Effect of the co-digestion of agricultural lignocellulosic residues with manure from South American camelids

Article *in* Biofuels Bioproducts and Biorefining · January 2021

DOI: 10.1002/bbb.2177

Orlando Meneses Quelal
Universitat Politècnica de València

B. Velázquez-Martí
Universitat Politècnica de València

Juan Alberto Gaibor
Universidad Estatal de Bolívar

Zulay Niño
Universidad Regional Amazónica IKIAM

Original Article

Biofpr
Biofuels Bioproducts & Biorefining

Effect of the co-digestion of agricultural lignocellulosic residues with manure from South American camelids

Orlando Meneses-Quelal, Borja Velázquez-Martí, Departamento de Ingeniería Rural y Agroalimentaria, Universitat Politècnica de València, Valencia, Spain
Juan Gaibor-Chávez, Departamento de Investigación, Centro de Investigación del Ambiente, Universidad Estatal de Bolívar, Guaranda, Ecuador
Zulay Niño-Ruiz, Laboratorio de Biomasa. Biomass to Resources Group, Universidad Regional Amazónica Ikiam, Tena, Ecuador

Received September 19 2020; Revised October 31 2020; Accepted November 23 2020;
View online at Wiley Online Library (wileyonlinelibrary.com);
DOI: 10.1002/bbb.2177; *Biofuels, Bioprod. Bioref.* (2020)

Abstract: This study aims to evaluate the effects of the co-digestion of agricultural residues with manure from camelids from the Andean zone. Different combinations of llama manure (LM) and vicuñas (VM) were made with amaranth (AS), quinoa (QS), and wheat (WS) residues. They were fermented using sewage sludge as inoculum. The co-digestion was evaluated under mesophilic conditions for 40 days. The ratios of volatile substances of substrate / co-substrate evaluated were 0:100; 25:75; 50:50, 75:25, and 100:0. Two substrate / inoculum ratios (SIR 1:1 and SIR1:2) were also evaluated. The results indicate that the maximum methane accumulation rate is obtained in SIR 1:1 for a VM-AS ratio (25:75) with 540 mL/g volatile solid (VS). In general, the results did not increase with the increase in inoculum; rather, the tendency to improve methane yield is associated with an increase in the amount of agricultural residues, mainly AS. Regarding the kinetic modeling, the transfer model is the one that best adjusted the predicted values to those observed with an r^2 between 0.991 and 0.999, and an RMSE value between 2.06 and 13.62 mL/g (volatile solid) VS. Finally, all the trials presented synergistic effects in their co-digestion except the digesters formed by LM-AS, LM-QS and LM-WS of SIR 1:2. These presented antagonistic effects in which the addition of the co-substrate generated competition with the substrates, reducing methane production. © 2021 Society of Chemical Industry and John Wiley & Sons, Ltd

Key words: biogas; co-digestion; camelids; fermentation; methane; waste; synergy

---

Correspondence to: Borja Velázquez-Martí, Departamento de Ingeniería Rural y Agroalimentaria. Universitat Politècnica de València. Camino de Vera s/n, 46022 València, España. E-mail: borvemar@dmta.upv.es

SCI

Introduction

The Andean area of South America is an economically depressed area where the energy supply is deficient.[1] Furthermore, in the past year the supply of gas and fuel has become more expensive. This has led to the need to take advantage of all available resources efficiently to improve living conditions with the search for more competitive alternatives at the environmental, energy, economic and social level.[2] The native resources of the area can be taken advantage of to produce biogas from anaerobic digestion (AD); however, AD has been underused in this area due to ignorance of its potential. This means that there is a need to study the energy resources of the area to evaluate their potential and transform them into an engine of development in rural areas.[3]

The Andean communities are eminently agricultural; their development is based mainly on the livestock and production of typical crops of the area.[4] Livestock in rural and peasant areas is characterized by camelid grazing (CM) as the main means of subsistence.[5] The main CM of South America belong to the Lamini tribe and are divided into four species of which two are domestic, the llamas (*Lama glama)* and the alpacas (*Vicugna pacos*), and two are wild, guanacos (*Lama guanicoe*) and vicuñas (*Vicugna vicugna*).[6] Camelid grazing resists the adverse environments of the Andean highlands, such as cold and altitude, which makes the economic production of other substitute livestock species difficult.[7] These adverse circumstances have made the CM constitute an important source of economic income for the livestock sector, as they provide products such as fiber, meat (jerky), skin (tacllas), milk, manure (fuel), and leather.[8] Furthermore, many farmers depend on their own agricultural production as a primary source of food and food security.[9] This agriculture is based on the production of typical crops of the area such as amaranth, quinoa, wheat, etc. According to FAOSTAT[10] in the Andean areas of Ecuador, 2048 and 3149 ha of quinoa and wheat crops were cultivated, respectively, which makes these crops the basis of their food diet.

Agricultural and livestock activities in the region generate large amounts of agricultural residues that have not yet been used effectively. This is waste that could provide energy (in the form of biogas), avoiding the use of local biomass (deforestation).[11] The use of CM manure, mainly llama manure (LM), due to its high content of volatile solids and its high content of nitrogen and phosphorus, would make it an ideal raw material for the production of methane.[12] Similarly, vicuña manure (VM) is complementary to other camelid manure as it is used by the local inhabitants as a biofuel.[13] In general, in rural communities it is very common to use dried animal manure for cooking, because it serves as a substitute for firewood.[11] Camelid manure is generally easy to manage. Domestic camelids defecate in stables, and wild camelids, despite living free, defecate in well defined places. This makes it possible for farmers to carry manure from anywhere to the fermenter using trucks with manual or mechanical shovels.[14,15] Similarly, residues of amaranth straw (AS), quinoa straw (QS), and wheat straw (WS) could be used as co-substrates in the digestion of CM manure. Many agricultural residues in rural communities are not properly managed, as they are burned after each harvest.[16] The transformation of agricultural waste into biogas would not only provide energy benefits; it would imply the generation of digestate as a fertilizer for crops,[17] and the reduction of environmental pollution through a more efficient management of waste.[18,19]

The use of monosubstrates in AD could have problems of insufficient nutrients such as carbon and nitrogen.[20] However, anaerobic co-digestion of different materials would improve the efficiency of simple digestion.[21] Co-digestion could be the most cost-effective way to balance nutrients (C/N ratio, macro- and micronutrients) and reduce the accumulation of inhibitors / toxic compounds that prevent improved biogas production.[22] In this way, with the co-digestion of LM, VM manure with the AS, QS and WS residues, mixtures could be obtained that correct the inhibitory effects between agricultural and livestock residues. Many studies have focused on the co-digestion of manure and crop residues.[23,24] However, not all types of manure and agricultural crop residues have been addressed. This creates a scientific gap in the study of residues from Andean agriculture and livestock.

This research assesses the energy potential of totally new materials, becoming the starting point for future research on either a pilot or industrial scale. In this work a chemical characterization of the new materials is approached. The energy potential of biogas is evaluated, through the biochemical potential of methane (BMP) both for the monodigestion of individual materials and for the co-digestion of mixtures between CM manure and agricultural residues of AS, QS and WS. In addition, the optimal relation between the main substrate and the co-substrate is analyzed. Finally, the kinetics, the synergistic and antagonistic effects, and the relation between theoretical and experimental performance are determined.

Materials and methods

Substrates, co-substrates and inoculum used

Pre-treatment and conservation of materials

The evaluated materials were divided into substrates and co-substrates. Thus, llama manure (LM) and vicuña manure

(VM) were used as substrates, and lignocellulosic residues of Andean character, such as amaranth straw (AS), quinoa (QS), and wheat (WS) were used as co-substrates. The LM was collected from the rural communities of Guaranda, Ecuador, and the VM was collected from the plains near the Chimborazo volcano (latitude 1° S, longitude 78° W, at an altitude of approximately 4000 m above mean sea level). The lignocellulosic residues of AS, QS, and WS were collected from the farms of the State University of Bolívar. The main substrate samples were collected, and immediately stored in a refrigerator at approximately 6 °C in polyethylene bags for preservation purposes. The co-substrates, on the other hand, were dried at room temperature, which varied between 10 °C (at night) and 25 °C (at day) for 7 days. Once dry they were cut and ground, using a universal cutter mill, into small particles less than 3 mm in size and then kept at 6 °C.

The inoculum used for all the tests was collected from the urban wastewater treatment station (EDAR) of the city of San Miguel de Ibarra (Ecuador). It was extracted from the primary sludge of the anaerobic digester operating under mesophilic conditions (temperature between 35–37 °C approximately). Following the recommendations of Hafner and Astals,[25] the inoculum was incubated at 37 °C for 5 days before starting the experiments to reduce endogenous CH_4 production.

Characteristics of materials

Substrates, co-substrates, and inoculum were characterized according to their total and volatile solids (VS) content, and their elemental composition. The total solid (TS) and VS content of the substrates and co-substrates were determined in accordance with the UNE-EN 18134 and UNE-EN ISO 18123 standards. The VS and TS of the inoculum were determined following the American Public Health Association method 2540A-2540G.[26] Elemental analysis of C, H, N, O, and S was performed using a Vario Macro Cube elemental analyzer. Finally, the pH of all the samples tested was measured using the Hach HQ 40D portable meter.

The characteristics of the substrates tested in this study, including the co-substrates and inoculum, are shown in Table 1. All parameters were determined in triplicate.

Experimental methodology

BMP assays of anaerobic digestion

In this study, biochemical methane potential (BMP) assays were performed to evaluate the differences in methane production from camelid residues (LM and VM) when combined with lignocellulosic crop residues (AS, QS, WS). The BMP tests were performed under mesophilic conditions of 38 °C in 311 mL digesters with a working volume of 186 mL. The C/N ratios varied depending on the mixing ratio between the substrate and co-substrate (Table 2). Two substrate ratios were applied: substrate to inoculum ratio (SIR) of 1:2 (g/g VS) and 1:1 (g/g VS). In the SIR (1:1) all the digesters were started at a concentration (mixture of substrate and co-substrate) of 9 g VS/l, while in the SIR (1:2) the digestion started with a concentration of 12 g VS/l. All batch digesters were run in triplicate according to the suggestions of Holliger *et al.*[27] As the bacterial inoculum could also contain biodegradable material, the gas that would originate from it was considered.[28] In this way, three additional blank (control) trials were performed, containing only inoculum.[29,30]

Experimental design

The experimental design of the present study comprises a five-factor mixture design based on the amount of VS (Table 2). Each mixture (Mi and Ni) is composed of pure fractions and binary mixtures of a substrate (LM, VM) and a co-substrate (AS, QS, and WS). The digesters M1–M4 and

Table 1. Main characteristics of the substrates, co-substrates, and inoculum.

Parameters	Units	LM	VM	AS	QS	WS	IN
TS	%	50.6 (1.0)	57.4 (0.5)	88.2 (0.1)	87.0 (0.1)	92.6 (0.1)	3.9 (0.1)
VS (% TS)	%	75.6 (0.4)	72.2 (1.6)	74.8 (0.3)	78.4 (1.5)	77.2 (0.9)	58.5 (0.5)
Ashes	%	25.5 (0.3)	27.6 (1.8)	8.4 (0.1)	30.3 (1.4)	11.8 (0.1)	55.6 (0.2)
N	%	2.2 (0.1)	2.6 (0.4)	3.3 (0.9)	2.2 (0.9)	1.7 (0.7)	3.4 (0.1)
C	%	40.7 (1.2)	40.3 (1.1)	42.9 (1.9)	30.7 (1.7)	48.9 (1.6)	25.0 (1.2)
H	%	4.5 (0.2)	5.1 (0.3)	6.5 (0.8)	6.4 (0.9)	6.1 (0.5)	2.1 (0.1)
O	%	27.0 (1.2)	23.9 (1.1)	38.6 (1.9)	29.8 (1.7)	31.1 (1.6)	12.9 (1.2)
S	%	0.2 (0.0)	0.4 (0.0)	0.2 (0.0)	0.6 (0.1)	0.5 (0.0)	0.7 (0.0)
C/N	-	17.4 (0.9)	15.4 (0.7)	12.9 (0.8)	12.0 (0.9)	29.6 (0.8)	7.5 (0.7)

LM, Llama manure; VM, Vicuña manure; WS, Wheat straw; AS, Amaranth straw; QS, Quinoa straw; IN, inoculum; WWTP sludge. The data in brackets are the standard deviations.

Table 2. Mixing compositions and experimental setups for co-digestion assays.

