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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 the anaerobic co-digestion of guinea pig manure (CY) with Andean agricultural residues such as amaranth (AM), quinoa (QU) and wheat (TR) in batch biodigesters under mesophilic conditions (37°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 the treatments that contained AM and QU as co-substrate and an ISR of 2. Thus, the highest methane production occurred in the CY:AM biodigesters (25:75) and CY:QU (25:75) with 341.86 mlCH₄/g VS and 341.05 mlCH₄/g VS, respectively. On the other hand, the results showed that methane production with an ISR of 2 was more feasible for guinea pig waste, where the methane fraction of the biogas generated was in a range of 57 and 69%. The kinetics of methane production from these raw materials was studied using five kinetic models: modified Gompertz, logistic equation, transfer, cone, and Richards. The cone model was the one that best adjusted the experimental values with those observed with an r^2 of 0.999 and an RMSE of 1.16 mlCH₄/g VS. Finally, the highest biodegradability was obtained in the CY-AM biodigesters (25:75) with 67.92%.

Keywords: fermentation, lignocellulosic waste, biogas, co-substrate, guinea pig, inoculum, kinetic model.

1. Introduction

This work has been carried out 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, many people in these areas still depend exclusively on organic fuels from their agricultural and livestock activities, such as firewood and dried manure, to meet their daily heating and cooking needs (He et al., 2010). The improvement of techniques to optimize its performance is necessary, under the conditions of economic, social and environmental sustainability, since it has to be integrated into a traditional way of life, and that these are socially accepted by users (Garfí et al., 2016). Increasing access to technified rural energy is essential to counteract the problems of these depressed areas and offer development possibilities (Sheinbaum and Ruiz, 2012). In the same way, deforestation and the reduction of greenhouse gas emissions would be avoided (Azevedo 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 et al., 2020; Melby et al., 2020). Its agricultural activities are of an agropastoral nature and are developed in semi-arid areas at high altitude where there is a great variety of microclimatic and ecosystems (Góngora, 2003). In the higher areas, the raising of guinea pigs (*Cavia porcellus*) constitutes one of the main agricultural activities. The guinea pig (CY) is one of the most common animals in rural communities in the Andes (Garfí et al., 2011; Kouakou et al., 2013). It is distributed by the countries of Peru, Ecuador, Bolivia and Colombia, and was domesticated between 2,500 and 3,600 years ago (Cedano et al., 2020; Sánchez et al., 2018). In this sense, the production of guinea pigs and their use has a high degree of

interest for the sustainability of the area, associated with its traditional and ethnic/regional character (Góngora, 2003; García, 2019). At present, CY manure has been little explored for energy purposes, which has made these resources undervalued (Gonzalez et al., 2014; Garfí et al., 2019). The conversion of this waste into bioenergy is of special interest in this scenario. One way to address the energy needs of Andean communities is through the production of biogas through the anaerobic digestion (AD) of agricultural and livestock waste.

The application of anaerobic digestion to guinea pig manures has been little studied. In this the main reference has been Garfí et al. (2011), who warned of the scientific interest in the characterization of this process for the production and use of biogas in the Andean context. Above all, because CY manure has a high content of nutrients (P-P₂O₅, K-K₂O, N-NH₄), and is a potential waste with multiple benefits, especially in the production of biogas and in the production of organic fertilizer (Boronat, 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, 2017). Thus, anaerobic digestion (AD) represents a potential possibility to reduce the amount of waste from farms and, at the same time, constitutes an alternative to meet local energy needs by transforming CY manure into biogas (Mata 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. (2011) investigated the digestion of CY manure, to produce biogas, under psychrophilic conditions and with continuous digesters at high altitude. On the other hand, they also analysed the co-digestion of CY manure with cow manure, without any additional inoculum, in tubular digesters evaluating the effect of temperature at high altitude.

