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Research Article

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ANAEROBIC CODIGESTION OF SLAUGHTER RESIDUES WITH AGRICULTURAL WASTE OF AMARANTH QUINOA AND WHEAT

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Abstract

The objective of this research is to experimentally evaluate the anaerobic co-digestion of slaughterhouse residues in the city of Guaranda with straw residues from agriculture, such as: amaranth, quinoa and wheat. The study was carried out on a laboratory scale using 311 ml biodigesters under mesophilic conditions of 37 °C. Anaerobic co-digestion resulted in methane yields of 407 ml CH_4/g VS, with a methane content in the biogas of 77% for the mixture of slaughterhouse waste and quinoa (RM-QU (25:75)). The increase in inoculum in the mixtures composed of slaughterhouse residues and quinoa increased the biodegradability between 17 and 22%. However, in the mixtures of slaughterhouse waste and amaranth (RM-AM (0:100)), a further increase in inoculum decreased biodegradability by 5%. To predict and simulate methane production, 5 kinetic models were used: modified Gompertz, logistic equation, transfer, cone and Richards. The cone model was the one that best adjusted the experimental values with those predicted with an R^2 of 0.982 to 0.999 and RMSE of 0.61 to 6.92 ml CH_4/g VS. The calculation of the theoretical yield was carried out by stoichiometry and elemental analysis of the samples. Theoretical yields ranged between 480-564 ml CH_4/g VS for all mixtures of RM with agricultural residues.

Keywords: methane, co-digestion, slaughterhouse waste, agricultural waste, kinetics, biodegradability.

1. Introduction

Efficient management of slaughterhouse waste is one of the most critical problems in developing countries [1]. This means that many wastes not properly treated cause major pollution problems. In the city of Guaranda, Ecuador, the municipal slaughterhouse dumps its waste into the Guaranda River, which causes all agricultural and livestock activities downstream to be significantly affected. In addition, the slaughterhouse does not have a treatment plant to reduce the polluting load of the waste, which means that the discharges have a direct impact on the river. Untreated slaughterhouse waste can create serious problems, due to its high biological oxygen demand (BOD) and chemical oxygen demand (COD) [2]. Hence, there is a prevailing need to reduce the dumping of waste from slaughterhouses and thus avoid contamination from open dumps [3]. On the other hand, the by-products of cattle and pigs that come from the agro-industrial processing of the Guaranda slaughterhouse contain different materials and organic compositions. These materials contain a high energy potential and a high C/N ratio due to their high fat and protein content [4]. However, the accumulation of waste from the Guaranda slaughterhouse has been little used as an energy-generating raw material, especially to produce biogas and methane.

Anaerobic co-digestion can be an alternative to treat slaughterhouse waste (RM), through the production of biogas and methane. This technology enables the transformation of RM into energy, constituting an energy-environmental paradigm in waste management. In addition, due to the large amount of residues from agriculture in the region, the digestion process can be optimized through anaerobic co-digestion between the RM and typical agricultural residues of the area: amaranth straw (AM), straw from quinoa (QU) and wheat straw (TR). Anaerobic co-digestion notably improves methane production increasing the biodegradability of RM, since they generate synergistic effects in the mixtures reducing the bioresistant, recalcitrant and poorly biodegradable effects [5]. In this sense, the co-digestion of more than one substrate can compensate for the deficiencies of mono-digestion [6]. Mixing different substrates can have a high synergistic effect on methane production as the nutrient content can be balanced. In this way, co-digestion contributes to eliminating the influence of toxic compounds in the digestion process, giving a higher yield of biogas from biomass [7,8].

The Guaranda slaughterhouse produces a large amount of organic waste, such as manure, ruminal content, viscera, hair, blood, hooves, wastewater, among others, which are accumulated or eliminated without any treatment, which increases the generation of bad odors, gases and leachates [9]. . All these residues constitute 25% of the total weight of the live animal within the slaughterhouses. Cattle produce in the slaughterhouse 7.5 to 30 kg of manure, mostly semi-liquid, 30 to 35 litres of blood, 66 kg of bones and 40 to 80 kg of stomach contents [10]. In addition, as in other slaughterhouses, the Guaranda slaughterhouse generates large volumes of waste with high organic resistance due to the presence of oils, fats and proteins derived from adipose tissue and blood, as well as the energy consumption associated with refrigeration and water heating [11]. More than 3,667 head of cattle are slaughtered annually, generating a large amount of waste that pollutes the environment.

At present there is a diversity of slaughterhouses, which depends on the type, quantity and variety of animals treated. The Guaranda slaughterhouse processes cattle and pigs. Most of the research in the literature addresses the anaerobic digestion of previously pre-treated RM, in which the contaminant load has been reduced. This makes the waste generated, as raw material in slaughterhouses, diverse and depends on the type of slaughterhouse to be treated. In this sense, this research addresses the anaerobic co-digestion of mixed RM not pre-treated with agricultural residues of AM, QU and TR. Furthermore, the effect of inoculum (sewage sludge) on methane yield is evaluated. The research process was carried out under mesophilic conditions and on a laboratory scale.

2. Materials and methods

2.1 Substrates, co-substrates and inoculum used.

RM and residues of lignocellulosic materials

Four materials were used for the biochemical methane potential (BMP) experiments: RM was used as the main substrate, the same materials that were collected from the Guaranda municipal slaughterhouse; and straw residues of AM, QU and TR were used as co-substrates, all residues were collected in the province of Bolívar (Ecuador). Once the samples were collected, they were stored at 4 °C in polyethylene bags, for conservation purposes. Once the co-substrates were harvested, they were subjected to mechanical pre-treatment using a universal cutter mill to reduce the size of the straw. Once the residues were crushed, they were sieved, to obtain a homogeneity of the samples, and at the same time obtain a particle size of less than 3 mm. The inoculum (anaerobic biomass) was obtained from the anaerobic digester of the municipal WWTP of Ibarra (Ecuador).

Characterization of substrates, co-substrates and inoculum.

The total solids (TS) and the volatile solids (VS) of the waste were measured in triplicate according to the UNE-EN 18134 and UNE-EN ISO 18123 standards. While the TS and VS content of the inoculum was determined in accordance with American Public Health Association methods 2540A-2540G [12]. A portable digital multimeter potentiometer (HACH HQ 40D) was used to determine the pH of the biodigester samples. Elemental analysis (C, H, N, O and S) was performed using a VARIO MACRO CUBE elemental analyser.

2.2 Theoretical methane production

Theoretical methane production is limited by stoichiometry, which means that it can be determined from the elemental composition of the different substrates and co-substrates [13]. In this sense, according to stoichiometry and elemental analysis, the theoretical methane potential (γ_{teo}) can be determined according to **Equations 1** and **2** proposed by Buswell and Boyle [14-16].

$$C_aH_bO_cN_d + \left(\frac{4a - b - 2c + 3d + 2e}{4}\right)H_2O \rightarrow \left(\frac{4a + b - 2c - 3d - 2c}{8}\right)CH_4 + \left(\frac{4a + b + 2c + 3d + 2e}{8}\right)CO_2 + dNH_3 + eH_2S \quad \text{Eq. 1}$$

$$\gamma_{teo}\left(\frac{\text{ml } CH_4}{\text{g VS}}\right) = \frac{22\,400 * (4a + b - 2c - 3d - 2e)}{(12a + b + 16c + 14d + 32e) * 8} \quad \text{Eq. 2}$$

Furthermore, starting from the theoretical chemical oxygen demand (CODt), the methane production (γ_{CODt}) can be determined using **Equation 3** [17,18].

$$\gamma_{CODt}\left(\frac{\text{ml } CH_4}{\text{g VS}}\right) = \frac{n_{CH4}.RT}{P.VS} \quad \text{Eq. 3}$$

where γ_{CODt} is the theoretical production, R is the gas constant (R = 0.082 atm l/mol K), T is the biodigester temperature (298 K), P is the atmospheric pressure (1atm), VS added (g) are the volatile solids of the substrate and n_{CH4} is the amount of molecular methane (mol).