SIR 1:1		SIR 1:2		C/N	Mixing ratios				
Mixture	pH	Mixture	pH		LM %VS	VM % VS	AS % VS	QS % VS	WS % VS
M1	7.49	N1	7.48	17.41	100	0	0	0	0
M2	7.80	N2	7.78	15.38	0	100	0	0	0
M3	8.02	N3	7.99	12.00	0	0	100	0	0
M4	7.50	N4	7.49	29.61	0	0	0	100	0
M5	7.27	N5	7.03	12.93	0	0	0	0	100
M6	7.41	N6	7.40	16.00	75	0	25	0	0
M7	7.40	N7	7.53	19.43	75	0	0	25	0
M8	7.36	N8	7.37	16.10	75	0	0	0	25
M9	7.46	N9	7.55	14.82	0	75	25	0	0
M10	7.58	N10	7.61	16.81	0	75	0	25	0
M11	7.70	N11	7.71	14.91	0	75	0	0	25
M12	7.33	N12	7.33	14.63	50	0	50	0	0
M13	7.49	N13	7.55	21.95	50	0	0	50	0
M14	7.33	N14	7.38	14.92	50	0	0	50	50
M15	7.40	N15	7.76	14.12	0	50	50	0	0
M16	7.45	N16	7.59	18.96	0	50	0	50	0
M17	7.60	N17	7.68	14.36	0	50	0	0	50
M18	7.40	N18	7.41	13.30	25	0	75	0	0
M18	7.39	N19	7.42	25.22	25	0	0	75	0
M20	7.29	N20	7.31	13.86	25	0	0	0	75
M21	7.38	N21	7.40	13.21	0	25	25	0	0
M22	7.39	N22	7.62	22.52	0	25	0	25	0
M23	7.57	N23	7.64	13.71	0	25	0	0	25

% VS: percentage of each individual fraction within the volatile solids (VS) content of the mixture.
LM, llama manure; VM, vicuña manure; AS, amaranth straw; QS, quinoa straw; WS, wheat straw.

N1–N4 represent the individual fractions of each factor, whereas the mixtures M5–M23 and N5–N23 represent the binary combinations. The design allows the synergistic or antagonistic interactions to be evaluated according to the individual or mixed fractions supplied in each digester.

Measurement and characterization of biogas

Biogas production was measured daily for 40 days. Measurements were performed manually using the manometric method to quantify the pressure in the headspace of the biodigesters.[29] The pressure was determined using the Delta OHM HD 2124.2 pressure gauge, adapted to a 100 bar sensor (Delta TP 704). The biogas volume of each biodigester was calculated using Eqn (Error! Reference source not found.). The biogas was normalized to standard conditions (25 °C and 1 atm) and expressed as ml/g VS:

$$V_{BIOGAS}\left(STP\right)=\frac{P_{ABS}V_{G}T_{STP}}{P_{STP}T_{1}} \qquad (1)$$

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).

The determination of the biogas components (CH_4, H_2S, CO_2, and O_2) was carried out with the Geotech Biogás GA-5000 analyzer. The BMP tests were terminated when the amount of methane was undetectable, and the volume extracted in each digester was less than 5% of the accumulated volume.

Theoretical methane potential

The theoretical methane potential (γ_{th}) of all the residues was determined under standard conditions (STP) – that is, at a temperature and pressure of 25 °C and 1 atm, respectively. The γ_{th} was estimated through its elemental composition and the stoichiometry of the degradation reaction, Eqn (2), considering Buswell's formula and Boyle's equation, Eqn ((3).[31–33]

$$C_aH_bO_cN_d + \left(\frac{4a-b-2c+3d+2e}{4}\right)\mathrm{H_2O} \rightarrow \left(\frac{4a+b-2c-3d-2c}{8}\right)CH_4 + \left(\frac{4a+b+2c+3d+2e}{8}\right)CO_2 + \mathrm{d}NH_3 + \mathrm{eH_2S} \tag{2}$$

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

Buswell's formula does not differentiate between degradable and non-degradable material because it assumes that all donated electrons are used exclusively for metabolic energy – that is, cellular synthesis is neglected.[34]

Biodegradability and synergistic and antagonistic effects of substrates

The biological efficiency (ε) of the anaerobic process was determined by Eqn (4).[35]

$$\varepsilon = \frac{\gamma_{(\exp)}}{\gamma_{(teo)}}.100\% \tag{4}$$

The synergistic and antagonistic effects can be obtained as the relationship between the experimental performance (γ_{exp}) and the weighted performance (γ_{pond}) in Eqn (5). The experimental performance is the result of the BMP tests for each mixture of the co-digestion, and the weighted performance (γ_{pond}) is the weighting between the experimental performance obtained by monodigestion of the substrate and co-substrate with their respective VS.[36,37]

$$\alpha = \frac{\gamma_{\exp}}{\gamma_{pond}} \tag{5}$$

The result of α indicates:

$\alpha > 1$: the mixture has a synergistic effect on the final production;

$\alpha = 1$: substrates function independently of the mixture of substrate and co-substrate;

$\alpha < 1$: the mixture presents antagonistic or competitive effects in the final production.

The (γ_{pond}) can be estimated using Eqn (6):

$$\gamma_{pond} = \frac{\gamma_{sp}.\lambda + \gamma_{cs}.\beta}{\lambda + \beta} \tag{6}$$

where, γ_{sp} refers to the production obtained from the digestion of the main substrate individually. On the other hand, γ_{cs} is the production obtained from the digestion of the different co-substrates separately. Furthermore, the sum of the λ and β values corresponds to the VS fractions added by the main substrates and the co-substrates.

Kinetic fit models

Methane production was modeled by fitting the data with five kinetic models through non-linear regression, using the statistical package Statistica 10. The feasibility of the fit was evaluated considering both the residual sum of squares (RMSE) and the values of the coefficient of determination (r^2).

The exponential models of two phases, logistics, transfer (reaction curve) and modified from Gompertz[31] and the Cone and Richards models[38,39] were used, which are described in Eqn ((7)–Eqn ((11).

$$M = M_{\mathrm{e}}.\exp\left\{-\exp\left[\frac{v_{\max} * \mathrm{e}}{M_{\mathrm{e}}}\left(\mathrm{t}_{lag} - \mathrm{t}\right) + 1\right]\right\} \tag{7}$$

$$M = M_{\mathrm{e}}\left\{1 - \exp\left[-\frac{v_{\max}}{M_{\mathrm{e}}}\left(\mathrm{t} - \mathrm{t}_{lag}\right)\right]\right\} \tag{8}$$

$$M = \frac{M_{\mathrm{e}}}{1 + \exp\left[\dfrac{4v_{\max}\left(\mathrm{t}_{lag} - \mathrm{t}\right)}{M_{\mathrm{e}}} + 2\right]} \tag{9}$$

$$M = \frac{M_{\mathrm{e}}}{1 + \left(\mathrm{k.t}\right)^{-\mathrm{n}}} \tag{10}$$

$$M = M_{\mathrm{e}}\left\{\begin{matrix} 1 + \mathrm{d}.\exp\left(1 + \mathrm{d}\right) \\ \times \exp\left[\dfrac{v_{\max} * \mathrm{e}}{\mathrm{M_e}}\left(1 + \mathrm{d}\right)\left(1 + \dfrac{1}{\mathrm{d}}\right)\left(t_{lag} - 1\right)\right]\end{matrix}\right\}^{\frac{1}{\mathrm{d}}} \tag{11}$$

where M is the specific methane yield accumulated at time t (ml CH_4 g^{-1} VS), M_e is the maximum methane yield (ml CH_4 .g^{-1} VS), t is the digestion time (d), k is the first order decomposition constant (d^{-1}), $\nu_{\max}$ is the maximum specific rate of methane production (ml CH_4 .g^{-1} VS. d^{-1}), is the t_{lag} dormancy or latency time (d), and n is the order of the factor.

Results

Characterization of the raw material

Main substrates used

The characterization data of the llama and vicuña manure were analyzed with respect to the VS/TS and C/N ratio and are presented in Table 3. The camelid manure (CM) had a solids content between 50–57%, which made digestion dry. Nasir *et al.*[40] consider that the process can be considered dry digestion if the solids content is between 25% and 40%, while a solid content below 15% makes the digestion wet.

The VS/TS ratio is a parameter that allows evaluating the organic content in substrates.[41] In general terms, substrates with a higher VS/TS ratio contain a high content of biodegradable material and are more suitable to produce biogas.[42–44] Similarly, a higher C/N ratio can balance the carbon and nitrogen of the raw material efficiently for a better optimization of methane production.[45] Table 3 compares LM and VM residues with other types of manure residues from the literature. Generally, the types of manure most used in the production of biogas have been cow, pig, and poultry manure;[46] this is due to the fact that their average VS/TS ratios are 80.37%, 74.75%, and 62.19%, respectively. To a lesser extent, other authors have also considered that llama manure has enormous potential in biogas production;[11,12,47,50] as its average VS/TS ratio is 68.80%.

Table 3. Comparison of camelid manure with other types of manure in the literature.

Manure	VS (%)	TS (%)	VS/TS (%)	C/N	References
Cow	13.64	16.12	84.62	16.10	48
	11.58	14.40	80.42	9.00	49
	13.39	17.60	76.08	19.07	50
Poultry	28.29	40.50	69.85	10.00	51
	17.47	26.70	65.43	11.52	52
	18.32	35.71	51.30	-	53
Pig	24.80	31.80	77.99	9.80	54
	12.04	15.88	75.82	8.13	55
	15.85	22.50	70.44	-	56
Llama	40.93	67.00	61.09	-	47
	41.33	58.3	70.90	-	11
	44.27	59.5	74.40	-	12
	33.69	17.6	76.10	19.01	50
LM	38.25	50.60	75.60	17.40	Data from this study
VM	41.44	57.40	72.20	15.40	Data from this study

In this research the relationship obtained from VS/TS for LM and VM was 75.60% and 72.20% respectively, which indicates that it is a substrate that contains a high level of organic matter and, therefore, is suitable for the AD. Table 3 shows that the VS/TS ratio of the LM is greater than pig manure by 1.1%, and greater than poultry by 17.7%; while VM is higher than poultry manure by 19.9%.

Regarding the C/N ratio, in this study, the results were the following: LM (17.40) and VM (15.40). As can be seen, the results were very promising, since a C/N ratio in a range of 15–30 is optimal for biogas production.[42] Furthermore, the results of this study reveal that they are better than those of cow (14.72), pig (8.97) and poultry (10.76). The results were even much higher than the C/N ratio of food waste (14.6–15.4) obtained by Han and Shin.[57]

Co-substrates used

The organic fraction of the co-substrates presented very favorable values for AD. The tabulated data of WS, AS, and QS presented a VS/TS ratio of 77.0%, 75.0%, and 58.0% respectively. However, the results were lower than those in the literature, where values of 84.0%, 80.0%, and 88.0% were recorded for the WS, AS, and QS residuals respectively.[58,59]

Regarding the C/N ratio, the WS, AS, and QS residues presented values of 29.6, 12.9, 12.0, respectively. These results are very consistent with those of other scientific articles. Korai *et al.*[60] found values of 30.31 for the WS samples. Similarly, Minzanova *et al.*[61] registered values of 10.7 for AS materials.

Generation and methane potential from camelid waste (CM)

Comparison of SIR from BMP tests

Figures 1 and 2 show the temporal evolutions resulting from the accumulated methane production of the batch tests. Two tests are distinguished: first the influence of the inoculum is evaluated for a SIR1:1, and then the influence of the inoculum for a SIR1:2. The results demonstrated that methane production was higher at SIR1:2 for both monodigestion and co-digestion (Fig. 3). That the results have been better for a SIR1:2, is in accordance with the recommendations of the German VDI standard (Verein Deutscher Ingenieure).[62] The standard states that the use of a SIR1:2 can better balance the buffer capacity (pH value) and prevent inhibition in the biodegradation process during testing.[63] Similarly, Holliger *et al.*[27] also consider that the use of a SIR1:2 is adequate to reduce the formation of acids and avoid inhibition problems during the fermentation process.