The work presented here expands on Garfi's work comparing the processes of simple anaerobic digestion of CY manure with anaerobic digestion of guinea pig with inoculum from sewage sludge and the co-digestion of guinea pig with lignocellulosic materials typical of Andean agriculture, accessible in these communities. rural areas such as quinoa straw (QU), wheat (TR) and amaranth (AM). Thus, the high carbon content of the residues from crops and the 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 influence the speed of the process (Bortolini et al., 2020; Parra et al., 2018; Holliger et al., 2016) affecting not only biodegradability but also the CH₄ production rate (Moset et al., 2015; Raposo et al., 2011). Therefore, it is necessary to investigate the performance of CY manure digestion with other co-substrates and inoculum to observe the influence of biogas production synergy. It is intended to investigate the influence of the substrate / inoculum relationship in the improvement and optimization of the anaerobic co-digestion system with lignocellulosic materials.

2. Materials and methods

2.1 Substrates and inoculum

In this study, the CY 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, the 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 were mixed with a kitchen blender to ensure that the experimental samples are uniform. As inoculum, sludge from a mesophilic anaerobic digester from the municipal wastewater treatment facility in Ibarra (Ecuador) was used. 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 at 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

2.2 Experimental setup and procedure

Batch digestion tests were carried out in triplicate using 311 ml anaerobic biodigesters with an effective volume of 186 ml at 37°C. CY manure co-digestion was performed under three substrate/co-substrate ratio: CY-AM (25:75), CY-AM (50:50) and CY-AM (75:25). In addition, two relationships 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 effective volume was completed with distilled water. The amount of added VS of inoculum was the same in all batches. The biodigesters were then hermetically sealed with rubber septa and aluminium plugs. To mix the contents, the biodigesters were shaken with an orbital shaker for 2 min before their 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 correct for the biogas produced solely by the inoculum.

2.3 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). To measure the pressure, 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: the biodigester, the portable pressure gauge and a syringe for the extraction of the biogas.

This connection system was carried out with a three-way valve and simultaneously. At the beginning of each extraction, the pressure generated by in the head space of each biodigester was measured. Biogas extraction was completed when the pressure inside the biogas equalized to atmospheric pressure. The biogas volume of each biodigester was calculated through (**Equation 1**). Finally, the cumulative biogas and methane yields (ml/g VS) were calculated by dividing the corrected amount of the cumulative gases (after subtracting the average amount of gas produced from the blank reactors) by the amount of VS used at the beginning of the tests. digestion tests (da Borso et al., 2021; Pearse et al., 2018). Each biodigester was shaken once a day and the volume of biogas was measured once a day.

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

where,

$V_{BIOGAS}(STP)$	total volume of methane 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 (CH₄, O₂, CO₂, H₂S content) was measured using Geotech's BIOGAS GA-5000 meter. In this way, using a 200 ml airtight syringe, 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 two

minutes at 100 rev/min. The composition of the biogas was measured once a day until the end of digestion.

2.4 Characterization of the substrate and inoculum

The total solids (TS) and volatile solids (VS) of the residues 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 according to 2540A-2540G methods of the American Public Health Association (APHA, 2005).. A portable digital multi-meter potentiometer (HACH HQ 40D) was used to determine the pH of the samples from the biodigesters. Elemental analysis (C, H, N, O and S) was performed using the VARIO MACRO CUBE elemental analyser.

2.5 Theoretical BMP

The methods described below are designed to determine the production of methane from co-digestion from its characterization of the theoretical chemical oxygen demand (COD_t), elemental composition or composition of organic fraction. The two methods calculate the theoretical methane potential of all residues under standard conditions (STP) at a temperature of 25 °C and a pressure of 1 atm.

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

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

$$\gamma_{\text{CODt}} \left(\frac{\text{ml CH}_4}{\text{g VS}} \right) = \frac{n_{\text{CH}_4} \cdot RT}{P \cdot VS} \quad (2)$$

where γ_{CODt} is the theoretical production, R is the gas constant ($R = 0.082 \text{ atm l/mol K}$), T is the temperature of the biodigester (298 K), P is the atmospheric pressure (1 atm), VS aggregate (g) are the volatile solids in the substrate and n_{CH_4} is the amount of molecular methane (mol).