The value of n_{CH4} has been determined from **Equation 4** [19].

$$n_{CH4} = \frac{CODt}{64\left(\frac{g}{mol}\right)} \qquad \text{Ec. 4}$$

The CODt of all substrates and co-substrates was estimated through their elemental composition and the stoichiometry of the oxidation reaction (Eq. 5), using equation (Eq. 6) [15].

$$C_aH_bO_cN_d + \left(\frac{4a + b - 2c - 3d + 2e}{4}\right)O_2 \rightarrow aCO_2\left(\frac{b - 3d}{2}\right)CH_4 + eH_2O + dNH_3 \qquad \text{Eq. 5}$$

$$CODt\left(\frac{ml\ O_4}{g\ VS}\right) = \frac{\left(2a + \frac{b}{2} - c - \frac{3d}{2}\right) * 16}{(12a + b + 16c + 14d)} * 1000 \qquad \text{Eq. 6}$$

2.3 Biodegradability of anaerobic co-digestion

The biodegradability was calculated from the experimental methane yield (γ_{exp}) and the theoretical methane yields (γteo and γ_{COD}), the anaerobic biodegradability (ε) of the substrate could be calculated according to the equation. **Equation 7** which estimates the calculation of biodegradability [20,21].

$$\varepsilon = \frac{\gamma_{(exp)}}{\gamma_{(teo)}}.100\% \qquad \text{Eq. 7}$$

To determine the influence of the substrate and the co-substrates on the biodegradability of the biodigesters, their synergistic and antagonistic effects were estimated. The parameter α allows evaluating the effect of the co-substrate and co-substrates in the mixtures to be co-digest. α was determined according to the experimental yield and the weighted methane yield (**Equation 8**) [17].

$$\alpha = \frac{\gamma_{\mathrm{exp}}}{\gamma_{\mathrm{pond}}} \qquad \text{Eq. 8}$$

Where γ_{exp} refers to the experimental performance obtained by the BMP and γ_{pond} corresponds to the weighted experimental performance.

γ_{pon} is determined by **Equation 9** [22].

$$\gamma_{\mathrm{pond}} = \frac{\gamma_{\mathrm{sp}}.\lambda + \gamma_{\mathrm{cs}}.\beta}{\lambda + \beta} \qquad \text{Eq. 9}$$

Where, γ_{sp} refers to the methane production obtained from the digestion of the main substrate calculated as monosubstrate. On the other hand, γ_{cs} is the production obtained through the singular digestion of the different co-substrates. The values of λ and β correspond to the VS fractions of the main substrates and the co-substrates.

2.4 Experimental setup and procedure

Initial conditions of co-digestion

Nine co-digestion conditions between the RM manure substrate and the AM, QU and TR co-substrates were tested, using different substrate:co-substrate ratios. For both the RM:AM, RM:QU and RM:TR ratios, three volatile solids proportionality ratios were used: 25:75, 50:50 and 75:25. Two substrate/inoculum ratios (SIR) were performed for all experiments: SIR 1:1 (g: g VS) and SIR 1:2 (g: g VS). The C/N ratio was determined based on elemental analysis and varied depending on the amount of VS mixture between the substrate and co-substrate (**Table 1**).

Anaerobic Co-digestion Biochemical Methane Potential (BMP) Assays

BMP experiments were used to determine the influence of co-substrates and inoculum on methane yield during anaerobic co-digestion of RM. All BMP experiments were performed in triplicate, in 311ml glass biodigesters filled with 60% working volume. The proportions of the substrates and co-substrates before being put into the biodigester were mixed with a kitchen blender to ensure that the experimental samples are uniform. Once the co-digestion mixtures had been made, the batch biodigesters were closed with rubber septa and aluminium lids to guarantee anaerobic conditions inside. The experiments were carried out for 40 days and 37 °C. Distilled water was added to obtain a final working volume of 60% of the volume of the biodigesters when necessary. As controls, three blank biodigesters containing only inoculum and distilled water were also incubated under the same conditions as the rest of the biodigesters. The biogas yield from these blank biodigesters was used to correct for the biogas produced solely by the inoculum.

The volume of biogas produced in each biodigester was calculated daily by measuring the pressure in the headspace of each biodigester using a portable pressure gauge (Delta OHM HD 2124.2) (**Figure 1**). The pressure in the head space of the biodigester was measured after the insertion of a syringe needle through the rubber stopper. The composition of the biogas (content of CH_4, O_2, CO_2, H_2S) was measured using the BIOGAS GA-5000 meter from Geotech. In this way, using a 200 ml hermetic syringe, biogas samples were taken from the headspace of each biodigester after releasing the gas. Before measuring the biogas composition in the headspace, the reactors were shaken for two minutes at 100 rev/min. The composition of the biogas was measured once a day until the end of the fermentation.

The maximum methane yield was expressed as the maximum volumetric yield of methane per gram of initial substrate VS added (ml CH_4/g VS). Each trial was performed in triplicate, and the results were obtained as the average of these.

2.5 Experimental modelling of the data to estimate the BMP.

Five kinetic models were selected, that is, the modified Gompertz kinetic model (**Equation (10)**), the transfer model (**Equation (11)**), the logistic function model (**Equation (12)**), the cone model (**Equation (13)**), and the modified Richards model (**Equation (14)**) to fit the cumulative methane production obtained from the experimental data.

The most suitable kinetic model was selected not only to predict the efficiency of the biodigesters used, but also to correctly analyse the metabolic pathways and the mechanisms involved during AD of the co-digestion of slaughterhouse waste with lignocellulosic waste [23]. However, all five kinetic models have individual specific benefits. The cone model is the simplest model and provides information on the degradation of substrates during the hydrolysis phase through the hydrolysis rate coefficient (k; d^{-1}) [24]. The modified Gompertz, logistic, transfer and Richards model are more sophisticated, since they take into account the phenomenon of the latency phase (t_{lag}; d) and the maximum specific methane production rate (ν_{max}) [25]. Therefore, the five kinetic models were used in this study to determine the cumulative biogas production potential, the hydrolysis kinetics, the lag phase duration, and the maximum methane production. All the parameters of the kinetic models were determined by fitting between the experimental and estimated data through the statistical tool STATISTISCA 10. To evaluate the performance of the models, the coefficient of determination (R^2) and the percentage of squared error were used. medium (RMSE; %). These coefficients were calculated to provide additional information on the goodness of fit of the different models.

If the model accurately predicts the kinetic coefficient, R^2 should be close to 1 and the RMSE should be as close to 0.

