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RESEARCH ARTICLE

Evaluation of methane production from the anaerobic co-digestion of manure of guinea pig with lignocellulosic Andean residues

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Abstract

The objective of this research was to evaluate anaerobic co-digestion of guinea pig manure (GP) with Andean agricultural residues such as amaranth (AM), quinoa (QU) and wheat (TR) in batch biodigesters under mesophilic conditions (37 ^{0}C) for 40 days. As microbial inoculum, sewage treatment sludge was used in two inoculum/substrate ratios (ISR of 1 and 2). In terms of methane production, the best results occurred in treatments containing AM and QU as co-substrate and an ISR of 2. Thus, the highest methane production yield in the GP:AM biodigesters (25:75) and GP:QU (25:75) with 341.86 mlCH_4/g VS added and 341.05 mlCH_4/g VS added, respectively. On the other hand, the results showed that methane production with an ISR of 2 generated higher yields for guinea pig waste and the methane fraction of the biogas generated was in a range from 57 to 69%. Methane production kinetics from these raw materials was studied using five kinetic models: modified Gompertz, logistic equation, transfer, cone and Richards. The cone model adjusted best to the experimental values with those observed with r^2 of 0.999 and RMSE of 1.16 mlCH_4/g VS added. Finally, the highest biodegradability (experimental yield/theoretical yield) was obtained in the GP-AM biodigesters (25:75) with 67.92%.

Keywords Anaerobic digestion · Lignocellulosic waste · Biogas · Co-substrate · Synergy · Inoculum · Kinetic model

Introduction

This work has been carried out in order to analyse applicable technologies in Andean areas of South America where the conventional energy supply is deficient, both in electricity and gas, often non-existent (Omambia et al. 2017). Currently, residents of these areas still depend exclusively on organic fuels from their agricultural and livestock activities, firewood and dried manure, to meet their daily heating and cooking needs (He et al. 2010). Optimizing performance techniques are necessary under conditions of economic, social and environmental sustainability, since it has to be integrated into a traditional way of life being socially accepted by users (Garfí et al. 2016). Increasing access to "*technified*" rural energy is essential to counteract problems these deficient areas face and offer development possibilities (Sheinbaum-Pardo and Ruiz 2012). In the same way, deforestation and the reduction of greenhouse gas emissions would be avoided (Azevedo-Ramos and Moutinho 2018; Pérez et al. 2014).

Most of the Andean communities base their economy mainly on self-sufficient agriculture and family farming (Garfí et al. 2019; Rivera-Parra and Peña-Loyola 2020; Melby et al. 2020). Their agricultural activities from agropastoral nature are developed in semi-arid areas at high altitude where there is a great variety of microclimatic areas as well as ecosystems. (Góngora 2003). In the higher areas, the raising of guinea pigs (*Cavia porcellus*) constitutes one of the main agricultural activities. The guinea pig (GP) is one of the most common animals in rural communities in the Andes (Garfí et al. 2011a, b; Kouakou et al. 2013). They are found in Peru, Ecuador, Bolivia and Colombia, having been

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domesticated between 2500 and 3600 years ago (Cedano-Castro et al. 2020; Sánchez-Macías et al. 2018). In this respect the production and use of guinea pigs represents significant interest for the sustainability of the area, associated with its traditional and ethnic/regional character (Góngora 2003; García 2019). At present, GP manure has been little explored in terms of energy purposes, undervaluing these resources (Gonzalez-Salazar et al. 2014; Garfí et al. 2019). Bioenergy conversion of this particular waste is of special interest. One way to address the energy needs of the Andean communities is through the production of biogas agricultural and livestock waste made possible by anaerobic digestion (AD).

The application of anaerobic digestion to guinea pig manures has been little studied. However, Garfí et al. (2011a, b), who paid particular scientific interest in the characterization of this process for the production and use of biogas in the Andean context. Above all, because of GP's high manure nutrient-content (P-P_2O_5, K-K_2O, N-NH_4), functions as potential waste with multiple benefits, especially in biogas and organic fertilizer production (Boronat Gil 2013). Manure contains a C/N ratio of 14–17, values very similar to those of sheep manure (C/N=16) and higher than those of poultry manure (C/N=12) (Barreros Chiluisa 2017). Thus, anaerobic digestion (AD) represents the potential possibility of reducing the amount of waste from farms and at the same time constitutes an alternative to meet local energy needs by transforming GP manure into biogas Mata-Alvarez et al. 2000.

In the literature there is little information on the use of guinea pig manure as a raw material for biogas production. Garfí et al. (2011a, b) investigated the digestion of GP manure, to produce biogas, under psychrophilic conditions and with continuous digesters at high altitude. Additionally, GP manure co-digestion with cow manure was analysed with no additional inoculum in tubular digesters, thus assessing the effects of high-altitude temperature.

The work presented here expands on Garfí's work comparing simple anaerobic digestion GP manure processes to guinea pig anaerobic digestion with inoculum from sewage sludge. Also, guinea pig co-digestion with lignocellulosic materials typical from Andean agriculture is found in these rural areas such as quinoa straw (QU), wheat (TR) and amaranth (AM). Thus, high carbon content residues from crops and rich nitrogen content of animal manure make for an optimal and balanced C/N ratio (Wei et al. 2014). In the same way, the use of an inoculum in AD can have an effect on the speed of the process (Bortolini et al. 2020; Parra-Orobio et al. 2018; Holliger et al. 2016) affecting not only biodegradability but also the CH_4 production rate (Moset et al. 2015; Raposo et al. 2011). Therefore, it is necessary to investigate GP manure digestion performance with other co-substrates and inoculum to observe the effects on biogas production synergy. Eventually, effects of the substrate/inoculum ratio to improve the anaerobic co-digestion system with lignocellulosic materials should also closely looked at.

Materials and methods

Substrates and inoculum

In this study, the GP manure, collected from the farms of the Bolívar State University, was analysed in co-digestion with three co-substrates: AM, QU and TR straw residues. As soon as the samples were collected, they were stored at 4 °C in polyethylene bags, for conservation purposes. Before co-digestion, AM, QU and TR residues were ground to a particle size of less than 3 mm, using a universal cutter mill. The proportions of the substrates and co-substrates before being put into the biodigester have to be mixed in a kitchen blender to ensure that the experimental samples are uniform. Next, sludge from a mesophilic anaerobic digester from the municipal wastewater treatment facility in Ibarra (Ecuador) was used as inoculum. Before the start of the fermentation tests, the inoculum was pre-incubated for 5 days at room temperature (10 °C at night and 25 °C during the day) to volatilize the residual biogas and deplete the easily available residual organic material. VDI 4630 (2006) prescribes inoculum incubation to limit methane production from targets

Experimental setup and procedure

Batch digestion tests were carried out in triplicate using 311-ml anaerobic biodigesters with an effective volume 186 ml at 37 °C. GP manure co-digestion was performed under three substrate/co-substrate ratio: GP-AM (25:75), GP-AM (50:50) and GP-AM (75:25). In addition, a two-way relationship between substrate and inoculum were established: ISR of 1 and ISR of 2. After the inoculum was mixed with the substrate in the biodigesters, the volume was completed with distilled water. The biodigesters were then hermetically sealed with a rubber septa and aluminium plugs. To mix the contents, the biodigesters were shaken with an orbital shaker for 2 min before the start of incubation. 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 investigate biogas produced solely by the inoculum.

