodegradation. Usually, before feeding full-scale AD plants with a specific substrate, BMP values are previously determined, in order to optimise the environmental conditions (such as temperature, organic loading rate, size of digester, etc.) and thus the methane yield of the converted organic matter. When upgraded to full-scale plant, the energy from a substrate is evaluated using its calorific value. The latter parameter can be compared to the related efficiency of the biological process [3], although few studies played attention on this issue.

The methane yield of a substrate or a mixed feedstock can be estimated using several methods. In addition to the BMP tests, other experimental and theoretical methods have been proposed [4, 5]. Spectrometry [6] and, in particular, near infrared (NIR) spectroscopy predict the enzymatically-digestible organic matter (the organic matter that can be digested under anaerobic conditions) and, therefore, the methane yield of a biomass through prediction models [5, 7, 8]. NIR spectrometry applied to several biomasses (such as municipal green waste, energy crops [9], municipal solid waste [10], household waste [11], plant biomasses [5] and straw and manure [12]) gave accurate BMP predictions. However, NIR spectral information may be influenced by the biomass composition, especially the water content and particle size, and this influence reduces the reliability of the NIR prediction models [5].

The analysis of the chemical composition of the digested substrates is an alternative method to predict the final methane production, since it is influenced by the biomass chemical characteristics [5]. Buswell [13, 14] and Tchobanoglous equations [15], which are based on the elemental composition (C, H, O, and N) of the substrate, are among the best predictors of the final methane production. These stoichiometric equations are extremely accurate in applications to easily-biodegradable substrates (e.g., cellulose) [4]. However, these equations are less or not reliable to predict the BMP of slowly-degradable compounds (such as the lignocellulosic biomasses) [16]. Therefore, these equations are mainly used to evaluate the biodegradability of a substrate, by comparing the methane yield of an experimental BMP batch test and the theoretical stoichiometric value derived by the formulas.

Other theoretical models, based on the complete characterisation of the substrate, have been developed to evaluate the variable level of biodegradability of a given substrate. For instance, the BMP of a lignocellulosic substrate can be predicted by measuring its lipid, protein and carbohydrate contents [17] (e.g., the model proposed by Raposo et al. [18]). Catenacci et al. [19] developed BMP prediction models to measure the sludge bio-methanisation based on its chemical composition. These authors found that the soluble organic nitrogen is an influential parameter, in addition to the organic fraction composition. In contrast, the chemical oxygen demand (COD) does not give information on the content of the biodegradable organic matter, since the chemical oxidation does not separate the effects of degradable and non-degradable organic matter [2]. Angelidaki and Sanders [20] used the COD to VS ratio as an indicator of the anaerobic bio-degradability of a substrate. Biological oxygen demand (BOD) is generally well correlated to the BMP [2], but this parameter is not appropriate for lignocellulosic substrates [21]. Models based on physico-chemical properties of the substrate could provide reliable predictions of BMP, but much effort must be paid to the accurate characterisation of the biomass, because each organic fraction shows different microbial accessibility and degradability [19]. In addition, the theoretical models hardly simulate the problems of the AD process, due to the substrate characteristics and environmental conditions.

A BMP laboratory test is still considered as the most accurate method to evaluate the methane yield of a given substrate [22]. This test is simpler compared to full-scale applications, and somehow allows the simulation of anaerobic digesters on commercial scale [4]. However, the BMP tests are expensive (requiring replications to statistically process the test results) and time-consuming (up to 3 months, with a minimum duration of 1 month) [22, 23].

Much effort has been paid and many studies have been conducted so far, in order to develop standard procedures for BMP tests [24–35]. In contrast, to the authors' best knowledge, little research is available on how to reduce the test duration as well as the number of replicates. Only Strömberg et al. [36] and Ponsá et al. [37] defined mathematical methods for the prediction of the BMP at an early stage of the AD test. Ponsá et al. [37] found a good correlation between the methane produced in the early stage of AD and the final methane yield of the organic fraction of municipal solid waste. In contrast, these authors reported the need of longer time (2 weeks or more) to fit well the final production of mixed (more heterogeneous) municipal waste. Again Strömberg et al. [36], analysing data from more than 100 BMP tests on several substrates, reported accurate predictions of the final BMP after a 1-week AD test using optimised algorithms. From this short analysis of the-state-of-the-art, it is evident that the research question is still open, and therefore more investigations are needed to setup quick and accurate methods for BMP estimations.

