UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Doctoral Program in Science and Technology of Animal Production

UNIVERSITAT
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DE VALÈNCIA

DOCTORAL THESIS

OPTIMIZATION OF ANAEROBIC CODIGESTION PROCESSES OF LIGNOCELLULOSIC MATERIALS OF DIFFICULT DEGRADATION WITH RESIDUES FROM ANDEAN LIVESTOCK

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To my family,

And especially my son Javier.

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ABSTRACT

Access to modern energy sources in rural areas of the Andean region is one of the main factors to reduce poverty, since its access would provide environmental, economic and social benefits. Despite efforts to find alternative sources to correct the energy deficit, there are still millions of people who suffer from the lack of accessibility to modern energy sources, a situation that is due to the high levels of poverty under which they are immersed. Along with this inconvenience is added the enormous increase in agricultural residues in the Andean communities. Waste that comes from agricultural activities, and that could be harmful to the environment if adequate measures are not taken. Unfortunately, in many developing countries where large amounts of these wastes are generated, little is known about their potential risks and benefits if not managed properly. One of the most interesting approaches to address this problem is the development of sustainable management of agricultural organic waste in the region, transforming it into resources for the generation of renewable energy (biogas) and organic fertilizers (digestate). This solution would allow to give an energetic recovery to the agricultural residues of the area, on which they base their economy, and at the same time would contribute to a better management of the residues avoiding the increase of environmental pollution.

To contribute to energy development and improve the paradigm of waste management in the Andean area of Guaranda (Ecuador), this Doctoral Thesis addresses the evaluation of the biochemical potential of methane (BPM) of agricultural organic waste in the region. A systematic quantification of biogas production is carried out through the biochemical transformation of agricultural organic waste that includes main substrates (vicuña, llama and guinea pig manure residues, and cattle slaughterhouse residues) and co-substrates (amaranth straw residues), quinoa and wheat). The general objective of this doctoral research has been carried out in four phases: (I) characterization of the raw material through elemental and proximal analysis through which the theoretical performance and biodegradability of substrates and co-substrates were estimated, (II) Performance of the co-digestion of agricultural organic waste with mixtures of sewage sludge in batch biodigesters, (III) Analysis of synergistic and antagonistic effects during monodigestion and co-digestion of raw materials and (IV) Evaluation of microbial kinetics of anaerobic digestion using modified Gompertz models, transfer, logistic equation, cone model and modified Richards.

In the physicochemical characterization it was determined that the VS/TS ratios of the substrates and co-substrates ranged between 58 and 77% with a C/N ratio between 12 and 102, which indicated that these wastes are suitable raw materials to produce methane. In all the tests an increase in the amount of inoculum improved the biodegradability of the substrates and consequently the methane production; thus, in monodigestion there were increases of up to 90% and in co-digestion increases of 71%. All the mixtures produced synergistic effects, where the highest percentages of methane occurred when the mixtures of amaranth, quinoa and wheat residues were 50 and 75% volatile solids. Regardless of SIR1:1 and SIR 1:2, the production of methane from co-digestion was improved by increasing the percentage of co-substrate, especially amaranth and quinoa residues. The best results of all the tests carried out were obtained in the biodigesters composed of slaughterhouse waste and quinoa waste, where methane productions between 581 and 555 ml/g VS were obtained. Regarding the kinetic modelling of the anaerobic digestion

process, it was found that all the models fit the experimental values quite well with the predicted ones. In the monodigestion, in all the logistic models, the calculated asymptotes were adjusted very precisely for the specific yield (M_e), which made them not vary more than 7.06% with respect to the experimental data, while the cone model generated differences between the experimental production of methane and Me of the order of 26%. Likewise, in co-digestion, the cone model generated large differences (20 and 30%) between the experimental production and M_e. Of all the logistic and complex models, the transfer model adjusted the results quite well since in many tests an R^2 greater than 99% and RMSE values less than 2 ml/g VS were obtained. However, the methane prediction from the kinetic models depended on the raw material used, since not all the mixtures had the same behaviour.

RESUMEN

El acceso a fuentes de energía moderna en las áreas rurales de la región andina es uno de los factores principales para disminuir la pobreza ya que su acceso proporcionaría beneficios ambientales, económicos y sociales. Pese a los esfuerzos de buscar fuentes alternativas para subsanar el déficit energético, aún existen millones de personas que sufren la falta de accesibilidad a fuentes de energía moderna, situación que se debe a los altos niveles de pobreza bajo los cuales se encuentran inmersos. Junto a este inconveniente se suma el enorme incremento de residuos agrícolas en las comunidades andinas. Residuos que provienen de las actividades agrícolas, y que podrían ser perjudiciales para el medio ambiente si no se toman medidas adecuadas. Lamentablemente, en muchos países en desarrollo donde se generan grandes cantidades de estos residuos, se sabe poco sobre sus posibles riesgos y beneficios si no se gestionan adecuadamente. Uno de los enfoques más interesantes para abordar esta problemática, es el desarrollo de la gestión sostenible de los residuos orgánicos agrícolas de la región, transformándolos en recursos para la generación de energía renovable (biogás) y fertilizantes orgánicos (digestato). Esta solución permitiría dar una valorización energética a los residuos de la agricultura de la zona, sobre la cual basan su economía, y a la vez contribuiría a una mayor gestión de los residuos evitando el incremento de la contaminación ambiental.

Con la finalidad de contribuir al desarrollo energético y mejorar el paradigma de la gestión de residuos en el área andina de Guaranda (Ecuador), la presente Tesis Doctoral aborda la evaluación del potencial bioquímico de metano (BMP) de los residuos orgánicos agrícolas de la región. Se realiza una cuantificación sistemática de la producción de biogás mediante la transformación bioquímica de residuos orgánicos agrícolas, que comprenden: sustratos principales (residuos de estiércol de vicuña, llama y cuy, y residuos de matadero de ganado vacuno) y cosustratos (residuos de paja de amaranto, quinua y trigo). El objetivo general de esta investigación de doctorado se ha llevado a cabo en cuatro fases: (I) Caracterización de la materia prima mediante el análisis elemental y proximal a través de los cuáles se estimó el rendimiento teórico y la biodegradabilidad de los sustratos y cosustratos, (II) Rendimiento de la codigestión de residuos orgánicos agrícolas con mezclas de lodos de aguas residuales en biodigestores batch, (III) Análisis de los efectos sinérgicos y antagónicos durante la monodigestión y codigestión de las materias primas y (IV) Evaluación de la cinética microbiana de la digestión anaerobia mediante los modelos de Gompertz modificado, transferencia, ecuación logística, modelo del cono y Richards modificado.

En la caracterización fisicoquímica se determinó que las relaciones SV/ST de los sustratos y cosustratos oscilaron entre 58 y 77% con una relación C/N entre 12 y 102, lo que indicó que estos residuos son materias primas adecuadas para la producción de metano. En todos los ensayos un aumento de la cantidad de inóculo mejoró la biodegradabilidad de los sustratos y por consiguiente la producción metano; así, en la monodigestión se tuvo incrementos de hasta 90% y en la codigestión incrementos del 71%. Todas las mezclas produjeron efectos sinérgicos, donde los mayores porcentajes de metano se dieron cuando las mezclas de residuos de amaranto, quinua y trigo fueron del 50 y 75% de sólidos volátiles. Independientemente de la SIR1:1 y la SIR 1:2 se mejoró la producción de metano de la codigestión al incrementar el porcentaje de cosustrato especialmente de residuos de amaranto y quinua. Los mejores resultados de todos los ensayos realizados se obtuvieron en los biodigestores compuestos por residuos de matadero y residuos de

quinua, donde se obtuvieron producciones de metano entre 581 y 555 ml/g VS. En lo que respecta al modelado cinético del proceso de digestión anaerobia se pudo comprobar que todos los modelos ajustaron bastante bien los valores experimentales con los pronosticados. En la monodigestión, en todos los modelos logísticos, las asíntotas calculadas se ajustaron con mucha precisión al rendimiento específico (M_e) lo que hizo que no varíen más del 7,06% con respecto a los datos experimentales, mientras que el modelo del cono generó diferencias entre la producción experimental de metano y M_e del orden del 26%. Igualmente, en la codigestión, el modelo cono generó grandes diferencias (20 y 30%) entre la producción experimental y M_e. De todos los modelos logísticos y complejos el modelo de la transferencia ajustó bastante bien los resultados ya que en muchos ensayos se obtuvo un R^2 superior al 99% y valores de RMSE inferiores al 2 ml/g SV. Sin embargo, la predicción de metano de los modelos cinéticos dependió de la materia prima empleada, ya que no todas las mezclas tuvieron el mismo comportamiento.

RESUM

L'accés a fonts d'energia moderna en les àrees rurals de la regió andina és un dels factors principals per a disminuir la pobresa ja que el seu accés proporcionaria beneficis ambientals, econòmics i socials. Malgrat els esforços de buscar fonts alternatives per a esmenar el dèficit energètic, encara existeixen milions de persones que pateixen la falta d'accessibilitat a fonts d'energia moderna, situació que es deu als alts nivells de pobresa sota els quals es troben immersos. Al costat d'aquest inconvenient se suma l'enorme increment de residus agrícoles en les comunitats andines. Residus que provenen de les activitats agrícoles, i que podrien ser perjudicials per al medi ambient si no es prenen mesures adequades. Lamentablement, en molts països en desenvolupament on es generen grans quantitats d'aquests residus, se sap poc sobre els seus possibles riscos i beneficis si no es gestionen adequadament. Un dels enfocaments més interessants per a abordar aquesta problemàtica, és el desenvolupament de la gestió sostenible dels residus orgànics agrícoles de la regió, transformant-los en recursos per a la generació d'energia renovable (biogàs) i fertilitzants orgànics (digestato). Aquesta solució permetria donar una valorització energètica als residus de l'agricultura de la zona, sobre la qual basen la seua economia, i alhora contribuiria a una major gestió dels residus evitant l'increment de la contaminació ambiental.

Amb la finalitat de contribuir al desenvolupament energètic i millorar el paradigma de la gestió de residus en l'àrea andina de Guaranda (l'Equador), la present Tesi Doctoral aborda l'avaluació del potencial bioquímic de metà (BMP) dels residus orgànics agrícoles de la regió. Es realitza una quantificació sistemàtica de la producció de biogàs mitjançant la transformació bioquímica de residus orgànics agrícoles que comprenen: substrats principals (residus de fem de vicunya, flama i cuy, i residus d'escorxador de bestiar boví), *cosustratos (residus de palla d'amarant, quinua i blat). L'objectiu general d'aquesta investigació de doctorat s'ha dut a terme en quatre fases: (I) caracterització de la matèria primera mitjançant l'anàlisi elemental i proximal a través dels quals es va estimar el rendiment teòric i la biodegradabilitat dels substrats i cosustratos, (II) Rendiment de la codigestión de residus orgànics agrícoles amb mescles de llots d'aigües residuals en biodigestores batch, (III) Anàlisis dels efectes sinèrgics i antagònics durant la monodigestión i codigestión de les matèries primeres i (IV) Avaluació de la cinètica microbiana de la digestió anaeròbia mitjançant els models de Gompertz modificat, transferència, equació logística, model del con i Richards modificat.

En la caracterització fisicoquímica es va determinar que les relacions SV/ST dels substrats i cosustratos van oscil·lar entre 58 i 77% amb una relació C/N entre 12 i 102, la qual cosa va indicar que aquests residus són matèries primeres adequades per a la producció de metà. En tots els assajos un augment de la quantitat d'inòcul va millorar la biodegradabilitat dels substrats i per consegüent la producció metà; així, en la monodigestión es va tindre increments de fins a 90% i en la codigestión increments del 71%. Totes les mescles van produir efectes sinèrgics, on els majors percentatges de metà es van donar quan les mescles de residus d'amarant, quinua i blat van ser del 50 i 75% de sòlids volàtils. Independentment de la SIR1:1 i la SIR 1:2 es va millorar la producció de metà de la codigestión en incrementar el percentatge de cosustrato especialment de residus d'amarant i quinua. Els millors resultats de tots els assajos realitzats es van obtindre en els biodigestores compostos per residus d'escorxador i residus de quinua, on es van obtindre produccions de metà entre 581 i 555 ml/g VS. Pel que fa al modelatge cinètic del procés de digestió anaeròbia es va poder comprovar que tots els models van

ajustar bastant bé els valors experimentals amb els pronosticats. En la monodigestión, en tots els models logístics, les asímptotes calculades es van ajustar amb molta precisió el rendiment específic (M_e) el que va fer que no varien més del 7,06% respecte a les dades experimentals, mentre que el model del con va generar diferències entre la producció experimental de metà i Me de l'ordre del 26%. Igualment, en la codigestión, el model con va generar grans diferències (20 i 30%) entre la producció experimental i Em. De tots els models logístics i complexos el model de la transferència va ajustar bastant bé els resultats ja que en molts assajos es va obtindre un R^2 superior al 99% i valors de RMSE inferiors al 2 ml/g SV. No obstant això, la predicció de metà dels models cinètics va dependre de la matèria primera emprada, ja que no totes les mescles van tindre el mateix comportament.