The value of n_{CH_4} has been determined from the CODt (**Equation 3**) (Maletić et al., 2018). The CODt for methane is 64 g of oxygen per mole of methane. 1 mole of methane per 64 g of CODt is, therefore, the maximum amount of methane that can be obtained if all the CODt is converted to methane (Heidrich et al., 2011).

$$n_{\text{CH}_4} = \frac{\text{CODt}}{64 \left(\frac{\text{g}}{\text{mol}} \right)} \quad (3)$$

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

$$\text{CODt} \left(\frac{\text{ml O}_2}{\text{g VS}} \right) = \frac{\left(2a + \frac{b}{2} - c - \frac{3d}{2} \right) * 16}{(12a + b + 16c + 14d)} * 1000 \quad (5)$$

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

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

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

2.6 Biodegradability and synergy

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

$$\varepsilon = \frac{\gamma_{(exp)}}{\gamma_{(teo)}} \cdot 100\% \quad (8)$$

Mixing a substrate with one or more substrates, through co-digestion, causes three types of internal reactions of its components: greater production of methane (synergistic effects), less production of methane (antagonistic effects) or simply not increase or decrease production regarding the individual production of a substrate or co-substrate (independence of waste from the coDA). To evaluate the synergy, antagonism and independence that occurs in the biodegradation process, (**Equation 9**) was used (Nielfa et al., 2015).

$$\alpha = \frac{\gamma_{\text{exp}}}{\gamma_{\text{pond}}} \quad (9)$$

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

$$\gamma_{\text{pond}} = \frac{\gamma_{\text{sp}} \cdot \lambda + \gamma_{\text{cs}} \cdot \beta}{\lambda + \beta} \quad (10)$$

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.

2.7 Kinetic Models to Predict BMP

A mathematical equation can describe the kinetics of the biodegradation process of the substrates. Thus, experimental performance, digestion time and biodegradation kinetics can help predict methane production from a specific substrate (Cecchi et al., 1991). In this work, the methane potential of the co-digested mixtures was predicted using 5 mathematical models applied to the BMP experimental tests. The following models were used: modified Gompertz **(Equation 11)** (Lay et al., 1997; Zou et al., 2018); Lima et al., 2018; Zwietering et al., 1990; Wang et al., 2021), transfer model **(Equation 12)** (Li et al., 2012; Ugwu and Enweremadu, 2019), logistic equation **(Equation 13)** (Deepanraj et al., 2015; Ware and Power, 2017; Ugwu and Enweremadu, 2019), cone models **(Equation**

14) (Pitt et al., 1999; Lima et al., 2018; Groot et al., 1996) and modified Richards model
(Equation 15) (Pitt et al., 1999; Ware and Power, 2017).

$$M = M_e \cdot \exp \left\{ -\exp \left[\frac{v_{\max} * e}{M_e} (t_{\text{lag}} - t) + 1 \right] \right\} \quad (11)$$

$$M = M_e \left\{ 1 - \exp \left[-\frac{v_{\max}}{M_e} (t - t_{\text{lag}}) \right] \right\} \quad (12)$$

$$M = \frac{M_e}{1 + \exp \left[\frac{4v_{\max}(t_{\text{lag}} - t)}{M_e} + 2 \right]} \quad (13)$$

$$M = \frac{M_e}{1 + (k \cdot t)^{-n}} \quad (14)$$

$$M = M_e \left\{ 1 + d \cdot \exp \left(1 + d \exp \left[\frac{v_{\max} * e}{M_e} (1 + d) \left(1 + \frac{1}{d} \right) (t_{\text{lag}} - 1) \right] \right)^{\frac{1}{d}} \right\} \quad (15)$$

Where M is the yield of the specific methane accumulated in time t (mlCH₄.g⁻¹ VS), M_e is the maximum yield of methane (mlCH₄.g⁻¹ VS), t is the digestion time (d), k is the first order decomposition constant (d⁻¹), v_{max} is the maximum specific rate of methane production (mlCH₄.g⁻¹ VS. d⁻¹), t_{lag} is the lethargy or latency time (d) , and n is the shape facto.