To fill these gaps, this study evaluates the accuracy of the "first-order kinetic", "logistic" and "Gompertz" models in predicting the BMP values. The model parameters are calibrated using the data of BMP tests that are shorter (i.e., the first 5, 7, 10, 14 and 21 days) than usually done (28–30 days or more). Moreover, the influence of the number of replicates (i.e., two or three) on the model prediction accuracy is also evaluated. A database from 32 BMP tests, previously carried out on different substrates throughout six years (2015–2021) in the same laboratory and under very similar experimental conditions, was adopted for the BMP tests of the current study. This database has been prepared using AD substrates of different origin (such as orange peel waste, bioplastics, market waste, anchovy residues, prickly pear cactus), which were tested under a variety of conditions (e.g., as mesophilic or thermophilic temperature, co-digestion) and or pre-treatments (such as ensiling or chemical conditioning). For most of the tests (about 90%), the inoculum was derived from the same full-scale mesophilic digested fed with manure and agro-waste.

Materials and Methods

Description of Substrates and Inocula

The substrates of the experimental dataset (Fig. 1), whose characterisation is reported in Table 1, are by-products or waste of the agro-industrial sector as well as municipal waste:

- Orange peel waste (OPW), the residue of juice production;
- anchovy sludge (AS), the by-product of fish after oil extraction;
- *Opuntia Ficus-indica* (OFI), a cactus commonly known as "prickly pear" [17];
- Mater-Bi, compostable bags [38].

In more detail, the OPW is the most abundant residue from orange industry, and consists of seeds (0–9%), peels (60–75%) and membrane residues (23–33%) [39, 40]. OPW has a noticeable water content (> 80%) and a very low pH (3–5), and contains essential oil, mainly composed of D-limonene, in variable concentrations [41, 42]; the latter is toxic for the AD biomass [43]. However, OPW use as a substrate for AD is one of the most promising valorisation patterns [39, 44]. In these BMP tests, OPW was digested as a raw (fresh) substrate or ensiled under three conditions [45]: (i) natural ensiling, (ii) wet ensiling (water, 20% w/w, added to OPW); (iii) dry ensiling (OPW placed over a drainage system made of quartz gravel). In one test, after ensiling, OPW was chemically treated by ethanol addition and then centrifuged, or simply centrifuged without previous ethanol addition [45]. The duration of the ensiling process was also analysed: different samples of OPW were ensiled for increasingly longer periods (0, 7, 14, 21 and 37 days) and then used as AD substrates [46]. To verify the bacteria adaptation, BMP tests on ensiled OPW were carried out by modifying the substrate to inoculum ratio (0.3 and 1, respectively) [47].

Anchovy sludge derived from fish oil extraction by novel process [48] was digested alone or with a co-substrate mimicking fruit and vegetable market waste (MW) [49], composed of 49.0% (w/w) of potatoes, 44.4% of apples, and 6.6% of carrots [48].

OFI is a biomass that is potentially worth to be used as substrate in AD, since it is an excellent source of lignocellulosic substrate, with a yield of 10–50 Mg of dry mass per year and ha [17]. OFI was co-digested alone or with

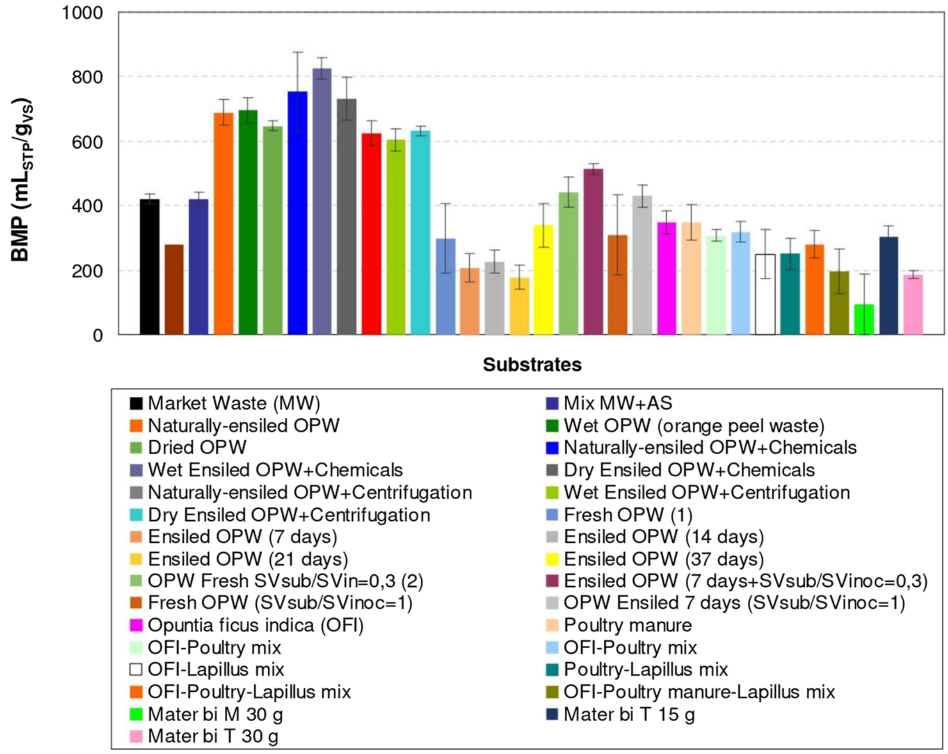


Fig. 1 Specific biochemical methane potential (BMP) values of different substrates in the experimental database. *sub* substrate, *inoc* inoculum)

poultry manure and lapillus (unpublished data). Lapillus is an unconsolidated volcanic fragment, which consists of fresh magma, solid magma from prior eruption or basement rocks passed by the eruption.