Biogas measurements and estimation of its composition

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) (**Fig. 1**). First, a 100-bar pressure sensor (Delta TP 704) was used, which remained connected to the portable pressure gauge. The measurement process consisted of setting up a system, in which three devices were connected: biodigester, a portable pressure gauge and a syringe for the extraction of the biogas. This connection system was set up with a three-way valve simultaneously. At the beginning of each extraction, the pressure generated in the head space of each biodigester was measured. Biogas extraction was completed when the pressure inside the biodigester equalled atmospheric pressure. Next, biogas volume of each biodigester was calculated through **Eq. 1**. Finally, the cumulative biogas and methane yields (ml/g VS added) were calculated by dividing the corrected amount of the cumulative gas (after subtracting the average amount of gas produced from the blank reactors) by the amount of VS used at the beginning of the digestion tests (da Borso et al. 2021; Pearse et al. 2018). The volume of biogas was measured daily after shaking the biodigesters.

$$V_{BIOGAS}(STP) = \frac{P_{ABS} V_G T_{STP}}{P_{STP} T_1} \tag{1}$$

where,

V_{BIOGAS} (STP)	Methane total volume under standard conditions
P_{ABS}	Absolute pressure generated by overpressure of the digester
T_{STP}	Temperature in standard conditions (298 K)
T_1	Experiment test temperature (311 K)
P_{STP}	Pressure under standard conditions (1 atm)
V_G	Digester head space volume (0.124 l)

Biogas composition (CH_4, O_2, CO_2, H_2S content) was measured using Geotech's BIOGAS GA-5000 meter, using a 200-ml airtight syringe, and biogas samples were taken from the headspace of each biodigester after the gas was released. Before measuring the biogas composition in the headspace, the reactors were stirred for 2 min at 100 rev/min. The composition of the biogas was measured once a day until the end of digestion.

Substrate and inoculum characterization

Total solids (TS) and volatile solids (VS) residues were measured in triplicate according to UNE-EN 18134 and UNE-EN ISO 18123 standards, while TS and VS inoculum content was defined according to 2540A-2540G the American Public Health Association methods (APHA 2018). A portable digital multi-meter potentiometer (HACH HQ 40D) was used to obtain biodigesters pH samples. Elemental analysis (C, H, N, O and S*) was performed using VARIO MACRO CUBE elemental analyser.*

Theoretical BMP

The methods described below are designed to estimate methane co-digestion production from a theoretical chemical oxygen demand (CODt), elemental composition or organic fraction composition. The two methods calculate the theoretical methane potential of all residues under standard conditions (STP) at 0 °C and 1 atm pressure.

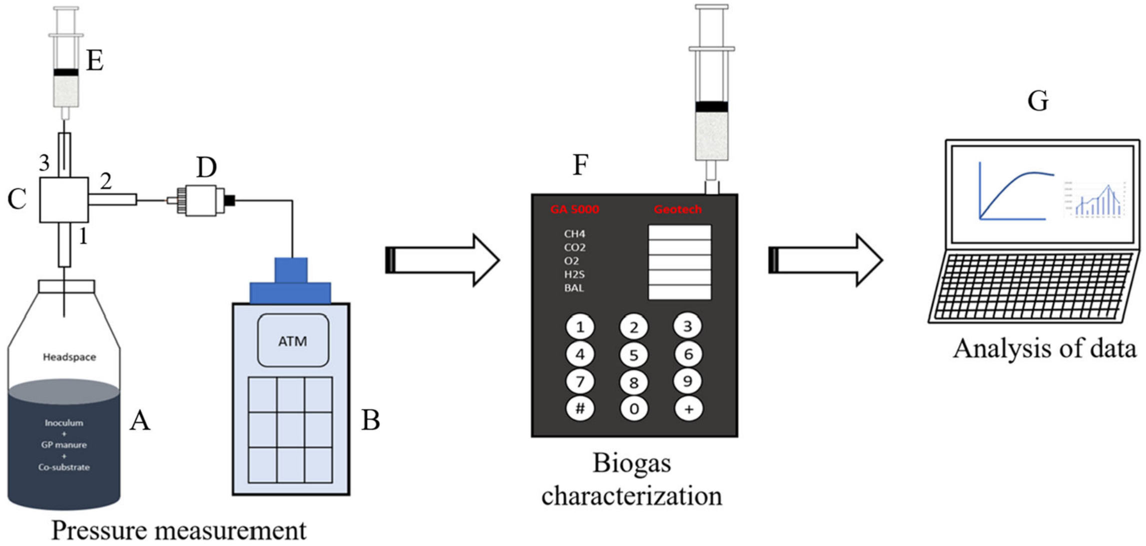

Fig. 1 Obtención y caracterización del biogas. **a** (biodigester), **b** (Delta OHM HD 2124.2 portable pressure gauge), **c** (Three-way valve), **d** (Delta TP 704 100 bar pressure sensor), **e** (200 ml syringe), **f** (GA-5000 BIOGAS meter from Geotech) and **g** (computer to process the data)

Methane production from the theoretical chemical oxygen demand (γ_{CODt})

Equation 2 allows the maximum methane yield calculated from the amount of material and the CODt concentration, assuming its validity for any type of substrate (Nielfa et al. 2015; Liu et al. 2016).

$$\gamma_{CODt}\left(\frac{ml\ CH_4}{g\ VS}\right)=\frac{n_{CH4}.\mathrm{RT}}{P.\mathrm{VS}} \tag{2}$$

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

The value of n_{CH4} has been determined from CODt (**Eq. 3**) (Maletić et al. 2018). CODt for methane is 64 g of oxygen per methane mole, while 1 mole of methane per 64 CODt grams is, therefore, the maximum amount of methane that can be obtained if the whole CODt is converted to methane (Heidrich et al. 2011).

$$n_{CH4}=\frac{CODt}{64\left(\frac{g}{mol}\right)} \tag{3}$$

CODt of all substrates and co-substrates was estimated through their elemental composition and stoichiometry oxidation reaction (**Eq. 4**), using the equation (Eq. 5) (Pellera and Gidarakos 2016). The calculation of CODt based on the atomic composition provides an attractive and easy alternative to obtain solid substrate organic resistance (Raposo et al. 2011).

$$\begin{aligned}&\mathrm{C_aH_bO_cN_d}\\&+\left(\frac{4\mathrm{a}+\mathrm{b}-2\mathrm{c}-3\mathrm{d}+2\mathrm{e}}{4}\right)\mathrm{O_2}\rightarrow\mathrm{aCO_2}\left(\frac{\mathrm{b}-3\mathrm{d}}{2}\right)\mathrm{CH_4}\\&+\mathrm{eH_2O}+\mathrm{dNH_3}\end{aligned} \tag{4}$$

$$CODt\left(\frac{ml\ O_2}{g\ VS}\right)=\frac{\left(2a+\frac{b}{2}-c-\frac{3d}{2}\right)*16}{(12a+b+16c+14d)}*1000 \tag{5}$$

Methane production from the analysis of elemental composition (γ_{teo})

Another way to determine the theoretical yield (γ_{teo}) is through the reaction of (**Eq. 6**), using the Buswell equation (**Eq. 7**). These stoichiometric equations take into account the elemental analysis of C, O, H and N elements in the different substrates and co-substrates (Pellera and Gidarakos 2016; Boulanger et al. 2012; Roberts et al. 2016).

$$\begin{aligned}&\mathrm{C_aH_bO_cN_d}+\left(\frac{4\mathrm{a}-\mathrm{b}-2\mathrm{c}+3\mathrm{d}+2\mathrm{e}}{4}\right)\mathrm{H_2O}\rightarrow\left(\frac{4\mathrm{a}+\mathrm{b}-2\mathrm{c}-3\mathrm{d}-2\mathrm{c}}{8}\right)\mathrm{CH_4}\\&+\left(\frac{4\mathrm{a}+\mathrm{b}+2\mathrm{c}+3\mathrm{d}+2\mathrm{e}}{8}\right)\mathrm{CO_2}+\mathrm{dNH_3}+\mathrm{eH_2S}\end{aligned} \tag{6}$$

$$\gamma_{\mathrm{teo}}\left(\frac{\mathrm{ml\ CH_4}}{\mathrm{g\ VS}}\right)=\frac{22\,400*(4\mathrm{a}+\mathrm{b}-2\mathrm{c}-3\mathrm{d}-2\mathrm{e})}{(12\mathrm{a}+\mathrm{b}+16\mathrm{c}+14\mathrm{d}+32\mathrm{e})*8} \tag{7}$$