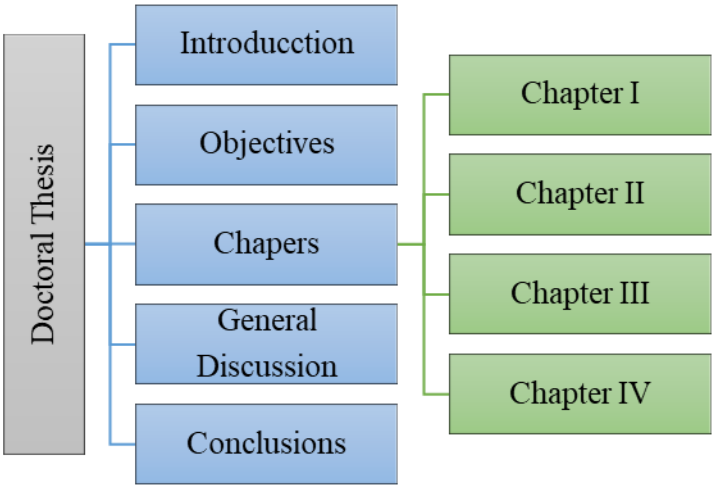
PREFACE

DISSERTATION OUTLINE

This Doctoral Thesis is divided into five sections: introduction, objectives, chapters, general discussion and conclusions.

The **introduction** section analyses the state of the art of current technologies and pre-treatments used in the anaerobic digestion of cow, pig and poultry manure, analysing their main pre-treatments: physical, chemical and biological. In addition, the advantages and disadvantages of its applicability are highlighted since the effects of pre-treatments are complex and generally depend on the characteristics of the animal manure and the operational parameters. All these aspects have been examined in a review entitled “Pre-treatment of animal manure biomass to improve biogas production: A review”. The introduction also includes a specific discussion on the mathematical modelling of the kinetic behaviour of anaerobic fermentation to predict the evolution of the system over time, establish characteristic parameters of the raw material, fermentation speed and establish the optimal conditions of the process performance. This discussion corresponds to a book chapter entitled "Review of mathematical models for the anaerobic digestion process".

The **objectives** section presents the general and specific objectives of the Thesis, which focuses on the analysis of biogas production from typical raw materials in the Andean region of Guaranda.



The results obtained are organized into four chapters, each one corresponding to a scientific publication with the usual sections: introduction, materials and methods, results and discussion, and conclusions.

Chapter 1, entitled “Biochemical potential of methane (BMP) of camelid waste and the Andean region agricultural crops”, analyses the processes of anaerobic monodigestion of agricultural wastes of amaranth, quinoa, and wheat, residues of llama, vicuña and guinea pig manure, and residues of cattle slaughterhouses of the municipal slaughterhouse of Guaranda. The results showed that the highest cumulative maximum methane production rate was achieved from flame manure residues and quinoa straw for a substrate inoculum ratio (SIR) 1:2 with a production of 376.08 ml CH_4/g VS and 377.02 ml CH_4/g VS,

respectively. On these materials, tests with a SIR1:2 improved methane production by 22.56% and 37.54% compared to tests with a SIR1:1.

Chapter 2, entitled "Effect of the co-digestion of agricultural lignocellulosic residues with manure from South American camelids", aimed to analyse the effect of the co-digestion of agricultural residues with manure from camelids from the Andean zone. Different combinations of llama and vicuña manure were made with amaranth, quinoa and wheat residues. The co-digestion was evaluated in 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. The results indicated that the maximum methane accumulation rate is obtained in the SIR (1:1) for a vicuña/amaranth ratio (25:75) with a production of 540 ml CH_4/g VS.

Chapter 3, entitled "Anaerobic co-digestion of slaughter residues with agricultural waste of amaranth quinoa and wheat", analysed anaerobic co-digestion of slaughterhouse residues from cattle with straw residues from agriculture, such as: amaranth, quinoa and wheat. Anaerobic co-digestion resulted in methane yields of 407 ml CH_4/g VS, with a biogas methane content of 77% for the slaughterhouse waste and quinoa mixture (25:75). The increase in inoculum in the mixtures composed of slaughterhouse waste and quinoa increased the biodegradability between 17 and 22%.

Chapter 4, entitled "Evaluation of methane production from the anaerobic co-digestion of manure of guinea pig with lignocellulosic Andean's residues", focused on the evaluation of the anaerobic co-digestion of guinea pig manure with Andean agricultural residues such as amaranth, quinoa and wheat in batch biodigesters. In terms of methane production, the best results were given in the treatments that contained amaranth and quinoa residues as co-substrate and a SIR1:2. Thus, the highest methane production occurred in the guinea pig/amaranth (25:75) and guinea pig/quinoa (25:75) biodigesters with 341.86 mlCH_4/g VS and 341.05 mlCH_4/g VS, respectively.

In the **general discussion** section, the main results obtained in the different chapters were analysed together, from a global perspective.

Finally, the last section shows the most relevant conclusions of the Thesis.

DISSEMINATION OF RESULTS

INTERNATIONAL JOURNALS JCR

Published

Review:

"Pre-treatment of Animal Manure Biomass to Improve Biogas Production: A Review". Meneses-Quelal O., Velázquez-Martí B., Gaibor-Chávez J and Niño-Ruíz Z. Energies (2020), 13(14), 3573. https://doi.org/10.3390/en13143573

Chapter:

"Review of Mathematical Models for the Anaerobic Digestion Process". Meneses-Quelal O., Velázquez-Martí B., Gaibor-Chávez J and Niño-Ruíz Z. Anaerobic Digestion, J. Rajesh Banu, IntechOpen (2018), 13(14), 3573.DOI: 10.5772/intechopen.80815.

Research articles:

"Effect of the co-digestion of agricultural lignocellulosic residues with manure from South American camelids". Meneses-Quelal O., Velázquez-Martí B., Gaibor-Chávez J and Niño-Ruíz Z. Biofuels, Bioprod. Biorefining (2021). https://doi.org/https://doi.org/10.1002/bbb.2177

"Biochemical potential of methane (BMP) of camelid waste and the Andean region agricultural crops". Meneses-Quelal O., Velázquez-Martí B., Gaibor-Chávez J and Niño-Ruíz Z. Renew. Energy 168, 406–415. https://doi.org/10.1016/j.renene.2020.12.071

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Research article:

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"Evaluation of methane production from the anaerobic co-digestion of manure of guinea pig with lignocellulosic Andean's residues". Meneses-Quelal O., Velázquez-Martí B., Gaibor-Chávez J., Niño-Ruíz Z and Ferrer-Gisbert A. Environmental Science and Pollution Research

COMMUNICATIONS IN INTERNATIONAL CONGRESSES

Oral communication:

"Evaluation of the generation of methane from the anaerobic co-digestion of lignocellulosic materials of difficult gradation with residues of the Andean livestock". Meneses-Quelal O., Velázquez-Martí B., Gaibor-Chávez J and Niño-Ruíz Z. "V International Congress of Sciences, Technology, Innovation and preneurship". Guaranda, Ecuador (2018).

"Methane production from slaughterhouse waste and wheat straw: influence of concentration". Meneses-Quelal O., Velázquez-Martí B. "I International Congress of Science and Technology Morona Santiago CICTMS". Macas, Ecuador (2020).

COMMUNICATION IN SCIENTIFIC EVENTS

Poster:

Meneses-Quelal O., Velázquez-Martí B. **Optimización de procesos de co-digestión anaerobia de materiales lignocelulósicos de difícil degradación con residuos de la ganadería andina.** *VI Encuentro de Estudiantes de Doctorado de la Universitat Politècnica de València*. Valencia, Spain (2019).

PREDOCTORAL STAYS AT FOREIGN INSTITUTIONS

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INTRODUCTION

1. Pre-treatment of Animal Manure Biomass to Improve Biogas Production: A Review

Excessive organic waste agricultural accumulation, especially animal manure, can be a source of contamination of land, water and air [1]. In this sense, there are many efforts to transform these wastes into clean and renewable energy, such as the use of anaerobic digestion (AD) to produce biogas. Animal manure is considered very attractive for the production of renewable energy, since it is a natural resource that can additionally replace industrial fertilizers and improve soil fertility [2]. However, manure has some limitations, since it has a low C/N ratio, little volatile solids (VS) and many materials of difficult degradability, such as lignocellulosic biomass making biogas production unsatisfactory [3,4,5,6]. This limitation results from cattle diet based on pasture residues that include a significant content of lignocellulosic materials [7,8]. Hence, in recent years, there has been great interest on the part of many researchers in improving AD animal manure processes [9,10].

The hydrolysis stage is one of the limiting factors of AD due to the difficult degradation of lignocellulosic materials [11]. Generally, these materials are composed of cellulose, hemicellulose, lignin, and various inorganic materials [12]. Cellulose represents between 40 and 50%, hemicelluloses between 25 and 35% and lignin between 15 and 20%; materials that are extremely resistant to enzymatic digestion [13]. The conversion of lignocellulosic biomass residues, mainly from agricultural waste, municipal waste, animal manure, etc., into biofuels is very complex [14]. In many of these residues, lignin is usually the material that causes the most inconvenience in digestion [15]. It has been shown that the higher the lignin content, the greater the resistance of biomass to degradation [16].

Therefore, it is necessary to look for new technologies aimed at addressing the AD process to optimize it and eliminate the bottleneck generated in the hydrolysis process [17]. The proposed alternatives contemplate the inclusion of a pre-treatment stage prior to the AD process [10]. Pre-treating the substrate makes for a more efficient conversion of hardly degradable biomass, accelerating the hydrolysis process, and therefore improve biogas production [18]. However, each type of manure has its own biodegradability process, which makes the pre-treatments that are proposed to optimize fermentation have their own specificity and are diverse.

A large number of investigations are focused on seeking pre-treatments to improve the biogas production of agricultural residues such as cereals, pruning remains, sewage sludge, etc. However, in regard to animal manure, especially cow, pig and poultry, there are few studies in the literature examining their adaptability to anaerobic biodegradability. Hence, there is a special interest in compiling the most widely used pre-treatment methods in the fermentation of livestock waste.

Pre-treatments prepare the substrates to facilitate the action of microorganisms reducing size and molecular composition of the pre-treated substrate, making it more accessible to bacterial consortia present in a reactor [19]. Atelge et al. [20] deem that pre-treatments increase the substrate's surface area so that enzyme activity is enhanced, causing biomass de-crystallization resulting in increased digestibility [21]. In addition, pre-treatments intensify porosity in the substrates, causing greater microbial accessibility [22]. Similarly, some pre-treatments contribute to hemicellulose removal and lignin from the substrate; this elimination increases the accessibility to cellulose, facilitating the degradation process [21]. For these reasons, the development of new technologies and various methods for biomass pre-treatment continue. Likewise, the applicability of different pre-

treatments cannot be generalized for all substrates since there is a lack of common and standardized protocols to evaluate their efficacy [23].
The objective of this review is to present the foundations and current states of various pre-treatments applied to anaerobic digestion of cattle, pig and poultry livestock waste. The successes obtained and the existing difficulties of the techniques used in maximizing biogas production are highlighted. Moreover, the composition of the lignocellulosic material is described, giving an overview of its incidence in the hydrolysis phase of the AD process.

1.1 Hydrolysis in Anaerobic Digestion of Animal Waste

Relating the content of cellulose, hemicellulose and lignin present in animal manure with its methane production is very important, since through this it can be known which lignocellulosic component has the greatest influence on the biodegradability of the substrate. The AD process is clearly complex and depends on many factors; however, knowing the lignocellulosic composition of each type of manure, a type of pre-treatment can be applied to each of them.

Table 1. Results of monodigestion of pig, cow and poultry manure with different fibre compositions.