Compostable bags made of Mater Bi® are designed to deliver organic residues to household waste collection systems. The bags used in the BMP tests were made up of starch and its derivatives (over 60%), and a synthetic resin that is hydrophilic and biodegradable (for the residual 40%) [38, 50].

The *inoculum* for BMP tests under mesophilic conditions was a liquid digestate collected before each test, from the second stage of a full-scale, two stages, mesophilic (35 °C) anaerobic digester fed with cattle and chicken manure, and several agro-industry residues (e.g., orange peel waste, greenhouse horticulture residues).

Generally, after collection, the *inoculum* was sieved and stored for about a week at 35 °C to reduce the non-specific biogas production. During the thermophilic tests, the *inoculum* from the same full-scale plant was subjected to the same pre-treatment, but it was progressively adapted to thermophilic conditions by increasing temperature from 35 to 55 °C [38].

BMP Tests

The same method, as described below, was followed for the BMP tests on the substrates of the experimental dataset. The tests were carried out in triplicate in 1-L glass reactors by mixing a proper amount of inoculum and substrate. The nutrient solutions were routinely added following the procedures set by the Italian norm on BMP tests (UNI 1601755—Method for the assessment of potential production of methane from anaerobic digestion in wet conditions [52]) during the tests carried out since the release of the same norm (Table 1). The norm requires the use of three different nutrient solutions defined as Solution A, B and C respectively. Solution A contains specified quantities of KH_2PO_4, $Na_2HPO_4 \cdot 12H_2O$, NH_4Cl, distilled water while the amount to be used is 5% of the final volume of the mixture subjected to BMP test. Solution B contains $CaCl_2 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$, distilled water and the amount to be used is 5% of the final volume. Solution C contains $MnCl_2 \cdot 4H_2O$, H_3BO_3, $ZnCl_2$, $CuCl_2$, $Na_2MoO_4 \cdot 2H_2O$, $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, Na_2SeO_3, distilled water and the amount to be used is 1% of the final volume of the blend. Blanks (reactors containing only inoculum) were used to evaluate the endogenous methane production (that is, the production due to the digestion of the inoculum). The biogas was periodically measured (on average three times a week) and its methane content was estimated by the fluid displacement method [49, 53, 54]. For this estimation, a three-neck bottle with an alkaline solution (3 M NaOH) was used, and this allows the precipitation of the carbon dioxide in the biogas. From one neck of the bottle, the biogas was transferred to the solution of sodium hydroxide; the pressure in

Table 1 Characterisation of substrates and BMP test duration

Substrate	(Pre)treatment	Condition	pH	TS (%)	VS (%)	F/M ratio (gVS_{sub}/gVS_{in})	Use of nutrient solutions	Test duration (d)	BMP (mL_{STP}/g_{VS})	References
OPW	Natural ensiling	MP	n.a.	11.1	93.8	0.3	Yes	27	688	[45]
	Wet ensiling			9.2	94.7	0.3			696	[45]
	Dry ensiling			13.1	94.7	0.3			647	
	Natural ensiling + centrifugation			13.9	95.1	0.3			625	
	Wet ensiling + centrifugation			12.7	95.1	0.3			604	
	Dry ensiling + centrifugation			14.6	95.4	0.3			632	
	Natural ensiling + chemical addition			14.2	95.1	0.3			753	
	Wet ensiling + chemical addition			13.1	95.7	0.3			826	
	Dry ensiling + chemical addition			14.7	95.3	0.3			732	
AS	Anchovy sludge	MP	6.85	20.1	66.7	0.3	Yes	24	278	[51]
MW	Market waste		5.26	19.4	93.3	0.3	Yes		421	[49]
AS + MW mix	(95% MW and 5% AS)		8.10	19.4	92.0	0.3	Yes		420	
OPW	Fresh	MP	3.50	17.0	96.8	0.31	No	31	297	[46]
	Ensiling (7 days)		3.24	16.8	96.5	0.34	No		207	[47]
	Ensiling (14 days)		3.14	14.1	95.9	0.33	No		228	
	Ensiling (21 days)		3.10	15.2	96.2	0.32	No		178	
	Ensiling (37 days)		3.06	13.3	95.9	0.33	No		338	
Mater Bi	Sub = 30 gr	MP	n.a.	97.7	99.5	0.5	Yes	30	95	[38]
		TP	n.a.	97.7	99.5	0.5	Yes		186	
OFI	Raw	MP	4.77	5.69	80.6	0.13	No		348	U.D
Poultry manure	Raw		4.66	8.29	80.6	0.18	No		348	
OFI-Poultry manure mix	60% OFI and 40% poultry manure		n.a.	n.a.	n.a.	0.22	No		308	
	80% OFI and 20% poultry manure					0.22	No		318	
OFI—Lapillus mix	OFI—Lapillus					0.13	No		250	
Poultry Manure—Lapillus mix	Poultry manure—Lapillus					0.18	No		251	
OFI—Poultry Manure—Lapillus	60% OFI—40% Poultry manure-Lapillus					0.32	No		280	
OFI—Poultry Manure—Lapillus mix	80% OFI—20% Poultry manure-Lapillus					0.35	No		196	
OPW	Fresh + VSsub/VSin = 0,3	MP	3.4	17.4	96.8	0.3	No	31	443	[47]
	Ensiling (7 days) + VSsub/VSin = 0,3		3.3	17.0	96.7	0.3	No		514	
	Fresh + VSsub/VSin = 1		3.4	17.4	96.8	1	No		310	
	Ensiling (7 days) + VSsub/SVinoc = 1		3,3	17.0	96.7	1	No		430	