Biodegradability and synergy

The experimental performance of methane (γ_{exp}) can be used to calculate anaerobic biological efficiency (ε) under defined test conditions compared to its theoretical value (γ_{teo}), through **Eq. 8** (Shen et al. 2019).

$$\varepsilon=\frac{\gamma_{(\mathrm{exp})}}{\gamma_{(\mathrm{teo})}}.100\% \tag{8}$$

Mixing a substrate with one or more substrates, through co-digestion, causes three types of internal component reactions: methane greater production (synergistic effects), less methane production (antagonistic effects) or simply neither, an increase nor a decrease production in terms of a substrate or co-substrate individual production (independence of waste from the co-digestion). To evaluate the synergy, antagonism and independence that occur in the biodegradation process, **Eq. 9** was used (Yilmaz et al. 2021).

$$\alpha=\frac{\gamma_{exp}}{\gamma_{pond}} \tag{9}$$

γ_{exp} refers to the experimental performance obtained by the BMP. γ_{pond} corresponds to the weighted average yield using (**Equation 10**) (Castro-Molano et al. 2018). If α>1, the mixture has synergistic effects. If α<1, the mixture had antagonistic effects. If α=1, the mixture has independence effects between the substrate and co-substrate.

$$\gamma_{pond}=\frac{\gamma_{sp}.\lambda+\gamma_{cs}.\beta}{\lambda+\beta} \tag{10}$$

γ_{sp} refers to methane production obtained from main substrate digestion of the calculated mono-substrate. 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 of the mixture, respectively.

Kinetic Models to Predict BMP

A mathematical equation can describe the substrates kinetics biodegradation processes. Thus, experimental performance, digestion time and biodegradation kinetics can help predict methane production from a specific substrate (Cecchi et al. 1991). In this experiment, co-digested mixtures methane potential was predicted using 5 mathematical models applied to BMP experimental tests. The following models were used: modified Gompertz (**Eq. 11**) (Zou et al. 2018; Lima et al. 2018; Wang et al. 2021), transfer model (**Eq. 12**) (Li et al. 2012; Ugwu and Enweremadu 2019), logistic equation (**Eq. 13**) (Deepanraj et al. 2015; Ware and Power 2017; Ugwu and Enweremadu 2019), cone models (**Eq. 14**) (Pitt et al. 1999; Lima et al. 2018; Groot et al. 1996) and modified Richards model (**Eq. 15**) (Pitt et al. 1999; Ware and Power 2017).

$$M = M_{\mathrm{e}}.exp\left\{-exp\left[\frac{\nu_{\max}{}^{*}e}{M_{\mathrm{e}}}\left(t_{lag}-\mathrm{t}\right)+1\right]\right\} \tag{11}$$

$$M = M_{\mathrm{e}}\left\{1-exp\left[-\frac{\nu_{\max}}{M_{\mathrm{e}}}\left(t-\mathrm{t}_{\mathrm{lag}}\right)\right]\right\} \tag{12}$$

$$M = \frac{M_{\mathrm{e}}}{1+exp\left[\frac{4\nu_{\max}\left(t_{lag}-t\right)}{M_{\mathrm{e}}}+2\right]} \tag{13}$$

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

$$\mathrm{M} = \mathrm{M_e}\left\{1+\mathrm{d.exp}(1+\mathrm{d})\mathrm{exp}\left[\frac{\nu_{\max}{}^{*}\mathrm{e}}{\mathrm{M_e}}(1+\mathrm{d})\left(1+\frac{1}{\mathrm{d}}\right)\left(\mathrm{t_{lag}}-1\right)\right]\right\}^{\frac{1}{\mathrm{d}}} \tag{15}$$

M is the yield of specific methane accumulated in time t ($\mathrm{mlCH_4.g^{-1}}$ VS), $\mathrm{M_e}$ is the maximum methane yield ($\mathrm{mlCH_4.g^{-1}}$ VS), t is the digestion time (d), k is the first-order decomposition constant ($\mathrm{d^{-1}}$), $\nu_{\max}$ is the maximum methane production specific rate ($\mathrm{mlCH_4.g^{-1}}$ VS. $\mathrm{d^{-1}}$), $\mathrm{t_{lag}}$ is the lethargy or latency time (d) and n is the facto shape.

Statistical analysis

To compare the effect of inoculum and the effect of codigestion of different AD groups, the differences in the experimental data between the results obtained were evaluated by means of the one-way variance analysis (ANOVA). Results were considered significant only if the *p* value was less than 0.05 (i.e., $p < 0.05$). In addition, to determine the degree of suitability between the experimental and predicted values, the mean absolute error MAE, the coefficient of determination (r^2) and the root mean square error (RMSE) were used. Through these statistical parameters, it was determined the model that best predicts the kinetics of raw materials evaluated. All statistical calculations such as determination of the kinetic parameters were carried out with the STATISTICA 10 package.

Results

Raw material physicochemical property characterization

Substrate analysis results, co-substrate and inoculum physicochemical characteristics are presented in **Table 1.** GP manure TS and VS content were 33.9 and 24.6%, respectively. Results were lower than TS and VS content compared with other studies reported in the literature, which varied between 68.51 and 27.82%, respectively (Garfí et al. 2011a, b). Variations TS and VS composition can be attributed to possible changes in nutrition and animals age, as well as changes in manure handling, storage conditions and sample time.(Masse et al. 2003).

All lignocellulosic residues used as a co-substrate presented high percentages of VS and TS. Also, TR residuals were characterized by having the highest TS values (92.6%), VS (71.5%) and VS/TS (0.77). However, results were lower than those obtained by Sun et al. (2019), who obtained TS, VS and VS/TS values of 74.1; 62.9 and 0.84, respectively. Co-substrate of AM presented similar VS characteristics (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. (2013), who reported TS and VS values of 18.0 and 14.4%, respectively. Finally, QU co-substrate presented a high TS value (87.0%), low VS values (50.8%) and VS/TS (0.58). Thus, results of TS, VS and VS/TS of QU were lower than those obtained by Alvarez and Lidén (2008), who obtained values of 95.3, 91.9 and 0.88%, respectively.

The high VS content indicated that raw materials contained a large amount of organic matter. Substrates VS/TS ratio and co-substrates ranged from 0.58 to 0.57, which indicated that raw materials are potential energy waste (Jeung et al. 2019). Similarly, the C/N ratio of GP manure was very similar to animal manure values previously analysed in other studies (5-30) (Liew et al. 2011; Sánchez-García et al., 2015). However, the AM and QU co-substrates had a lower C/N ratio than most lignocellulosic residues, which is usually greater than 50 (Brown et al. 2012). This means that these type of co-substrates need to be investigated to clarify their true energy potential.

Finally, the inoculum (IN) had TS of 3.9%, VS of 2.3% and a VS/TS ratio of 0.59. The IN values were similar to those used by Sun et al. (2019), who reported TS, VS and VS/TS of 5.9, 3.19 and 0.58%. Likewise, IN results were comparable to

Table 1 Substrate, co-substrates and inoculum characterization

Parameters	Units	Substrate	Co-substrates			Inoculum
		GP	AM	QU	TR	IN
TS	%	33.9 (1.7)	88.2 (0.1)	87.0 (0.1)	92.6 (0.1)	3.9 (0.1)
VS	%	24.6 (0.9)	65.9 (0.8)	50.8 (0.7)	71.5 (0.7)	2.3 (0.7)
VS/TS	-	0.73	0.75	0.58	0.77	0.59
Ashes	%	13.1 (0.1)	8.4 (0.1)	30.3 (1.4)	11.8 (0.1)	55.6 (0.2)
N	%	2.3 (1.0)	3.3 (0.9)	2.2 (0.9)	1.7 (0.7)	3.4 (0.1)
C	%	39.5 (1.2)	42.9 (1.9)	30.7 (1.7)	48.9 (1.6)	25.0 (1.2)
H	%	4.6 (0.5	6.5 (0.8)	6.4 (0.9)	6.1 (0.5)	2.1 (0.1)
O	%	39.7 (1.2)	38.6 (1.9)	29.8 (1.7)	31.1 (1.6)	12.9 (1.2)
S	%	0.4 (0.0)	0.2 (0.0)	0.6 (0.1)	0.5 (0.0)	0.7 (0.0)
C/N	-	15.3 (0.8)	12.9 (0.8)	12.0 (0.9)	29.6 (0.8)	7.5 (0.7)

those from Pellera and Gidarakos (2016), reporting 2.7, 1.7% and 0.62 TS, VS and VS/TS, respectively.