Feedstock	Cellulose (%)	Lignin (%)	Hemicellulose (%)	CH_4 mL/g VS	Inoculum	References
Pig manure	32.4	18.4	14.6	191.4	a	[26]
Pig manure	15.9	1.8	16.7	377.0	b	[24]
Pig manure	22.0	9.8	22.0	111.0	b	[27]
Pig manure	11.9	7.7	18.8	178.7	b	[28]
Pig manure	18.2	4.8	21.5	187.7	b	[29]
Pig manure	23.6	8.4	21.7	245.1	b	[30]
Cow manure	21.2	11.6	30.4	37.5	c	[31]
Cow manure	23.5	8.0	12.8	270.0	b	[24]
Cow manure	17.9	18.2	15.7	206.9	b	[29]
Cow manure	22.9	8.1	22.9	112.1	d	[32]
Poultry manure	37.2	8.4	25.5	163.2	a	[33]
Poultry manure	44.0	1.7	11.8	410.0	a	[24]
Poultry manure	20.0	2.3	23.2	260.8	a	[34]
Poultry manure	4.4	4.2	19	158.0	a	[35]
Poultry manure	14.9	3.3	24.3	273.9	a	[29]
Poultry manure	24.3	5.1	9.9	261.7	e	[36]

Table 1 shows cow, pig and poultry manure residue fibre content mainly used in recent years. Recorded values show a high dispersion, although the same type of manure is compared. This is due to the fact that the digestibility of the animals is varied in the different parts of the world, which makes the percentages of lignocellulosic material vary with very wide ranges among themselves [24]. In the table, the methane production and the inoculum used in the anaerobic digestion process are also presented. In most investigations, sludge from wastewater treatment plants of various raw materials is used as inoculum. The inclusion of an inoculum has been key in the start-up of the digesters. The quality and quantity are determiners in defining the start-up period duration and

digester performance, since this is where the active biomass grows and acquires vital properties necessary for organic matter removal, consequently reducing digestion time [25].

In Table 1, pig manure presents averages of cellulose, hemicellulose and lignin of 20.67%, 19.22%, and 8.48%, respectively; cow manure, on the other hand, has ranges of 21.38%; 20.45% and 11.48%, respectively. Finally, poultry manure contains cellulose, hemicellulose and lignin of 24.13%, 18.95% and 4.17%, respectively. The lignocellulosic composition of other organic wastes is also similarly formed. Thus, for example, cereal residues contain 30–45% cellulose, 10–40% hemicellulose, and 5–25% lignin [37]. Lawn waste contains 25–39% cellulose, 17–32% hemicellulose, and 9–20% lignin [38]. Alternatively, fruit waste has varied compositions and depends mainly on the relative proportion of skin and seeds of individual sources [39].

Although the minor lignocellulosic component is lignin; this is the material that generates the most inconvenience in the digestion of animal manure. The highest percentage of lignin was registered in cow manure (9.8%), then in pig manure (8.5%) and finally in poultry manure (4.2%). As the value of lignin decreases, methane production increases both for cow manure and for pig and poultry manure (Figure 1), demonstrating that the recalcitrant content of lignin mostly inhibits methane production. Thus, the average value of methane production from the monodigestion of pig, cow and poultry manure is 215 mL/g VS, 168 mL/g VS, and 255 mL/g VS, respectively.

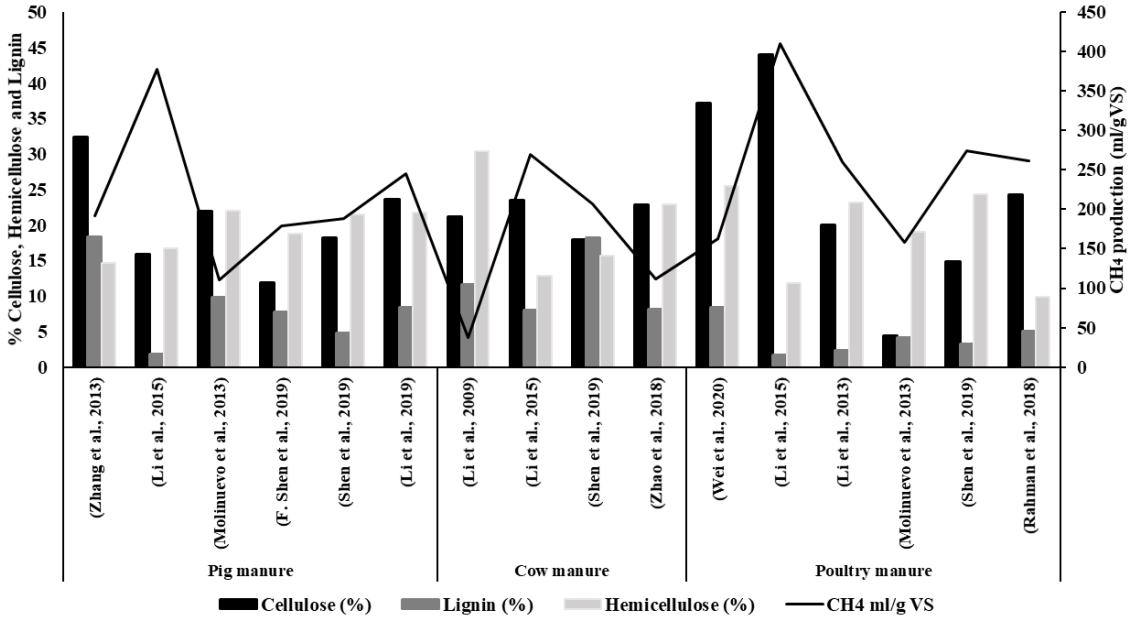

Figure 1. Production of methane from livestock residues and influence of cellulose, hemicellulose and lignin content.

The above-mentioned results have been carried out in batch digesters, using sewage sludge, sludge from a beer waste treatment plant and sludge from an anaerobic livestock waste digester. The results of the monodigestion of the latter are low; the reasons for its poor performance are diverse. For instance, the higher the lignan content, the greater the biomass resistance to degradation [16]. Additionally, because the concentration of volatile solids in animal manure is very low, it accounts for significantly reduced substrates production [11].

The conversion of cellulose and hemicellulose into energy also generates low efficiency in the production of biogas due to the intra and intermolecular hydrogen bonds of the

hydroxyl groups, producing a supramolecular structure with a high degree of polymerization [16]. Thus, hydrogen bonding causes cellulose crystallinity to occur, making digestion difficult during enzymatic hydrolysis [40]. In short, the presence of lignocellulosic material affects the hydrolysis process, creating a barrier or shield that prevents the action of microorganisms in substrate degradation.

1.2 Pre-treatments and Techniques to Improve the Digestion of Animal Manure

One of the techniques traditionally used to overcome the limitation of hydrolysis is the solubilization and degradation of the hemicelluloses and lignin parts of the substrate [41]. The objective of the pre-treatment process is to eliminate lignin and hemicelluloses, reducing the amount of crystalline cellulose and increasing the porosity of lignocellulosic materials [42]. There are different types of pre-treatments to remove lignocellulosic material, all of which are related through the use of physical, chemical, physicochemical, and biological procedures [43,44].

1.2.1 Physical Pre-treatments

Physical pre-treatments break cells through physical force, allowing them to increase the surface area of the biomass by reducing particle size. This reduction in size can improve biomass accessibility and increase its susceptibility to microbial and enzymatic attacks, promoting biomass digestion during AD [21]. Furthermore, physical pre-treatment does not produce secondary inhibitory substances, suggesting that they might be suitable to produce methane or any other bioprocess. It is classified into two groups: mechanical, which includes milling and extrusion; and thermal [11,45].
In general, mechanical pre-treatments and their combination with thermal ones cut, grind, and reduce cellulose crystallinity, but, above all, they reduce particle size, facilitating the activity of microorganisms in the degradation of biomass [46]. These are highly effective methods, but their applicability is expensive and demands high energy, in addition to making extrapolation challenging on an industrial scale.

1.2.1.1 Mechanical Pre-treatment

Milling is a pre-treatment that reduces the crystallinity of the cellulose, increasing the digestibility of the particles [42]. The choice of techniques depends on the moisture content of the biomass [21]. However, milling has the limitation that it does not eliminate lignin, being an unsuitable option for those substrates that have a large amount of lignin [47]. Extrusion, on the other hand, is a method where compression and shear forces improve the degree of softening that causes greater access by microorganisms [11]. The duration of applicability of the pre-treatment depends on the type of biomass treated, which means that its application cannot be standardized [45].

1.2.1.2 Heat Pre-treatment

Thermal treatments consist of reaching temperatures between 150 and 250 °C. The most common treatments are usually cooking and radiation. Both require a closed and hermetic bottle that allows them to reach those temperatures.
Cooking or treatment with liquid hot water (LHW) consists of heating the manure while maintaining the liquid state of the water by increasing the pressure by 5 MPa [12,47]. In

this pre-treatment, hemicellulose is depolymerized and the products dissolve in the liquid phase, while cellulose is completely retained in the solid phase [48].
Radiation is usually with microwaves on wet manures or infrared on drier manures. One of the advantages of microwave irradiation is the degradation of lignocellulose materials into more brittle fibres and low molecular weight oligosaccharides; degradation that is obtained through the dissociation of glycoside bonds [42,49].
The application of heat has the disadvantage of compromising energy balance since many pre-treatments demand high energy costs, as the degradation of lignin requires high temperatures to dissolve [50].

1.2.2 Physicochemical Pre-treatments

1.2.2.1 Steam Explosion

This pre-treatment consists of placing the biomass in a reactor with saturated steam under conditions of temperatures and pressure of 160–200 °C and 0.69–4.83 MPa, respectively [51]. Once the steam condenses and penetrates the pre-treated biomass, it is suddenly depressurized. The glycosidic hemicellulose bonds are then broken and its solubilization occurs [52]. In this way, the pressure is gradually released and the steam expands through the lignocellulosic material of the organic matter, breaking the cell wall [53].

1.2.2.2 Plasma

Plasma pre-treatment consists of applying ozone (O_3) to the biomass composed of lignocellulosic materials. The application of ozone causes an alteration of the biomass and radioactive compounds such as HO and H_2O_2 are generated. In this way, the interaction of these compounds in the biomass contributes to a degradation of the lignocellulosic materials and simpler compounds (such as glucose) are obtained as a product. In short, the surface of the pre-treated biomass is altered and the action of the macro-organisms is facilitated, producing an acceleration of the hydrolysis process [52,54].

1.2.2.3 CO_2 Explosion

The application of CO_2 as pre-treatment of biomass in the anaerobic digestion process is a process in which CO_2 is used as a green solvent to treat biomass before hydrolysis. Their procedure consists of applying CO_2 to the biomass in the presence of water to accelerate the enzymatic digestibility [55]. CO_2 acts as a solvent in the pre-treated biomass transforming it into glucose through the enzymatic hydrolysis of cellulose from the exploited materials [56]. An upside of this pre-treatment is that it requires little temperature and it is easy to separate the solvent from the pre-treated biomass. Finally, it does not generate flammable or corrosive products in its applicability [47].

1.2.2.5 Ammonia Fibre Expansion (AFEX)

In this pre-treatment, the biomass is subjected to the application of ammonia at relatively high temperatures (90–100 °C) [12]. This process normally adds ammonia to a reactor containing lignocellulosic material at high pressure and temperature for approximately 30 min. Once the pre-treatment has begun, the pressure is gradually decreased until the degradation of hemicellulose in oligomeric sugars is achieved [47]. An advantage of this

pre-treatment is that deacetylation of the pre-treated material is achieved and, on the other hand, ammonia can be recovered for reuse in the next procedures. However, this pre-treatment does not alter lignin, which makes the hydrolyzation of cellulose and hemicellulose possible [57].

1.2.3 Chemical Pre-treatments

1.2.3.1 Alkaline Hydrolysis

This pre-treatment consists of adding alkaline compounds (NaOH, Ca $(OH)_2$, NH_3, etc.) to the biomass to accelerate the hydrolysis process. The choice of the type of alkaline solution is made based on cost and its possibility of recovery. Thus, for example, $Ca(OH)_2$ is the least expensive, and in addition calcium can be recovered in insoluble calcium carbonate by neutralizing calcium with carbon dioxide [47]. This pre-treatment is very useful in the solubilization of lignin [58]. According to Janker et al. [59], NaOH causes the interruption of the hydrogen bond in cellulose and hemicellulose; breaking the ester bonds between lignin and xylan and causing the deprotonation of phenolic groups. Many researchers consider that the application of NaOH as a pre-treatment generates better biomass digestibility results than the application of $Ca(OH)_2$ [11].

1.2.3.2 Acid Hydrolysis

This pre-treatment consists of treating the biomass at high and low temperatures with the following compounds: sulfuric acid (H_2SO_4), hydrochloric acid (HCl), acetic acid (CH_3COOH) and nitric acid (HNO_3). Pre-treatment can be performed with dilute acid (low concentration and high temperature) and with concentrated acid (high concentration and low temperature) [12]. The application of this pre-treatment contributes to the elimination of lignin, causing better cellulose degradation by different enzymes and microorganisms. Li et al. [60] consider that acid pre-treatment causes the interruption of van der Waals forces, hydrogen bonds and covalent bonds that hold the components of the biomass together, causing the solubilization of hemicellulose and the reduction in cellulose.
However, the main disadvantages of applying this pre-treatment is the high cost of equipment resistant to corrosive acids, and the need to recover and recycle some chemicals or solvents [11]. Thus, the high cost of the necessary equipment and the need for additional energy for the thermal process make it unprofitable [61].