F/M ratio Food to Microorganisms ratio, *n.a.* not available, *MP* mesophilic, *TM* thermophilic, *TS* total solids; *VS* volatile solids, sub substrate, *in* inoculum, *OPW* orange peel waste, *OFI Opuntia Ficus-indica*, *AS* anchovy sludge, *MW* market waste, *U.D.* unpublished data the bottle a corresponding volume of the alkaline solution displaced through the second neck into a graduated cylinder. Hypothesising that the biogas is only composed of methane and carbon dioxide, the methane volume of biogas can be considered equal to the volume of the alkaline solution displaced in the cylinder (as the CO_2 was trapped in the solution). The test ended when the methane production throughout three consecutive days was less than 1% of the cumulated volume. The endogenous methane production was subtracted to the production of the blend of inoculum and substrate (which estimates the "net" methane production), and then normalised to standard pressure and temperature conditions (1 bar and 0 °C, respectively). Finally, the cumulative methane production was referred to the weight of substrate added (in terms of VS), to obtain the specific production (hereafter simply indicated as "BMP" and measured in L or its submultiple under standard temperature and pressure, STP).

Detailed BMP tests settings (such as temperature or food to microorganisms' ratio) as well as possible pre-treatment types and conditions, are explained in the related reference (Table 1).

Modelling

Three well known models [55] (e.g., the first-order kinetics, the modified Gompertz equation and the logistic model) have been used for the purposes of this research. Hereafter, the first-order kinetics model will be simply indicated as "first order" model. The three models are commonly used to estimate the kinetic constants of the AD process and predict the specific cumulative methane production from BMP tests. Their analytical expression is reported in Eqs. (1), (2), and (3), respectively:

$$B = B_0 \times [1 - exp(-k \times t)] \tag{1}$$

$$B = P \times exp\{-exp[\frac{Rme}{P}(\lambda - t) + 1]\} \tag{2}$$

$$B = \frac{B_0}{1 + b \times (-k \times t)} \tag{3}$$

where:

B_0 (or P), B ($mL_{STP}\ g_{VS}^{-1}$) = asymptotic and daily cumulative methane yields, respectively
k (d^{-1}) = kinetic constant
Rm ($mL_{STP} \cdot g_{VS}^{-1} \cdot d^{-1}$) = maximum methane production rate
λ (d) = duration of the lag phase
b = numeric constant of the model (3)
t (d) = hydraulic retention time.

P, Rm, λ in Eq. (2), k and B_0 in Eq. (1) and B_0, b and k in Eq. (3) were determined through the Excel tool "Solver" by minimizing the sum of square errors between the model and the experimental mean values. In our study, B is assumed as the model prediction of the observed BMP.

In order to estimate the influence of test duration on the model's prediction accuracy, the BMP was estimated, using the data related to the first 5, 10, 14 and 21 days of the BMP tests, respectively; this value will be indicated hereafter as "BMP at i-th day". The final BMP value calculated using a given model and all the available data (that is, for the total duration) was used as reference value ("control"), in order to evaluate the model performance.

To compare the model's accuracy using two or three replicates, four combinations of replicates for the test with three original replicates (indicated as R1, R2 and R3) were prepared (R1-R2, R2-R3 and R1-R3) and the BMP was estimated by each model, using the data related to each couple of replicates or three replicates (R1-R2-R3) as reference.

Statistical Analysis

Preliminarily, all the final BMP values estimated by modelling that were higher than the stoichiometric methane production for lipids (equal to 1014 $mL_{STP}\ gVS^{-1}$) were removed from the experimental dataset. In fact, this yield is the maximum value that is theoretically possible for a given substrate [20]. Then, a one-way ANOVA was used to identify significant differences ($p < 0.05$) among the three models (Gompertz, first order and logistic) and between the mean BMP predicted by each model and the corresponding reference BMP (control, calculated by the same model but using all the data available).