Effect of ISR on biomethane potential and biodigester stability

Daily methane production rates of different mixtures are presented under two substrate-inoculum ratios (ISR of 1 and ISR of 2) (**Fig. 2**). In both proportions, the methanogenic activity began immediately shortly after the start of the incubation, causing rapid microorganisms' adaptability. Furthermore, regardless of ISR, it was observed that methane curves showed a similar pattern yielding higher production during the first days. At ISR of 1 and ISR of 2, maximum methane rates were 32.33 ml CH_4/g VS added and 32.39 mlCH_4/g VS added, respectively. As a result, increasing the amount of inoculum from 50 to 66.7%, production decreased slightly. However, in both proportions, the highest methane peaks occurred in mixtures of GP-AM and GP-QU.

For the ISR of 1, more than half of the total methane produced was obtained during the first 10 days. During this period, production varied between 62 and 76%. Between days 11 and 20, methane production varied between 13 and 24%. On the other hand, in the interval between days 21 and 30, methane production decreased by 5–10%. Finally, in 31- and 40-day intervals, digesters hardly produced any amounts of methane, from 1 to 8%. When the amount of inoculum increased, that is, when it went from ISR of 1 to ISR of 2, matter digested faster causing an increase of accumulated methane production. Thus, in the first 10 days, 54–67% percentages were obtained. In the interval from days 11 to day 20, production percentages from 17 to 31% were obtained. Between days 21 and 30, percentages decreased dramatically from 8 to 14%. Finally, in the last co-digestion stage (31–40), methane production decreased to 1–7%.

Maximum accumulated methane production was obtained after 960 h of digestion (40 days) as daily methane productions were 1% of the total accumulated production (Zhao et al. 2019). The results showed that an increase in the amount of inoculum contributed to samples increasing methane production. When comparing ISR of 1 methane production with ISR of 2, trials showed significant differences ($P > 0.05$) according to the Tukey test; except for GP-QU (50:50) mixture that did not present significant figures ($P < 0.05$). A considerable improvement from 9 to 31% in ISR of 2 methane rates took place compared with ISR of 1, suggesting that a high ISR > 1 ratio favour methane production in GP manure co-digestion with AM, QU and TR residues.

Effect of lignocellulosic residues on co-digestion

Several studies have shown that methane production from animal manure can be improved by co-digestion with a variety of co-substrates of agricultural origin (Shrestha et al. 2017). However, the increase in methane production depends on the proper ratio between the main substrate and co-substrate. In this study, mixtures that produced the highest methane rate were those with the highest concentration of co-substrate (AM, QU and TR). Thus, all tests with 75% co-substrate significantly improved compared with those mixtures containing 25 and 50% co-substrate. The highest amount of methane was obtained in GP-AM (25:75), GP-AM (50:50), GP-QU (25:75), GP-QU (50:50) and GP-TR (25:75) mixtures: 341.86, 333.91, 341.05, 315.24 and 315.92 ml/g VS, respectively. However, results revealed that when using 75 or 50% of co-substrate in the mixtures, methane production rates did not present significant values ($P < 0.05$). In addition, it was proven that by increasing the amount of co-substrate from 25 to 75%, mixtures increased methane production between 20 and 26%. Likewise, when the amount of co-substrate

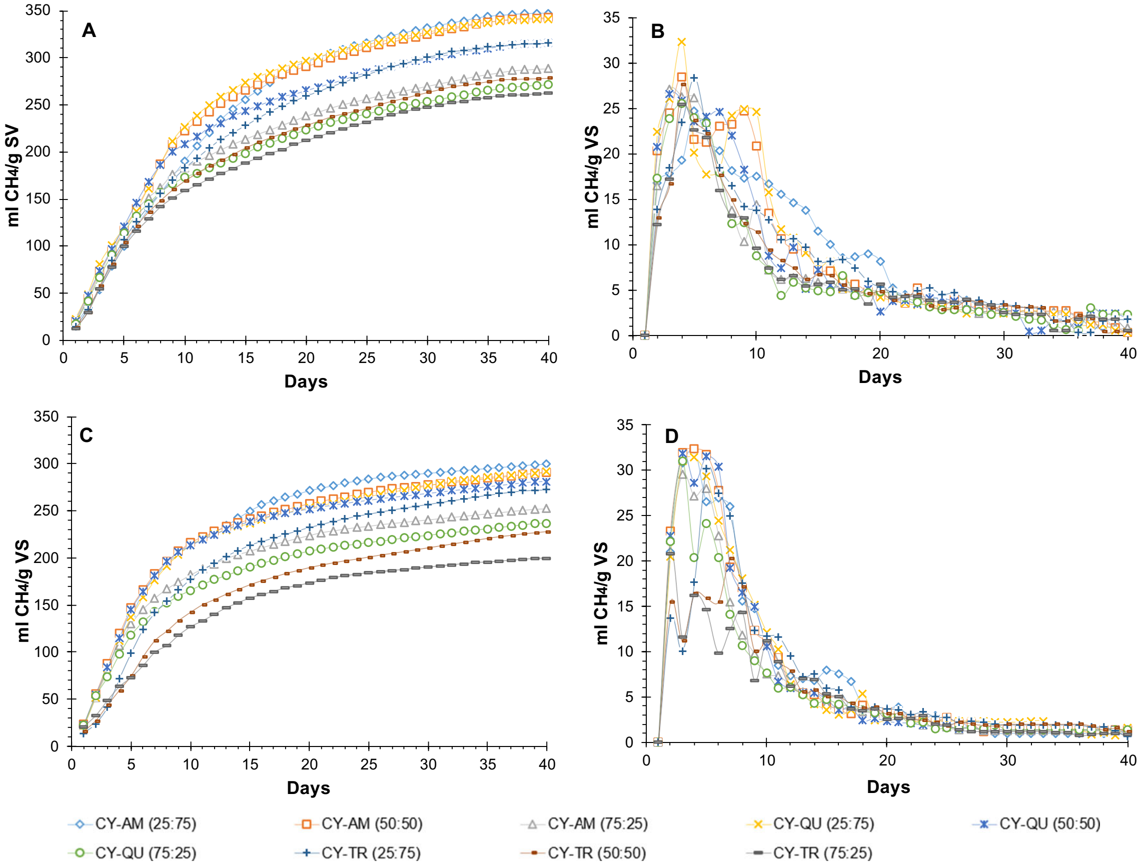

Fig. 2 Daily and cumulative profiles of CH$_4$ production as a function of time, for trials with different IRS. **a** (Cumulative methane production for SIR 1:2), **b** (Daily methane production for SIR 1:2), **c** (Cumulative methane production for SIR 1:1) and **d** (Daily methane production for SIR 1:1) SIR 1:1)

increased from 25 to 50%, methane production in the biodigesters rose between 16 and 20%. It was also found that all lignocellulosic residues were valuable substrates in enhancing GP manure digestion. Such tests are justified since all were performed with 50 and 75% co-substrate concentration and did not present significant figures ($P < 0.05$) in methane generation.