The results of this study revealed that the individual fractions of the main substrates of LM and VM are influenced by

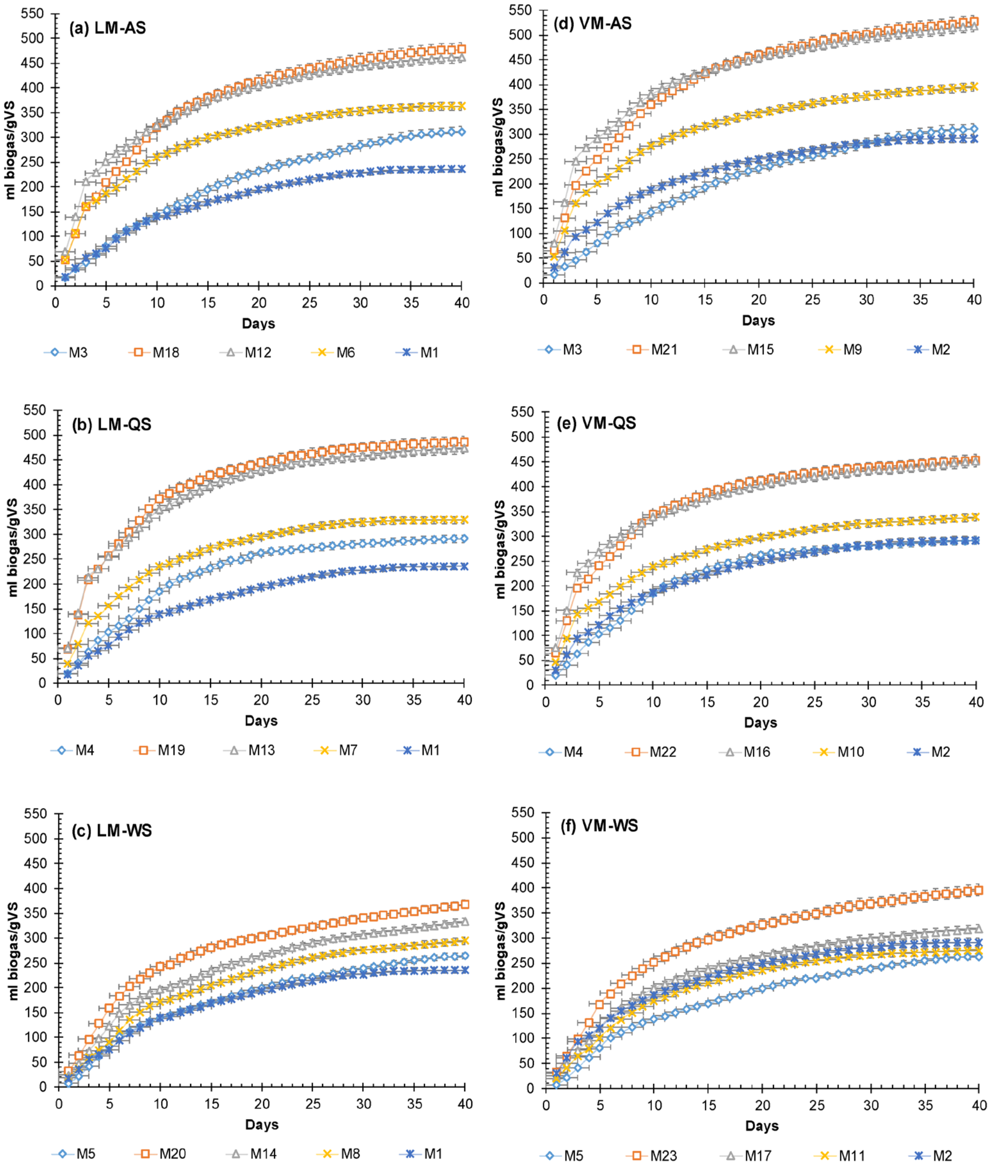

Figure 1. Cumulative profiles of CH_4 production as a function of time, for SIR1:1 assay. Note: LM = llama manure; VM = vicuña manure; WS = wheat straw; AS = amaranth straw; QS = quinoa straw. The tests M1–M5 represent the biodigesters of monodigestion and the tests M6–M23 represent the biodigesters of co-digestion.

the inoculum. Thus, for a SIR1:1, M1 and M2 produced a cumulative methane accumulation of 235 mL CH_4/g VS and 292 mL CH_4/g VS, respectively. The increase in the amount of inoculum to a SIR1:2 supposed that the digesters M1 and M2 improved their production at N1 = 377 mL CH_4/g VS and N2 = 300 mL CH_4/g VS, respectively. However, only N1 presented significant differences ($P < 0.05$) when the inoculum increased. Similarly, the individual fractions of the AS (M3), QS (M4) and M5 (WS) co-substrates had a similar behavior to the previous substrates. Thus, for a SIR1:1, the mixtures M3, M4, and M5 had a production of 310.68; 291.23, and 264.10 mL CH_4/g VS respectively; while for a SIR1:2, its production increased to N3 = 381 mL CH_4/g VS, N4 = 376 mL CH_4/g VS and N5 = 268 mL CH_4/g VS. Even though all the co-substrate mixtures improved their methane production with the increase in inoculum, only N3 and N4 showed significant differences ($P < 0.05$). The co-digestion tests, with a SIR1:2, also increased methane production with respect to the SIR1:1.

The methane results of the individual fractions of LM and VM were very competitive when compared with other types of manure reported in the literature. Thus, for example, the methane production of LM and VM was 2 and 1.5 times

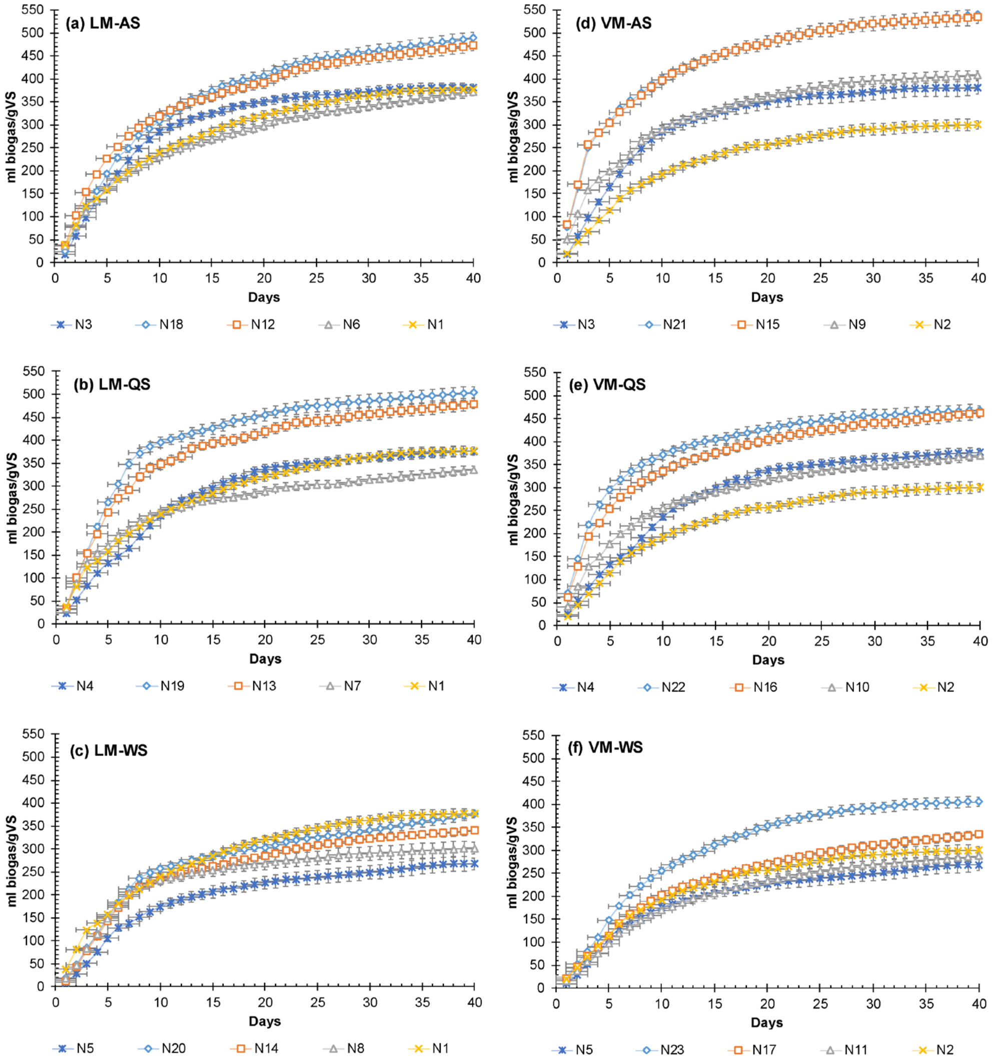

Figure 2. Cumulative profiles of CH_4 production as a function of time, for SIR1:2 assays. Note: LM = llama manure; VM = vicuña manure; WS = wheat straw; AS = amaranth straw; QS = quinoa straw. The tests N1–N5 represent the biodigesters of monodigestion and the tests N6–N23 represent the biodigesters of co-digestion.

the value obtained by Zhang *et al.*,[64] who studied methane production from pig manure. Li *et al.*[65] carried out a digestion study to produce methane from cow manure and obtained a production of 270.0 mL CH_4/g VS; however, the LM and VM values were 1.4 and 1.1 times more than the previous study. In another study, Wei *et al.*[66] conducted an investigation to obtain methane from poultry manure and obtained a production of 163.2 mL CH_4/g VS; however, the methane obtained by the LM and VM residues was 2.3 and 1.8 times more than the previous study. The data from this study have also been contrasted with other data that have been reported in various scientific articles, being higher or showing similar values.[67–72]

The co-digestion tests, with a SIR 1:2, also increased the methane production with respect to the SIR 1:1. Thus, the co-digestion of LM and VM with AS, QS and WS co-substrates (N6–N23 mixtures) improved methane production in a range of 1.37–9.32%, although only the N10 treatment presented significant differences ($P < 0.05$). Even though different SIR is recommended in the literature, these vary depending on the characteristics of the substrate and the inoculum.[73] For this reason, Lesteur *et al.*[74] recommend defining, for each substrate and inoculum, a proportion that guarantees the highest methane production.

Influence of co-substrates on the co-digestion of BMP assays

In this study, different combinations of substrates and co-substrates were tested to assess the methane potential of

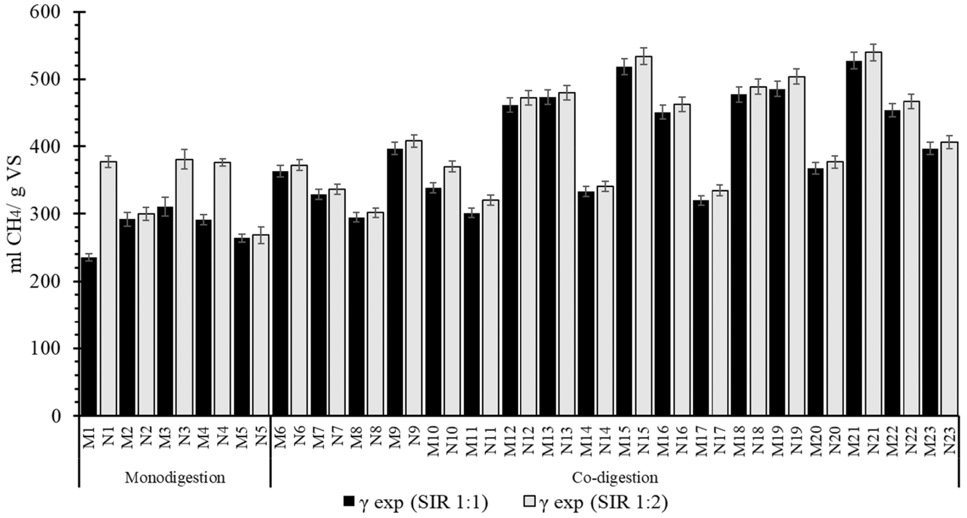

Figure 3. Influence of inoculum on methane production by comparing two SIRs (1:1 and 1:2). Note: The left part of the figure shows the variability of methane for monodigestion and the right part shows the variability of methane in co-digestion.

a wide range of mixtures and, more important, to identify mixtures that generate synergy in terms of higher methane yields. The co-digestion of organic waste involves the mixing of different materials in variable proportions. If all other factors, such as physical parameters, are kept constant, the methane yield (ml/g VS) and the percentage of VS degradation are functions only of the proportions used.[75] As expected, the co-digestion showed dependence on the mixing ratio of the digested co-substrates, improving significantly with respect to the individual substrates of LM and VM. The tests increased the methane yield in most of their mixtures, especially those with the highest concentration of AS and QS. It should be noted that the highest production was obtained in the mixtures that operated with concentrations of 50 and 75% of co-substrate (Fig. 4). The mixtures with WS, on the other hand, generated little methane. According to Korai *et al.*,[60] the biodegradation of some samples of agricultural waste, especially WS, usually affects the AD of some substrates. Certain effects are usually due to the hydrophobic bonds between lignin and hemicellulose, limiting the access of anaerobic microorganisms to the organic portion of the biomass.[76]

For a SIR1:1 the best methane results were obtained for the mixtures M21 and M15 with 527 and 519 mL CH_4/g VS, respectively. These results correspond to the co-digestion of VM with a mixture of 75% and 50% of AS. Mixtures M19 (486 mL CH_4/g VS) and M18 (477 mL CH_4/g VS) also produced high levels of methane. The above mixes corresponded to 75% of QS and AS. Methane increases in co-digestion represented improvements of 25% to 124% with respect to LM and improvements of 1 to 80% with respect to VM. All the mixtures showed significant differences, except those with mixtures of 50% and 75% of AS and QS.