2.8 Statistical analysis

To compare the effect of the inoculum and the effect of codigestion of the different AD groups, the differences in the experimental data between the results obtained were evaluated by means of the one-way analysis of variance (ANOVA). The results were considered significant only if the p value was less than 0.05 (ie, $p < 0.05$). In addition, to determine the degree of fit 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 which is the model that best predicts the kinetics of the raw materials evaluated. All calculations were performed with the STATISTICA 10 statistical package.

3. Results

3.1 Characterization of the physicochemical properties of the raw material

The results of the analysis of the physicochemical characteristics of the substrate, co-substrate and inoculum are presented in **Table 1**. The TS and VS content of the CY were 33.9% and 24.6% respectively. These results were lower than the TS and VS content of other studies reported in the literature, where they varied between 68.51% and 27.82% respectively ([Garfí et al., 2011](#)). Variations in the composition of TS and VS can be attributed to possible changes in the nutrition and age of the animals, as well as changes in manure management, storage conditions, and sampling time ([Masse et al., 2003](#)).

All lignocellulosic residues used as a co-substrate presented high percentages of VS and TS. Also, the TR residuals 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. ([2019](#)), who obtained TS, VS and VS/TS values of 74.1%; 62.9% and 0.84 respectively. The co-substrate of AM 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. (2013), who reported TS and VS values of 18.0% and 14.4% respectively. 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 (2008), who obtained values of 95.3%; 91.9% and 0.88 respectively.

Finally, the inoculum (IN) presented 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, the IN results were comparable to those of Pellerá & Gidarakos (2016), who reported TS, VS and VS/TS of 2.7%; 1.7% and 0.62 respectively.

3.2 Effects of inoculum on biogas production

The daily methane production rates of the different mixtures are presented under two substrate-inoculum ratios (ISR of 1 and ISR of 2) (**Figure 1**). In both proportions, the methanogenic activity began immediately shortly after the start of the incubation, causing a rapid adaptability of the microorganisms to occur. Furthermore, regardless of the ISR, it is observed that the methane curves showed a similar pattern, with a higher production in the first days. At the ISR of 1 and ISR of 2, the maximum methane rates were 32.33 ml CH₄/g VS and 32.39 mlCH₄/g VS, respectively. With the increase in the amount of inoculum from 50 to 66.7%, the daily productions decreased slightly. However, in both proportions the highest methane peaks occurred in the mixtures of CY-AM and CY-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 productions varied between 13 and 24%. On the other hand, in the interval between days 21 and 30, methane production decreased by 5 to 10% percentages.

Finally, in the interval of days 31 and 40 the digesters produced very low amounts of methane of 1 to 8%. When the amount of inoculum was increased, that is, when it went from an ISR of 1 to an ISR of 2, the material in the digesters digested faster which caused the accumulated methane production to increase. Thus, in the first 10 days, percentages of 54 to 67% were obtained. In the interval from day 11 to day 20, production percentages of 17 to 31% were obtained. Between days 21 and 30, it decreased dramatically to percentages of 8 to 14%. Finally, in the last stage of co-digestion (31-40), methane production decreased to percentages of 1 to 7%.

The maximum accumulated methane production was obtained after 960h of digestion (40 days) when the 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 the samples increasing their methane production. When comparing the methane production of the ISR of 1 with the ISR of 2, all the trials showed significant differences ($P > 0.05$) according to the Tukey test; Except for the CY-QU (50:50) mixture that did not present significant figures ($P < 0.05$). The increase in the improvement of the methane rate of the ISR of 2 compared to the ISR of 1 was around 9 and 31%. These results suggested that a high $ISR > 1$ ratio favour methane production in manure co-digestion of CY with residues of AM, QU and TR.