1.2.3.3 Organosolv

This method is generally used to extract lignin from lignocellulosic raw materials. This extraction causes the cellulose fibres to be exposed to enzyme activity, causing further acceleration of the hydrolysis phase. This extraction exposes cellulose fibres to enzyme activity, inducing further acceleration of the hydrolysis phase. Furthermore, aqueous organic solvents (methanol, acetone, ethanol, and ethylene glycol) can be used to remove or decompose part of the hemicellulose [62]. The use of these solvents has the advantage of being easy to recover and recycle; its recovery can be carried out through a distillation process once the pre-treatment has finished. Furthermore, the pre-treatment with Organosolv is implanted in a catalyst (a salt, an acid or a base) with temperatures below 200 °C; although, it generally depends on the type of biomass that is being pre-treated [47]. There are many catalysts used in the literature, including acid, sodium hydroxide,

and magnesium sulphate. Of all of them, sulfuric acid and sodium hydroxide have proven to be very effective in improving digestibility; whereas sulfuric acid is highly toxic and inhibitory in biogas production [63].

1.2.3.4 Wet Oxidation

This treatment consists of applying oxygen to the manure with high temperature and pressure [64]. The temperatures necessary for pre-treatment are around 140–200 °C with an approximate time of 30 min [65]. Wet oxidation makes the biomass susceptible to enzymatic hydrolysis, and pre-treatment separates the raw material into cellulose, lignin, and hemicellulose fractions through its solubilization and degradation [12,66]. Wet oxidation is an alternative to steam explosion. Within chemical pre-treatments, wet oxidation is more efficient to treat lignocellulosic materials, since the crystalline structure of cellulose opens during the process [67]. Organic molecules, including lignin, are broken down into CO_2, H_2O, and simpler and more oxidized organic compounds, mainly into low molecular weight carboxylic acids [68].

1.2.3.5 Alkaline Peroxide

Hydrogen peroxide (H_2O_2) pre-treatment is a low-cost pre-treatment that results in increased accessibility of enzymes to the surface of the lignocellulosic material. Hydrogen peroxide removes and breaks the lignin walls that make up the biomass outer shell, making it more exposed to enzyme activity [69]. In this method, lignocelluloses are immersed in pH adjusted water (e.g., pH 11–12 with NaOH) containing H_2O_2 at room temperature within 6 to 24 h period [12].

1.2.4 Biological Thermal Pre-treatments

Currently, there are several biological pre-treatments that are used to pre-treat biomass and obtain higher biogas yields. However, all pre-treatments employ microorganisms (white and soft rot fungi, actinomycetes, and bacteria) to degrade the recalcitrant material of lignocelluloses [70]. Biological pre-treatment to improve biogas production in anaerobic digestion has mainly focused on fungus, microbial consortium pre-treatment, and enzyme pre-treatment [71].

White or brown rot fungi degrade lignin, and to a lesser extent cellulose and hemicellulose through a family of extracellular enzymes collectively called “lignases”, such as lignin peroxidase, manganese peroxidase, and laccase [72,73]. White rot fungi break down a broad spectrum of environmentally persistent xenobiotics and organic pollutants [74]. Thus, over a long period of time, biomass is inoculated with fungi lignolytic enzymes to degrade lignocellulosic material. Moreover, in biological pre-treatment, several enzymes are required to achieve greater efficiency in biomass degradation. Mixtures of different enzymes cause greater synergy to expand small pores and increase access to the cell wall [46,75]. Although there is a diversity of fungi used in biological pre-treatment, the most widely used are: Phanerochaete chrysosporium, Trametes versicolor, Ceriporiopsis subvermispora, Pleurotus ostreatus, Ceriporia lacerata, Pycnoporus cinnabarinus, Cyathus cinnabarinus, Bjerkandera adusta, Ganoderma versceumum, Irpex lacteus, Lepista nuda and Phanerochaete chrysosporium, Sporotrichum, Aspergillus, Fusarium, Penicillum, etc. [12,47].

Biological pre-treatment through a microbial consortium mainly attacks cellulose and hemicellulose. Generally, microbes are extracted from natural environments, such as

decomposing straw and thermophilic landfills [76]. The biodegradation of cellulose and hemicellulose under these microbial consortiums has turned out to be a very efficient pre-treatment for biotechnological application, since it avoids the problems of regulation by feedback and repression of metabolites posed by isolated strains [77]. Finally, biological pre-treatment also uses enzymes with hydrolytic activity that include cellulase and hemicellulase [78]. Many studies suggest that the addition of enzymes used in the pre-treatment of manure can improve the performance of anaerobic digestion systems [79]. In general, biological pre-treatments are not as expensive; however, they are slow and require a large space with fairly controlled environments to make their application more efficient [80]. Furthermore, for biological pre-treatments to be feasible in the application of commercial biogas production, additional research is needed to address some key issues such as cost, selectivity, and efficiency [71].

Table 2. Types of pre-treatments most used to improve biogas production.

Pre-treatments	Effects on Lignocellulosic Structure			References
	Cellulose	Hemicellulose	Lignin	
Physical				
Milling	Reduces crystallinity			[42,47]
Extrusion	Decreases the degree of polymerization			[11]
Microwave irradiation	Increases substrate availability for enzymes			[42,49]
Physicochemicals				
Steam explosion		Greater solubilization	Solubilization Alteration of the structure	[53]
Plasma	Degrades it into glucose			[52,54]
CO_2 explosion	Break the structures	Break the structures		[56]
Liquid hot water (LHW)		Increased solubilization and depolymerization		
Ammonia fibre Expansion (AFEX)		Degradation in oligomeric sugars Deacetylation		[47]
Chemical				
Alkaline hydrolysis		Solubilization of hemicellulose	Decompose, alter and breakdown of lignin	[58,59]
Acid hydrolysis		Solubilization of hemicellulose	Decompose, alter and breakdown of lignin	[60]
Organosolv process		Solubilization of hemicellulose	Lignin solubilization	[63]
Wet oxidation			Lignin solubilization	[12,66]
Alkaline peroxide			Altered lignin structure	[69]
Biological				
Pre-treatment with microbial consortia, fungi and enzymes	Degrade cellulose	Degrades hemicellulose	Degrades lignin	[46,75]

Table 2 summarizes the different types of pre-treatments most used. Most of them affect all lignocellulosic material; however, some affect more a part than the rest of the lignocellulose composition. The main effects of pre-treatments on cellulose, hemicellulose and lignin are presented.

1.3 Application of Pre-treatments to Livestock Waste

1.3.1 Pre-treatments Applied to Cow Manure

Cow manure has provided low methane yield results [81], since it is made of highly undegradable material inhibiting the biogas production process. However, cow manure is highly available and has many advantages due to its synergistic nature to balance pH, C/N ratio and nutrient content [2]. Table 3 shows some pre-treatments used in the monodigestion of cow manure to accelerate the hydrolysis phase. Most of the pre-treatments analyzed are carried out in batch reactors and using inoculum to start the AD process.

Angelidaki and Ahring [82] conducted a study on biological pre-treatment through B4 bacteria to degrade hemicellulose from cow manure. Digested manure from a laboratory reactor under thermophilic conditions was used as inoculum. Results showed that monodigestion can improve methane production by 30%, which implies a methane production of 300 mL CH_4/g VS. However, not many studies have been conducted on the anaerobic biodegradability of monodigestion from cow manure with biological pre-treatments.

In another study, Ferreira et al. [88] pre-treated cow manure through a physicochemical (thermal) pretreatment. In this experiment the sample was pre-treated at 125 °C. The results were positive, obtaining 450 mL CH_4/g VS; that is, 35% more than the control tests. Similarly, Qiao et al. [84] carried out a study with cow manure, pre-treating it in boilers at 170 °C. The results were not so favorable (130.2 mL CH_4/g VS), which meant a decrease of 7% compared to the untreated material. The fact that methane production was low may be because no inoculum was used. Nielsen et al. [3] also used heat to pre-treat cow manure. They carried out an experiment at 68 °C, using digestion of cow manure sludge from a laboratory scale digester as inoculum. They concluded that methane production can improve up to 56%. However, the accumulated methane production was 260 mL CH_4/g VS.

As a physical pre-treatment, Angelidaki and Ahring [82] mechanically pre-treated cow manure. They macerated the manure to decrease the particle size to 0.35 mm, pressurizing it to 100 atm. In the test they used digested manure as the inoculum and obtained a methane production of 276 mL CH_4/g VS. Through this pre-treatment, methane production increased by 20%. In another experiment by Coarita et al. [85], they also pre-treated cow manure under mechanical techniques. They used mobile hammer mills to grind the manure and decrease its size. They wet-sieved the samples at different particle size calibrations (0.25–31.5 mm). During the digestion process, they used sludge from an anaerobic digester of a treatment plant as inoculum and obtained productions of 316 mL CH_4/g VS with improvements of 15%. Similarly, Tsapekos et al. [4] carried out studies on mechanical pre-treatments with cow manure. They used a combination of three plates: aluminum, sandpaper, and stainless steel. The combination of these plates allowed them to apply shear forces on the samples and decrease the size of the manure. As in the previous case, they used anaerobic sludge from a sewage digester as inoculum. As a result of the pre-treatment, they obtained a methane production of 168 mL CH_4/g VS. Mechanical pre-treatment showed a positive effect on the digestibility of the fibres, which caused a four-fold production improvement.

Table 3. Effects of the different pre-treatments applied to cow manure.

Pre-treatment	Process	Inoculum	Initials Condition	CH_4 (mL/g VS)	Methane Enhancement (%)	References
Biological	Incubation (7 days, 70 °C with B4 bacteria to degrade hemicellulose)	Digested manure from a thermophilic laboratory reactor	Vr = 0.117 L; TRH = 40–60 d; T = 55 °C	300.0	30	[82]
Physiochemical	125 °C, 37.5 min and 24 h	Digestate from a wastewater plant	TS = 16.12%; VS = 13.64%; pH= 7.85; C/N = 16.1; Vr = 2 L; TRH = 40 d	450.0	35	[83]
Physiochemical	Boiler 1l (170 °C at 1 h)	-	TS = 34.66%; VS= 19.52%; pH= 8.57; Vr = 0.250 L; TRH = d; T = 37 °C	130.2	−7	[84]
Physiochemical	68 °C (36, 108 and 168 h)	Digested sludge from cattle manure of a laboratory scale digester	Vr = 116 L; TRH=70 d; T = 68–55 °C	260.0	56	[3]
Physical	Maceration with a blender <0.35 mm and pressurizing the manure to 100 atm	Digested manure from a thermophilic laboratory reactor	Vr = 0.117; TRH = 40–60d; T = 55 °C	276.0	20	[82]
Physical	Mobile hammer mills. Sieving	Sludge from an anaerobic digester from a WWTP	TS = 19.6%; VS = 17.32%; pH = 8.23; Vr = 1 L; TRH = 39 d; T = 35 °C	316.3	15	[85]
Physical	Combination of three plates: aluminum, sandpaper and stainless steel	Sludge from an anaerobic digester from a WWTP	TS = 223.59 g/kg; VS = 191.87 g/kg; pH = 8.32; Vr = 0.164 L, TRH = 30 d; T = 53 °C	168.0	-	[4]
Chemical	$Ca(OH)_2$, 60 °C, 12 and pH of 12	Sludge from an anaerobic digester from a WWTP	Vr = 0.118 L; TRH = 45 d; T = 37 °C	225.0	76	[86]
Chemical	Calcium oxide (CaO)	Sludge from an anaerobic digester from a WWTP; sludge from an agro-industrial cow manure digester	TS = 9.84%; VS = 8.34%; pH = 7.15; Vr = 1.6 L; T = 38 °C	168.2	26	[87]
Chemical	Peracetic Acid ($C_2H_4O_3$)	Sludge from an anaerobic digester from a WWTP; sludge from an agro-industrial cow manure digester	TS = 9.84%; VS = 8.34%; pH = 7.15; Vr = 1.6 L; TRH = 43 d T = 38 °C	182.4	39	[87]
Chemical and Physiochemical	NaOH 6% *p*/*p* TS 121 °C, 20 min	Sludge from a WWTP anaerobic digester	TS = 223.59 g/kg; VS = 191.87 g/kg; pH = 8.32; Vr = 0.164 L; T = 53 °C	168.0	155	[4]

Table 4. Effects of the different pre-treatments applied to pig manure.