Modified Gompertz model [26]:

$$M = M_{\mathrm{e}}.exp\left\{-exp\left[\frac{\nu_{\max} * e}{M_{\mathrm{e}}}\left(t_{lag} - \mathrm{t}\right) + 1\right]\right\} \quad \text{Eq. 10}$$

Transfer model [27]:

$$M = M_{\mathrm{e}}\left\{1 - exp\left[-\frac{\nu_{\max}}{M_{\mathrm{e}}}\left(t - \mathrm{t_{lag}}\right)\right]\right\} \quad \text{Eq. 11}$$

Logistics function model [27]:

$$M = \frac{M_{\mathrm{e}}}{1 + exp\left[\frac{4\nu_{\max}\left(t_{lag} - t\right)}{M_{\mathrm{e}}} + 2\right]} \quad \text{Eq. 12}$$

Cone model [28]:

$$\mathrm{M} = \frac{\mathrm{M_e}}{1 + (\mathrm{k.t})^{-\mathrm{n}}} \quad \text{Eq. 13}$$

Modified Richard model [28]:

$$\mathrm{M} = \frac{\mathrm{M_e}}{1 + (\mathrm{k.t})^{-\mathrm{n}}} \quad \text{Eq. 14}$$

Where,

M is the amount of methane (ml/g VS_{added}) with respect to time t (days),

M_e is the maximum methane potential of the substrate (ml/g VS_{added}),

k is the hydrolysis rate constant (d^{-1}),

t is the digestion time (days),

v_{max} is the maximum biogas production rate (ml/g VS_{added} .d),

t_{lag} is the time of the lag phase (days),

e is the Euler function equal to 2.7183.

3. Results

3.1 Characteristics of the raw material

Table 2 shows the characterization of the RM manure, used as the main substrate, and the three lignocellulosic biomasses used as co-substrates. Through this characterization, the great difference between the selected biomasses stands out, mainly due to the different percentages of its components: TS, VS, VS/TS and their C/N ratio. When analysing the MR substrate, it was obtained that the values of TS, VS and VS/TS were 9.6%, 6.8% and 0.70, respectively. However, the RM results were lower than those obtained by Álvarez and Liden [29], who obtained TS of 18.8%, VS of 20% and an VS/TS ratio of 0.94.

On the other hand, the three co-substrates analysed (AM, QU and TR), presented a high content of TS, that is, 88.2; 87.0 and 92.6% respectively. In the same way, they had a high content of VS, that is, 65.9; 50.8 and 71.5% respectively, compared to the RM.

The TR residues were characterized by having the highest values of TS (92.6%), VS (71.5%) and VS/TS (0.77). However, these results were lower than those obtained by Sun et al. [30], who obtained values of TS, VS and VS/TS of 74.1%; 62.9% and 0.84, respectively. For its part, the AM co-substrate presented similar characteristics of VS (88.2%), TS (65.9%) and VS/TS (0.75) to those of TR. Furthermore, the AM results were superior to those obtained by Seppälä et al. [31], who reported TS and VS values of 18.0% and 14.4% respectively; however, they obtained a higher VS/TS ratio (0.80). Finally, the QU co-substrate presented a high value of TS (87.0%) and low values of VS (50.8%) and VS/TS (0.58). Thus, the results of TS, VS and VS/TS of QU, were lower than those obtained by Alvarez & Lidén [29], who obtained values of 95.3%; 91.9% and 0.88, respectively. On the other hand, the results of TS, VS and VS/TS of QU, were superior to those of Pabón [32], who obtained data of TS and VS of 22% and 19% respectively; however, he obtained a higher VS/TS ratio (0.86).

The RM and TR residues were characterized by presenting the highest C/N contents, 101.9 and 29.6 respectively, while the QU (12,9) and AM residues showed a lower and similar C/N ratio. Thus, the high C/N ratio of the RM and TR residues could compensate for the low C/N ratios of the QU and AM residues through the co-digestion process. The mixture of different residues allows an optimal digestion process between the different substrates and co-substrates tested. On the other hand, having a fairly high C/N value as is the case of RM (101,9) does not significantly affect the efficiency of digestion [33], since not all the carbon and nitrogen in the matter raw are available for anaerobic digestion [29]. In this sense, the biodegradable C/N ratios are lower than the total C/N ratios of the substrates and co-substrates [34].

Even though the inoculum (IN) presented a low solids content (3.9% and 2.3% in TS and VS, respectively). The IN values were like those presented by Sun et al. [30], who reported TS, VS and VS/TS of 5.9%; 3.19% and 0.58. Similarly, IN results were comparable to those of Pellera & Gidarakos [15], who reported TS, VS and VS/TS of 2.7%; 1.7% and 0.62, respectively.

3.2 Potential methane production

Daily and cumulative methane production

The daily and cumulative production of biogas from slaughterhouse waste with amaranth, quinoa and wheat straw waste are shown in **Figure 2**. It is observed that the evolution of methane production from slaughterhouse waste is influenced by two factors: the influence of the substrate and inoculum ratio, and the influence of agricultural residues (AM, QU and TR).

Increasing the amount of inoculum from a SIR1:1 to a SIR1:2 increased the daily methane yield in most biodigesters during the first days of anaerobic digestion (AD). For a SIR1:1, the amount of methane, during the first 10 days, was between 46.80% and 68.70% of the total amount of accumulated methane. In contrast, when the inoculum was increased to a SIR1:2, the methane production increased slightly in a range of 46.17-74.58% on day 10. According to Fernández et al. [35], an increase in inoculum can increase the degradation capacity of microbial populations on the organic load, thus avoiding the accumulation of volatile fatty acids (VFA) and the inhibition of methanogenesis; causing methane production to increase. Furthermore, the behaviour of daily production was determined by the type of co-substrate used. The highest peaks of daily methane production were obtained in the mixtures of slaughterhouse waste with quinoa straw. Thus, during day 2, the RM-AM (25:75), RM-QU (50:50) mixtures experienced the highest methane peaks (34.46 ml CH_4/g VS and 41.11 ml CH_4/g VS) for a SIR1:1 and a SIR1:2, respectively.

The highest cumulative methane yields were found in trials using a SIR1:2, especially in the RM and QU mixtures. Thus, the mixtures RM-QU (25:75) and RM-QU (25:75) generated results of 406.86 and 391.45 ml CH_4/g VS, respectively. Similarly, the RM-AM mixture (25:75) generated high amounts of methane (379.38 ml CH_4/g VS). The percentages of improvement in methane production, when increasing the inoculum from a SIR1:1 to a SIR1:2, were 0.6-23%; however, the individual substrate of RM decreased by 5% with increasing inoculum. Co-digestion also enhanced methane production from individual RM substrates. For a SIR1:1 co-digestion increased methane production by 1-14%; and for a SIR1:2 production increased by 0.5-22%.

The results obtained in this study are similar to those of other authors in the literature [36-39], who carried out the co-digestion of RM with various crops (straw and fruit and vegetable waste) and obtained methane productions from 461, 499, 208 and 380 ml CH_4/g VS respectively. Similarly, the RM yields are in the same line with the results obtained by Cuentos et al. [40], who obtained yields of 400 ml CH_4/g VS when they co-digested liquid waste from poultry slaughterhouses and solid urban waste. Furthermore, the RM results obtained are much higher than those obtained by Álvarez y Lidén [29], who reported that the co-digestion of pig slaughterhouse waste with pig manure produces specific methane yields of 260 ml CH_4/g VS. The results obtained were also greater than the results reported by Rosenwinkel and Meyer [41], who obtained 230 ml CH_4/g VS when they co-digested slaughterhouse waste (stomach content of pigs and cows) with sewage sludge. However, the results were somewhat lower than those reported by Luste and Luostarinen [4], who obtained results of 430 ml CH_4/g VS when they worked on the co-digestion of livestock waste (pig slaughterhouse) with sewage sludge.