Finally, two-way ANOVA was applied to evaluate the statistical significance (at $p < 0.05$) of the differences in the BMP (considered as dependent variables) among the number of replicates and test duration (independent factors) as well as their interaction.

For both tests, which were applied to each prediction model, the data were subjected to normality test or square root-transformed whenever necessary, in order to satisfy the assumptions of the statistical tests (equality of variance and normal distribution).

All the statistical tests were carried out by the XLSTAT software (release 2019).

Results and Discussions

Comparing the performance of the models with reference to the number of replicates, the Gompertz model gave the largest difference (34.3 ± 46.0%) between the mean predicted BMP compared to the control using two replicates,

Fig. 2 Comparison of mean modelled BMP values among the number of replicates (**a**) and the test duration (**b**) for the three models. Different letters indicate significant differences either in the number of replicates or the test duration among the three models after Tukey's test ($p < 0.05$)

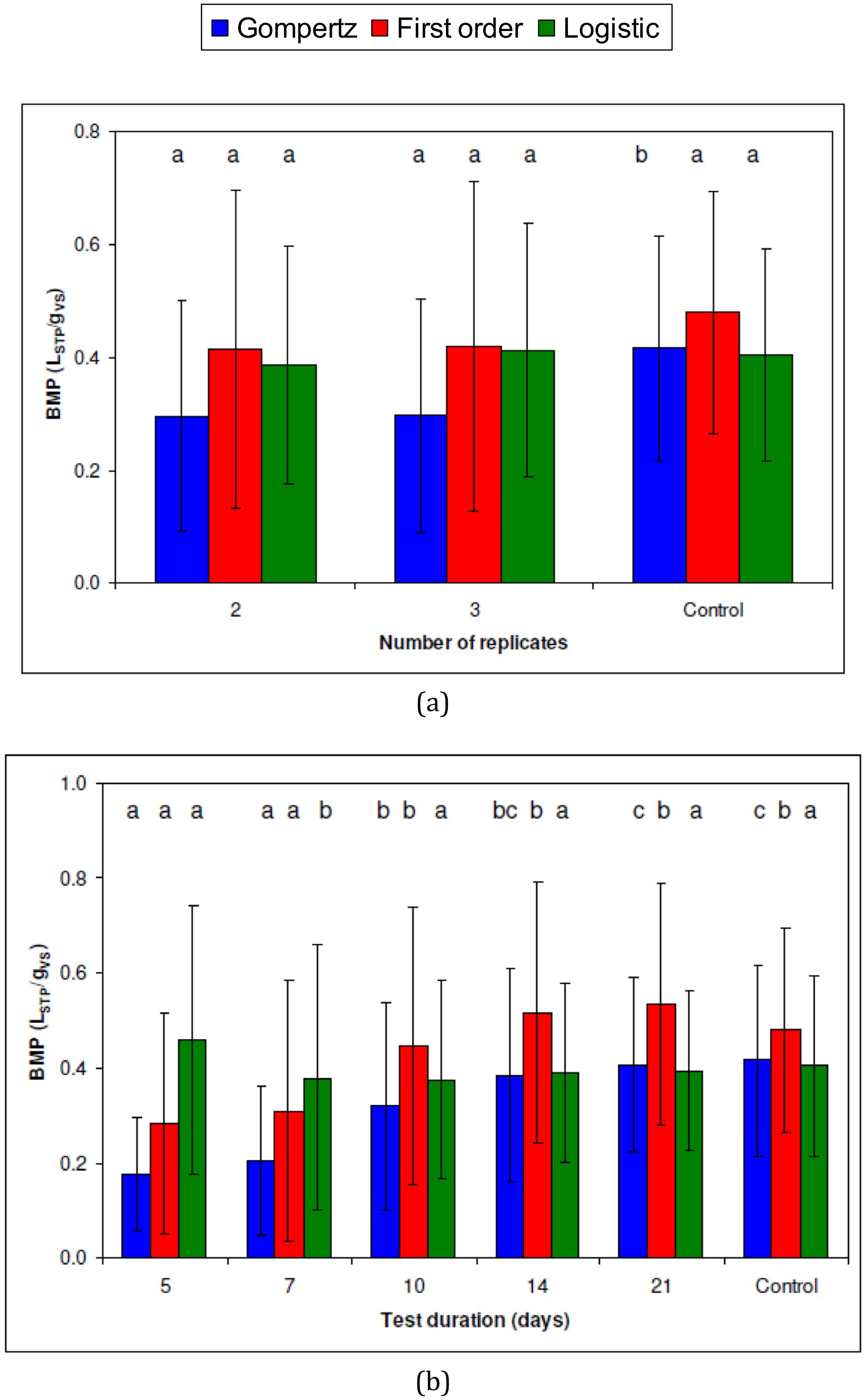


Table 2 Absolute percent variation (mean ± std. dev.) of the BMP value predicted by each model compared to the reference value (n = 32 BMP tests)