Anaerobic co-digestion synergistic effects

In **Fig. 3**, methane production results, mono-digestion synergy and GP manure residues co-digestion, including AM, QU and TR agricultural residues, are shown. Mono-digestion data incorporated in this article has already been calculated in another article (Meneses-Quelal et al. 2021) in which the same methodology in this research has been followed. This allows the individual performance of GP manure to be compared with the performance of substrate and co-substrate mixtures.

The value of the synergistic effect (α) in GP manure mono-digestion of and agricultural AM, QU and TR residues was determined as 1, since the values of α have been estimated from the mixing proportions and the individual yields of the substrate and co-substrate. Manure mono-digestion methane yields of GP (ISR of 1) and GP (ISR of 2) were 211.07 and 174.27 ml/g VS, respectively. Co-digestion mixtures improved mono-digestion methane production regardless of the ISR used, increasing from 8 to 42% in ISR of 1 and between 50 and 96% in ISR of 2. All comparisons between mono-digestion and co-digestion data showed significant difference ($P > 0.05$), except for ISR of 1 GP-TR (25:75) mixture that did not show significant difference ($P < 0.05$).

Figure 3 shows that α values from ISR of 1, for GP-AM (75:25), GP-QU (75:25), GP-TR (50:50) and GP-TR (75:25) mixtures ranged from 0.957 to 0.988, suggesting that the co-digestion of these mixtures is independent from substrate and co-substrates since the value of α was close to 1. However, GP-AM and GP-QU mixtures containing of 50 and 75% co-substrate had synergistic effects ($\alpha > 1$) with values between 1.11 and 1.17. In the ISR of 2, the synergistic effects were much more promising since their values ranged from 1.13 to 1.50; except for GP-QU (25:75) mixture which α was 0.975. In any event, in the last mixture, there were no synergistic effects, nor were completely antagonistic effects, since α was close to 1.

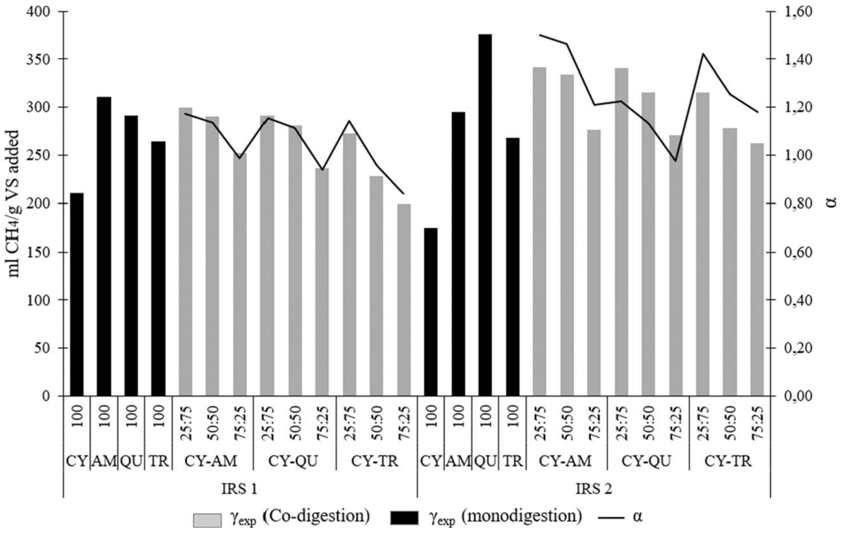

Fig. 3 Synergy index of the co-digestion of guinea pig manure with different lignocellulosic co-substrates. $\alpha > 1$ indicates synergistic effect and $\alpha < 1$ indicates antagonistic effect
(Source: Adapted from Meneses-Quelal et al. (2021))

High content of AM, QU and TR mixtures showed stronger synergistic effects regardless of ISR used. Results are consistent with methane yields, suggesting that a high proportion of co-substrates added to GP manure digestion could have positive effects on the co-digestion yield.

Biogas composition of GP waste

In **Fig. 4 and Table 2**, biogas composition of different combinations between co-substrates and inoculum is shown. Results showed CH_4 and H_2S percentages increased by the rising of inoculum. On the contrary, CO_2 production decreased as the amount of inoculum increased. GP-QU composed mixtures generated the highest percentage of methane regardless of ISR used. In trials with ISR of 2, the mixtures formed by GP-QU experienced a rise of 2.26–4.52% compared with GP-AM combinations and improvements of 2.68–5.68% compared GP-TR-structured biodigesters. In the same way, the biodigesters formed by GP-QU of the ISR of 1 generated higher percentages of CH_4. The difference was 9.89–10.58% and 12-84-14.59% with respect to GP-AM and GP-TR biodigesters, respectively.

In this study, CO_2 was between 29 and 42%, H_2S was almost negligible with percentages of 0.40–1.70%. On the contrary, CH_4 average percentage was 57–69%. Results obtained were similar to those of other investigations in the literature. Thus, for example, Garfí et al. (2011a, b) in a study on anaerobic digestion guinea pig manure, obtained values 63–65% for CH_4 and 0.19% for H_2S. Similarly, in another study on guinea pig manure, Garfí et al. (2011a, b) obtained 59 and 0.15% for CH_4 and H_2S, respectively. Also, Ferrer et al. (2011) recorded values of 60% of the methane fraction in previous bio-methanization studies

Table 2 Production of methane and energy from the substrate and co-substrate

IRS	Feedstock	Composition	CH_4 (%)	γ_{exp} (ml/g VS)	α_1
IRS 1	GP	100	53.50	211.07	
	AM	100	62.57	310.68	
	QU	100	50.84	291.23	
	TR	100	56.79	264.10	
	GP-AM	25:75	59.71	299.68	1.173
		50:50	59.51	290.56	1.137
		75:25	60.17	252.35	0.988
	GP-QU	25:75	62.75	291.29	1.154
		50:50	66.38	281.13	1.114
		75:25	66.78	236.78	0.938
	GP-TR	25:75	58.21	272.32	1.144
		50:50	58.21	227.74	0.957
		75:25	57.04	199.62	0.839
IRS 2	GP	100	44.17	174.27	
	AM	100	59.41	294.99	
	QU	100	65.65	376.08	
	TR	100	57.68	268.23	
	GP-AM	25:75	66.56	341.86	1.499
		50:50	68.14	333.91	1.464
		75:25	67.94	276.32	1.212
	GP-QU	25:75	68.14	341.05	1.226
		50:50	69.71	315.24	1.133
		75:25	68.50	271.37	0.975
	GP-TR	25:75	65.75	315.92	1.423
		50:50	66.78	278.43	1.255
		75:25	67.84	262.09	1.181

(Source: Adapted from Meneses-Quelal et al. (2021)).

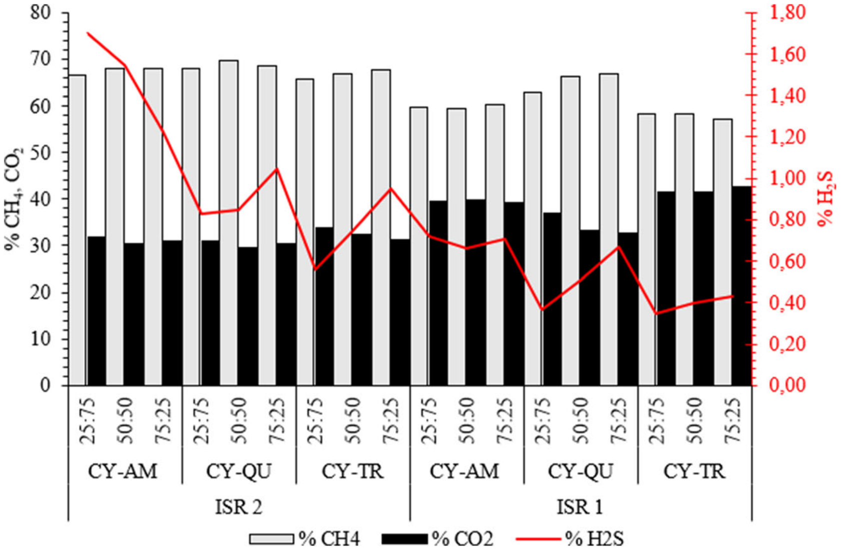

Fig. 4 Percentages of CH_4, CO_2 and H_2S from GP manure biogas

Kinetic study

Estimation of kinetic parameters

Kinetic modelling parameters were calculated using the Richards, logistic and modified Gompertz equations including the Cone model presented in **Table 3**.