In the mixtures operated with a SIR1:2, the methane production behavior was also more influenced by the effect of the high concentrations of AS and QS. Thus, mixtures N21 and N16 produced 540 and 534 mL CH_4/g VS, respectively. Similarly, mixtures of N19 (504 mL CH_4/g VS) and N18 (489 mL CH_4/g VS) produced high methane yields for 75% AS and QS. However, the increase in inoculum meant that the improvements in co-digestion were not as high as in the SIR1:1. In this case, improvements of 43% and 80% were experienced with respect to LM and VM. Despite the decrease in methane production, all the treatments showed significant differences, except the mixtures with 50 and 75% of AS and QS.

The improvements in methane production due to co-digestion of LM and VM are due to the fact that anaerobic co-digestion can increase the efficiency of the process due to a healthier balance of nutrients and carbon.[75,77,78] The fact that the mixtures increase the production of methane with the increase in the co-substrate concentration may be due to the fact that an increase in manure leads to an eventual accumulation of Volatile Fatty Acids (VFA), producing an acidification in the composition of the digesters.[31]

Biodegradability and synergistic effects

Figure 5 presents the results of the synergistic effects, biodegradability and percentage of methane from the different mixtures. In Fig. 5(a) it is observed that, for a SIR1:1, all the mixtures show synergistic effects ($\alpha > 1$) on

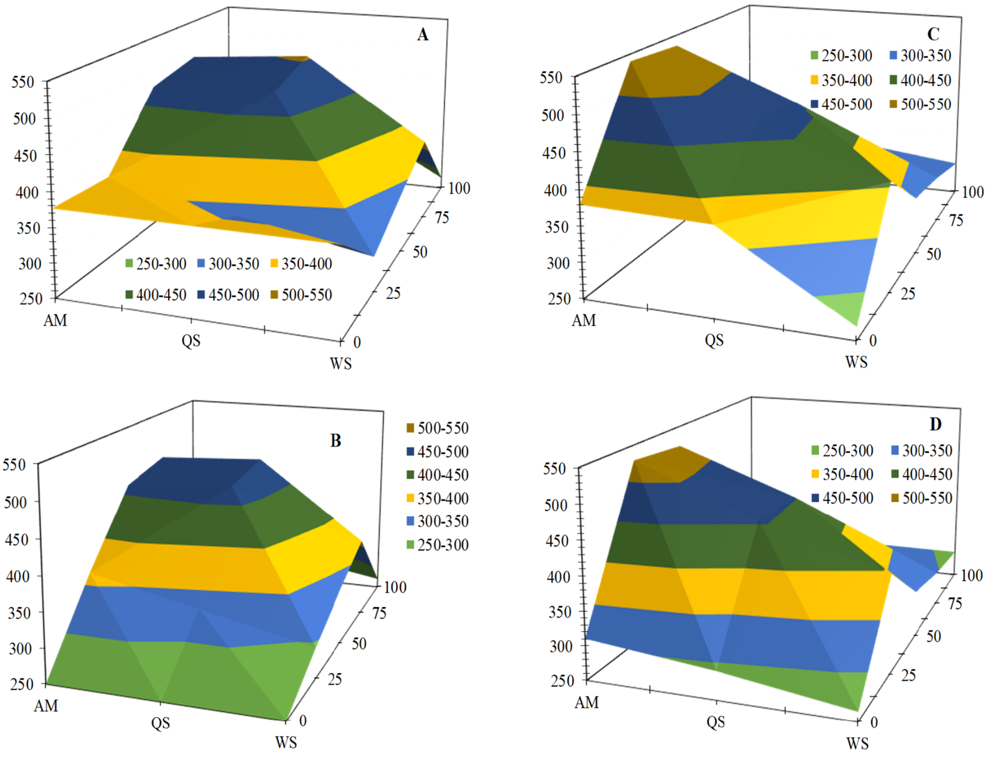

Figure 4. Mixing of substrates and methane potential through co-digestion of llama manure (VM) and vicuña manure (VM) with different agricultural co-substrates. Note: A = LM with SIR 1:2, B = LM with SIR 1:1, C = VM with SIR1:2, D = VM with SIR 1:1.

co-digestion. The α values oscillate in a range of 1.01–1.82. The highest values are recorded in the mixtures M21, M18, M15, and M12, which correspond to the digesters that had 50% and 75% AS. Similarly, biodegradability (ε) follows the same behavior as α – that is, for higher concentrations of AS and QS, their values are higher than those with lower concentrations of co-substrate. The synergy has been reflected in the increase in the CH_4 yield of some co-digestion mixtures, especially with the increase in the concentrations of the agricultural residue co-substrates.

For a SIR1:2 (Fig. 5(b)), not all mixtures exhibited synergistic effects; specifically, mixtures of the LM configuration that produced lower methane yield. According to Nielfa *et al.*,[36] the generation of less methane in co-digestion compared to monodigestion is evidenced due to the antagonistic effects of the mixture. Thus, for example, the mixtures N6–N8, N12–N14, and N18–N20 did not generate more methane than the monodigestion of the individual substrate LM, which caused antagonistic effects (α <1) to occur in theco-digestion of the aforementioned mixtures. However, with or without antagonistic effects, all the mixtures improved methane production over the SIR1:1 mixture. In contrast, mixtures of VM with AS, QS, and WS produced synergistic effects, which ranged from 1.08 to 1.74. The increase in inoculum also caused the mixtures to increase their ε. In this case, the biodegradability ranged between 51 and 95%.

Regarding the composition of the biogas, all the mixtures produced had a methane concentration higher than 50% for both SIR1:1 and SIR1:2. The mixtures with 75% and 50% AS, QS, and WS reached amounts greater than 60%, especially the M21 and N21 fractions whose content had 75% AS. The treatments with the highest methane production provided the highest methane values. However, no treatment presented significant differences.

Kinetics

Effects on latency (t_{lag})

All the kinetic models studied had a negative t_{lag}, except the transfer model. The digesters that experienced a t_{lag}, in the transfer model were those that were formed by the WS co-substrate. For example, in SIR1:1, the LM-WS mixtures generated t_{lag} between 0.42 days and 0.68 days, while those of VM-WS generated t_{lag} between 0.123 days and 0.557 days (Table 4). With the increase in inoculum (SIR 1:2), the t_{lag} remained negative in all models, except in the transfer model (Table 5). However, at SIR 1:2, the t_{lag} decreases

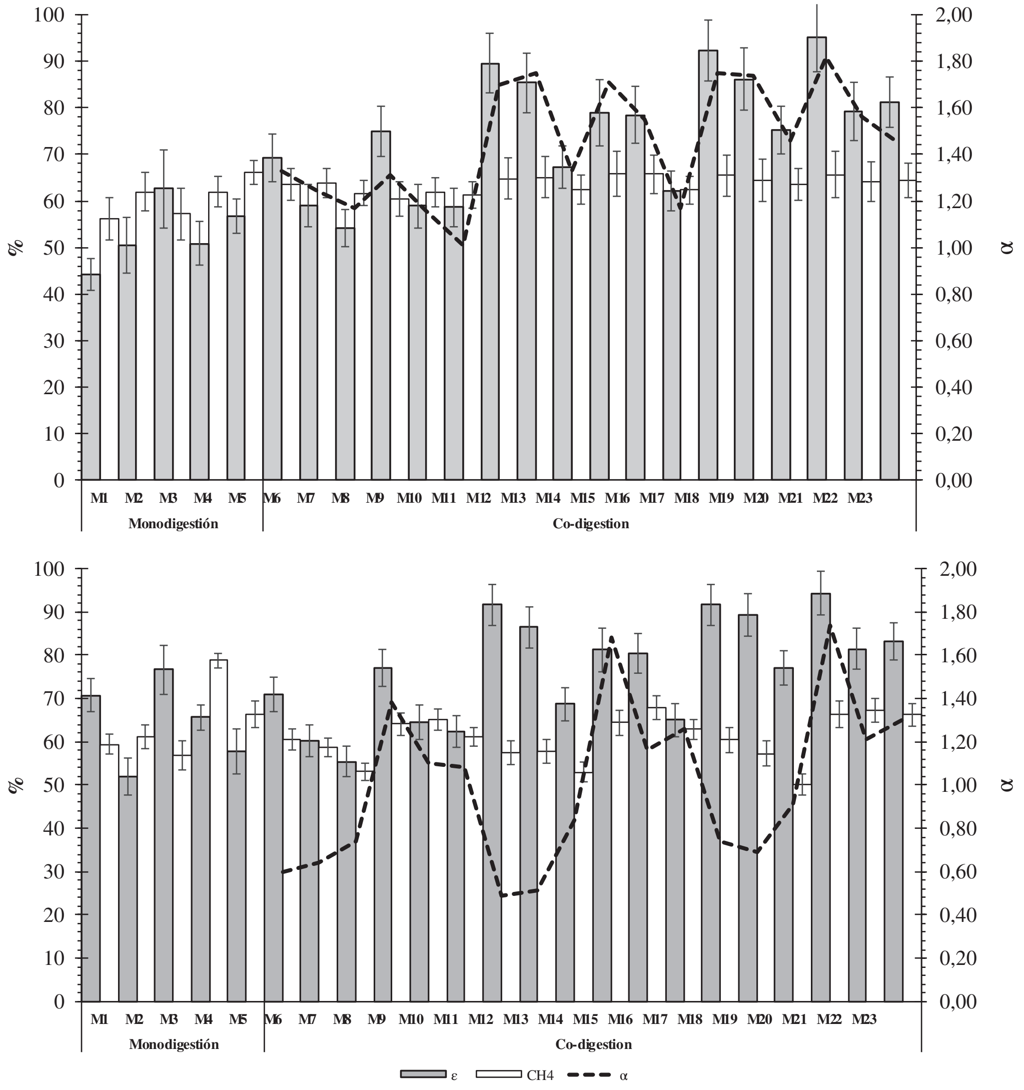

Figure 5. Evolution of methane, biodegradability and synergistic effects of co-digestion of llama and vicuña manure. Note: ε, is the biodegradability, α, is the synergistic effect of the mixture and CH_4 is the percentage of methane generated in the process.

relative to SIR 1:1. The fact that the t_{lag} was reduced with the increase in inoculum is due to the presence of activated sludge, which has a high content of organic matter for energy production.[79] In this sense, the introduction of sufficient active microorganisms in the digesters led to a direct initiation of methanogenesis without a measurable latency period. Boulanger *et al.*[32] showed that for SIR of 1:2 and 1:4, the latency is minimal and for SIR greater than 1:4 it is no longer interesting to measure the t_{lag}, as it would give values close to 0 and possibly negative values. In this case, the methane production curves with the least amount of inoculum experienced more sigmoid behavior than the curves with more inoculum, which were more oval.

Effects on hydrolysis and on the maximum rate of methane production (ν_{max})

The cone model was used to observe the behavior of the hydrolysis of organic matter, through the disintegration rate constant of the first order (k).[38] According to Labatut *et al.*,[34] the physicochemical characteristics, such as particle size, lignin content, or degree of crystallinity of the lignocellulosic matrix affect the kinetics of the hydrolysis stage. Furthermore, according to Brulé *et al.*,[80] if there are no inhibitory effects during digestion, the cumulative yields of methane or biogas generation usually follow a first-order accumulation pattern. Tables 4 and 5 show that many digesters experienced an

Table 4. Kinetic parameters of methane from camelid co-digestion SIR 1:1.