Pre-treatment	Process	Feedstock	Inoculum	Initials Condition	CH_4 (mL/g VS)	Methane Enhancement (%)	References
Physiochemical	170 °C at 1 h	Pig manure	-	TS = 28.14%; VS = 22.26%; pH = 6.91; Vr = 0.250 L; TRH = 43 d; T = 37 °C	290.8	14.6	[84]
Physiochemical	Thermal steam explosion (170 °C and 30 min)	Pig manure	Sludge from a WWTP anaerobic digester	TS = 46.6 g/kg; VS = 36.8 g/kg; C/N = 8.5; Vr = 0.300 L; T = 35.1 °C	329	206.9	[88]
Physiochemical	(100 °C) 1h	Dehydrated pig manure	Sludge from an anaerobic digester from a WWTP	TS = 46.6 g/kg; VS = 36.8 g/kg; C/N = 8.5; Vr = 0.300 L; TRH = 29 d; T = 35.1 °C	237.5	28	[93]
Chemical	$Ca(OH)_2$ al 5%, 2 h and neutralization of pH with HCl	Dehydrated pig manure	Sludge from an anaerobic digester from a WWTP	TS = 46.6 g/kg; VS = 36.8 g/kg; C/N =8.5; Vr = 0.300 L; TRH = 29 d; T = 35.1 °C	204.74	12	[93]
Chemical	6% NaOH (*p*/*p*)	Pig manure	Anaerobic sludge from a beer plant	TS = 84.5%; VS = 67.76%; Vr = 0.500 L; T = 35 °C	232.4	21.4	[29]
Chemical	$Ca(OH)_2$,1 h (70 °C)	Dehydrated pig manure	Sludge from an anaerobic digester from a WWTP	TS = 46.6 g/kg; VS = 36.8 g/kg; C/N = 8.5; Vr = 0.300 L; TRH = 29 d; T = 35.1 °C	345	72	[93]
Biological	Microbial community cell biocatalyst to accelerate degradation of antibiotics	Pig manure	-	TS = 28.14 %; VS = 22.26 %; pH = 6.91; Vr = 0.420 L; TRH = 7 d	98.7	93.2	[94]
Physical	Liquid and solid matrix separation using a 0.25mm pore size screen	Pig waste slurry	Sludge from an anaerobic digester from a WWTP	TS= 11.4%; VS = 9.34%; Vr = 1 L; TRH = 30 d; T=32 °C	251 mL/g DQO	−2.33	[95]
Physiochemical	Power at 600 W. The temperature increased with a ramp of 10 °C/min until reaching 80 °C and was maintained for 15 min supplemented with C	Pig manure	Sludge from an anaerobic digester from a WWTP	TS = 23.1g/l; VS = 15.2g/L; pH = 6.9 C/N = 10.9; Vr = 0.250 L; TRH = 30 d; T = 35 °C	433.2	39	[96]

* In all tests a batch reactor was experimented. Vr is the volume of the reactor

Another type of pre-treatment that has been widely used in the literature is the chemical pre-treatment, using either alkaline or acidic compounds [89]. For one thing, alkaline pre-treatment involves the use of bases such as sodium, potassium, calcium and ammonium hydroxide, for the pre-treatment of livestock manure [11]. Generally, the accessibility to carbohydrates of lignocellulosic biomass is limited, but can be improved with alkaline pre-treatment [90]. Seyedy et al. [86] in an experimental study showed the possibility of improving biogas production from cow dung with $Ca(OH)_2$ lime as a pre-treatment. Their studies contain the pre-treatment of cow manure in different alkaline conditions at a pH of 12 for 12 h. The alkaline pre-treatment results achieved a 76% improvement in methane production with respect to the untreated material; this was 225 mL of mL CH_4/g VS. Seyedy et al. [88] used calcium oxide (CaO) to pre-treat cow manure. They showed that its monodigestion markedly improves methane production by up to 26%. They used sewage sludge mixed with sludge from an agro-industrial cow manure digester to optimize the process, obtaining 168.2 mL CH_4/g VS of methane. Similarly, Ramos et al. [87] considered that the optimal conditions for alkaline pre-treatment are based on using sodium hydroxide (NaOH) at a concentration of 6% p/p of total solids with a temperature of 121 °C for 20 min. During co-digestion, they used sewage sludge and managed to obtain 168 mL CH_4/g VS of methane, which represents an increase of 155% compared to the untreated samples. Another way to apply chemical pre-treatment is through acidic compounds since it has a high selectivity with lignin [91]. A commonly used chemical compound is peracetic acid (PAA) as it solubilizes lignin by cleaving bonds resulting in lignin cleavage [92]. Ramos et al. [90] used peracetic acid (PAA) to improve methane production from cow manure. They carried out an experiment where they used as an inoculum mud from an anaerobic digester of a wastewater treatment plant (WWTP). They obtained 182.4 mL CH_4/g VS, which meant a 39% improvement in methane production with respect to the untreated material.

Overall, studies show that pre-treatments solubilize cow manure by increasing biodegradability and methane production. The most widely used treatments combine more than one pre-treatment, as is the case of physicochemical by the addition of heat. Regarding chemical pre-treatments, alkali compounds of NaOH and Ca $(OH)_2$ are commonly used. While temperature improves the production of biogas, temperature above 200 °C inhibits the fermentation process, decreasing biogas production.

1.3.2 Pre-treatments Applied to Pig Manure

Pig manure as raw material has great potential in production of biogas. However, it requires methods to optimize its biodegradation process and eliminate difficult-to-decompose materials impeding the hydrolysis acceleration process. Table 4 shows some pre-treatments used to improve the biogas production of AD from this raw material. In a study on anaerobic digestion, Qiao et al. [84] evaluated the biogas production from pig manure residues with and without hydrothermal pre-treatment. The pre-treatment was carried out in eight stainless boilers applying 170 °C for one hour. The researchers obtained a methane productivity of 290.8 mL CH_4/g VS, resulting in a 14.6% increase. Ferreira et al. [88] applied a thermal pre-treatment to a pig manure mixture by means of a thermal steam explosion. They evaluated the methane yield of the separated solid fraction of pig manure under different combinations of temperature and duration. They determined that the optimal temperature–time combinations of the pre-treatment were 170 °C and 30 min. They managed to double the methane production from 159 to 329 mL of CH_4/g VS, which represented an improvement of 206.9%. They demonstrated that temperature has a greater effect on methane yield than pre-treatment time. Rafique et al.

[93] used heat pre-treatment on dehydrated pig manure. They demonstrated that the maximum amount of biogas is obtained when the substrates were pre-treated with temperatures of 100 °C; however, above this temperature, production decreased rapidly. During monodigestion, they used sludge from an anaerobic digester from a WWTP as inoculum. After pre-treatment, they obtained 25% improvements, with a production of 237.5 mL of CH_4/g VS.

Another class of pre-treatments that are useful for improving methane production from pig manure are chemical pre-treatments. The use of compounds such as NaOH are highly efficient in improving pig manure fermentation through the solubilization of hemicellulose [97]. Zhang et al. [29] used NaOH with a concentration of 6% based on the total solids of the sample. After pre-treatment, the content of lignin, cellulose and hemicellulose decreased from the respective values of 18.36%, 32.36%, and 14.6% to 17.10%, 30.07%, and 10.65%. During the digestion process, they used anaerobic sludge from a beer plant as an inoculum, which reduced the amount of TS and VS by 48.5% and 70.4%, respectively. With the application of this pre-treatment, they obtained a methane production of 232.4 mL of CH_4/g VS, which meant an improvement of 21.4% compared to the untreated materials. Meanwhile, Rafique et al. [93] used a chemical pre-treatment on pig manure, focused on alkaline compounds. The samples were pre-treated with $Ca(OH)_2$ with a concentration of 5% for 2h; furthermore, before starting the AD process, they added hydrochloric acid (HCl) to the pig manure digesters to neutralize their pH. In the fermentation process, as inoculum, they used sewage sludge in mesophilic conditions for 29 days. At the end of the digestion time, they obtained a production of 204.74 mL of CH_4/g VS with an improvement of 12% compared to the controls. Furthermore, under the same conditions as above, they carried out another test using $Ca(OH)_2$ as a pre-treatment for pig manure, but applying a temperature of 70 °C. In this case, methane production was remarkably increased, reaching 345 mL CH_4/g VS, meaning a 72% increase. They showed that the use of temperatures not higher than 70 °C during the alkaline pre-treatment optimizes the methane production.

Another type of pre-treatment that improves methane production is biological. According to Feng et al. [98], many of the antibiotics administered to pigs are usually released through their droppings. In this sense, Liu et al. [94] carried out a study to eliminate β-lactam antibiotics present in pig manure in a biological way. They demonstrated that removing antibiotics from pig manure can greatly improve methane production. They carried out the biological pre-treatment using a biocatalyst made up of a microbial community that accelerates antibiotics degradation. With pre-treatment, penicillin, cefamezine, and amoxicillin were completely degraded by the biocatalyst for 1 h. Pre-treatment increased methane production by 93.2% when pre-treatment was performed for 3 days.

Table 4. Effects of the different pre-treatments applied to pig manure.

Pre-treatment	Process	Feedstock	Inoculum	Initials Condition	CH_4 (mL/g VS)	Methane Enhancement (%)	References
Physiochemical	170 °C at 1 h	Pig manure	-	TS = 28.14%; VS = 22.26%; pH = 6.91; Vr = 0.250 L; TRH = 43 d; T = 37 °C	290.8	14.6	[84]
Physiochemical	Thermal steam explosion (170 °C and 30 min)	Pig manure	Sludge from a WWTP anaerobic digester	TS = 46.6 g/kg; VS = 36.8 g/kg; C/N = 8.5; Vr = 0.300 L; T = 35.1 °C	329	206.9	[88]
Physiochemical	(100 °C) 1h	Dehydrated pig manure	Sludge from an anaerobic digester from a WWTP	TS = 46.6 g/kg; VS = 36.8 g/kg; C/N = 8.5; Vr = 0.300 L; TRH = 29 d; T = 35.1 °C	237.5	28	[93]
Chemical	$Ca(OH)_2$ al 5%, 2 h and neutralization of pH with HCl	Dehydrated pig manure	Sludge from an anaerobic digester from a WWTP	TS = 46.6 g/kg; VS = 36.8 g/kg; C/N =8.5; Vr = 0.300 L; TRH = 29 d; T = 35.1 °C	204.74	12	[93]
Chemical	6% NaOH (*p*/*p*)	Pig manure	Anaerobic sludge from a beer plant	TS = 84.5%; VS = 67.76%; Vr = 0.500 L; T = 35 °C	232.4	21.4	[29]
Chemical	$Ca(OH)_2$,1 h (70 °C)	Dehydrated pig manure	Sludge from an anaerobic digester from a WWTP	TS = 46.6 g/kg; VS = 36.8 g/kg; C/N = 8.5; Vr = 0.300 L; TRH = 29 d; T = 35.1 °C	345	72	[93]
Biological	Microbial community cell biocatalyst to accelerate degradation of antibiotics	Pig manure	-	TS = 28.14 %; VS = 22.26 %; pH = 6.91; Vr = 0.420 L; TRH = 7 d	98.7	93.2	[94]
Physical	Liquid and solid matrix separation using a 0.25mm pore size screen	Pig waste slurry	Sludge from an anaerobic digester from a WWTP	TS= 11.4%; VS = 9.34%; Vr = 1 L; TRH = 30 d; T=32 °C	251 mL/g DQO	−2.33	[95]
Physiochemical	Power at 600 W. The temperature increased with a ramp of 10 °C/min until reaching 80 °C and was maintained for 15 min supplemented with C	Pig manure	Sludge from an anaerobic digester from a WWTP	TS = 23.1g/l; VS = 15.2g/L; pH = 6.9 C/N = 10.9; Vr = 0.250 L; TRH = 30 d; T = 35 °C	433.2	39	[96]

To further improve the anaerobic biodegradability of pig manure, there are pre-treatments that have emphasized mechanical pre-treatment through sample screening. González et al. [95] designed an experiment to improve methane production through liquid and solid separation of pig manure. The particles were separated from the samples using a 0.25 mm pore size screen. However, the application of this method was not very successful in improving methane. Production decreased to 251 mL/g COD, which meant a 2.33% decrease compared to the controls. Another widely used technique is the pre-treatment by microwave irradiation, as carried out by Gómez et al. [96]. The test was carried out by setting a power of 600 W (maximum efficiency of 80%). The temperature was increased with an interval of 10 °C/min until reaching 80 °C and they kept it that way for 15 min. At the end of the digestion process, they obtained a methane production of 433.2 mL CH_4/g VS, improving production by 39% compared to the tests without pre-treatment.
To sum up, pig manure physical pre-treatments by milling and extrusion did not exactly improve methane production; however, microwave irradiation had more effect on improving substrate biodegradability. On the other hand, there are a few biological pre-treatment studies that focus on increasing pig manure production. For that matter, the application of thermal and alkaline pre-treatment enhances anaerobic digestion, significantly yielding higher biogas and methane. Alkaline heat pre-treatments were proven more effective than acid pre-treatments in pig manure hydrolysis.