Synergistic effects of agricultural co-substrates.

Agricultural residues from AM, QU and TR had a significant influence on methane production. The synergistic effects of agricultural residues are reflected in the improvement of the methane yield of the individual mixtures of the RM. It was shown that mixtures with a higher amount of agricultural residues increase methane yield regardless of the type of SIR used. However, the highest productions were obtained when 25% RM and 75% AM, QU and TR residues were used. Thus, for the SIR1:1 the mixtures RM-AM (25:75), RM-QU (25:75) and RM-TR (25:75) generated 363.17; 335.94 and 301.61 CH_4/g VS, respectively. Similarly, for a SIR1:2 the mixtures RM-AM (25:75), RM-QU (25:75) and RM-TR (25:75) generated 379.78; 406.86 and 303.71 CH_4/g VS, respectively (**Figure 3**).

The average methane content of the biogas produced in all the reactors varied between 54.31% and 68.74% for the SIR1:1 and between 54.42% and 76.55% for the SIR1:2. However, the increase in inoculum increased methane production in most of the biodigesters, except in the RM-AM (75:25), RM-AM (50:50) and RM-TR (75:25) mixtures in which decreased by 1.4; 0.46 and 0.54%. The percentages of methane obtained in this study were very similar to those reported by other authors in the literature. Thus, for example, Borowski [42] found methane content in biogas between 55% and 60% for the monodigestion of municipal solid waste and between 58% and 66% for the co-digestion of municipal solid waste and sewage sludge. Regarding fruit and vegetable residues, Bouallagui et al. [43] reported a methane content in biogas of 64%, while Scano et al. [44] reported average methane content of 75%. Lin et al. [45] reported percentages of methane between 53.7% and 63.8% on the co-digestion of fruit and vegetable residues, and food waste.

In addition, **Figure 3** shows the biodegradability (ε_{teo} and ε_{COD}) for all the mixtures used. The results ranged from 46-73% for the SIR1:1 and between 56 and 77% for the SIR1:2. Thus, an increase in the amount of inoculum increased the biodegradability in a range of 0.20-18%. The data showed considerable concordance between ε_{teo} and ε_{COD}, showing that the theoretical methane production values obtained by Buswell's stoichiometric method (γt_{eo}) and elemental analysis of CODt (ε_{COD}) were similar (**Figure 4**).

Biodegradability values were correlated with experimental methane production. This agreement resulted in a coefficient of determination greater than 95% being obtained for both the SIR1:1 and the SIR1:2.

3.3 Kinetic study of the anaerobic digestion of slaughterhouse waste

The modified Gompertz, transfer, logistic equation, cone and Richards models were evaluated in all biodigesters in the SIR 1:1 and SIR 1:2 assays. The kinetic parameters (maximum specific methane production rate (ν_{max}), rate constant (k), lag phase time ($t l_{ag}$) and specific maximum methane production (M_e)), as well as the statistical parameters (coefficient of determination (R^2) and mean square error (RMSE)) are shown in **Table 3** and **Table 4**.

Maximum specified rate of methane production

The νmax values were maximum in the SIR 1:2, specifically in the mixtures RM-AM (0:100) both for the Gompertz model (21.19 ml CH_4/g VS d), logistic equation (31.34 ml CH_4/g VS d) and blot pattern (41.23 ml CH_4/g VS d). While Richard's model had maximums of 43.75 and 33.05 ml CH_4/g VS d in the RM-QU (25:75) and RM-AM (25:75) mixtures, respectively. In general, the results showed that ν_{max} is more homogeneous in the modified Gompertz sigmoidal models and in the logistic equation. However, in the Richards model, νmax was not highly correlated with the transfer model and the two previous sigmoidal models. This is because the Richards equation is generally flawed due to its inconsistent properties [46]. This means that the behaviour of the Richards equation is exponential in small ranges or low densities. In this way, the parameters of different curves fitted using the Richards growth model are not necessarily equivalent.

Specific Maximum Methane Production

The results of the asymptote M_e of the sigmoidal models were not like each other. The fact that M_e is not fully correlated with all kinetic models is because M_e differed from experimentally obtained methane production. The predicted and observed values of the sigmoidal models registered differences of 0.25-19.48% (modified Gompertz), 0.32-18.22% (logistic equation), 0.85% and 12.69% (model of transfer), cone model (20.06-36.97%) and 0.40-19.42% (Richards). However, the mean differences obtained between the experimental performance and M_e were like those obtained by Ware and Power [47], who obtained differences for poultry slaughterhouse residues of 0.54 and 27.07%. On the other hand, the differences between the experimental performance and M_e of this study were higher than those of Patil et al. [48] who obtained 8.7% results when predicting the water hyacinth yield. Similarly, the results of this study were superior to the results of Raposo et al. [49] who reported differences of 10% when predicting the yield of the sunflower oil cake when using first-order kinetic models.

Delay phase time

Regarding the latency period (t_{lag}), the RM co-digestion recorded null latency periods for all models, except for the transfer model, which presented delay phases of 1.16 and 0.77d for the trials RM-AM (0:100) and RM-TR (25:75), respectively. The fact that there are zero latency phases means that the biodegradability of the raw materials is very high and there is little presence of inhibitors [50]. Furthermore, according to Kafle et al. [51] the low duration of the lag phase in the digestion processes can be attributed to a low content of proteins and fats in the substrates.

First order constant

The hydrolysis constant (k) was much higher as the amount of inoculum in the mixtures increased. Thus, in the SIR1:1, k varied between 0.05-0.14 d^{-1}, while in the SIR1:2, k varied between 0.06-0.18 d^{-1}. Furthermore, the constant k increased for biodigesters composed of RM-QU and decreased for biodigesters composed of RM-TR. The results of this study were inferior to other studies in the literature. So, for example, Song and Clarke. [52] found k of 0.45 d^{-1} for cellulose in a mixed culture enriched with landfill waste. Hu and Yu [53] used ruminal microorganisms to improve the anaerobic digestion of the corn cob and estimated that k was 0.94 d^{-1}. On the other hand, in studies on the co-digestion of microalgae biomass with sludge, values of k between 0.25–0.28 d^{-1} have been obtained [54]. Similarly, in microalgae mono-digestion tests, k values of 0.07 d^{-1} have been obtained [55].

3. Discussion

In this research, the daily methane production remained constant during the first three days, subsequently it decreased continuously and remained at very low levels. The early onset of microbial activity caused the mixtures to generate more than 70% methane during the first 10 days. According to Zhang et al. [56] consider that around 80% of the methane can be obtained during the first ten days of digestion. Furthermore, many authors in the literature suggest that some of the BMP trials require short treatment periods [57]. A possible reason why a high generation of methane has been obtained during the first days is because the inoculum and the methanogenic microorganisms immediately acclimatized to the mixtures used in the tests [58,59]. The methane accumulation curves also reflected a rapid adaptation of the microorganisms, since it caused very small and even zero lag periods (tlag) to be shown. In general, the accumulation curves showed a rapid exponential growth during the start of digestion. According to Remigi & Buckley [60], the rapid growth of the methane accumulation curves is due to three factors: use of easily biodegradable materials, immediate production of methane when starting the AD process, and the presence of a stationary phase as the biodegradable material is depleted.