When analysing the ν_{max} parameter, the modified Gompertz models and logistic equation correlate having the most similarity, since their ν_{max} values are between 11.98 and 20.80 mlCH$_4$/g VS d and between 10.69 and 19.52 mlCH$_4$/g VS d, respectively. On the other hand, the methanogenic activity occurred at a faster rate in the transfer model, since; for this model, ν_{max} ranged between 21.86 and 31.31 mlCH$_4$/g VS d. The model that differs the most from the rest is the Richards model, where the range of ν_{max} was between 2.13 and 13.76 mlCH$_4$/g VS d. In contrast to other investigations, ν_{max} values in this study are lower than those reported for food residues (28.03–174.63 mlCH$_4$/g VS d) (Li et al. 2018) and those reported for manure chicken (19.4–48.9 mlCH$_4$/g VS d) (Li et al. 2013a, b). Additionally, ν_{max} results of this study are similar to those reported for corn stubble (16.3–32.1 mlCH$_4$/g VS d) (Li et al. 2013a, b) and higher than pig manure co-digestion with sewage sludge (4.8–14.0 mlCH$_4$/g VS d) (Zhang et al. 2014).

Regarding the specific experimental methane yield, ISR of 2 results best fit the kinetic parameter M$_e$. Thus, the mean difference between the observed and predicted values is around 0.16–5.53% (modified Gompertz), 1.04–8.30% (transfer), 2.40–7.04% (equation logistics) and between 0.32 and 5.32% (Richards). These trends suggest that these models are suitable for representing variables of the digestion process and estimating AD yield and kinetic parameters. On the other hand, in the cone model, the differences between the predicted and observed values were more overestimated ranging between 5.85 and 18.95%. The fact that there are discrepancies in the mean differences between the experimental performance and the predicted ones is due to the types of kinetic models used, raw material, conditions used and the digestion of more complex residues (co-digestion). However, the average differences obtained between specific performance and M$_e$ were in line with those obtained by Ware and Power (2017), who obtained differences of 0.54 and 27.07%.

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Regarding the latency period (t_{lag}), many of the digesters experienced very short periods, even 0 days, indicating organic compound high bioavailability within substrates (Ware and Power 2017). In this context, GP manure co-digestion experienced zero periods in the latency phase, except for GP-AM digesters (25:75), whose maximum periods were approximately 0.41 days (modified Gompertz), 0.98 days (transfer), 0.71 days (logistics) and 0.42 days (Richards). The fact that there were low latency periods in these trials indicates that there was a rapid microorganism adaptation process response

Table 3 Kinetic parameters of methane from guinea pig manure co-digestion

Model	Parameters	ISR of 2									ISR of 1								
		GP-AM			GP-QU			GP-TR			GP-AM			GP-QU			GP-TR		
		25:75	50:50	75:25	25:75	50:50	75:25	25:75	50:50	75:25	25:75	50:50	75:25	25:75	50:50	75:25	25:75	50:50	75:25
Gompertz	$\mathbf{M_e}$	341,3	324,1	273,9	323,9	298,7	259,2	302,1	270,6	253,7	180,7	174,1	150,2	184,5	149,9	140,9	160,7	153,3	124,6
	ν_{max}	17,13	19,09	13,67	20,8	17,02	13,02	14,86	13,75	11,98	13,27	13,63	10,62	14,67	13,01	8,85	11,02	9,12	7,3
	$\mathbf{t_{lag}}$	0,41	−0,89	−2,72	−0,53	−1,76	−2,72	−1,09	−1,61	−2,16	−1,13	−1,5	−1,78	−1,37	−0,88	−2,43	−0,27	−0,86	−1,34
	$\mathbf{r^2}$	0,998	0,993	0,986	0,995	0,989	0,987	0,992	0,99	0,988	0,984	0,969	0,977	0,965	0,974	0,975	0,981	0,985	0,995
	RMSE	5,88	10,41	11,31	8,65	11,09	10,27	10,18	10,07	10,05	5,48	7	5,31	7,95	5,61	5,09	5,91	5,04	2,16
Transference	$\mathbf{M_e}$	372,8	*	283,1	*	308,6	267,4	319,8	282,4	264,9	184,2	176,7	152,8	187,5	152,4	143,5	166,1	159,6	129,1
	ν_{max}	25,95	*	25,86	*	31,31	24,74	25,45	24,69	21,86	24,94	26,51	20,63	28,2	24,36	17,38	19,14	15,87	12,96
	$\mathbf{t_{lag}}$	0,98	*	−0,28	*	0,12	−0,29	0,57	0,33	0,09	0,35	0,13	0,01	0,18	0,38	−0,3	0,87	0,53	0,17
	$\mathbf{r^2}$	0,995	*	0,995	*	0,996	0,995	0,999	0,998	0,996	0,998	0,993	0,996	0,992	0,995	0,995	0,997	0,997	0,999
	RMSE	10,04	*	7,04	*	6,33	6,26	4,31	4,96	5,58	2,48	4,52	3,11	5,5	3,61	3,12	3,46	2,93	1,12
Logistic	$\mathbf{M_e}$	331,7	318,6	269,8	318,7	294,5	255,6	295,6	265,9	249,3	179,1	172,7	148,9	182,9	148,7	139,5	158,6	150,8	122,8
	ν_{max}	16,83	17,66	12,01	19,52	15,2	11,4	13,7	12,37	10,69	11,79	11,92	9,27	13	11,67	7,66	10,12	8,38	6,68
	$\mathbf{t_{lag}}$	0,71	−1,26	−3,8	−0,71	−2,52	−3,83	−1,54	−2,32	−3,05	−1,74	−2,23	−2,6	−2	−1,35	−3,45	−0,61	−1,28	−1,78
	$\mathbf{r^2}$	0,997	0,986	0,978	0,99	0,982	0,979	0,986	0,983	0,981	0,985	0,976	0,981	0,974	0,979	0,98	0,983	0,986	0,994
	RMSE	7,52	14,3	14,11	12,18	14,47	12,88	14,02	13,31	12,85	7,51	8,68	6,79	9,63	7,09	6,42	7,96	6,92	3,58
Cone	$\mathbf{M_e}$	394,8	372,5	340,9	362,3	350,5	319,9	368,3	327,6	318,6	198,2	191,2	167,4	203,3	161,7	162	176,9	175,1	143
	k	0,08	0,12	0,1	0,13	0,12	0,11	0,09	0,1	0,09	0,17	0,2	0,18	0,2	0,2	0,16	0,14	0,12	0,12
	n	1,61	1,4	1,08	1,51	1,25	1,09	1,3	1,24	1,14	1,43	1,34	1,29	1,34	1,48	1,17	1,59	1,42	1,35
	$\mathbf{r^2}$	0,997	0,986	0,978	0,99	0,982	0,979	0,986	0,983	0,981	1	0,998	0,999	0,997	0,998	0,999	0,998	0,999	0,999
	RMSE	8,85	4,6	4,4	5,13	4,06	3,93	3,35	3,62	4,13	1,16	2,53	1,46	3,51	2,19	1,44	2,62	2,1	1,37
Richards	$\mathbf{M_e}$	340,76	324,23	275,07	323,84	298,86	259,33	301,88	271,07	253,45	180,72	173,97	150,62	184,54	150,49	140,84	160,75	153,25	124,68
	d	0,008	0,004	0,002	0,005	0,004	0,004	0,005	0,005	0,001	0,005	0,002	0,001	0,003	−0,028	0,004	0,004	0,004	0,005
	ν_{max}	12,14	6,78	2,13	10,75	7,22	4,63	6,72	6,1	1,07	5,98	3,23	0,56	3,7	−35,95	3,95	4,48	3,35	3,91
	$\mathbf{t_{lag}}$	0,42	−0,92	−2,88	−0,53	−1,83	−2,78	−1,09	−1,7	−2,13	−1,15	−1,51	−1,96	−1,4	−1,14	−2,44	−0,3	−0,89	−1,37
	$\mathbf{r^2}$	0,998	0,993	0,986	0,995	0,989	0,987	0,992	0,99	0,988	0,998	0,984	0,988	0,982	0,987	0,987	0,991	0,992	0,998
	RMSE	5,87	10,43	11,33	8,67	11,12	10,28	10,2	10,09	10,05	5,49	7,01	5,33	7,96	5,59	5,1	5,92	5,05	2,16