Model	Test duration (days)*					Number of replicates**	
	5	7	10	14	21	2	3
Gompertz	49.8 ± 21.7	46.3 ± 31.3	34.0 ± 56.0	28.2 ± 61.5	9.6 ± 19.6	34.3 ± 46.0	31.4 ± 40.2
First order	40.9 ± 60.7	38.8 ± 66.5	37.7 ± 109.5	24.7 ± 71.6	20.1 ± 48.1	32.3 ± 57.7	32.9 ± 74.1
Logistic	33.3 ± 243.0	62.9 ± 170.7	43.1 ± 133.8	22.9 ± 48.3	9.4 ± 23.4	31.5 ± 85.0	42.7 ± 118.3

*For all replicates; ** for all test durations

and this difference decreased to 31.4 ± 40.2% in the tests with three replicates. The lowest difference between the predicted BMP and the control were found for the logistic model (31.5 ± 85.0%) in the tests with two replicates, while, surprisingly, the same model gave the highest error (42.7 ± 118.3%) in the tests with three replicates (Fig. 2a and Table 2).

It is worth mentioning that, for the first order and logistic models, the variability is significantly larger than the standard deviation of the control.

Figures 3 report examples of comparisons between the predicted BMP (using 2 or 3 replicates, and different test duration) and the control values for a selection of five substrates. As previously reported, outliers obtained by applying the modelled tests at different time were excluded from the study. A high number of outliers (over 74% of the available data) were observed applying the first order model to the substrate poultry manure in the first 2 weeks of the experiment (Fig. 3), indicating a low reliability of the predictive model at the first stage of the test for some substrate.

The two-way ANOVA shows that the test duration significantly influences the estimations of the BMP values predicted by the Gompertz and first order models, while this factor is not significant for the logistic model (Fig. 2b and Table 3).

More specifically, the accuracy of the BMP estimations on average increases with the test duration for the Gompertz and first order models. This is shown by the monotonic decrease in the differences among the durations (from 49.85 ± 21.7% and 40.9 ± 60.7% with test duration of 5 days down to 9.6 ± 19.6% for BMP at the 21-th day, respectively). Moreover, for the first order and logistic models, the BMP estimations are more reliable with the increase in the test duration, although the trend is not always monotonic. The tests with durations of seven and 10 days are two exceptions for the logistic model, since the errors in the estimation are higher compared to a shorter test (Fig. 2b and Table 2).

It is worth to notice that tests with duration of 14 days give estimations of the mean BMP values with mean errors close to 25% and never higher than 30% (28.2 ± 61.5% for the Gompertz model, 24.7 ± 71.6% for the first order, and 22.9 ± 48.3% for the logistic), and this error decreases to only 9.6 ± 19.6% and 9.4 ± 23.4% for the Gompertz and logistic models, respectively. In contrast, the error in the BMP at the 21-st day using the first order model is 20.1 ± 48.1% (Fig. 2b and Table 2). Moreover, the standard deviation of the BMP values at the 14-th and 21-st day for the first order model is noticeably higher compared to the other models. However, these BMP values are statistically similar as the control.

The scatterplots (Figs. 1SM, 2SM, 3SM and 4SM) clearly demonstrate how the accuracy of the Gompertz model based on a test duration of 21 days is very close to the control.

Table 4 clearly shows that the number of outliers (i.e., predicted values higher than 1.014 L_{STP} gVS^{-1}) is minimum for the Gompertz model; for this model only 10–20% of the estimated values must be discarded.

The fact that, for the logistic model based on a test duration of 5 days, about 75% of the predictions was not reliable reduces its apparent accuracy for the lowest duration. The number of outliers produced by the Gompertz and logistic models based on durations of 14 and 21 days is very similar and even higher compared to the first order model.

Overall, the evaluation of the accuracy and potential usage of the BMP predictions using tests with shorter duration and/or a minimum number of replicates must consider the intrinsic difficulty of BMP tests and their uncertainty, linked to the complexity of AD biochemistry. This complexity is fully acknowledged by the regulations on the BMP tests in view of their practical applications. For example, the Italian Norm (UNI 1601755—Method for the assessment of potential production of methane from anaerobic digestion in wet conditions) tolerates variations of ± 25% in the experimental value of the BMP for microcrystalline cellulose; the latter is a very simple and biodegradable substrate, which is used as a feasible control for the whole procedure of a BMP test. This means that BMP estimates with errors close to this tolerance can be generally accepted.