3.3 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 the co-substrate. In this study, the mixtures that generated 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 to

those mixtures with 25 and 50% co-substrate. The highest amount of methane was obtained in the mixtures of CY-AM (25:75), CY AM (50:50), CY-QU (25:75), CY-QU (50:50) and CY-TR (25:75) with 341.86; 333.91; 341.05; 315.24 and 315.92 ml/g VS, respectively. However, the results revealed that when using 75% or 50% of co-substrate in the mixtures, the methane production rates did not present significant figures ($P < 0.05$). In addition, it was found that by increasing the amount of co-substrate from 25% to 75%, the mixtures increased their methane production between 20 and 26%. Likewise, when the amount of co-substrate was increased from 25 to 50%, methane production in the biodigesters improved between 16 and 20%. It was also found that all lignocellulosic residues used were good co-substrates to improve the digestion of CY manure. This is justified, since all the tests with a 50 and 75% concentration of co-substrate did not present significant figures ($P < 0.05$) in methane generation.

Synergistic effects of anaerobic co-digestion

In **Figure 2**, the results of the methane production and the synergy of the mono-digestion and co-digestion of the manure residues of CY and the agricultural residues of AM, QU and TR are presented. The mono-digestion data incorporated in this article has already been calculated in another article ([Meneses et al., 2021](#)) in which the same methodology of this research has been followed. This allows the individual performance of CY manure to be compared with the performance of substrate and co-substrate mixtures.

The value of the synergistic effect (α) in the mono-digestion of the manure of CY and the agricultural residues of AM, QU and TR was assumed as 1, since the values of α have been estimated from the mixing proportions and the individual yields. of the substrate and co-substrate. The manure mono-digestion methane yields of CY (ISR of 1) and CY (ISR of 2) were 211.07 and 174.27 ml/g VS, respectively. The co-digestion mixtures improved the methane production of the mono-digestion regardless of the ISR used. The increments

of improvement ranged from 8 to 42% for the ISR of 1 and between 50 and 96% for the ISR of 2. All comparisons between mono-digestion and co-digestion data showed significant differences ($P>0.05$), Except for the CY-TR (25:75) mixture of the ISR of 1 that did not present significant differences ($P<0.05$).

Figure 2 shows that the values of α , of the ISR of 1, for the mixtures of CY-AM (75:25), CY-QU (75:25), CY-TR (50:50) and CY-TR (75:25) ranged from 0.957 to 0.988, which suggests that the co-digestion of these mixtures is independent of the substrate and co-substrates used since the value of α was close to 1. However, the mixtures of CY-AM and CY-QU, with a content of 50 and 75% of co-substrate, had synergistic effects ($\alpha>1$) with values that ranged between 1.11 and 1.17. In the ISR of 2, the synergistic effects were much more promising since their values ranged between 1.13 and 1.50; except for the CY-QU (25:75) mixture where α was 0.975. In which case in this last mixture there were no synergistic effects, but neither were completely antagonistic effects, since α was close to 1.

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

3.4 Biogas composition of CY waste

In **Figure 3**, the biogas composition of the different combinations between cosubstrates and inoculum is shown. The results showed that the percentages of CH_4 and H_2S increased with the increase of inoculum; on the contrary, the generation of CO_2 decreased as the amount of inoculum increased. The mixtures composed of CY-QU were the ones that generated the highest percentage of methane regardless of the ISR used. In the trials with

an ISR of 2, the mixtures formed by CY-QU experienced improvements of 2.26-4.52% compared to the combinations of CY-AM and improvements of 2.68-5.68% compared to the biodigesters formed by CY-TR. In the same way, the biodigesters formed by CY-QU of the ISR of 1, generated higher percentages of CH₄. These differences were 9.89-10.58% and 12.84-14.59% with respect to the biodigesters formed by CY-AM and CY-TR, respectively.