The use of straw residues from amaranth, quinoa and wheat increased methane production from slaughterhouse residues. According to Vivekanand et al. [61] a mixture has a synergistic effect if more methane is produced relative to an estimate based on methane yields from single substrate digestions. In this case, the simultaneous presence of RMs with various co-substrates (AM, QU and TR) improved the co-digestion process, due to the synergistic interactions of the mixtures [62]. In this way, a mixture of different substrate fractions with different characteristics can provide all the nutrients and trace elements that microorganisms need [37] This fact is justified, since the catalytic centers of the enzymes involved in the methanogenic pathways depend to a great extent on the micronutrients [63]. In addition, the synergistic effects of mixtures can contribute trace elements, nutrients, enzymes, or any other amendment that a substrate alone may lack [64]. In short, the mixture of many heterogeneous substrates increases the activity of microorganisms and, therefore, stimulates AD. In this study, the most relevant findings were the following: a higher concentration of SV of the co-substrates (AM, QU and TR) in the mixtures caused the production of methane to increase up to 22% in the individual mixtures of the RM; in addition, the co-digestion of the RM-QU and RM-AM mixtures generated the highest methane productions regardless of their SIR, and finally, the concentrations of 50-75% of AM and QU were optimal to improve methane production. In the characterization of the raw materials, the VS of the slaughterhouse RM were 6.8 while the VS of the straw waste of AM, QU and TR were higher with 66%, 51% and 72% respectively. In this case, the use of agricultural residues helped to balance the physicochemical properties of the RM by improving the biodegradability of the VS of the mixtures [65-67]. In this way, the addition of agricultural residues provided a better substrate for methanogenic bacteria, causing them to accelerate the fermentation process and increase methane production [68,69]

For a SIR1:2, the co-digestion of the RM-QU and RM-AM mixtures generated the highest amount of methane with ranges of 378-407 and 320-380 ml/g VS, respectively. However, the RM-QU (25:75) mixtures generated 7% more than the RM-AM (25:75) mixtures. Similarly, the RM-QU (50:50) mixtures generated 13% more than the RM-AM (50:50) mixtures. These results were very similar to other studies in the scientific literature. Thus, in the co-digestion of urban solid waste, Mojapelo et al. [70] and Kubaska et al. [71] reported 386 ml/g VS and 385 ml/g VS, respectively. Salminen et al. [72], by fermenting solid waste from poultry slaughterhouses, they obtained 550 to 670 ml/g VS. Li, et al. [73], presented yields of 300 ml/g VS for the AD of lignocellulosic biomass of agricultural residues. Similarly, Mussgnug et al. [74], reported methane productions for the anaerobic digestion of 6 different microalgae between 218 and 387 ml/g VS. Although the reported results were comparable with other previous studies, the methane yields were of medium production. According to Velázquez et al. [75] digestion processes can be classified into three groups according to methane production potential: low production processes (150 and 300 ml/g VS), medium production processes (300 and 450 ml/g VS) and processes high production (more than 450 ml/g VS).

According to Raposo et al. [76] the experimental methane yield can be used to calculate the level of anaerobic biodegradability under the defined test conditions compared to its theoretical value. In this study, theoretical calculations provided a rough first estimate of methane production. However, it was found that the theoretical yield was much higher than the experimental one. According to Herrmann & Rath [77], the theoretical estimates are usually much higher than the experimental yield because in the theoretical analysis all biomass is biodegradable. On the other hand, in obtaining experimental methane, the suitability of fermentation decreases with the lignification of the substrate, since lignin is not degraded in the fermenter and makes the degradation of other components of the cell wall difficult [78]. Furthermore, in experimental trials there is a wide variety of substances that can inhibit anaerobic processes [79]. In short, the conversion of organic substances into methane, in the experimental tests, is lower than in the theoretical estimates since the ideal conditions cannot be met [80]. The tests of this research showed that the data for obtaining biodegradability are adequate, since the results of biodegradability and experimental performance showed a concordance of more than 95% in their coefficient of determination (R^2) (**Figure 4**). This concordance between biodegradability and experimental performance was superior to the tests performed by Labatut et al. [64] on digestion of complex substrates.

For the RM methane production kinetics, several kinetic models were used: modified Gompertz model, logistic equation, modified Richards model, transfer model and cone model. Models widely used in anaerobic digestion to produce methane [81,49]. It is worth noting that the convenience and precision of the models always depends on the experimental conditions, the operating parameters, as well as the origin of the inoculum and the type of substrates used [82]. In this study, all the models experienced an R^2 above 0.95 (**Tables 3** and **4**), however, none of them provided a precise fit to the experimental data. In general, all models consist of monotonically increasing functions that always increase and are never equal to zero or decrease [83]. Furthermore, all equations have a single point of inflection, where the curvature changes from concave to convex or vice versa [84]. This has meant that the models do not fully describe the kinetic behaviour of the tests.

The kinetic model with the highest R^2 (0.982-0.999) and the lowest RMSE (0.61-6.92) ml CH_4/g VS) was the cone model. Similarly, the blot model fitted the data with an R^2 (0.990-0.999) and an RMSE of (1.54-8.78 ml CH_4/g VS). While the model of the logistic equation is the one that best adjusted the values observed with the models, since the value of R^2 and the RMSE ranged between (0.957-0.996) and (7.43-13.35 ml CH_4/g VS) respectively. On the other hand, the modified Gompertz and Richards models had a lot of similarity to each other. In the modified Gompertz model, the correlation coefficient presented an R^2 of 0.977 to 0.999 and an RMSE of 4.09 to 11.39 ml CH_4/g VS); while in the Richards model it presented an R^2 of 0.978 to 0.999 and RMSE between 4.11 and 11.40 ml CH_4/g VS. The similarity between the Richards model and the modified Gompertz model is justified by the fact that the parameter "d" of the Richards model is very small (0.001-0.022). In this sense, the smaller the parameter "d", the more similarity there is between the two models [81]. The Richards model gives some flexibility to the curve, allowing it to be adjustable in the event of partial inhibition of the digestion process [47]. Based on the R2 and RMSE values, the Cone model was the best model to adjust the measured and predicted methane yields. Similarly, in other digestion studies, they considered that the cone and first-order models are the most recommended and that best adjust methane yields [85,86].

Conclusions

BMP was investigated using RM as the main substrate in co-digestion with agricultural crop residues (co-substrates). It was determined that the proportions of the mixtures between the substrate and the co-substrates play a key role in the rate of degradation of organic matter. Furthermore, it is concluded that SIR has a significant influence on methane production and biodegradability of the raw materials used. Increasing inoculum from 50% to 66.33% caused all mixes to increase methane production by up to 22%. Concentrations of 50-75% of AM and QU were optimal to improve methane production with ranges of 320-407 ml/g VS. It was shown that the higher the concentration of the co-substrate, the higher the methane production. The RM kinetic study revealed that the lag phase was zero in all tests for the Gompertz, Richards and logistic equation sigmoidal models. While the transfer model experiment resulted in latency phases of 1.16 days. The differences in methane production between the predicted and observed values of the sigmoidal models were 0.25-19.48% (modified Gompertz), 0.32-18.22% (logistic equation) and 0.40- 19.42% (Richards). For its part, the cone model experienced differences between 20 and 36% and the transfer model experienced a difference between 0.85% and 12.69%. The model that best adjusted the observed and predicted values was the cone model with an R^2 of 0.982 to 0.999 and RMSE of 0.61 to 6.92 CH_4/g VS.