Mezcla	GOMPERTZ					TRANSFERENCE					LOGISTIC					CONE					RICHARDS					
	M_e	ν_{max}	t_{lag}	r^2	RMSE	M_e	ν_{max}	t_{lag}	r^2	RMSE	M_e	ν_{max}	t_{lag}	r^2	RMSE	M_e	k	n	r^2	RMSE	M_e	d	ν_{max}	t_{lag}	r^2	RMSE
M1	238.2	10.8	−2.2	0.988	0.72	251.6	18.9	−0.1	0.997	0.12	233.3	9.8	−2.8	0.979	1.26	303.4	0.08	1.1	0.998	1.24	239.1	0.03	0.3	−2.3	0.988	0.73
M2	290.6	14.1	−3.2	0.988	0.94	300.1	26.7	−0.7	0.998	0.15	286.2	12.5	−4.2	0.976	1.38	366.3	0.10	1.0	0.999	0.39	290.5	0.01	0.1	−3.2	0.988	0.96
M3	317.5	12.0	−1.4	0.991	1.19	358.4	18.6	0.1	0.998	0.35	304.9	11.5	−1.5	0.979	1.61	454.5	0.05	1.1	0.999	0.14	317.4	0.01	0.1	−1.4	0.991	1.19
M4	286.5	17.8	−0.5	0.997	1.11	297.5	30.5	0.6	0.997	0.23	282.3	16.6	−0.7	0.990	1.85	318.9	0.12	1.5	0.997	0.65	286.6	0.00	0.1	−0.5	0.997	1.12
M5	211.0	9.0	−3.0	0.977	2.24	221.4	16.4	−0.4	0.993	1.36	206.9	8.0	−4.1	0.961	2.72	284.2	0.07	1.0	0.996	0.82	211.4	0.00	0.0	−3.1	0.977	2.23
M6	358.1	19.3	−4.1	0.981	1.03	364.4	39.2	−1.3	0.996	0.47	354.6	16.5	−5.5	0.971	1.43	437.0	0.15	0.9	0.0995	0.82	*	*	*	*	*	*
M7	326.4	19.1	−2.7	0.988	0.60	333.2	37.3	−0.5	0.999	0.06	323.1	16.7	−3.7	0.978	1.02	380.8	0.15	1.1	0.998	1.09	326.4	0.00	8.5	−2.7	0.988	0.60
M8	289.4	13.4	−1.9	0.987	1.48	305.5	23.5	0.1	0.998	0.55	283.6	12.2	−2.6	0.975	1.99	363.2	0.09	1.2	0.994	0.01	290.4	0.00	5.4	−2.0	0.987	1.36
M9	384.8	20.4	−4.1	0.978	2.13	392.2	41.2	−1.2	0.995	1.48	380.8	17.4	−5.5	0.967	2.58	473.5	0.14	0.9	0.991	0.09	385.7	0.00	0.3	−4.2	0.978	2.02
M10	331.3	17.6	−4.1	0.982	1.39	337.5	35.7	−1.3	0.996	0.85	328.0	15.1	−5.4	0.972	1.76	406.0	0.14	0.9	0.995	0.33	331.7	0.00	6.7	−4.1	0.982	1.33
M11	271.5	15.1	−1.4	0.991	0.95	281.8	27.0	0.3	0.998	0.09	267.5	13.6	−2.0	0.98	1.43	316.1	0.12	1.3	0.992	0.44	271.6	0.00	5.6	−1.5	0.991	0.93
M12	453.3	22.9	−5.0	0.974	1.72	460.7	47.5	−1.8	0.993	1.13	449.1	19.2	−6.7	0.964	2.15	569.4	0.14	0.9	0.997	0.51	453.3	0.01	13.9	−5.0	0.974	1.73
M13	459.2	28.7	−3.4	0.983	1.45	465.1	59.0	−0.9	0.997	0.80	455.9	24.3	−4.6	0.973	1.88	528.7	0.19	1.1	0.995	0.83	459.5	0.01	16.9	−3.4	0.983	1.41
M14	324.4	14.5	−2.9	0.98	2.13	339.1	26.6	−0.4	0.997	1.31	318.4	12.9	−3.9	0.967	2.62	423.5	0.08	1.1	0.993	0.47	323.5	0.00	5.7	−2.8	0.98	2.24
M15	501.0	28.2	−4.5	0.968	3.01	507.3	59.6	−1.5	0.991	2.39	497.2	23.5	−6.2	0.956	3.47	601.4	0.18	0.9	0.997	0.48	502.1	0.00	10.4	−4.8	0.968	2.87
M16	435.4	26.2	−4.4	0.964	2.60	439.6	56.4	−1.5	0.989	2.14	432.9	21.5	−6.2	0.951	2.92	508.5	0.21	0.9	0.996	0.49	435.4	0.01	13.5	−4.4	0.964	2.60
M17	308.8	16.2	−2.0	0.986	2.06	320.5	29.6	−0.1	0.999	1.14	304.0	14.6	−2.8	0.973	2.60	371.6	0.11	1.2	0.991	0.38	310.6	0.06	89.8	−2.5	0.985	1.82
M18	466.7	25.4	−2.7	0.989	2.05	479.8	48.1	−0.6	0.999	0.92	460.4	22.6	−3.6	0.98	2.79	562.6	0.13	1.1	0.993	0.53	467.3	0.01	11.9	−2.8	0.989	1.97
M19	475.1	32.0	−2.7	0.986	1.78	481.7	64.3	−0.6	0.998	0.98	471.4	27.7	−3.7	0.976	2.30	537.2	0.20	1.1	0.998	0.67	475.3	0.02	56.8	−2.7	0.985	1.74
M20	348.4	19.4	−2.5	0.975	3.29	358.3	36.8	−0.3	0.995	2.38	343.8	17.0	−3.4	0.96	3.84	416.0	0.13	1.1	0.991	1.29	349.3	0.00	8.2	−2.5	0.975	3.15
M21	513.8	27.9	−3.4	0.986	2.53	525.3	54.8	−0.9	0.998	1.51	507.9	24.3	−4.5	0.976	3.21	623.4	0.14	1.0	0.992	0.24	514.4	0.01	13.0	−3.5	0.986	2.45
M22	440.6	29.3	−2.8	0.985	2.19	446.7	59.0	−0.7	0.997	1.48	437.2	25.2	−3.8	0.975	2.67	500.5	0.19	1.1	0.997	0.08	440.6	0.01	15.7	−2.8	0.985	2.20
M23	380.1	20.0	−2.5	0.979	2.98	392.4	37.7	−0.3	0.997	1.98	374.7	17.7	−3.4	0.965	3.58	460.7	0.12	1.1	0.996	0.87	380.0	0.01	9.6	−2.5	0.979	2.98

The (*) means that for this biodigester the model was not adjusted and was not suitable.

Table 5. Kinetic parameters of methane from camelid co-digestion SIR 1:2.

Mezcla	GOMPERTZ					TRANSFERENCE					LOGISTIC					CONE					RICHARDS					
	M_e		t_{lag}	r^2	RMSE	M_e	ν_{max}	t_{lag}	r^2	RMSE	M_e	ν_{max}	t_{lag}	r^2	RMSE	M_e	k	n	r^2	RMSE	M_e	d	ν_{max}	t_{lag}	r^2	RMSE
N1	376.5	17.7	−3.5	0.988	9.67	389.5	33.4	−0.9	0.997	4.99	370.5	15.6	−4.5	0.979	12.96	485.5	0.10	0.98	0.998	1.98	376.4	0.00	0.1	−3.5	0.988	9.69
N2	258.1	14.8	−1.4	0.988	7.25	266.9	26.8	0.3	0.998	2.79	254.5	13.3	−2.0	0.976	10.37	297.9	0.12	1.33	0.999	3.46	258.0	0.00	0.1	−1.4	0.988	7.27
N3	287.6	23.2	−0.2	0.991	7.07	294.0	41.2	0.8	0.998	3.02	284.8	21.3	−0.5	0.979	10.60	308.3	0.17	1.67	0.999	4.30	287.6	0.00	0.1	−0.2	0.991	7.09
N4	370.3	22.6	−0.5	0.997	5.47	385.0	38.6	0.6	0.997	5.34	364.6	21.0	−0.7	0.990	9.73	414.3	0.12	1.53	0.997	1.67	370.2	0.01	0.1	−0.5	0.997	5.50
N5	254.7	16.2	−0.8	0.977	10.15	263.2	28.9	0.7	0.993	5.66	251.2	14.7	−1.3	0.961	13.27	287.8	0.13	1.43	0.996	0.61	254.8	0.00	0.0	−0.8	0.977	10.16
N6	130.8	5.8	−4.2	0.977	1.00	135.3	11.1	−1.2	0.995	0.74	128.8	5.0	−5.6	0.965	1.17	177.7	0.09	0.89	0.998	0.31	*	*	*	*	*	*
N7	157.3	10.3	−2.8	0.97	1.47	159.7	20.8	−0.6	0.993	1.19	156.0	8.8	−3.9	0.956	1.65	180.4	0.18	1.11	0.997	0.59	157.3	0.00	4.6	−2.8	0.97	1.46
N8	223.3	20.9	−0.2	0.974	1.98	227.7	37.0	0.7	0.994	1.35	221.2	19.5	−0.4	0.959	2.31	238.2	0.20	1.65	0.994	0.85	223.5	0.00	6.0	−0.2	0.974	1.96
N9	380.1	21.8	−3.2	0.984	1.49	387.4	43.3	−0.8	0.997	0.78	376.3	18.9	−4.4	0.973	1.96	450.0	0.15	1.05	0.998	0.52	380.1	0.01	12.0	−3.2	0.984	1.48
N10	354.1	20.3	−3.0	0.978	2.72	361.4	40.0	−0.6	0.996	2.00	350.4	17.5	−4.2	0.965	3.17	420.4	0.15	1.07	0.999	0.77	354.1	0.01	10.4	−3.1	0.978	2.72
N11	279.0	14.0	−1.6	0.987	1.69	292.3	24.8	0.2	0.999	0.77	273.9	12.7	−2.2	0.975	2.21	338.1	0.10	1.24	0.999	0.22	*	*	*	*	*	*
N12	160.0	8.0	−4.2	0.955	1.14	162.8	16.4	−1.1	0.987	0.92	158.6	6.6	−6.1	0.94	1.26	199.1	0.13	0.94	0.989	0.34	159.9	0.00	3.0	−4.3	0.955	1.14
N13	182.7	12.9	−1.7	0.974	1.48	186.0	25.0	0.1	0.995	1.07	181.1	11.3	−2.5	0.958	1.72	204.1	0.18	1.29	0.997	0.47	182.7	0.00	5.7	−1.7	0.974	1.48
N14	252.1	16.5	−1.2	0.967	2.26	258.8	30.7	0.5	0.993	1.51	249.2	14.6	−1.9	0.949	2.65	284.3	0.14	1.37	0.992	0.84	252.0	0.00	5.0	−1.2	0.967	2.27
N15	461.6	27.5	−4.1	0.971	1.89	466.8	58.1	−1.3	0.992	1.34	458.5	22.9	−5.7	0.959	2.28	540.4	0.20	0.96	0.996	0.38	461.9	0.00	5.7	−4.2	0.971	1.84
N16	375.4	22.6	−3.7	0.968	2.79	380.5	46.8	−1.0	0.992	2.24	372.6	18.9	−5.1	0.954	3.15	439.6	0.18	1.01	0.997	0.82	375.3	0.01	10.8	−3.7	0.968	2.81
N17	322.9	16.3	−1.8	0.984	2.29	337.6	29.1	0.1	0.998	1.24	317.2	14.7	−2.5	0.971	2.88	392.9	0.10	1.21	0.998	0.55	322.9	0.01	8.3	−1.8	0.984	2.29
N18	241.0	13.6	−1.7	0.984	1.72	248.9	25.0	0.2	0.998	1.03	237.7	12.2	−2.4	0.971	2.13	282.2	0.12	1.26	0.999	0.41	241.0	0.00	5.8	−1.7	0.984	1.72
N19	245.7	23.1	−0.5	0.974	2.02	249.8	42.2	0.6	0.995	1.42	243.6	21.3	−0.7	0.959	2.35	262.2	0.21	1.59	0.995	0.79	245.7	0.00	7.6	−0.5	0.974	2.02
N20	265.4	18.0	−1.1	0.961	3.90	273.2	32.6	0.4	0.99	2.98	261.8	16.4	−1.6	0.942	4.42	300.6	0.15	1.36	0.988	2.24	265.3	0.00	7.2	−1.1	0.961	3.90
N21	473.7	29.0	−3.9	0.967	2.84	478.9	61.4	−1.1	0.991	2.26	470.7	24.1	−5.5	0.954	3.23	549.5	0.20	1.00	0.996	0.48	473.6	0.01	14.2	−3.9	0.967	2.84
N22	392.3	31.2	−2.6	0.963	2.28	395.8	65.2	−0.5	0.991	1.79	390.2	26.1	−3.7	0.948	2.59	430.8	0.26	1.17	0.996	0.33	395.7	−0.99	12.1	−0.6	0.981	1.81
N23	341.8	19.8	−0.9	0.991	1.04	354.8	34.9	0.6	0.999	0.10	336.9	18.0	−1.4	0.98	1.64	389.4	0.12	1.42	0.999	0.64	341.8	0.00	7.1	−0.9	0.991	1.03