In this study, CO₂ was between 29-42%, H₂S was almost negligible with percentages of 0.40-1.70%, on the contrary, the percentage of CH₄ was around 57-69%. The results obtained were similar to those of other investigations in the literature. Thus, for example, Garfí et al. (2011) in a study on the AD of guinea pig manure, obtained values of 63-65% for CH₄, and values of 0.19% for H₂S. Similarly, in another study on guinea pig manure, Garfí et al. (2011) obtained percentages of 59% and 0.15% for CH₄ and H₂S, respectively. Also, Ferrer. (2011), recorded values of 60% of the methane fraction in previous biomethanization studies.

3.5 Kinetic study

Estimation of kinetic parameters

The kinetic modelling parameters were calculated using the Richards, logistic, modified Gompertz equations and the Cone model presented in **Table 2**.

When analysing the $v_{\max}$ parameter, it is verified that the modified Gompertz models and the logistic equation are the ones that correlate the most and have the most similarity, since their $v_{\max}$ values are between 11.98-20.80 mlCH₄/g VS d and between 10.69-19.52 mlCH₄/g VS d, respectively. On the other hand, the methanogenic activity occurred at a faster rate in the transfer model, since, for this model, $v_{\max}$ ranged between 21.86 and 31.31 mlCH₄/g VS d. The model that differs the most from the rest of the models is that

of Richards, where the range of $v_{\max}$ was between 2.13 and 13.76 mlCH₄/g VS d. In contrast, with other investigations, the $v_{\max}$ values of this study are lower than those reported for food residues (28.03 to 174.63 mlCH₄/g VS d) (Li et al., 2018) and those reported for manure chicken (19.4 to 48.9 mlCH₄/g VS d) (Li et al., 2013). However, the $v_{\max}$ results of this study are similar to those reported for corn stubble (16.3 to 32.1 mlCH₄/g VS d) (Li et al., 2013) and higher than those of manure co-digestion of pig with sewage sludge (4.8–14.0 mlCH₄/g VS d) (Zhang et al., 2014).

Regarding the specific experimental methane yield, the results of the ISR of 2 are those that best fit the kinetic parameter M_e . Thus, the mean difference between the observed and predicted values are around 0.16-5.53% (modified Gompertz), 1.04-8.30% (transfer), 2.40-7.04% (equation logistics) and between 0.32-5.32% (Richards). These trends suggest that these models are suitable for representing the variables of the digestion process and estimating the AD yield and kinetic parameters. On the other hand, in the cone model, the differences between the predicted and observed values were more overestimated since they ranged between 5.85-18.95%. 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%.

Regarding the latency period (t_{lag}), many of the digesters experienced very short periods, even 0 days, which indicates the high bioavailability of organic compounds within the substrates (Ware and Power, 2017). In this sense, CY co-digestion experienced zero periods in its latency phase, except for CY-AM digesters (25:75), whose maximum periods were around 0.41 days (modified Gompertz); 0.98 days (transfer); 0.71 days

(logistics) and 0.42 days (Richards). The fact that there are low latency periods in these trials indicates that there was a rapid response of the microorganisms in their process of adaptation to the environment and conditions of the experiment. Furthermore, the low t_{lag} values demonstrated the simple nature of the substrates and co-substrates, and their high biodegradability. Finally, it is important to note that, compared to other authors who previously reported latency periods of 0.50 days (Zhang et al., 2017) and 12.3 days (Fang et al., 2014), the t_{lag} of some biodigesters of this study were relatively similar and even shorter.

Evaluation and comparison of the different kinetic models

According to **Figure 4**, the kinetic model that has the highest correlation coefficient r^2 (0.992-0.999) and the lowest RMSE (1.37-10.04 mlCH₄/g VS) is the transfer model. Similarly, the cone model fit the data quite well with an r^2 (0.978-0.999) and an RMSE of (1.16-8.85 mlCH₄/g VS). While the model of the logistic equation is the one that best adjusts 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 mlCH₄/g VS) respectively. On the other hand, the modified Gompertz and Richards models have a lot of similarity yes. Thus, 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 mlCH₄/g VS); while in the Richards model r^2 is between (0.982-0.999) and RMSE between (2.16-11.33 mlCH₄/g VS). This similarity between these two models is due to the fact that 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), when they described the sigmoidal growth of the curves, they had a higher RMSE.