The (*) means that for these mixtures the model does not converge. $\mathbf{M_e}$ is the maximum yield of methane, **k** is the first order decomposition constant, $\boldsymbol{\nu}_{\mathbf{max}}$ is the maximum specific rate of methane production, $\mathbf{t_{lag}}$ is the lethargy or latency time and **n** is the shape factor.

to the experiment environmental conditions. Furthermore, low t_{lag} values demonstrated the simple nature of substrates and co-substrates and their high biodegradability levels. Finally, it is important to note that, compared with other authors who previously reported latency periods of 0.50 days (Zhang et al. 2017) and 12.3 days (Fang et al. 2014), t_{lag} of some biodigesters in this study were relatively similar and even shorter.

Evaluation and comparison of the different kinetic models

According to **Fig. 5**, the kinetic model with the highest correlation coefficient r^2 (0.992-0.999) and the lowest RMSE (1.37–10.04 mlCH_4/g VS) is the transfer model. Similarly, the cone model fits the data quite well with r^2 (0.978–0.999) and (1.16–8.85 mlCH_4/g VS) RMSE. While the logistic equation model best adjusts to the values observed with the models, since the value of r^2 and the RMSE range between (0.974–0.997) and (3.58–14.30 mlCH_4/g VS), respectively. Subsequently, modified Gompertz and Richards models are very similar. In the modified Gompertz model the correlation coefficient is in an interval of (0.965–0.999) and the RMSE in an interval of (2.16–11.31 mlCH_4/g VS), while in the Richards model r^2 is between (0.982–0.999) and RMSE between (2.16–11.33 mlCH_4/g VS). The similarity between these models is because the Richards model tends to transform into the modified Gompertz model, since its parameter “d” tends to reduce to 0. Furthermore, the sigmoidal models (modified Gompertz, logistic, equation and Richards) (Altaş 2009) had a higher RMSE as the sigmoidal growth of curves was described.

Although, the transfer model did not show total convergence between the observed and predicted values when the non-linear regression was performed. No convergence for the entire duration of co-digestion meant that there were no predicted values in the biodigesters tested. Accordingly, this model did not provide the necessary information for the correct evaluation nor evaluation of data.

It should be noted that the suitability and precision of models always vary considerably depending on the experimental conditions, operating parameters, as well as inoculum origin and type of substrate used (Zhen et al. 2015). In this study, out of every proposed model, the cone model best adjusted to the real evolution of methane production. Similarly, El-Mashad (2013) demonstrated that the cone model provided a more realistic experimental methane yields simulation. It is fascinating that the cone model surpasses methane production expectations since many studies have traditionally considered the Gompertz model to be the most suitable (Zhen et al. 2014; Lu et al. 2014). On the contrary, other authors (Pitt et al. 1999) have considered that the cone model does not adequately produce methane production. Despite low credibility in the cone model, its high precision may be due to many authors’ unfamiliarity with this model (Zhen et al. 2015).

Discussion

Effect of ISR on biomethane potential and biodigester stability

In the current study, results showed that methane yields increased at a higher ISR and are equivalent to previous studies using different substrates (da Silva et al. 2020; Córdoba et al. 2018; Raposo et al. 2009; Wei et al. 2014). For an optimal ISR in a biodigester, it should contain a balanced amount of anaerobic microorganisms for primary and intermediate product digestion (Eskicioglu and Ghorbani 2011). Furthermore, an adequate inoculum can increase degradation rate, improve biogas production, shorten the start-up time and make the digestion process more stable (Quintero et al. 2012). However, determining ISR optimal values is not an easy step,

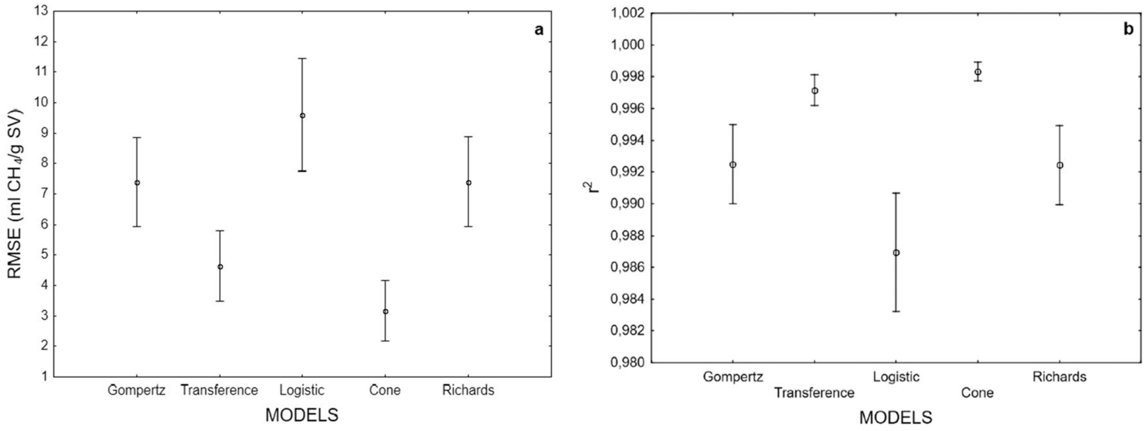

Fig. 5 LSD (Least significant difference) intervals variance analysis at the 95% confidence level for the comparison of RMSE, the r^2 of the different models applied GP manure co-digestion

especially when the substrates and co-substrates used are relatively unknown. Raposo et al. (2006) in a BMP test of corn waste used an ISR range of 3, 2, 1.5 and 1. They concluded that there is a slight variation in higher proportions. Caillet et al. (2019) in a sugarcane distillery wastewater bio-methanization test determined that ISR of 1 methane production rate was faster and higher than in 2, 2.6 and 3.9 proportions. The use of a high or low ISR can be decisive in BMP tests. While a very high ISR will primarily challenge the experimental setup due to relatively substrate low gas production (Caillet et al. 2019), a low ISR could cause an overload in microbial community, as it has already been shown in previous studies (Polizzi et al. 2017; Holliger et al., 2016, b). From the literature consulted, it can be concluded that methane production rates are specific to the substrate and the inoculum, so it is not always possible to generalize digestion performance.

In this study, the use of an ISR of 2 notably improved the biodegradability of matter compared to an ISR of 1. These results are consistent with other solid waste studies, such as Zhou et al. (2011) and Boulanger et al. (2011), who found that ISR ratios less than or equal to 0.5 negatively affect the anaerobic process under the conditions in this study. This phenomenon is associated with the inhibition of anaerobic microbial consortia due to the accumulation of VFA, since it has been shown that ISR ratios lower than 0.25, the substrate biodegradability begins to decline (Alexis et al. 2015). Another probable cause for the effect of ISR ratio on GP manure is hydrolysis. According to Bouallagui et al. (2005) there is a direct relationship between soluble organic matter (SOM) and hydrolysis, since higher SOM content means anaerobic digestion times are reduced, resulting in less fundamental substrates content; thus production of methane increases. In this study, the increase in the ISR ratio implied an increase in certain organic matter in the substrate.