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FIGURES

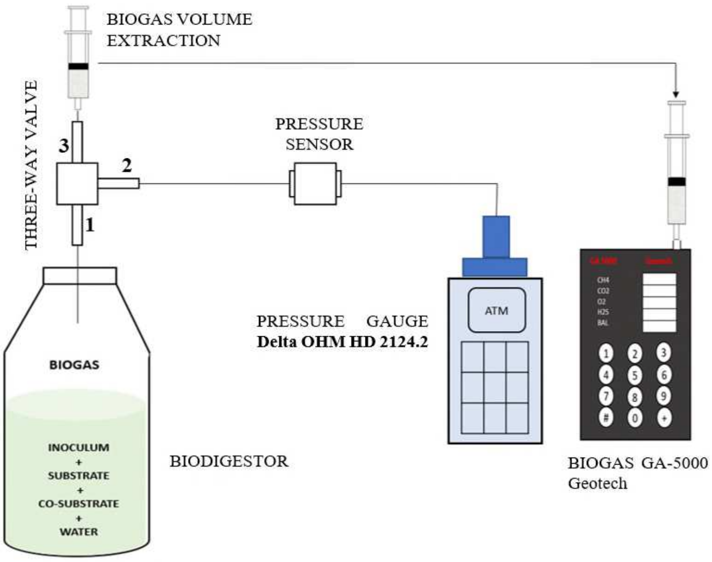

Figure 1. Manometric determination of the BMP of the co-digestion of slaughterhouse residues (RM) with lignocellulosic residues of agricultural origin

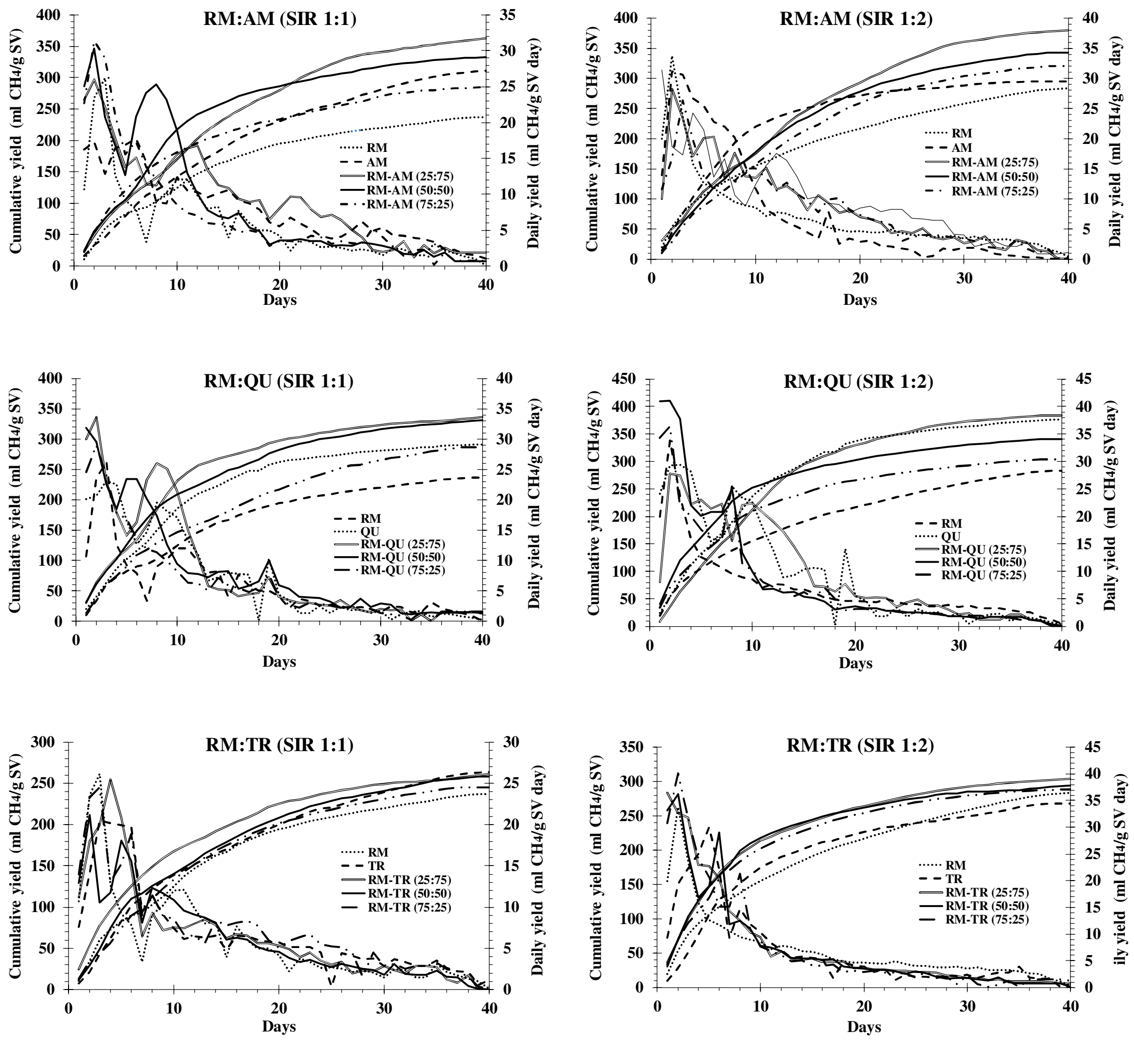

Figure 2. Daily and cumulative methane production for RM co-digestion for both SIR 1:1 and 1:2

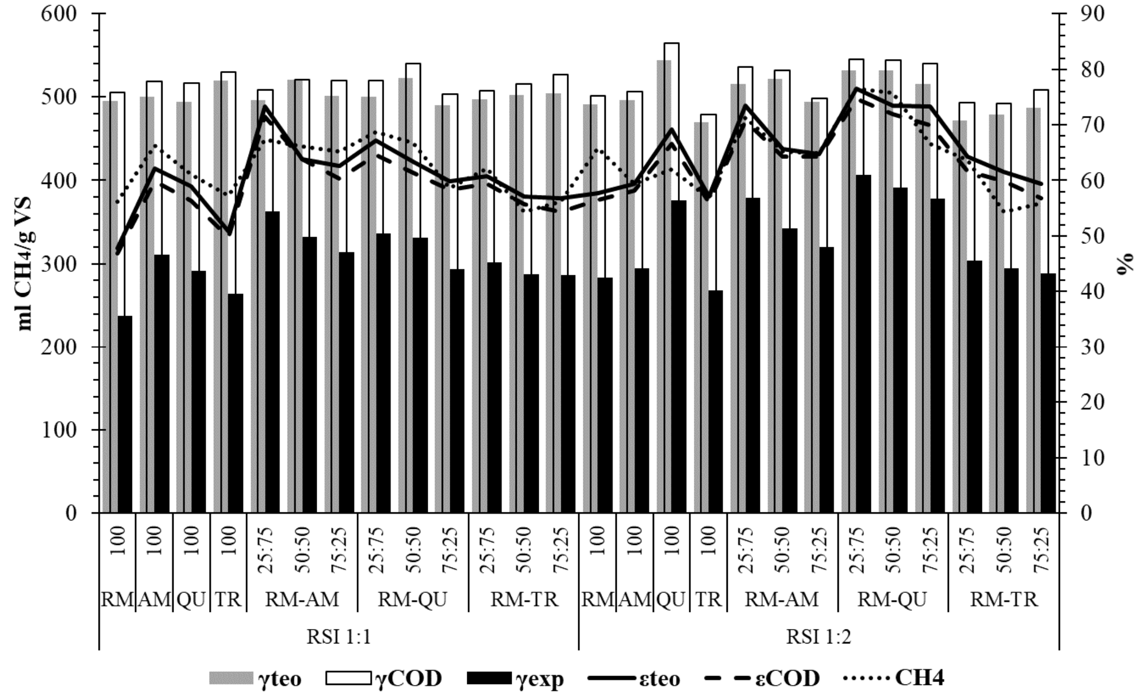

Figure 3. γ_{teo}: Theoretical maximum methane yield based on elementary analysis, γ_{COD}: Theoretical maximum methane yield based on CODt, ε_{teo}: biodegradability based on γ_{teo}, ε_{COD}: biodegradability based on CODt, CH_4: Percentage of methane from the biogas obtained.