The (*) means that for this biodigester the model was not adjusted and was not suitable.

improvement in the constant k with an increasing amount of inoculum. One possible reason for the improvement in the hydrolysis rates of some biodigesters is because they contained a greater quantity of microorganisms, and this accelerates the degradation of insoluble and complex particles. However, the mixtures of M18, M12, M6, M15, M17, and M11 decreased k by 7.69%, 7.14%, 40.00%, 14.29%, 9.09%, and 16.28%, respectively. The differences observed in these last biodigesters may be due to the level of destruction of the structures of the lignocellulosic material achieved in the physical pre-treatment, together with the increase in the concentration of substances easily assimilated by the microorganisms of the substrates and co-substrates.[81]

The values obtained for k were quite heterogeneous in all the trials, and ranged in SIR1:1 between 0.08 d^{-1} (M15) and 0.21 d^{-1} (M12), and in SIR1:2 between 0.09 d^{-1} (N21) and 0.26 d^{-1} (N6). The first-order hydrolysis rates for this study agreed with those of El-Mashad *et al.*,[81] who reported results of 0.09–0.18 d^{-1}. Furthermore, the results of k were superior to the studies by Pitt *et al.*,[83] who obtained ranges of 0.07–0.14 d^{-1}. It should be noted that hydrolysis is a surface process and requires contact between hydrolytic microorganisms or enzymes and the surface of substrates and co-substrates. Thus, when the bioavailable surface of substrates and co-substrates is completely covered by hydrolytic agents, the hydrolysis rate cannot be increased due to the increase in the concentration of microorganisms in the system.[82] In this context, the variability of the amount of substrate and co-substrate in the biodigester mixtures caused the hydrolysis constant to increase or decrease in each biodigester. Thus, the digesters containing QS had a faster acceleration in hydrolysis and the digesters with AS and WS experienced more delays in the AD process.

On the other hand, in this study, only the cone model was used to determine the influence of the first-order decay rate constant. This is because all the first-order models raised convergence objections in the nonlinear regression fits. In this way, the first-order model of the cone was the only one that provided convergence between the values of the observed and predicted yields. In this sense, it can be inferred that co-digestion produced high concentrations of AGVs, so the hydrolysis rate cannot be determined precisely from methane yields. Initial concentrations of AGVs are very common in manure.[85] In this case, the biodegradability and therefore the biogas potential of the substrates and co-substrates is complex and depends on the content of biodegradable carbohydrates (including cellulose, hemicellulose, and lignin fractions), proteins, and lipids.[86]

Regarding $\nu_{\max}$, its information helps to determine the quantitative generation of methane or biogas but it was also used to identify the rate-limiting process in anaerobic co-digestion. In this sense, this kinetic parameter is essential to identify the synergistic effects of co-digestion. In this study, the maximum methane production rate produced higher values using the transfer model. Thus, in SIR1:1, values of 64.34 mL/g VS day were recorded in the M19 digesters, and 62.23 mL/g VS day for SIR1:2. The lowest peaks were produced by the Richards model, while the modified Gompertz and logistic models produced more homogeneous and similar values to each other. On the other hand, with the increase of VS in the agricultural waste from 25% to 50% and 75%, the $\nu_{\max}$ decreased in all the digesters with all the models tested.

Effects on maximum methane yield (M_e)

When the maximum methane yield is analyzed, it is observed that the experimental values followed the same trend as the theoretical models.

However, sometimes the cone model overestimates the performance M_e. This would be related to the high initial concentrations of AGVs in substrate mixtures.[87] As most of the methane was generated during the first 5 days of digestion, the cone model does not correctly simulate the later period of slower generation of methane and biogas. For their part, the Gompertz, Logistic and Richards models estimated M_e in quantities lower than the values measured experimentally. Similarly, the transfer model estimated the value of M_e in lower quantities than the experimental one, except for the digesters of N14, N20, M23, N17, and N23 of the SIR 1:2.

To evaluate the robustness of the results of the different models, a comparison of the percentage differences between predicted and experimental values was made. The greatest percentage differences were observed in the cone model for the M14 digesters of the SIR1:1 and N9 in the SIR1:2, with percentages of 21.36% and 23.84% respectively. On the other hand, the transfer model is the one with the smallest percentage difference between the predicted and experimental values. Thus, for example, the values that best fit the SIR 1:1 are the digesters composed of M12 in which a difference of 0.20% was obtained while, in the SIR 1:2, the smallest differences were obtained in the M18 digesters with differences of 0.05%.

Evaluation of the different kinetic models of co-digestion

The r^2 results contribute to the validation of the different models tested and, together with the kinetic parameters, help to determine the model that best fits the experimental co-digestion data. According to Tables 4 and 5, the models that fit best are the transfer model and the cone model. For its part, in SIR1:1, the r^2 value in the transfer model ranged between 0.991 and 0.999 whereas for the cone model, the value of r^2 includes ranges between 0.995 and 0.999. On the other hand, for the SIR1:2, the transfer model had a value of r^2 between 0.987 and 0.999. However, for the cone model the value of r^2 was between 0.988 and 0.999. According to the results, the cone model has a slight value of r^2 a little higher than the rest of the models under the conditions tested. However, the cone model overestimated the value of M_e, and therefore yielded higher percentage differences between the predicted and experimental values.

Regarding the RMSE values, the Gompertz, logistic and Richards models generated much higher values than the cone and transfer models. A value of RMSE = 0 indicates a perfect fit between the observed series and the estimated series. Thus, for the SIR1:1, methane varied the RMSE value between 2.06 and 13.62 mL/g VS for the transfer model, and for the cone model it varied between 1.79 and 6.78 mL/g VS. Regarding the SIR1:2, methane varied the RMSE value between 2.96 and 12.67 mL/g VS for the transfer model, and between 1.49 and 7.68 for the cone model.

In general, due to their low RMSE values and the high coefficient of determination, they demonstrated that the transfer and cone models were capable of simulating the cumulative biogas and methane production curve well. However, the lower percentage difference in methane and biogas yield between the observed and the estimated values showed that the transfer model was better than the cone model. There were differences between the kinetic constants that were obtained in all the models. The biogas production potentials (M_e) in the cone model were higher than the rest of the models. The logistic equation model showed the lower values of M_e, while the lower values of $\nu_{\max}$ were obtained in the Richards model. For their part, all models experienced a negative latency phase, except for the transfer model, which had positive phases of up to 13 h.

At time $t = 0$ days, all models exhibited positive values for all digesters, including the transfer model, as its latency phase was only hours. This shows that the biogas production under test conditions is equal to the specific growth of methanogenic bacteria. For this reason, in this study, the digesters had a minimal or almost no period for the recognition, adaptation, and growth of methanogenic bacteria. In this sense, it is possible that the inoculum with the substrates and co-substrates from co-digestion kept their methanogenic bacterial population active.

Discussion

In this study, two scenarios were analyzed: the influence of the inoculum, which was examined by comparing the two SIRs (1:1 and 1:2), and the influence of the AS, QS and WS co-substrates on the digestion of camelids.

In the first scenario, the two SIRs did not present significant differences in methane production, except for the LM, AS, and QS, which improved with the increase in inoculum by 60%, 22% and 29% respectively. Owen *et al.*[88] have considered that a SIR1:1 is adequate, but Chynoweth *et al.*[89] state that an increase in SIR may be necessary for some types of substrates and have suggested a SIR1:2. However, determining the ratio of inoculum to substrate in BMP assays is not that straightforward; each substrate has an optimal SIR.[74] Anaerobic degradation processes are strongly influenced by the inherent characteristics of substrates,[90] which suggests that organic materials require specific studies on the effect of SIRs.[91] To evaluate the effect of the inoculum correctly, it is also necessary to know the type, incubation time, and origin of the inoculum used.[92] In this study, the small influence of the SIR on the methane yield may be due to the fact that, theoretically, the SIR has an effect only on the kinetics, and not on the final methane yield, which only depends on the content of organic matter.[91] In this case, only one type of inoculum (sewage sludge) was used in all treatments and only two SIR were performed. This suggests that, to have more data on the influence of the inoculum on the methane yield, treatments with more proportions between substrate and inoculum should be carried out.

The methane production of LM and VM constantly improved when mixed with agricultural residues, which is corroborated by other studies of co-digestion of animal manure.[93,94] The observed improvements in methane production can be attributed to the synergistic effects of agricultural crop residues[64], which have improved the load of the biodegradable substrate, the hygienic stabilization and the increase in the speed of the digestion process.[95] The increase in methane from co-digestion occurred mainly in the mixtures with AS and QS. The optimal amounts of volatile solids mixture between LM, VM and agricultural residues were in a 50:50 ratio. However, when the load of agricultural residues was increased to 75%, digestion improved slightly, although without significant figures. The best results obtained with SA mixtures, to a great extent, are due to the fact that some chemical characteristics (fiber, sugars, fats, proteins) of SA straw are similar to those of corn straw.[96] Many researchers have considered that corn straw residues constitute one of the main agricultural residues to generate high methane yields.[97,98] Similarly, the contribution of QS also generated high methane yields due to its high C/N ratio[29] and its high VS percentage (78%). However, the proper mixing ratios of multicomponent substrates between camelids and agricultural residues are largely unknown due to the limited study of these raw materials. This means that more research is needed to evaluate the synergistic effects in detail and the mixing ratios can be optimized to obtain more stable and robust systems that generate higher yields.

The improvements in methane production in the co-digestion of camelids were quite competitive, as maximum improvements of more than 120% were obtained with respect to the digestion of monosubstrates. The improvements of this study were superior to those of other investigations on co-digestion of animal residues. Ma *et al.*[99] carried out an investigation in which they reported the improvements in the co-digestion of pig manure, bobbin, and poultry manure residues; they determined that the co-digestion of these manures with other co-substrates improves methane production by 20, 38, and 22% respectively. On the other hand, the methane production obtained in this study for the LM and VM residues were in a range of 260–540 mL CH_4/g VS; results that were very similar to that of other studies. Nasir *et al.*[40] reported that the ranges for the co-digestion of manure from cattle, pigs and poultry are around 100–370, 100–440, and 100–500 mL CH_4/g VS, respectively. Finally, the methane productions generated by camelids correspond to a medium-high range according to the literature. Velázquez *et al.*[100] reported that methane productions of 150–300, 300–450, more than 450 mL CH_4/g VS corresponds to a low, medium, and high classification respectively.

The possibility of mixing raw materials and even obtaining synergistic effects is useful for countries like Ecuador, where all the raw materials used are available in much of the country. The present findings serve as the basis for future research, especially for continuous anaerobic digestion processes; however, further investigation is still required as continuous processes would be run in an industrial environment. Ultimately, the beneficial (synergistic) effects of small amounts of camelid manure with agricultural residues deserve special attention due to their enormous potential. Perhaps if mixtures of more than one co-substrate were made (combinations of two, three or four co-substrates with a main substrate), methane production could be further optimized.

Conclusions

In this work, methane potentials were obtained from the co-digestion of camelid manure mixed with amaranth, quinoa, and wheat residues from the Andean zone. The methane results obtained ranged between 260 and 540 CH_4/g VS. This study demonstrated that the increase from SIR1: 1 to SIR 1: 2 was not relevant, since the results showed that the differences in methane production between SIR1.1 and SIR1: 2 were not significant. On the other hand, increasing the proportion of VS from agricultural residues (AS, QS, and WS) increased the production of CH_4 from residues of LM and VM. Thus, regardless of the SIR, the increase of VS in the co-substrate (50–75%) improved methane production up to 120%. All the trials showed synergistic effects ($\alpha > 1$), except co-digestion with LM of the SIR (1:2), which presented antagonistic values ($\alpha < 1$). In most of the mixtures composed of AS, high biodegradability values were given, whose maximum values were 95%. All the kinetic models fit very well the methane production values between the experimental and predicted results, especially the transfer and cone models ($r^2 > 99\%$, RMSE <2 ml/g SV).