Despite the latter, more trials are needed with different ISR ratios (greater than 2 and less than 1) to fully evaluate the influence of inoculum; especially since co-digestion tests were carried out from easily degradable material (GP manure) and lignocellulosic matter. In addition, the materials used are little known, which means that there is little literature evaluating their energy potential.

Effect of co-digestion on biomethane potential and process stability

Generally, GP manure co-digestion with lignocellulosic residues repeatedly increased methane production. Furthermore, throughout the world traditionally animal manure has been used as a mono-substrate in most bio-methanization tests and co-digestion processes were dynamic (Wu et al. 2010). In this case, due to the inherent carbon deficiency in manure and the increase in the synergistic effects of co-digestion by AM, QU and TR, the biodegradability in the biodigester was increased (Himanshu et al. 2018; Khoufi et al. 2015). Results in this study were very similar to those of other authors and corroborated previous studies (**Table 4**). Unequalled results ranged from 300 to 340 CH_4/g VS, corresponding to average methane production. According to Velázquez-Martí et al. (2018), low methane productions range between 150 and 300 CH_4/g VS, the average productions between 300 and 450 CH_4/g VS and the high productions are higher than 450 CH_4/g VS.

GP manure mono-digestion production was low (around 170–211 CH_4/g VS) compared with previous cow, pig and poultry manure studies ranging from 238, 271 and 328 ml/g VS, respectively (Meneses-Quelal and Velázquez-Martí 2020). Low methane production can be attributed to the quality and management techniques of the organic matter in manure (Ferrer et al. 2011). In rural Andean areas, harsh climatic conditions and frost-tolerant forages are responsible for unconventional animal diets compared with other climates and conditions (Alvarez and Lidén 2009). The type of animal diet has an effect on GP manure as protein and lipid content may be low. Therefore, the amount of digesting material increases (Alvarez et al. 2006).

The proportions that generated the best results were those in which 50 and 75% of the co-substrate was used (based on VS content). Concentrations of 25% generated lower methane ranges (260–276 ml/g VS). Low efficiencies can be attributed to a higher content of lignin or other recalcitrant carbon in biodigester composition (Ebner et al. 2016). Ma et al. (2020) concluded that for a maximum pig and cow methane manure improvement yield, the recommended proportions of lignocellulosic residues should be approximately 30–50%. By contrast, co-substrate concentrations between 60 and 90% can produce low methane yields in co-digestion. However, the variations in co-substrates in co-digestion have very wide ranges and depend on the type of manure used (Vivekanand et al. 2018). Determining the appropriate ratio between substrate and co-substrate is essential to optimize the co-digestion processes (Jeung et al. 2019), above all because co-substrates volume in co-digestions vary greatly between different studies (Andriamanohiarisoamanana et al. 2018; Zahan et al. 2018).

The synergistic effects were closely related to methane production, the biodigesters with a greater amount of co-substrate had a greater synergistic effect and greater yield. Methane synergy biochemical potentials are directly related to substrate composition (Astals et al. 2014). Substrate composition determines the efficacy of the microbial population, which in turn greatly influences biogas yield, long-term process stability and solid degradation rate (Castro-Molano et al., 2018). The presence of antagonistic effects in some biodigesters (GP-TR (75:25; GP-QU (75:25)) is because in this study the co-digestion of binary mixtures was carried out, when there are mixtures of three and four substrates, greater synergy effects are achieved than in mixtures of two

Table 4 Co-digestion of animal manure residues with lignocellulosic residues present in the literature

Raw materials	Mixing ratio	Methane production ml/g VS	References
CMA:CS	25:75	218,8	(Li et al., 2013)
RS: KW: GW	57:29:14	303,4	(Chen et al. 2019)
DM:CS:TO	54:33:13	415,4	(Li et al., 2016)
PD:WS	70:30	330,1	(Rahman et al. 2017)
PD:MG	50:50	340,1	(Rahman et al. 2017)
ESBP: PM	25:75	212,4	(Gómez et al., 2019)
BS:TPM	50:50	152,3	(Wei et al. 2014)
BS:CM	50:50	120-125	(Wei et al. 2014)
GP-AM	25:75	341,9	Data from this study
GP-QU	25:75	341,1	Data from this study
GP-TR	25:75	315,9	Data from this study

CS (corn stover), CMA (chicken manure), RS (residual sludge), KW (kitchen waste), GW (green waste from Garden branches and leaves), TO (tomato residues), DM (dairy manure), WS (wheat Straw), MG (meadow grass), PD (Poultry droppings), PM (pig manure), ESBP (sugar beet pulp), BS (barley Straw), TPM (Tibet pig manure) and CM (cow manure)

substrates (Baquerizo Crespo et al. 2016). Finally, in biodigesters that present antagonism, binary mixtures have not been able to provide all the nutrients and trace elements necessary to that microorganisms have a higher methanogenic activity (Pagés-Díaz et al. 2014).

C/N ratio from GP substrate was 15.3 an AM and QU co-substrates was 12.9 and 12 respectively. According to Li et al. (2011) a C/N ratio of 20–30 is optimal for anaerobic digestion. A high C/N ratio would reduce biodegradation rate, while a low C/N ratio tends to produce excess VFA ammonia, which may cause inhibition in anaerobic digestion (Lin et al. 2019). However, in this study, a C/N ratio of 12–15 did not influence methane production. In fact, the best results were obtained from GP-AM and GP-QU mixtures. Lin et al. (2015) used low C/N ratios of 10–20 yielding satisfactory results, attributing high methane production to carbon biodegradability. Romano and Zhang (2008) recommended that the C/N ratio be kept at 15 for the co-digestion of onion juice and digested sludge. Zhu et al. (2009) inoculated corn stubble with digested sewage sludge, obtaining excellent results with a C/N of 15–18. In another study, Jeung et al. (2019) demonstrated that the optimal C/N ratio for sludge-based anaerobic co-digestion is approximately 15–20. For that matter, optimal C/N ratio varies with the type of raw material to be digested. In addition, solid results in study may be due to amaranth properties like protein, sugar, fat and fibre, very similar corn, which is an excellent substrate for producing biogas (Haag et al. 2015).

Anaerobic digestion (AD) of animal manure and lignocellulosic residues is gaining greater interest because of its wide availability, optimal physicochemical characteristics, high methane potential and absence of conflict with the human food chain compared with energy crops (Naik et al. 2010).

Conclusion

This study evaluated methane production by anaerobic codigestion of guinea pig manure (GP) from amaranth (AM), quinoa (QU) and wheat (TR) cosubstrates. In addition, the effect of an inoculum from sewage sludge on the biochemical potential of methane was investigated. A substrate-to-inoculum ratio (ISR) of 2 proved more suitable for GP manure codigestion. Specifically, ISR of 2 resulted in 341.86 ml CH_4/g VS for GP:AM biodigester (25:75). The influence of the co-substrates was notable in methane production, since improvements from 20 to 26% were obtained as co-substrate concentration increased from 25 to 75%. Finally, the results of the kinetic modelling concluded that the transfer and cone models are the most suitable for the stimulation of cumulative biogas and the methane production curve, since they provided r^2 of 0.999. However, in the transfer model not all the data converged between the observed and estimated values, especially in GP-AM (50:50) and GP-QU (50:50) biodigesters.

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Authors' contributions Contribution of each co-author is described below:

OWMQ: performed the conceptualization, methodology, validation, formal analysis, investigation and writing-original draft preparation.

BVM: performed the conceptualization, methodology, validation, formal analysis, investigation, writing—original draft preparation, writing review and editing and funding acquisition.

JGC: performed the formal analysis and funding acquisition.

ZNR: performed the methodology, validation, writing review and editing and funding acquisition.

AFG: performed the writing review and editing.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Availability of data and materials The datasets during the current study are available from the corresponding author on reasonable request.

Competing interests The authors declare that they have no competing interests.

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