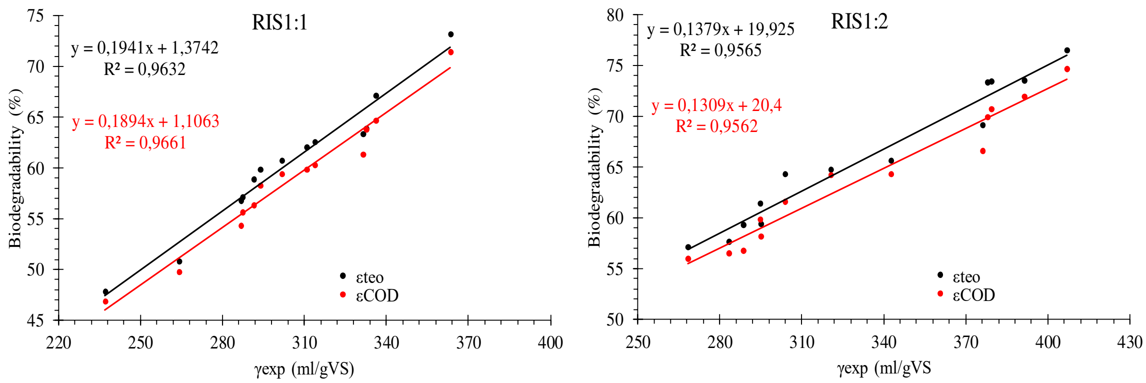

Figure 4. Effect of experimental performance γ_{exp} on biodegradability: ε_{teo}: biodegradability based on γ_{teo}, ε_{COD}: biodegradability based on CODt.

TABLES

Table 1. Composition of raw materials used in BMP tests.

Organic fractions	Composition (g/g VS)	CODt	Empirical formula	C/N	SIR 1:1		SIR 1:2	
					VS (g)	pH	VS (g)	pH
RM:TR	25:75	1429.13	$C_{22.05}H_{47.56}O_{11.79}N$	16.65	1.67	7.37	2.23	7.80
	50:50	1424.26	$C_{32.18}H_{66.85}O_{22.57}N$	23.26	1.67	7.44	2.23	7.75
	75:25	1419.92	$C_{52.97}H_{101.61}O_{12.31}N$	38.15	1.67	7.42	2.23	7.77
RM:AM	25:75	1590.40	$C_{41.06}H_{63.47}O_{21.49}N$	16.38	1.67	7.38	2.23	7.45
	50:50	1532.44	$C_{51.52}H_{83.38}O_{29.49}N$	23.98	1.67	7.47	2.23	7.30
	75:25	1474.32	$C_{70.99}H_{120.44}O_{44.38}N$	40.44	1.67	7.67	2.23	7.37
RM:QU	25:75	1351.52	$C_{19.18}H_{34.35}O_{12.98}N$	35.68	1.67	7.38	2.23	7.40
	50:50	1372.51	$C_{26.54}H_{47.45}O_{18.01}N$	45.23	1.67	7.56	2.23	7.49
	75:25	1394.01	$C_{43.33}H_{77.31}O_{29.47}N$	62.46	1.67	7.54	2.23	7.52

Table 2. Characterization of substrates, co-substrates and inoculum.

Parameters	Units	RM	AM	QU	TR	IN
TS	%	9.6 (1.3)	88.2 (0.1)	87.0 (0.1)	92.6 (0.1)	3.9 (0.1)
VS	%	6.8 (0.8)	65.9 (0.8)	50.8 (0.7)	71.5 (0.7)	2.3 (0.7)
VS/TS	-	0.70	0.75	0.58	0.77	0.59
Ash	%	12.8 (0.2)	8.4 (0.1)	30.3 (1.4)	11.8 (0.1)	55.6 (0.2)
N	%	0.4 (0.1)	3.3 (0.9)	2.2 (0.9)	1.7 (0.7)	3.4 (0.1)
C	%	42.2 (1.1)	42.9 (1.9)	30.7 (1.7)	48.9 (1.6)	25.0 (1.2)
H	%	6.3 (0.9)	6.5 (0.8)	6.4 (0.9)	6.1 (0.5)	2.1 (0.1)
O	%	38.3 (1.1)	38.6 (1.9)	29.8 (1.7)	31.1 (1.6)	12.9 (1.2)
S	%	0.0 (0.0)	0.2 (0.0)	0.6 (0.1)	0.5 (0.0)	0.7 (0.0)
C/N	-	101.9 (0.9)	12.9 (0.8)	12.0 (0.9)	29.6 (0.8)	7.5 (0.7)

Table 3. Kinetic parameters of slaughterhouse waste BMP tests SIR (1:1).