1.3.3 Pre-treatments Applied to Poultry Manure

It has been demonstrated that through different methods and pre-treatments, the lignocellulosic content of poultry manure can be decreased to accelerate the hydrolysis phase and improve the accumulated production of methane [11]. In this regard, increasing attention has been paid to the use of poultry manure, especially chicken litter, as an alternative source for bioenergy production [99]. Table 5 shows some pre-treatments aimed at improving biogas production from poultry manure. Costa et al. [100] studied the pre-treatment of sand for birds and chicken feathers with NaOH and $Ca(OH)_2$ at different temperatures and pressures. They carried out the pre-treatments applying the following conditions: $Ca(OH)_2$ at 90 °C with 1 bar pressure; $Ca(OH)_2$ at 90 °C and 1.27 bar pressure, and with NaOH at 90 °C and 1.27 bar pressure. They demonstrated that the best treatment was to pretreat the manure with $Ca(OH)_2$ at 90 °C and 1.27 bar pressure for 120 min. The anaerobic digestion process was carried out under mesophilic conditions (37 °C) with anaerobic sludge from a wastewater treatment plant used as the inoculum, obtaining 137 mL CH_4/g VS. Zahan and Othman [101] also conducted studies with chicken litter under alkaline conditions and using an alkaline–acid sequence. For alkaline conditions, they pre-treated the samples with 5% NaOH at 120 °C for 90 min, while for alkaline–acid conditions, they used 5% NaOH at 120 °C for 90 min and 3% H_2SO_4 at 120 °C for 90 min. They demonstrated that alkaline pre-treatment was the most appropriate and the one that provided the best results. After the anaerobic digestion process was completed, they obtained 481.5 mL CH_4/g VS, which represented an improvement of 50% compared to untreated testing.
On the other hand, many investigations focus on biological pre-treatment methods, which are sustainable, ecological and profitable to extract soluble keratins through the use of microorganisms [102]. Patinvoh et al. [106] used strains of bacteria (Bacillus sp.C4) to pre-treat chicken feathers and produce biogas. The samples were pre-treated for 2 to 8 days with concentrations of 5–20% of the total solids. They performed anaerobic digestion, using sludge from a wastewater treatment plant as inoculum and obtained improvements of 292%, producing 430 mL CH_4/g VS. In another study, Costa et al. [100]

performed the biological pre-treatment of organic poultry manure with Clostridium cellulolyticum, Caldicellulosiruptor saccharolyticum and Clostridium thermocellum as bioaccumulation strains. They used sewage sludge from a treatment plant as inoculum in the anaerobic digestion process. They concluded that biologically pre-treated manure allows methane productions of 102 mL CH_4/g VS to be obtained, which means an improvement of 15% compared to untreated manure.

Hydrolysis continues to be the limiting step in the fermentation process since it prevents optimal degradation of the lignocellulosic material. Furthermore, the accumulation of nitrogen and ammonia in the manure of the birds prevents efficient conversion of bioenergy [107]. For its part, chicken manure contains materials that produce alkalinity and ammonia accumulation, that is, proteins and uric acid [108]. Therefore, a technology that reduces the negative effects caused by the accumulation of ammonia in the anaerobic system is necessary to optimize the production of biogas. Yin et al. [104] launched a device to extract ammonia in the gas phase. They extracted ammonia from poultry manure by exposing the samples to 70 °C for 3 days. The fermentation was carried out, using sludge from an anaerobic chicken manure reactor and in a continuous stirred tank reactor (CSTR) as inoculum. At the end of the digestion process, they concluded that after applying the hyperthermophilic pre-treatment to the manure, it was possible to obtain a methane production of 518 mL CH_4/g VS, which represented an improvement of 54.6% compared to controls.

Although some studies have been conducted on the effect of high temperatures on chicken manure, few have focused on a wide range of temperatures, particularly temperatures of 200 °C and above. In this way, Raju et al. [105] pre-treated chicken manure under isochoric conditions for 15 min at temperatures between 100 and 225°C with intervals of 25 °C. After 27 days of incubation, in batch reactors, the methane production was 340 mL CH_4/g VS at 225 °C, which meant a decrease of 7.86%. Nevertheless, there were no significant changes at lower temperature compared unpretreated samples. Consequently, this pre-treatment process is considered unsuitable for this type of manure.

Out of all studies carried out, the thermochemical poultry manure pre-treatment has the most effective results regarding biogas and methane production. In addition, alkaline treatments with the use of NaOH and Ca $(OH)_2$, with the addition of heat, are the most widely used and are the ones that significantly improve the hydrolysis of poultry manure. For their part, biological pre-treatments have played a leading role in increasing production; up to 292% improvements have been obtained using fungi and enzymes. On the other hand, it was discovered that the pre-treatment isochoric conditions do not improve the yield; on the contrary, they decreased the amount of methane by up to 8%.

Table 5. Effects of the different pre-treatments applied to poultry manure.

Pre-treatment	Process	Feedstock	Inoculum	Initials Condition	CH_4 (mL/g VS)	Methane Enhancement (%)	References
Chemical	5% de NaOH 90 min 120 °C + 3% de H_2SO_4 90 min 120 °C	Chicken litter	Sludge from an anaerobic digester from a WWTP	TS = 77.2%; VS = 39.1%; pH = 8.15; C/N = 13.02; Vr = 1 L; T = 37 °C	481.5	50	[101]
Chemical	$Ca(OH)_2$ at 90 °C y 1.27 bar pressure	Chicken litter and chicken feathers	Anaerobic sludge from a wastewater treatment plant	TRH = 80 d; T = 37 °C	137	-	[100]
Biological	*Clostridium cellulolyticum*, *Clostridium saccharolyticum* and *Clostridium thermocellum* as bioaccumulation strains	Poultry manure	Sludge from an anaerobic digester from a WWTP	TS = 77%; VS = 70%; Vr = 0.05 L; T =37 °C	102	15%	[100]
Biological	2–8 days at total solid concentrations of 5–20% by *Bacillus sp*. C4	chicken feathers	Sludge from an anaerobic digester from a WWTP	TS = 92.05 %; VS = 89.78%; C/N = 3.66; Vr = 0.056 L; TRH = 55 d; T = 37 °C	430	292	[102]
Thermal	Pressure in a stirred tank 150 °C/5 min and 4.8 bar	Poultry manure	Digestate from a biogas plant from cattle manure and corn silage	TS = 52.73 %; VS = 37.25%; Vr = 0.05 L; T = 39 °C	288	14.4	[103]
Physiochemical	(70 °C) from chicken manure under 3-day HRT	Poultry manure	Sludge from an anaerobic chicken manure reactor	Reactor CSTR; Vr = 16 L; TRH = 120 d; T = 55 °C	518	54.6	[104]
Physiochemical	High pressure and temperature reactor (T = 200 °C, 15 min)	Poultry manure	Anaerobic sludge from anaerobic digester from cow, corn and grass manure	Vr = 0.500 L; TRH = 90 d; T = 35 °C	340	−7.86	[105]

* In all tests a batch reactor was experimented. Vr is the volume of the reactor

1.4 Summary of the Effects of Pre-treatment on Animal Manure

1.4.1 Comparison of the Main Pre-treatments

The physical, physicochemical, chemical and biological pre-treatments used in the literature are variable in their application, which means that each one has its own singularities: type of concentration, application times, temperature, etc. Furthermore, in the anaerobic digestion process, the researchers use various operating parameters (hydraulic retention time, digestion temperature, VS concentration, agitation, pH and C/N ratio). Many operating parameters, individually or together, are decisive; their choice is conditioned by their suitability and flexibility [109]. On the other hand, the characteristics of the elemental and proximal analysis of the raw material (animal manure) are not the same in each of the investigations consulted; there is a lot of variability between them, although the same type of substrate is analysed. In this sense, making a comparison between the pre-treatment methods is complex since it depends on various conditions and factors.
In Figure 2, a comparison is made between the different types of pre-treatments obtained in Table 3, Table 4 and Table 5; a rough quantitative assessment of its impact on methane production is shown. The figure shows the average methane production in cow, pig and poultry manure after applying a pre-treatment. Furthermore, the improvement in methane production between pre-treated and untreated raw materials is estimated.

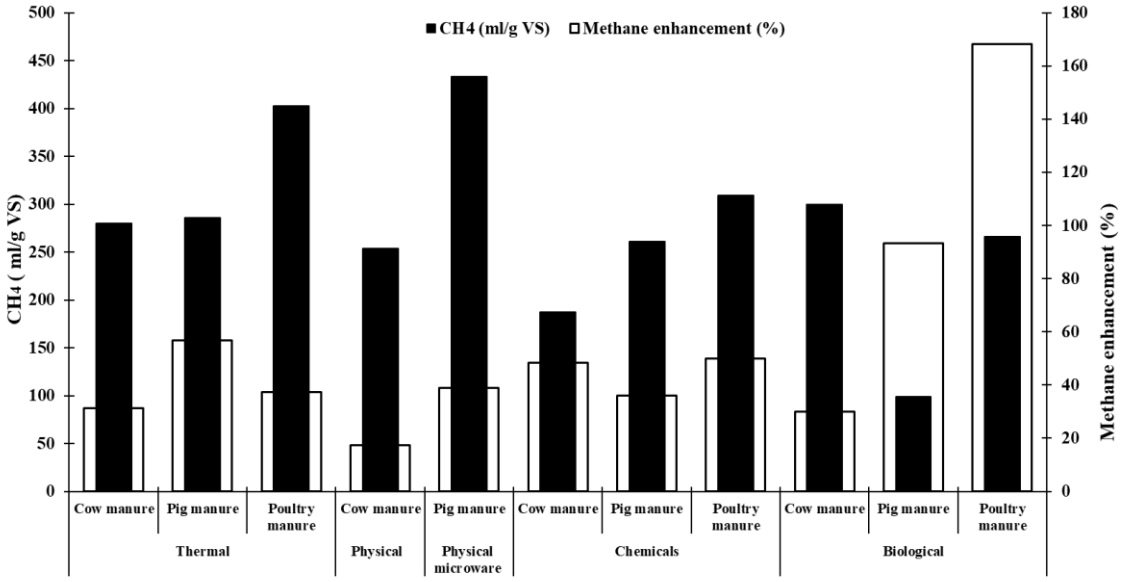

Figure 2. Methane production from cow, pig and poultry manure with respect to physical, chemical, thermal and biological pre-treatments.

Thermal and hydrothermal pre-treatments provide the most methane. They include production ranges between 130 to 450 mL/g VS for cow manure, 238 to 329 mL/g VS for pig manure and between 288 to 518 mL/g VS for poultry. They were more effective for pig manure with improvements of 12 and 206.9%. For their part, mechanical pre-treatments (microwave irradiation) have had more effect on pig manure with 433 mL/g VS and improvements of 39%. On the other hand, mechanical pre-treatments such as milling and extrusion have been used more in the pre-treatment of cow manure, obtaining methane productions from 168 to 316 mL/g VS with improvements of 15 to 20%. On the other hand, chemical pre-treatments have been the most widely used in the literature,

especially alkaline chemicals. Its influence on cow manure has resulted in methane productions of 168 to 225 mL/g VS, with improvements of 26 to 155%. Instead, its effect on pig manure is 205 to 345 mL/g VS with improvements of 12 to 72%. In poultry manure, more effective results were observed with values of 137 to 482 mL/g VS and improvements of 50%. Finally, biological pre-treatments also have positive effects on the pre-treatment of animal manure, although their use has been less frequent. Thus, in cow manure, methane productions of 300 mL/g VS and improvements of 30% have been obtained. In pig manure its effect has resulted in methane productions of 99 mL/g VS. In poultry, they have been very effective, as methane productions of 102–430 have been obtained with improvements of 15 to 292%.

Biological pre-treatments are those that best optimized the AD of the different types of animal manure, that is, they improved methane production by 74%. Its most effective application was in poultry manure since improvements of 168% were obtained in this raw material. In contrast, chemical pre-treatments experienced improvements of 45%; they had more effect on cow and poultry manure with improvements of 48% and 50%, respectively. Third, thermal pre-treatments registered improvements of 41%; they were more effective in treating pig and poultry manure, as they improved production by 57% and 37%, respectively. Finally, the application of physical pre-treatments had less effect on animal manure. These pre-treatments improved AD by 30%; however, they were more effective in pig manure, as they improved their production by 39%.