Conclusions

The accuracy of three models (“first order kinetic”, “logistic” and “Gompertz”) in predicting the BMP values has been verified, calibrating the model parameters with the data collected in BMP tests (i.e., the first 5, 7, 10, 14 and 21 days) that are shorter than usually done (28 days or

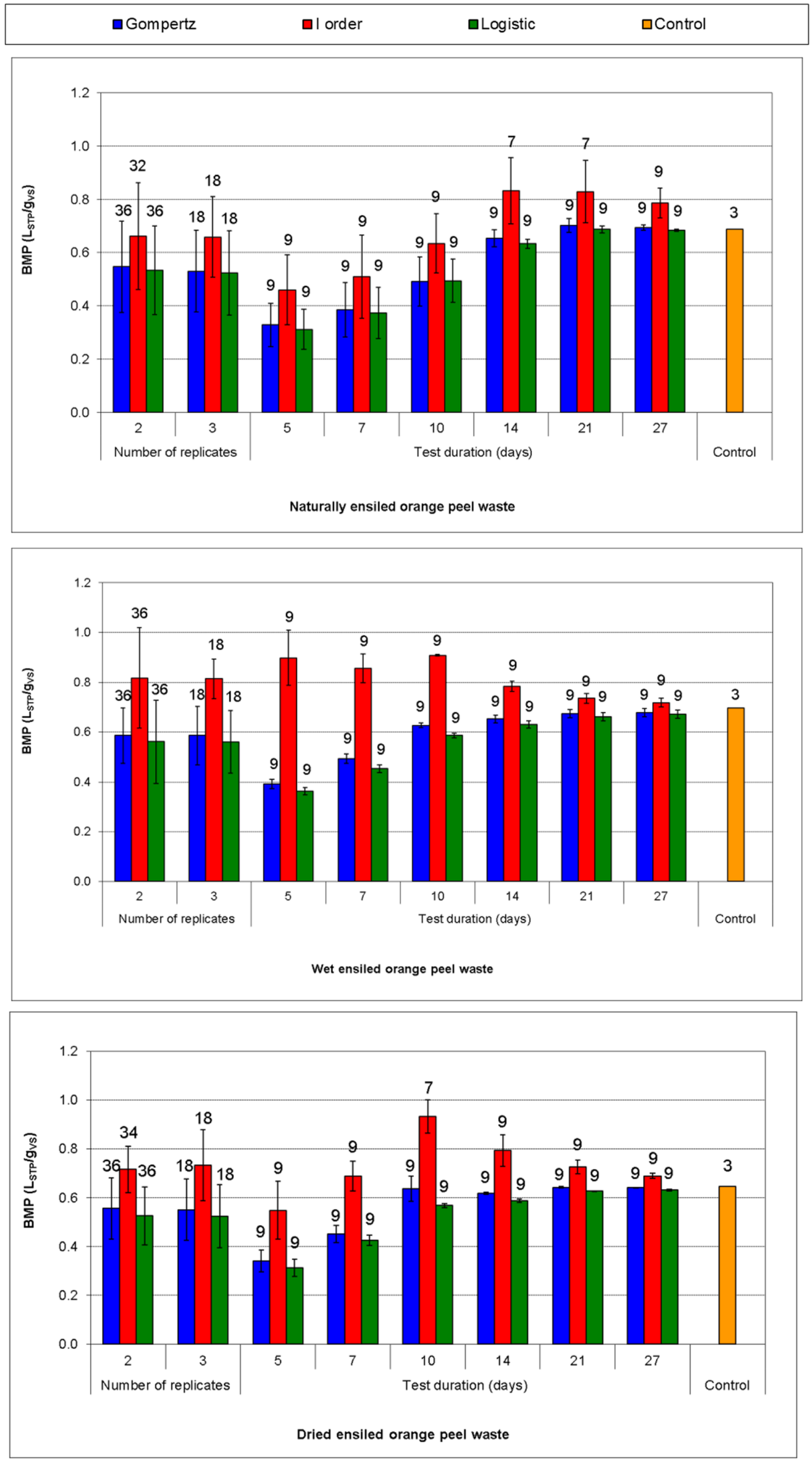
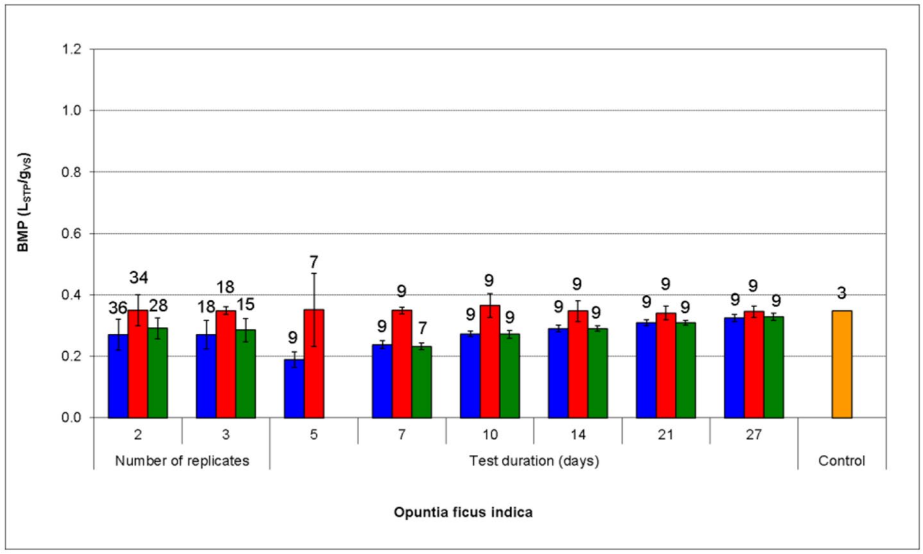