On the other hand, in the transfer model there was not a total convergence between the observed and predicted values when the non-linear regression was performed. The fact that there was no convergence for the entire duration of co-digestion meant that there were no predicted values in all the biodigesters tested. In this sense, this model did not provide all the information necessary for its correct evaluation and evaluation of the data.

It should be noted that the suitability and precision of the models always vary considerably depending on the experimental conditions, the operating parameters, as well as the origin of the inoculum and the type of substrate used (Zhen et al., 2015). In this study, of all the proposed models, the cone model best adjusted to the real evolution of methane production. Similarly, El-Mashad (2013) demonstrated the cone model makes a better realistic simulation of experimental methane yields. The fact that the cone model is the most suitable for making the best predictions on methane production is very interesting since many studies have traditionally considered the Gompertz model to be the most suitable (Zhen et al., 2014; Lu et al., 2014; Kacprzak, Krzystek et al., 2012). On the contrary, other authors (Pitt et al., 1999) have considered that the cone model does not adequately model the results of methane production. The low credibility in the cone model, despite its high precision, may be due to the little familiarity of many authors with this model (Zhen et al., 2015).

4. Discussion

4.1 Effect of ISR on biomethane potential and biodigester stability

In the current study, the results showed that methane yields increased at a higher ISR, and are in agreement with previous studies in which different substrates have been used (Silva et al., 2020; Córdoba et al., 2018; Raposo et al., 2009; Wei et al., 2014). An optimal ISR in the biodigester is considered to contain the balanced amount of anaerobic

microorganisms for the digestion of primary and intermediate products (Eskicioglu and Ghorbani, 2011). Furthermore, an adequate inoculum can increase the degradation rate, improve biogas production, shorten the start-up time, and make the digestion process more stable (Quintero et al., 2012). However, determining the optimal values of an ISR is not easy, 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 and concluded that their results presented a slight variation in higher proportions. Caillet et al. (2019) in a sugarcane distillery wastewater biomethanization test determined that the methane production rate with an ISR of 1 was faster and higher than in the proportions of 2; 2.6 and 3.9. 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 the relatively low gas production of the substrate (Caillet et al., 2019), a low ISR could cause an overload of the microbial community, as has already been shown in several studies. previous (Polizzi et al., 2017; Holliger et al., 2016). 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 make general statements about the performance of digestion.

In this study, the use of an ISR of 2 notably improved the biodegradability of the materials compared to an ISR of 1. Despite all this, more trials with different ISR proportions (greater than 2) are needed to fully evaluate the influence of the inoculum; especially since the co-digestion tests were carried out from easily degradable material (CY manure) and lignocellulosic material. In addition, the materials used are little known which means that there is little literature evaluating their energy potential.

4.2 Effect of co-digestion on biomethane potential and process stability

Co-digestion of CY manure with lignocellulosic residues, in general, constantly increased methane production. Co-digestion was very productive, despite the fact that traditionally, throughout the world, animal manure has been used as a mono-substrate in most biomethanization tests (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). The results obtained in this study were very similar to those of other authors and were corroborated with previous studies (Table 3). The best results ranged from 300-340 CH₄/g VS, which means that they correspond to an average methane production. According to Velázquez et al. (2018), the low methane productions range between 150 and 300 CH₄/g VS, the average productions between 300 and 450 CH₄/g VS and the high productions are higher than 450 CH₄/g VS.

CY manure mono-digestion production was low (around 170-211 CH₄/g VS) compared to previous studies of cow, pig, and poultry manure that ranged from 238, 271, and 328 ml/g VS, respectively (Meneses & Velázquez, 2020). The low production of methane 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 give an animal diet quite different from that of other climates and conditions (Alvarez and Lidén, 2009). The type of diet of the animal can influence so that the content of the CY manure has a lower content of proteins and lipids, and a greater amount of difficult to digest material (Alvarez & Lidén, 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 ranges of methane (260-276 ml/g VS). Low efficiencies can be attributed to a higher content of lignin or other recalcitrant carbon in the composition of the biodigester (Ebner et al.,

2016). Ma et al. (2020), concluded that for a maximum improvement of the methane yield of pig manure and cow manure, 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 the proportions of the co-substrates, in the co-digestions, vary greatly between different studies (Andriamanohiarisoamanana et al., 2018; Zahan et al., 2018).