Methane productions, expressed in mL CH_4/g VS, are the average of the data collected in Table 3, Table 4 and Table 5. In each type of pre-treatment, the average methane production in each of the cow, pig and poultry manure residues has been calculated. [2] The improvement in methane production, expressed in %, has been estimated from the methane productions in Table 3, Table 4 and Table 5. The improvement has been obtained by relating the methane averages of the untreated substrates with the pre-treated averages.

1.4.2 Effect of Pre-treatments on Cow, Pig and Poultry Manure

As in the previous case, this section analyses the methane results obtained in Table 3, Table 4 and Table 5 after applying the different types of pre-treatment. Figure 3 shows the influence of pre-treatments on animal manure waste. It is analysed in which type of manure (cow, pig and poultry) its methane production increases more easily.

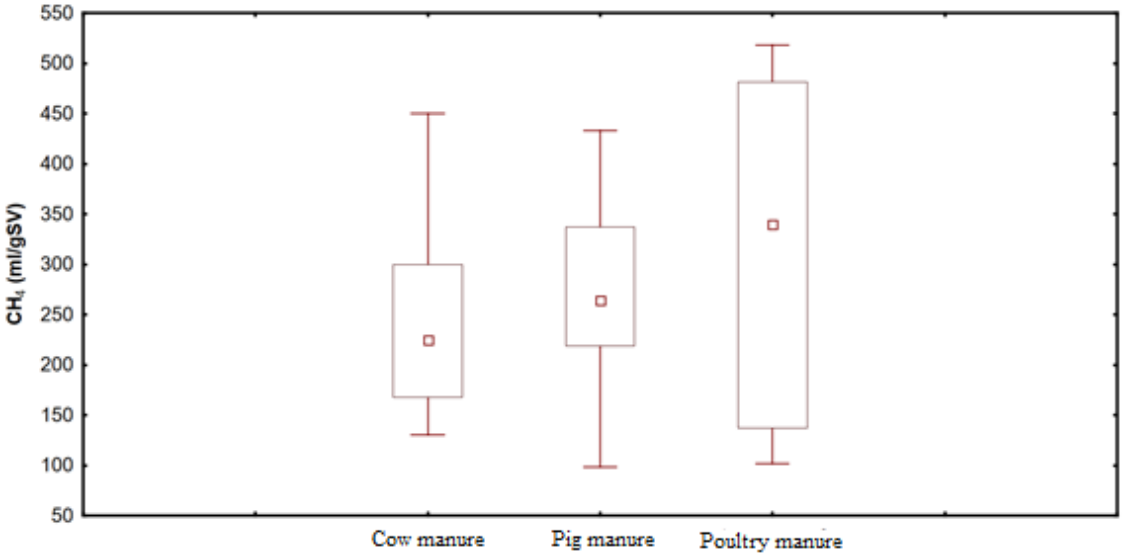

Figure 3. Box of whiskers from the production of pre-treated methane from cow, pig and poultry manure.

In general, the VS concentration of animal manure from the analysed data is not so high, which means that the average ranges to produce pre-treated methane from cow, pig and poultry manure are 238, 271 and 328 mL/g VS, respectively. According to Velázquez et al. [110], substrates with low, medium and high methane production are characterized by having productions between 150 and 300 mL/g VS, between 300 and 400 mL/g VS, and more than 450 mL/g VS, respectively. In this research, the average methane production of cow and pig manure corresponds to a low production, while the methane production of poultry corresponds to an average production.

The analysed data collected in this study show that the application of pre-treatments to cow manure improves the average yield of biogas and methane compared to untreated manure. Improvements for all registered pre-treatments ranged from 15 to 155%. Regarding pig manure, this had improvements between 12 and 206.9%. On the other hand, the behavior of the pre-treatments with respect to the manure and feather of poultry made it improve the production of methane. In this case, the improvements ranged between 14 and 292%. In general, animal manure is suitable to produce biogas. However, it should be borne in mind that the results of a pre-treatment is not always appropriate for any anaerobic digestion process [11]. No pre-treatment method is suitable for all anaerobic digestion processes and substrates; each pre-treatment has its own advantages and disadvantages [111]. The different pre-treatment technologies described above may be more suitable for a particular reactor design or size [112]. Thus, efforts to optimize the fermentation process should be aimed at finding the appropriate substrate composition and, at the same time, adequately characterizing the substrate so that its bioavailability can be increased through pre-treatment. This is because the lignocellulosic composition of each manure is very particular, which means that not all pre-treatments are adequate to accelerate its degradability process.

The box of whiskers was estimated from the results of Table 3, Table 4 and Table 5. The methane estimates from cow, pig and poultry manure include all pre-treatments (physical, physicochemical, chemical and biological).

1.5 Perspectives and Challenges of Animal Manure Pre-treatments

This document has reviewed the available pre-treatment methods for animal manure waste as a substrate prior to the AD process. It is highlighted that pre-treatments are a necessary process, and that they can significantly improve methane production. However, most pre-treatments lose their effectiveness due to the lignin content present in the waste. Thus, in the degradation of lignin from cow, pig and poultry manure residues, the solubilization and depolymerization of lignocellulosic components are the main obstacle during AD [52].

Each of the analysed technologies has its own associated advantages and disadvantages, depending on the biomass source, the methods used and the lignocellulosic composition [113]. The efficiency on the application of a pre-treatment is highly related to the characterization of the substrate. Thus, the biggest challenge to pre-treating substrates is to combine the ideal substrate composition with the most appropriate pre-treatment technique. Thus, for example, in this study it is revealed that physical pre-treatment methods have been used more frequently to treat cow manure. This is because physical pre-treatments are used in large-scale applications and one of their drawbacks is high energy demand and high maintenance costs [20]. While physicochemical pre-treatments are applied to all types of manure analysed, its efficacy is more closely related to the temperature and duration of the pre-treatment. However, the application of a physicochemical (thermal) pre-treatment generates higher methane production in poultry

manure. As regards chemical pre-treatments, the most widely used are alkalis, mainly because they more easily degrade the lignin content. The decision to use this type of pre-treatment will depend on the cost of the chemicals and the ability to control the inhibition of some compounds. Finally, biological pre-treatments provide environmental benefits and are profitable due to their low energy demand. However, the information in the literature shows that its application in pig manure has been little studied. One of the challenges is defining the correct enzyme set, since the composition of carbohydrates, lipids and proteins, as well as the lignin content, can be extremely variable in substrates [114].

The challenges of evaluating the effect of pre-treatment on improving AD have a huge gap between laboratory results and those of a pilot and industrial scale; most of the literature studies have been conducted on a small scale.

To date, the pre-treatment of livestock residues for biogas production has not been as widely studied as other organic substrates. In general, few pre-treatment methods have been explored, most of them only in Biochemical Methane Potential tests in laboratory.

Studies on the optimization of pre-treatments are focused on the solubilization of biomass and the increase in methane production. All these efforts have been very useful and interesting; however, the mechanisms that affect the complete solubilization of the cell wall structure are still not well understood.

Many studies collected from the literature lack an economic and environmental approach, which limits the most efficient proportion of results regarding the bioconversion of livestock residues to biofuel.

The evaluation of pre-treatments to improve performance could be optimized with the combination of several pre-treatments. The current literature on animal manure includes few studies in this regard; the combinations found are based solely on the contribution of heat to chemical pre-treatments.

Conclusions

The main pre-treatments (physical, chemical, physicochemical and biological) have the potential to increase enzyme accessibility by improving the susceptibility of animal manure to hydrolysis and subsequent anaerobic digestion. However, each technology has its own associated advantages and disadvantages, depending on the biomass source and the methods used.

In livestock waste treatments (cow, pig and poultry manure), biological pre-treatments improved methane production by 74%, chemical pre-treatments by 45%, thermal pre-treatments by 41% and physical pre-treatments by 30%.

The main bottleneck that prevents improving methane production from livestock waste is the lignin content, as it creates protective barriers that prevent microbial action and the development of hydrolysis. However, pre-treatment of the waste before anaerobic digestion significantly improves methane production.

Pre-treated methane production for cow manure was 238 mL/g VS, for pig manure 271 mL/g VS and for poultry manure 328 mL/g VS: with improvements of 32%, 45% and 46%, respectively.

Author Contributions

M.-Q.O.: Investigation, Data curation, Writing—original draft, Writing—review & editing; V.-M.B.: Conceptualization, Methodology, Investigation, Data curation Writing—review & editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influ-ence the work reported in this paper.

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2. Review of mathematical models for the anaerobic digestion process

Anaerobic digestion is a biological process in which the organic matter in the absence of oxygen, and through the action of a group of specific bacteria, is broken down into a set of gaseous products, called biogas, formed by CH_4, CO_2, H_2, H_2S, etc., and in a digestate, which is a mixture of mineral substances (N, P, K, Ca, etc.) and compounds of difficult degradation [1]. One of the objectives of anaerobic digestion is the production of methane, which can be used as fuel. Anaerobic digestion is considered one of the most important and advantageous processes in the treatment of livestock manure and sludge residues. It represents a possibility to reduce its environmental impact, while at the same time providing a biofuel for local energy needs [2]. This process has been known for hundreds of years; however, it is still the object of research due to the great variability of the conditions in which it can be produced, diversity of raw materials, and influential factors. **Table 1** shows some of the most recent research. In recent years there has been an increasing interest in new raw fermentation materials, mainly lignocellulosic materials from agriculture, or waste such as paper and cardboard. So, co-digestion processes are being analysed, which consist of improving methane production by mixing materials that ferment better together than separated due to the enriched microbial load this way, their nutritional needs are better complemented.

New inoculate are also being examined, such as the rumen, and its interaction with the raw material, together with nutritional requirements. Pre-treatment studies are being carried out along with thermal sequences in the processes, alternating thermophilic and mesophilic stages, evaluating the productivity, kinetics and net energy balance. The microbiological identification involved in the fermentation according to the substrate and the followed thermal process also acquires interest.

One of the most discussed aspects is mathematical modelling. The objective of the modelling is to be able to establish characteristic parameters of the raw material and process conditions to predict the system's evolution over time, the performance obtained and fermentation speed. In this study the most important models are evaluated.

Anaerobic digestion comprises a decomposition mechanism of organic matter based on three stages [3]: First a hydrolytic phase, in which polymers of long carbon chains are broken obtaining shorter acid chains; subsequently, an acetogenic phase, in which the short-chain acids obtained in the previous phase are transformed into acetic acid; and finally, a methanogenic phase, in which the acetic acid is transformed into methane.

Each of these stages is provided by a differentiated microbiological group. Each group takes as a substrate the product generated in the previous phase. When the evolution of a microbial group is analysed in a batch type reactor—in batches, the variation of cell concentration varies, as shown in **Figure 1**.

Initially, the concentration of microorganisms responsible of digestion is small and evolves very slowly in this stage because it needs time to adapt. This phase is called *lag phase* or *lethargy*. Subsequently there is a very rapid increase in cell concentration called the *growth phase*. The growth phase ends when cell compete for substrate, causing a number of cell replications to equal deaths, so the number of living cells is stabilized. This phase is called the *stationary phase*. The stationary phase ends when this battle for substrate causes a higher number of deaths than the number of reproductions, resulting in cell concentration to fall sharply. This phase is called the cell *death phase*.

Table 1. Values obtained from methane potential in various co-digestion processes.

Author	Material	Pre-treatment	Methane potential $m^3 kg^{-1}VS$
Bayrakdar et al. [4]	Chicken manure		0.272
Franco et al. [5]	Wheat straw + inoculum		0.229
Franco et al. [5]	Wheat straw + glucose + ac. Formic + inoculum *		0.276
Guo et al. [6]	Excessively withered corn straw + glucose		0.282
Li et al. [7]	Parton + sheep manure		0,152
Li et al. [7]	Paper + sheep manure		0,199
Mancini et al. [8]	Lignocellulose in general	N-methylmorpholine N-oxide	0.304
Martín Juárez et al. [9]	Microalgae + pig manure	Alkaline pretreatment with NAOH	0.377
Mustafa et al. [10]	Bagasse of sugarcane + inoculum *	Hydrothermal pretreatment	0.318
Vazifehkhoran et al. [11]	Wheat straw + sewage		0.314
Xu et al. [12]	Corn straw + Bacillus Subtilis	Microaerobic mesolithic	0.270
Zahan et al. [13]	Gallinaza (sawdust, wood shavings and rice or straw husk) with yoghurt serum		0.670
Aboudi et al. [14]	Dry sediment of sugar beet tails + pig manure		0.260
Dennehy et al. [15]	Food waste and pig manure		0.521
Glanpracha y Annachhatre, [16]	Cassava pulp with pig manure		0.380
Marin Batista et al. [17]	Vinasse and chicken manure (chicken dung)		0.650
Aboudi et al. [18]	Dry beet granules of sugar beet + cow dung		0.280
Belle et al. [19]	Fodder radish with cow dung		0.200
Cestonaro et al. [20]	Sheep litter (mixture of rice husk with feces and urine) + cattle manure		0.171
Di Maria et al. [21]	Sludge from wastewater with fruit and vegetable waste		0.216
Fu et al. [22]	Corn straw + inoculum *	Thermophilic microaerobic	0.326
Fu et al. [23]	Corn straw + inoculum *	Secondary thermophilic microaerobic	0.381
Agyeman and Tao [24]	Food waste + livestock manure		0.467

* Inoculum is material obtained from the effluent of a previous biogas plant that ferments raw materials, such as manure from pigs, cows, sheep, chickens and other animals, at mesophilic ranges.