References

1. Surendra KC, Takara D, Hashimoto AG and Khanal SK, Biogas as a sustainable energy source for developing countries: opportunities and challenges. *Renew Sustain Energy Rev* **31**:846–859 (2014). Available: http://www.sciencedirect.com/science/article/pii/S1364032113008290.
2. Pérez I, Garfí M, Cadena E and Ferrer I, Technical, economic and environmental assessment of household biogas digesters for rural communities. *Renew Energy* **62**:313–318 (2014). Available: http://www.sciencedirect.com/science/article/pii/S0960148113003625.
3. Ferrer I, Garfí M, Uggetti E, Ferrer-Martí L, Calderon A and Velo E, Biogas production in low-cost household digesters at the Peruvian Andes. *Biomass Bioenergy* **35**(5):1668–1674 (2011). Available: http://www.sciencedirect.com/science/article/pii/S0961953410004940.
4. Orsini F, Kahane R, Nono-Womdim R and Gianquinto G, Urban agriculture in the developing world: a review. *Agron Sustain Dev* **33**(4):695–720 (2013).
5. Martín Espada C, Pinto Jiménez CE and Cid Vázquez MD, Camélidos sudamericanos: estado sanitario de sus crías south american camelids: health status of their cria. *Rev Complut Ciencias Vet* **4**(1):37–50 (2010).
6. Dubey JP, A review of coccidiosis in south American camelids. *Parasitol Res* **117**(7):1999–2013 (2018).
7. Aparicio Porres JJ, Una aproximación al efecto del cambio climático en los camélidos domésticos en Bolivia. *Rev Investig e Innovación Agropecu y Recur Nat* **6**(1):87–93 (2019).
8. Esquivel A, Montero M and Barros M, Los Camélidos Sudamericanos: Productos Y Subproductos. *Aica* **11**:30–38 (2018).
9. Melby CL, Orozco F, Averett J, Muñoz F, Romero MJ and Barahona A, Agricultural food production diversity and dietary diversity among female small holder farmers in a region of the Ecuadorian Andes experiencing nutrition transition. *Nutrients* **12**(8):2454 (2020).
10. FAOSTAT. Food and Agriculture Organization of the United Nations [Internet]. 2018. Available: http://www.fao.org/faostat/en/#data/QC [24 October 2020]
11. Martí-Herrero J, Alvarez R, Cespedes R, Rojas MR, Conde V, Aliaga L *et al.*, Cow, sheep and llama manure at

psychrophilic anaerobic co-digestion with low cost tubular digesters in cold climate and high altitude. *Bioresour Technol* **181**:238–246 (2015). Available: http://www.sciencedirect.com/science/article/pii/S0960852415000838.

12. Alvarez R, Villca S and Lidén G, Biogas production from llama and cow manure at high altitude. *Biomass Bioenergy* **30**(1):66–75 (2006).
13. Yapu B. Determinación de la calidad de estiércol de vicuña en dos cultivares de lechuga (*Lactuca sativa*) bajo ambiente protegido en Patacamaya. Universidad Mayor de San Andrés, La Paz (Bolivia). Facultad de Agronomía; 2013.
14. Ottalagano F. Decoración experimental de cerámica aplicada al estudio de las técnicas incisas del área del Paraná. Intersecc en Antropol Num11. 2010 Dec 1;11.
15. Palamarczuk V, Cocción experimental de cerámica con estiércol de llama. *Intersecc en Antropol* **5**:119–127 (2004).
16. De Figueiredo EB and La Scala N, Greenhouse gas balance due to the conversion of sugarcane areas from burned to green harvest in Brazil. *Agric Ecosyst Environ* **141**(1):77–85 (2011). Available: http://www.sciencedirect.com/science/article/pii/S0167880911000521.
17. Zheng YH, Li ZF, Feng SF, Lucas M, Wu GL, Li Y *et al*., Biomass energy utilization in rural areas may contribute to alleviating energy crisis and global warming: a case study in a typical agro-village of Shandong, China. *Renew Sustain Energy Rev* **14**(9):3132–3139 (2010). Available: http://www.sciencedirect.com/science/article/pii/S1364032110002327.
18. Wang Y, Li G, Chi M, Sun Y, Zhang J, Jiang S *et al*., Effects of co-digestion of cucumber residues to corn Stover and pig manure ratio on methane production in solid state anaerobic digestion. *Bioresour Technol* **250**:328–336 (2018). Available: http://www.sciencedirect.com/science/article/pii/S0960852417320576.
19. Wang X, Yang G, Feng Y, Ren G and Han X, Optimizing feeding composition and carbon–nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. *Bioresour Technol* **120**:78–83 (2012). Available: http://www.sciencedirect.com/science/article/pii/S0960852412009625.
20. Wang M, Zhang X, Zhou J, Yuan Y, Dai Y, Li D *et al*., The dynamic changes and interactional networks of prokaryotic community between co-digestion and mono-digestions of corn stalk and pig manure. *Bioresour Technol* **225**:23–33 (2017). Available: http://www.sciencedirect.com/science/article/pii/S0960852416315139.
21. Wan C, Zhou Q, Fu G and Li Y, Semi-continuous anaerobic co-digestion of thickened waste activated sludge and fat, oil and grease. *Waste Manag* **31**(8):1752–1758 (2011). Available: http://www.sciencedirect.com/science/article/pii/S0956053X11001772.
22. Abouelenien F, Fujiwara W, Namba Y, Kosseva M, Nishio N and Nakashimada Y, Improved methane fermentation of chicken manure via ammonia removal by biogas recycle. *Bioresour Technol* **101**(16):6368–6373 (2010). Available: http://www.sciencedirect.com/science/article/pii/S0960852410005481.
23. Panichnumsin P, Nopharatana A, Ahring B and Chaiprasert P, Production of methane by co-digestion of cassava pulp with various concentrations of pig manure. *Biomass Bioenergy* **34**(8):1117–1124 (2010). Available: http://www.sciencedirect.com/science/article/pii/S0961953410000930.
24. Li Y, Feng L, Zhang R, He Y, Liu X, Xiao X *et al*., Influence of inoculum source and pre-incubation on bio-methane potential of chicken manure and corn Stover. *Appl Biochem Biotechnol* **171**(1):117–127 (2013).
25. Hafner SD and Astals S, Systematic error in manometric measurement of biochemical methane potential: sources and solutions. *Waste Manag* **91**:147–155 (2019). Available: http://www.sciencedirect.com/science/article/pii/S0956053X19302934.
26. Federation WE and Association APH, *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington, DC (2005).
27. Holliger C, Alves M, Andrade D, Angelidaki I, Astals S, Baier U *et al*., Towards a standardization of biomethane potential tests. *Water Sci Technol* **74**(11):2515–2522 (2016).
28. Strömberg S, Nistor M and Liu J, Towards eliminating systematic errors caused by the experimental conditions in biochemical methane potential (BMP) tests. *Waste Manag* **34**(11):1939–1948 (2014). Available: http://www.sciencedirect.com/science/article/pii/S0956053X14003237.
29. Hafner SD, Fruteau de Laclos H, Koch K and Holliger C, Improving inter-laboratory reproducibility in measurement of biochemical methane potential (BMP). *Water* **12**(6):1752 (2020).
30. Hansen TL, Schmidt JE, Angelidaki I, Marca E, Jansen J l C, Mosbæk H *et al*., Method for determination of methane potentials of solid organic waste. *Waste Manag [Internet].* **24**(4):393–400 (2004). Available: http://www.sciencedirect.com/science/article/pii/S0956053X0300223X.
31. Pellera F-M and Gidarakos E, Effect of substrate to inoculum ratio and inoculum type on the biochemical methane potential of solid agroindustrial waste. *J Environ Chem Eng [Internet]* **4**(3):3217–3229 (2016).
32. Boulanger A, Pinet E, Bouix M, Bouchez T and Mansour AA, Effect of inoculum to substrate ratio (I/S) on municipal solid waste anaerobic degradation kinetics and potential. *Waste Manag* **32**(12):2258–2265 (2012).
33. Roberts KP, Heaven S and Banks CJ, Comparative testing of energy yields from micro-algal biomass cultures processed via anaerobic digestion. *Renew Energy [Internet].* **87**:744–753 (2016).
34. Labatut RA, Angenent LT and Scott NR, Biochemical methane potential and biodegradability of complex organic substrates. *Bioresour Technol [Internet].* **102**(3):2255–2264 (2011).
35. Shen J, Zheng Q, Zhang R, Chen C and Liu G, Co-pretreatment of wheat straw by potassium hydroxide and calcium hydroxide: methane production, economics, and energy potential analysis. *J Environ Manage [Internet]* **236**:720–726 (2019).
36. Nielfa A, Cano R and Fdz-Polanco M, Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. *Biotechnol Rep* **5**:14–21 (2014).
37. Castro-Molano LP, Escalante-Hernández H, Lambis-Benítez LE and Marín-Batista JD, Synergistic effects in anaerobic codigestion of chicken manure with industrial wastes. *DYNA* **85**:135–141 (2018).
38. Achinas S, Krooneman J and Euverink GJW, Enhanced biogas production from the anaerobic batch treatment of Banana peels. *Engineering [Internet]* **5**(5):970–978 (2019).
39. Ware A and Power N, Modelling methane production kinetics of complex poultry slaughterhouse wastes using sigmoidal growth functions. *Renew Energy [Internet].* **104**:50–59 (2017).
40. Nasir IM, Mohd Ghazi TI and Omar R, Anaerobic digestion technology in livestock manure treatment for biogas production: a review. *Eng Life Sci [Internet]* **12**(3):258–269 (2012).
41. Wang C, Hong F, Lu Y, Li X and Liu H, Improved biogas production and biodegradation of oilseed rape straw by

using kitchen waste and duck droppings as co-substrates in two-phase anaerobic digestion. *PLoS One [Internet]* **12**(8):e0182361 (2017).