The synergistic effects were closely related to methane production; therefore, the biodigesters with a greater amount of co-substrate had a greater synergistic effect and greater yield. The biochemical potential of methane as well as the synergy are directly related to the composition of the substrate (Astals et al., 2014). The composition of the substrate determines the efficacy of the microbial population, which in turn greatly influences biogas yield, long-term process stability, and solids degradation rate (Castro et al., 2018). On the other hand, the presence of antagonistic effects in some biodigesters (CY-TR (75:25; CY-QU (75:25)) is due to the fact that in this study the co-digestion of binary mixtures was carried out, since when there are mixtures of three and four substrates, greater synergy effects are achieved than in mixtures of two substrates (Baquerizo et al., 2016). Finally, in biodigesters that present antagonism, binary mixtures have not been able to provide all the nutrients and trace elements necessary so that microorganisms have a higher methanogenic activity (Pagés et al., 2014).

Anaerobic digestion (AD) of animal manure and lignocellulosic residues is gaining greater interest as a result of its wide availability, optimal physicochemical

characteristics, high methane potential, and absence of conflict with the human food chain compared to energy crops (Naik et al., 2010).

Conclusions

This study evaluated methane production by anaerobic codigestion of guinea pig manure 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 was shown to be more suitable for manure codigestion of CY. Specifically, an ISR of 2 resulted in methane yields of 341.86 ml CH₄/g VS for the CY:AM biodigester (25:75). The influence of the co-substrates was notable in methane production, since improvements between 20 and 26% were obtained when the co-substrate concentration was increased from 25 to 75%. Finally, the results of the kinetic modeling concluded that the transfer and cone models are the most suitable to simulate the cumulative biogas and methane production curve, since they provided an r^2 of 0.999. However, in the transfer model, not all the data converged between the observed and estimated values, especially in the CY-AM (50:50) and CY-QU (50:50) biodigesters.

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Compliance with ethical standards

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FIGURES

Figure 1. Daily and cumulative profiles of CH₄ production as a function of time, for trials with different IRS.

Figure 2. 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 et al. (2021)).

Figure 3. Percentages of CH₄, CO₂ and H₂S from CY manure biogas

Figure 4. LSD (Least significant difference) intervals of the analysis of variance at the 95% confidence level for the comparison of the RMSE, the r^2 of the different models applied to the co-digestion of CY manure.

TABLES

Table 1. Characterization of substrates, co-substrates and inoculum

Parameters	Units	Substrates	Co-substrates			Inóculo
		CY	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)

Table 2. Kinetic parameters of methane from guinea pig manure co-digestion

Model	Parameters	ISR of 2									ISR of 1								
		CY-AM			CY-QU			CY-TR			CY-AM			CY-QU			CY-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	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
	v_{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
	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
	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	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
	v_{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
	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
	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	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
	v_{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
	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
	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	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
	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	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
	v_{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
	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
	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

NOTE: The (*) means that for these mixtures the model does not converge.

Table 3. 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)
CY-AM	25:75	341,9	Data from this study
CY-QU	25:75	341,1	Data from this study
CY-TR	25:75	315,9	Data from this study

Note: 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).

Figures

Figure 1

Daily and cumulative profiles of CH₄ production as a function of time, for trials with different IRS.

Figure 2

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 et al. (2021)).

Figure 3

Percentages of CH₄, CO₂ and H₂S from CY manure biogas

Figure 4

LSD (Least significant difference) intervals of the analysis of variance at the 95% confidence level for the comparison of the RMSE, the r² of the different models applied to the co-digestion of CY manure.