Model	Parameters	RM-AM					RM-QU					RM-TR				
		0:100	**25:75**	**50:50**	**75:25**	**100:0**	**0:100**	**25:75**	**50:50**	**75:25**	**100:0**	**0:100**	**25:75**	**50:50**	**75:25**	**100:0**
Modified Gompertz	M_e	317,47	371,6	323,5	279,4	235,36	286,540	326,6	325,5	256,1	235,36	262,500	257,1	244,0	295,3	235,36
	ν_{max}	11,96	15,13	19,90	13,34	10,63	17,820	21,19	16,58	13,02	10,63	10,600	11,41	11,75	10,80	10,63
	t_{lag}	-1,40	-1,31	-0,64	-3,32	-1,89	-0,460	-0,78	-2,34	-2,89	-1,89	-2,090	-2,11	-1,02	-2,79	-1,89
	R^2	0,994	0,999	0,996	0,989	0,992	0,997	0,997	0,995	0,994	0,992	0,980	0,993	0,998	0,995	0,992
	RMSE	6,53	4,80	7,40	9,99	5,56	4,09	6,85	8,22	6,70	5,56	9,70	8,02	4,69	7,70	5,56
Transfer	M_e	358,38	411,1	320,12	288,6	250,32	297,510	337,6	328,4	263,9	250,32	235,360	271,5	260,4	322,8	250,32
	ν_{max}	18,58	23,83	24,14	25,45	18,16	30,520	36,83	28,13	24,66	18,16	10,630	20,11	19,53	18,03	18,16
	t_{lag}	0,13	0,09	0,01	-0,68	-0,08	0,640	0,38	-0,38	-0,54	-0,08	-1,890	0,01	0,42	-0,53	-0,08
	R^2	0,999	0,999	0,998	0,996	0,996	0,997	0,997	0,998	0,999	0,996	0,990	0,998	0,999	0,999	0,996
	RMSE	1,96	5,40	5,48	6,04	3,76	4,06	6,74	4,12	3,13	3,76	4,08	4,05	1,64	4,07	3,76
Logistic equation	M_e	304,86	358,9	318,2	275,2	229,44	282,320	321,9	320,5	252,5	229,44	255,450	251,4	238,2	285,3	229,44
	ν_{max}	11,46	14,50	18,65	11,68	9,94	16,610	19,79	14,81	11,48	9,94	9,740	10,42	11,00	10,10	9,94
	t_{lag}	-1,48	-1,34	-0,85	-4,50	-2,23	-0,660	-1,00	-3,17	-3,88	-2,23	-2,710	-2,73	-1,29	-3,24	-2,23
	R^2	0,986	0,997	0,992	0,982	0,985	0,990	0,993	0,990	0,989	0,985	0,970	0,987	0,993	0,991	0,985
	RMSE	10,19	8,20	10,86	12,64	7,57	7,49	9,74	11,69	9,10	7,57	12,52	10,61	7,80	10,26	7,57
Cone	M_e	454,47	496,6	363,9	356,8	304,65	318,930	363,6	396,0	314,7	304,65	361,620	333,2	297,1	454,0	304,65
	k	0,05	0,06	0,12	0,10	0,08	0,120	0,14	0,11	0,11	0,08	0,060	0,08	0,09	0,05	0,08
	n	1,14	1,20	1,49	1,01	1,14	1,550	1,49	1,15	1,07	1,14	1,090	1,12	1,32	0,97	1,14
	R^2	0,999	0,997	0,992	0,982	0,995	0,997	0,993	0,990	0,989	0,995	0,996	0,987	0,993	0,991	0,995
	RMSE	2,04	6,45	5,71	3,16	4,17	4,24	6,92	2,93	2,11	4,17	4,23	3,50	1,75	3,53	4,17
Modified Richards	M_e	317,41	371,39	323,44	279,60	235,47	286,640	326,44	325,24	258,08	235,47	263,390	257,47	243,88	299,19	235,47
	d	0,01	0,009	0,005	0,005	0,01	0,000	0,005	0,004	0,005	0,01	0,000	0,004	0,005	0,008	0,01
	ν_{max}	13,55	13,76	9,41	6,56	12,49	20,950	9,62	7,27	6,81	12,49	9,990	4,51	6,32	8,16	12,49
	t_{lag}	-1,42	-1,32	-0,63	-3,37	-1,92	-0,510	-0,78	-2,31	-3,09	-1,92	-2,230	-2,19	-1,02	-3,02	-1,92
	R^2	0,994	0,999	0,996	0,989	0,992	0,997	0,997	0,995	0,994	0,992	0,981	0,993	0,997	0,995	0,992
	RMSE	6,56	4,83	7,42	10,00	5,57	4,11	6,86	8,24	6,77	5,57	9,72	8,04	4,71	7,80	5,57

Table 4. Kinetic parameters of slaughterhouse waste BMP tests SIR (1:2).

Model	Parameters	RM-AM					RM-QU					RM-TR				
		0:100	25:75	50:50	75:25	100:0	0:100	25:75	50:50	75:25	100:0	0:100	25:75	50:50	75:25	100:0
Modified Gompertz	M_e	287,60	393,0	267,4	238,2	282,46	370,25	283,6	252,1	227,9	282,46	254,65	323,5	342,6	379,5	282,46
	ν_{max}	23,19	15,36	15,60	14,10	8,58	22,57	19,53	17,06	13,58	8,58	16,15	14,79	16,08	22,27	8,58
	t_{lag}	-0,24	-1,62	-2,89	-2,62	-5,96	-0,49	-2,03	-2,08	-2,21	-5,96	-0,80	-0,44	-0,80	0,41	-5,96
	R^2	0,991	0,997	0,980	0,984	0,969	0,997	0,983	0,986	0,991	0,969	0,977	0,997	0,995	0,997	0,969
	RMSE	7,07	5,40	8,52	6,98	11,39	5,47	8,42	6,86	5,19	11,39	10,15	5,12	6,78	6,23	11,39
Transfer	M_e	293,95	398,4	272,9	243,5	307,94	384,97	288,5	256,7	233,8	307,94	263,16	352,4	367,8	401,5	307,94
	ν_{max}	41,23	29,15	30,68	27,32	15,01	38,59	38,06	32,92	25,54	15,01	28,87	23,44	26,42	35,71	15,01
	t_{lag}	0,77	-0,36	-0,57	-0,46	-2,42	0,63	-0,18	-0,25	-0,30	-2,42	0,66	0,71	0,59	1,16	-2,42
	R^2	0,998	0,997	0,997	0,998	0,982	0,997	0,997	0,998	0,999	0,982	0,993	0,999	0,999	0,998	0,982
	RMSE	3,02	3,56	4,90	3,81	8,78	5,34	4,55	3,79	2,46	8,78	5,66	3,62	1,54	6,20	8,78
Logistic equation	M_e	284,80	378,9	264,7	235,6	272,16	364,60	281,1	249,6	225,2	272,16	251,17	314,3	334,0	372,2	272,16
	ν_{max}	21,34	14,69	13,48	12,30	7,82	21,05	17,12	15,05	12,09	7,82	14,68	14,13	15,13	21,27	7,82
	t_{lag}	-0,50	-1,69	-4,02	-3,62	-7,16	-0,69	-2,84	-2,84	-2,96	-7,16	-1,29	-0,46	-1,00	0,43	-7,16
	R^2	0,979	0,996	0,983	0,986	0,957	0,990	0,985	0,987	0,990	0,957	0,961	0,995	0,993	0,995	0,957
	RMSE	10,6	9,01	11,01	9,25	13,35	9,73	11,09	9,18	7,43	13,35	13,27	9,04	11,14	11,43	13,35
Cone	M_e	308,30	544,3	314,1	278,2	716,77	414,30	318,3	284,4	264,8	716,77	287,83	397,2	420,2	423,2	716,77
	k	0,17	0,06	0,15	0,15	0,01	0,12	0,18	0,17	0,14	0,01	0,13	0,08	0,08	0,10	0,01
	n	1,67	1,14	1,10	1,13	0,66	1,53	1,24	1,23	1,19	0,66	1,43	1,38	1,33	1,69	0,66
	R^2	0,999	0,998	0,999	0,999	0,991	0,997	1,000	0,999	0,999	0,991	0,996	0,999	0,999	0,999	0,991
	RMSE	4,30	6,33	1,80	1,92	1,89	1,67	1,95	2,26	2,29	1,89	0,61	3,88	2,44	4,48	1,89
Modified Richards	M_e	287,58	392,79	267,64	238,36	283,04	370,21	283,66	252,08	227,91	283,04	254,78	323,34	342,74	379,44	283,04
	d	0,00	0,022	0,004	0,001	0,00	0,01	0,023	0,005	0,006	0,00	0,00	0,007	0,006	0,006	0,00
	ν_{max}	27,67	33,05	5,72	0,70	10,13	26,52	43,40	9,07	8,14	10,13	19,26	9,62	9,87	12,46	10,13
	t_{lag}	-0,24	-1,65	-2,95	-2,68	-6,13	-0,50	-2,07	-2,09	-2,23	-6,13	-0,84	-0,43	-0,82	0,41	-6,13
	R^2	0,991	0,999	0,990	0,992	0,969	0,997	0,991	0,993	0,995	0,969	0,978	0,998	0,997	0,998	0,969
	RMSE	7,09	5,49	8,53	6,98	11,4	5,50	8,50	6,88	5,21	11,4	10,16	5,15	6,81	6,26	11,4