42. Li Y, Zhang R, Liu X, Chen C, Xiao X, Feng L *et al*., Evaluating methane production from anaerobic mono- and co-digestion of kitchen waste, corn Stover, and chicken manure. *Energy Fuels [Internet].* **27**(4):2085–2091 (2013).
43. Castrillón L, Marañón E, Fernández-Nava Y, Ormaechea P and Quiroga G, Thermophilic co-digestion of cattle manure and food waste supplemented with crude glycerin in induced bed reactor (IBR). *Bioresour Technol [Internet].* **136**:73–77 (2013). Available: http://www.sciencedirect.com/science/article/pii/S0960852413003118.
44. Zhao M-X and Ruan W-Q, Biogas performance from co-digestion of Taihu algae and kitchen wastes. *Energy Convers Manag [Internet]* **75**:21–24 (2013).
45. Wang X, Lu X, Li F and Yang G, Effects of temperature and carbon-nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: focusing on ammonia inhibition. *PLoS One [Internet].* **9**(5):97265 (2014).
46. Orlando M-Q and Borja V-M, Pretreatment of animal manure biomass to improve biogas production: a review. *Energies* **13**(14):3573 (2020).
47. Alvarez R and Lidén G, Anaerobic co-digestion of aquatic flora and quinoa with manures from Bolivian Altiplano. *Waste Manag [Internet]* **28**(10):1933–1940 (2008).
48. McVoitte WPA and Clark OG, The effects of temperature and duration of thermal pretreatment on the solid-state anaerobic digestion of dairy cow manure. *Heliyon [Internet]* **5**(7):02140 (2019).
49. Oladejo OS, Dahunsi SO, Adesulu-Dahunsi AT, Ojo SO, Lawal AI, Idowu EO *et al*., Energy generation from anaerobic co-digestion of food waste, cow dung and piggery dung. *Bioresour Technol [Internet]* **313**:123694 (2020). Available: http://www.sciencedirect.com/science/article/pii/S0960852420309664.
50. Alvarez R and Lidén G, Low temperature anaerobic digestion of mixtures of llama, cow and sheep manure for improved methane production. *Biomass Bioenergy [Internet].* **33**(3):527–533 (2009).
51. Bhatnagar N, Ryan D, Murphy R and Enright A-M, Trace element supplementation and enzyme addition to enhance biogas production by anaerobic digestion of chicken litter. *Energies* **13**:3477 (2020).
52. Yılmaz Ş and Şahan T, Utilization of pumice for improving biogas production from poultry manure by anaerobic digestion: a modeling and process optimization study using response surface methodology. *Biomass Bioenergy [Internet]* **138**:105601 (2020).
53. Ramm P, Abendroth C, Latorre-Pérez A, Herrmann C, Sebök S, Geißler A *et al*., Ammonia removal during leach-bed acidification leads to optimized organic acid production from chicken manure. *Renew Energy [Internet]* **146**:1021–1030 (2020).
54. Zheng Z, Cai Y, Zhao Y, Meng X, Zhang Y, Lu C *et al*., Achieve clean and efficient biomethane production by matching between digestate recirculation and straw-to-manure feeding ratios. *J Clean Prod [Internet]* **263**:121414 (2020).
55. Guo H-G, Chen Q-L, Hu H-W and He J-Z, High-solid anaerobic co-digestion of pig manure with lignite promotes methane production. *J Clean Prod [Internet]* **258**:120695 (2020). Available: http://www.sciencedirect.com/science/article/pii/S0959652620307423.
56. Zheng X, Liu Y, Huang J, Du Z, Zhouyang S, Wang Y *et al*., The influence of variables on the bioavailability of heavy metals during the anaerobic digestion of swine manure. *Ecotoxicol Environ Saf [Internet]* **195**:110457 (2020). Available: http://www.sciencedirect.com/science/article/pii/S0147651320302967.
57. Han S-K and Shin H-S, Biohydrogen production by anaerobic fermentation of food waste. *Int J Hydrogen Energy [Internet]* **29**(6):569–577 (2004).
58. Sun J, Li Z, Zhou X, Wang X, Liu T and Cheng S, Investigation on methane yield of wheat husk anaerobic digestion and its enhancement effect by liquid digestate pretreatment. *Anaerobe [Internet]* **59**:92–99 (2019).
59. Seppälä M, Laine A and Rintala J, Screening of novel plants for biogas production in northern conditions. *Bioresour Technol [Internet].* **139**:355–362 (2013).
60. Korai RM, Wachemo AC, Yue L, Jaffar M, Li Z, Shahbaz M *et al*., Effect of ultrasonic application during KOH pretreatment and anaerobic process on digestion performance of wheat straw. *RSC Adv [Internet]* **10**(16):9290–9298 (2020).
61. Minzanova ST, Mironov V, Belostotskii DE, Mindubaev AZ, Mironova LG, Gins M *et al*., Materials derived from *Amaranthus cruentus* L. used as co-substrates can intensify methanogenesis during bioconversion of organic waste. *Sel'skokhozyaistvennaya Biol* **53**:209–217 (2018).
62. VDI, *Fermentation of Organic Materials–Characterisation of the Substrate, Sampling, Collection of Material Data, Fermentation Tests*. Verein Deutscher Ingenieure, Dusseldorf (2006).
63. Sohoo I, Ritzkowski M, Heerenklage J and Kuchta K, Biochemical methane potential assessment of municipal solid waste generated in Asian cities: a case study of Karachi, Pakistan. *Renew Sustain Energy Rev [Internet]* **135**:110175 (2021).
64. Zhang T, Liu L, Song Z, Ren G, Feng Y, Han X *et al*., Biogas production by co-digestion of goat manure with three crop residues. Du C, editor. *PLoS One [Internet]* **8**(6):66845 (2013).
65. Li K, Liu R and Sun C, Comparison of anaerobic digestion characteristics and kinetics of four livestock manures with different substrate concentrations. *Bioresour Technol [Internet].* **198**:133–140 (2015).
66. Wei Y, Yuan H, Wachemo AC and Li X, Impacts of modification of corn Stover on the synergistic effect and microbial community structure of co-digestion with chicken manure. *Energy Fuels [Internet]* **34**(1):401–411 (2020).
67. Molinuevo-Salces B, Mahdy A, Ballesteros M and González-Fernández C, From piggery wastewater nutrients to biogas: microalgae biomass revalorization through anaerobic digestion. *Renew Energy [Internet].* **96**:1103–1110 (2016).
68. Shen F, Zhong B, Wang Y, Xia X, Zhai Z and Zhang Q, Cellulolytic microflora pretreatment increases the efficiency of anaerobic co-digestion of Rice straw and pig manure. *BioEnergy Res [Internet]* **12**(3):703–713 (2019).
69. Li R, Tan W, Zhao X, Dang Q, Song Q, Xi B *et al*., Evaluation on the methane production potential of wood waste pretreated with NaOH and co-digested with pig manure. *Catalysts [Internet]* **9**(6):539 (2020).
70. Li Y, Zhang R, Chen C, Liu G, He Y and Liu X, Biogas production from co-digestion of corn Stover and chicken manure under anaerobic wet, hemi-solid, and solid-state conditions. *Bioresour Technol [Internet].* **149**:406–412 (2013).
71. Molinuevo-Salces B, Gómez X, Morán A and García-González MC, Anaerobic co-digestion of livestock and vegetable processing wastes: fibre degradation and digestate stability. *Waste Manag [Internet].* **33**(6):1332–1338 (2013).

72. Rahman MA, Møller HB, Saha CK, Alam MM, Wahid R and Feng L, Anaerobic co-digestion of poultry droppings and briquetted wheat straw at mesophilic and thermophilic conditions: influence of alkali pretreatment. *Renew Energy [Internet].* **128**:241–249 (2018).
73. Alexis P-OB, Patricia T-L, Fernando M-RL, Marcela C-CL, Carlos V-F, Alexander T-LW *et al.*, Efecto de la relación sustrato-inóculo sobre el potencial bioquímico de metano de biorresiduos de origen municipal. *Ing Investig y Tecnol [Internet]* **16**(4):515–526 (2015).
74. Lesteur M, Bellon-Maurel V, Gonzalez C, Latrille E, Roger JM, Junqua G *et al.*, Alternative methods for determining anaerobic biodegradability: a review. *Process Biochem [Internet].* **45**(4):431–440 (2010).
75. Parawira W, Murto M, Zvauya R and Mattiasson B, Anaerobic batch digestion of solid potato waste alone and in combination with sugar beet leaves. *Renew Energy [Internet].* **29**(11):1811–1823 (2004).
76. Yin C-R, Seo D-I, Kim M-K and Lee S-T, Inhibitory effect of hardwood lignin on acetate-utilizing methanogens in anaerobic digester sludge. *Biotechnol Lett [Internet]* **22**(19):1531–1535 (2000).
77. Batool N, Qazi JI, Aziz N, Hussain A and Shah SZH, Bio-methane production potential assays of organic waste by anaerobic digestion and co-digestion. *Pak J Zool* **52**(3):971 (2020).
78. Mshandete A, Kivaisi A, Rubindamayugi M and Mattiasson B, Anaerobic batch co-digestion of sisal pulp and fish wastes. *Bioresour Technol [Internet].* **95**(1):19–24 (2004).
79. Ebenezer AV, Kaliappan S, Adish Kumar S, Yeom I-T and Banu JR, Influence of deflocculation on microwave disintegration and anaerobic biodegradability of waste activated sludge. *Bioresour Technol [Internet]* **185**:194–201 (2015).
80. Brulé M, Oechsner H and Jungbluth T, Exponential model describing methane production kinetics in batch anaerobic digestion: a tool for evaluation of biochemical methane potential assays. *Bioprocess Biosyst Eng [Internet]* **37**(9):1759–1770 (2014).
81. El-Mashad HM, Kinetics of methane production from the codigestion of switchgrass and Spirulina platensis algae. *Bioresour Technol [Internet].* **132**:305–312 (2013).
82. El-Mashad HM, Zeeman G, van Loon WKP, Bot GPA and Lettinga G, Effect of temperature and temperature fluctuation on thermophilic anaerobic digestion of cattle manure. *Bioresour Technol [Internet].* **95**(2):191–201 (2004).
83. Pitt RE, Cross TL, Pell AN, Schofield P and Doane PH, Use of in vitro gas production models in ruminal kinetics. *Math Biosci [Internet]* **159**(2):145–163 (1999).
84. Song H and Clarke WP, Cellulose hydrolysis by a methanogenic culture enriched from landfill waste in a semi-continuous reactor. *Bioresour Technol [Internet].* **100**(3):1268–1273 (2009).
85. Xie S, Lawlor PG, Frost JP, Hu Z and Zhan X, Effect of pig manure to grass silage ratio on methane production in batch anaerobic co-digestion of concentrated pig manure and grass silage. *Bioresour Technol [Internet].* **102**(10):5728–5733 (2011).
86. Angelidaki I and Sanders W, Assessment of the anaerobic biodegradability of macropollutants. *Re/Views Environ Sci Bio/Technology* **3**(2):117–129 (2004).
87. Dennehy C, Lawlor PG, Croize T, Jiang Y, Morrison L, Gardiner GE *et al.*, Synergism and effect of high initial volatile fatty acid concentrations during food waste and pig manure anaerobic co-digestion. *Waste Manag [Internet].* **56**:173–180 (2016).
88. Owen WF, Stuckey DC, Healy JB Jr, Young LY and McCarty PL, Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Res* **13**(6):485–492 (1979).
89. Chynoweth DP, Turick CE, Owens JM, Jerger DE and Peck MW, Biochemical methane potential of biomass and waste feedstocks. *Biomass Bioenergy [Internet].* **5**(1):95–111 (1993).
90. Yoon Y-M, Kim S-H, Shin K-S and Kim C-H, Effects of substrate to inoculum ratio on the biochemical methane potential of piggery slaughterhouse wastes. *Asian-Austral J Anim Sci [Internet]* **27**(4):600–607 (2014).
91. Raposo F, Banks CJ, Siegert I, Heaven S and Borja R, Influence of inoculum to substrate ratio on the biochemical methane potential of maize in batch tests. *Process Biochem [Internet]* **41**(6):1444–1450 (2006).
92. Hülsemann B, Zhou L, Merkle W, Hassa J, Müller J and Oechsner H, Biomethane potential test: influence of inoculum and the digestion system. *Appl Sci* **10**(7):2589 (2020).
93. Khoufi S, Louhichi A and Sayadi S, Optimization of anaerobic co-digestion of olive mill wastewater and liquid poultry manure in batch condition and semi-continuous jet-loop reactor. *Bioresour Technol [Internet].* **182**:67–74 (2015).
94. Vivekanand V, Mulat DG, Eijsink VGH and Horn SJ, Synergistic effects of anaerobic co-digestion of whey, manure and fish ensilage. *Bioresour Technol [Internet].* **249**:35–41 (2018).
95. Tufaner F and Avşar Y, Effects of co-substrate on biogas production from cattle manure: a review. *Int J Environ Sci Technol [Internet]* **13**(9):2303–2312 (2016).
96. Martínez-Gutiérrez E, Biogas production from different lignocellulosic biomass sources: advances and perspectives. *3 Biotech [Internet]* **8**(5):233 (2018). Available: https://europepmc.org/articles/PMC5928011.
97. Mazurkiewicz J, Marczuk A, Pochwatka P and Kujawa S, Maize straw as a valuable energetic material for biogas plant feeding. *Mater (Basel, Switzerland) [Internet]* **12**(23):3848 (2019).
98. von Cossel M, Möhring J, Kiesel A and Lewandowski I, Methane yield performance of amaranth (*Amaranthus hypochondriacus* L.) and its suitability for legume intercropping in comparison to maize (*Zea mays* L.). *Ind Crops Prod [Internet]* **103**:107–121 (2017).
99. Ma G, Ndegwa P, Harrison JH and Chen Y, Methane yields during anaerobic co-digestion of animal manure with other feedstocks: a meta-analysis. *Sci Total Environ [Internet]* **728**:138224 (2020).
100. Velázquez-Martí B., Meneses-Quelal O., Gaibor-Chavez J., Niño-Ruiz Z. Review of Mathematical Models for the Anaerobic Digestion Process, Anaerobic Digestion, J. Rajesh Banu, IntechOpen (2018), DOI: 10.5772/intechopen.80815. Available from: https://www.intechopen.com/books/anaerobic-digestion/review-of-mathematical-models-for-the-anaerobic-digestion-process.

Orlando Meneses

Orlando Meneses is a doctoral student at the Institute of Animal Science and Technology of the Polytechnic University of Valencia (UPV). He holds an MSc in environmental engineering from the UPV. His current research is related to the anaerobic co-digestion of organic waste of animal and agricultural origin.

Borja Velázquez-Martí

Borja Velázquez-Martí has a PhD and is a professor at Universidad Politécnica de Valencia (Spain), where he teaches agricultural mechanization and bioenergy at the Department of Rural and Food Engineering. His research work is focused on quantification, logistics, and management of biomass for energy.

Zulay Niño Ruiz

Zulay Niño is a chemical engineer with a PhD in environmental process simulation. She is a professor at the Amazon Regional University Ikiam where she is in charge of the biomass laboratory. Her research interests are biomaterials, biomass, and biofuel with an emphasis on biogas.

Juan Gaibor-Chávez

Juan Gaibor-Chávez is an agro-industrial engineer with a PhD. He works at the Universidad Estatal de Bolívar in Ecuador, where he teaches thermodynamics and heat processing He is also a researcher leader of the biomass research group in this university.