Fig. 3 Comparison of mean modelled BMP values of selected substrates (naturally, wet and dried ensiled orange peel waste; *Opuntia ficus* indica; poultry manure) among the number of replicates and the test duration for the three models. The numbers on the bars are the data processed for each BMP test modelling

Fig. 3 (continued)



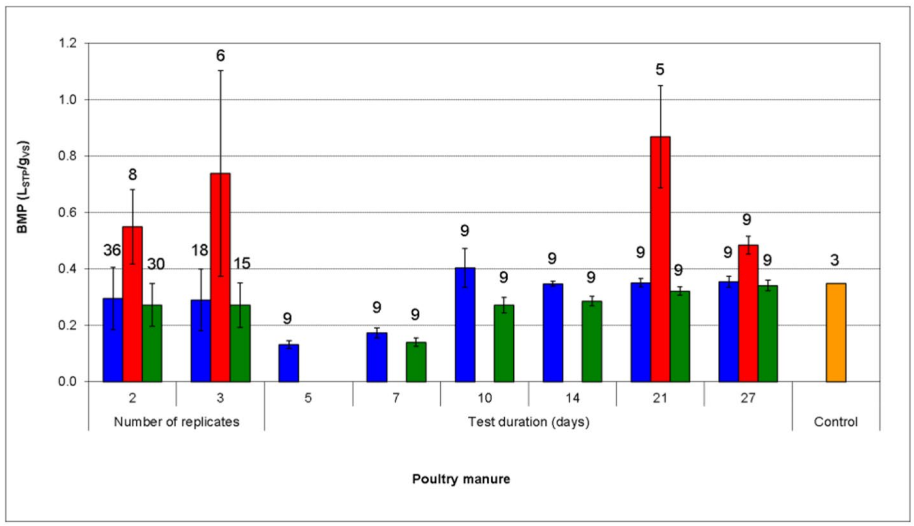


Table 3 Results of two-way ANOVA applied to the BMP values calculated using three prediction models (Gompertz, first order and logistic)

Factors	Degrees of freedom	Sum of squares	Mean squares	F	Pr > F
Gompertz					
Number of replicates	2	0.411	0.205	4.892	**0.008**
Test duration (days)	5	4.909	0.982	29.046	**< 0.0001**
First order					
Number of replicates	2	0.111	0.055	0.703	0.496
Test duration (days)	5	4.126	0.825	11.965	**< 0.0001**
Logistic					
Number of replicates	2	0.051	0.026	0.558	0.573
Test duration (days)	5	0.160	0.032	0.704	0.620

Values in bold are significant at a p-level < 0.05

Table 4 Outliers in the predictions by the models (for the three sets of two replicates the mean number is given)

Duration (days)	Model					
	Gompertz		First order		Logistic	
	2 Replicates (mean)	3 Replicates	2 Replicates (mean)	3 Replicates	2 Replicates (mean)	3 Replicates
5	3.33	3	9.00	10	23.33	23
7	3.33	5	8.33	9	14.33	12
10	6.33	5	11.67	10	10.33	11
14	5.33	6	12.00	11	6.00	6
21	3.67	5	8.00	9	3.67	4

more). Moreover, the influence of the number of replicates (i.e., two or three) on the model prediction accuracy was also evaluated.

The test duration significantly influences the prediction accuracy of Gompertz and first order models. In contrast, the number of replicates is only significant for the Gompertz model. Performing BMP in triplicates is however highly advisable especially when the failure of BMP tests is possible (e.g., acidification or presence of inhibiting agents).

The BMP model predictions based on tests with the shorter durations (less than 10 days) are not accurate, since the errors are generally higher than 30%. A dataset of 14 days reduces these errors to 20–25% for the first order and logistic models, and below 30% for Gompertz model.

Overall, this study has demonstrated that two of the evaluated models (i.e., first order and logistic models) provide BMP estimations with differences lower than 25% and a limited numbers of outliers compared to full-length tests, using as model's input parameters the data collected in tests of limited duration (i.e., more than 14 days) compared to the commonly adopted time (28 days or even more). The increase of this test duration to 21 days gives errors in BMP values below 10% (for the Gompertz and logistic models) and a further reduction of outliers.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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