From the practical point of view, it is only interesting to analyse the period between the beginnings of the fermentation to the stationary phase, appearing a curve similar to the sigmoid one. However, the sigmoid equation does not correctly fit the experimental results obtained.

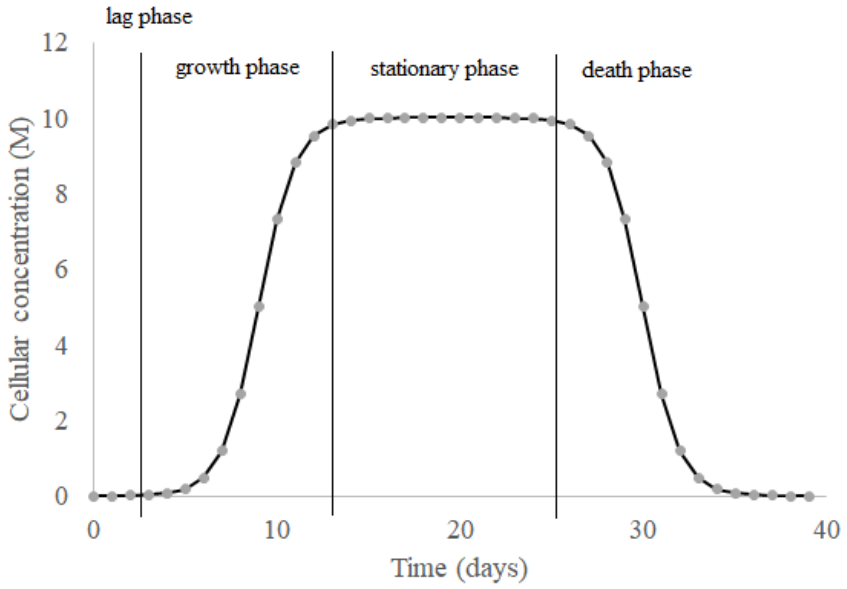

Figure 1. Variation of cell concentration over time in a batch reactor.

2.1 Exponential model

A model widely used to describe the variation of cell concentration in the growth phase has been the exponential model. This model is based on the hypothesis that the speed of growth in an instant is proportional to the concentration of cells existing at that moment. This is expressed mathematically by equation (1), where X is the concentration of cells, and μ is the constant of proportionality called *cell growth rate*.

$$\frac{dX}{dt} = \mu \cdot X \tag{1}$$

The development of equation (1) shows that in the growth phase the variation of cells follows an exponential curve.

$$\frac{dX}{X} = \mu \cdot dt$$

$$\int_{X_1}^{X_2} \frac{dX}{X} = \int_{t_{lag}}^{t} \mu \cdot dt$$

$$\ln \frac{X_2}{X_1} = \mu \cdot (t - t_{lag})$$

$$X_2 = X_1 e^{\mu \cdot (t - t_{lag})}$$

t_{lag} it is the lag time. The cell growth rate has as unit the inverse of time (d^{-1}) and can be calculated experimentally with equation (2).

$$\mu = \frac{X_2 - X_1}{X_1 \cdot (t - t_{lag})} \tag{2}$$

This model is not completely satisfactory because it has been verified that μ it is not constant and it varies as time goes by. As competition for the substrate increases, the curve in Figure 1 moves away from the exponential. To achieve a better fit, Monod [25] proposed a model for calculating the cell growth rate as a function of the substrate concentration according to equation (3), where S is the substrate concentration at a given time, μ_{max} is the maximum rate of cell growth, K_s is a constant called saturation.

$$\mu = \frac{\mu_{\max} \cdot S}{K_s + S} \tag{3}$$

The Monod model proposes the existence of a maximum cell growth rate and a saturation constant that are characteristics of microbial species growing under defined conditions. The maximum growth rate is the one that occurs initially in the growth phase exponentially. When the substrate begins to be scarce, the rate decreases with respect to the maximum.
Along with the Monod model there are others with the same style that can be observed in Table 2. In all of them the maximum rate value considered in the exponential phase is minorized when the substrate concentration is low.

Table 2. Variation models of the cell growth rate.

Type of model	Author	Model
Kinetic models without inhibition	Tessier [26]	$\mu = \mu_{\max} \cdot \left(1 - e^{-S/K_S}\right)$
	Moser [27]	$\mu = \mu_{\max} \frac{S^n}{K_S \cdot a + S^n}$
	Contois [28]	$\mu = \mu_{\max} \frac{S}{BX + S}$
Kinetic models with inhibition	Andrews y Noak [29]	$\mu = \mu_{\max} \frac{1}{K_s + S + \frac{S^2}{K_{is}}}$
	Webb [30]	$\mu = \mu_{\max} \frac{S \cdot \left(1 + \frac{\beta \cdot S}{K_{is}}\right)}{K_s + S + \frac{S^2}{K_{is}}}$
	Aiba et al. [31]	$\mu = \mu_{\max} \frac{S}{K_S + S} e^{-S/K_{si}}$
	Teissier [26]	$\mu = \mu_{\max} \left[e^{-S/K_{si}} - e^{-S/K_S}\right]$
	Tseng and Wymann [32]	$\mu = \mu_{\max} \frac{S}{K_S + S} - K_{si}\left(s - s_c\right)$

The relationship between the variation of cell concentration is always proportional to substrate consumption. The proportionality constant is called the *biomass/substrate yield* $Y_{x/s}$, and is defined by equation (4), where S_0 and S_1 are the initial and final substrate concentration; and X_0 and X_1 are the initial and final cell concentration.

$$Y_{x/s} = \frac{X_1 - X_0}{S_0 - S_1} \tag{4}$$

If the initial concentration of substrate *So* is known, the variation of cell mass during the process is obtained from the biomass/substrate ratio of the process $Y_{x/s}$. Limiting the decrease in the growth rate to a certain percentage of its maximum value allows calculating the time retention (*TR*) in a bioreactor batch.

$$z \cdot \mu_{\max} = \frac{\mu_{\max} S_1}{K_s + S_1} \quad (0 < z < 1) \rightarrow S_1 = \frac{z}{1-z} \cdot K_s$$

$$Y_{x/s} = \frac{X_1 - X_0}{S_0 - S_1} \quad \rightarrow \quad X_1 = X_0 + Y_{x/s} \cdot (S_0 - S_1)$$

$$\ln \frac{X_1}{X_o} = \mu_{\max} \cdot \left(TR - t_{lag}\right) \quad \rightarrow \quad TR = t_{lag} + \frac{1}{\mu_{\max}} \ln \frac{X_1}{X_o}$$

The amount of product generated per unit volume and time (*P*), methane in this case (*M*), is proportional to the variation of cell concentration (*X*). The proportionality constant $Y_{p/x}$ is called *product/biomass yield*.

$$Y_{p/x} = \frac{P_1 - P_0}{X_1 - X_0}$$

$$\frac{dM}{dt} = Y_{p/x} \cdot \frac{dX}{dt}$$

Since the variation of cell concentration is proportional to the concentration of cells at a given time, we must:

$$\frac{dM}{dt} = Y_{p/s} \cdot \mu X$$

By developing the variation of cell concentration over time, it has been demonstrated that the amount of product obtained (methane) follows an exponential growth during the exponential growth of microorganisms. That is the reason because working in this phase with batch-type bioreactors is preferred for optimum performance. To do this, you must adjust the retention time to the duration of this stage.

X_0 represents the initial cell concentration in the reactor; *X* represents cell concentration at a time *t*, t_{lag} is the time of lethargy or cellular adaptation.

$$\frac{dM}{dt} = Y_{p/s} \cdot \mu X_0 \cdot e^{\mu(t - tlag)}$$

$$M = Y_{p/s} \cdot X_0 \cdot \left(e^{\mu(t - tlag)} - 1\right)$$

Whereas the value of $Y_{p/s} \cdot X_0$ is negligible compared to the exponential, that is $Y_{p/s} \cdot X_0 <<< Y_{p/s} \cdot X_0 \cdot e^{\mu(t - tlag)}$, the accumulated volume obtained in each experiment can be graphically represented with the model of equation (1), calculating the cell growth rate, the productivity of the substrate, the optimum retention time for a greater use of energy.

$$M = Y_{p/s} \cdot X_0 \cdot e^{\mu(t - tlag)}$$

2.1.1 Model of Gomperzt

Despite the practicality of the exponential model when complemented by the Monod equation, it is not completely satisfactory because it does not describe well the variation of cell concentration as the substrate is being consumed and the stationary phase approaches. Knowing how cell growth behaves in this area is significantly relevant if you want to use high retention times.
To find an adequate adjustment function for all phases of the process, Winsor [33] proposes to use an equation developed by Gompertz [34] in human demography. This proposes a model that considers the variable cell growth rate, as shown in equation (5) and (6) where *a* and *c* are constants.

$$\frac{dX}{dt} = c \cdot \ln(a / X) \cdot X \tag{5}$$

$$\mu = c \cdot \ln(a / X) \tag{6}$$

According to the equation (6), Gomperzt moves radically away from the Monod approach, since the cell growth rate has no maximum. If there were a maximum, the derivative of equation (6) would be cancelled at some point, something that does not happen.

$$\lim_{X \to 0} \mu = \lim_{X \to 0} c \cdot \ln(a / X) = \infty$$
$$\lim_{X \to \infty} \mu = \lim_{X \to \infty} c \cdot \ln(a / X) = -\infty$$
$$\frac{d\mu}{dt} = c \frac{X}{a} \cdot \left(\frac{-a}{X^2} \right) = \frac{-c}{X}$$

To obtain the function of cell concentration in time according to Gomperzt, we must solve equation (5), which is a differential equation of separable variables.

$$\frac{dX}{X \cdot \ln(a / X)} = c \cdot dt$$
$$\int_{X0}^{X} \frac{dX}{X \cdot \ln(a / X)} = \int_{0}^{t} c \cdot dt$$
$$-\int_{X0}^{X} \frac{-dX}{X \cdot \ln(a / X)} = \int_{0}^{t} c \cdot dt$$
$$-\left[\ln\left(\ln \frac{a}{X} \right) - \ln\left(\ln \frac{a}{X_0} \right) \right] = ct$$
$$\ln\left(\frac{\ln \frac{a}{X_0}}{\ln \frac{a}{X}} \right) = ct$$
$$\frac{\ln \frac{a}{X_0}}{e^{ct}} = \ln \frac{a}{X}$$

Since a and X_0 are constant, the following consideration can be made:

$$\ln \frac{a}{X_0} = B = e^b$$

$$e^{e^{-ct+b}} = \frac{a}{X_0}$$

Therefore, equation (7) is obtained, which describes the cellular concentration in the reactor for each instant. This equation is the true contribution of the Gompertz.

$$X = a \cdot e^{\left[-e^{-ct+b}\right]} \tag{7}$$

When analyzing the limits in zero and infinity, we observe that the initial concentration of cells is $X1$, and that a represents an asymptote corresponding to the maximum cell potential, which would occur in the steady state.

$$\lim_{t \to 0} X = a \cdot e^{-B} = a \cdot e^{\ln \frac{X_0}{a}} = X_0$$

$$\lim_{t \to \infty} X = a$$

Considerations to the Gompertz model

If we accept the Gompertz model, Zwietering et al. [35] suggests modifications providing physical meaning to these variables. The rate of growth can be redefined as equation (8):

$$\frac{dX}{dt} = a \cdot e^{\left[-e^{-ct+b}\right]} \cdot \left(-e^{-ct+b}\right) \cdot -c = a \cdot c \cdot e^{\left[-e^{-ct+b}\right]} \cdot e^{-ct+b}$$

$$\frac{dX}{dt} = a \cdot c \cdot e^{\left[-e^{-ct+b}\right]} \cdot e^{-ct+b} \tag{8}$$

The instant in which the maximum growth velocity t_m occurs would be calculated from the first derivative of the velocity equal to zero, which is the same as the second derivative of the Gompertz equation (7). This implies that at that point where the growth speed is maximum, the Gompertz function has a turning point.

$$\frac{d^2X}{dt^2} = a \cdot c^2 \cdot e^{\left[-e^{-ct+b}\right]} \cdot \left(e^{-ct+b}\right)^2 - a \cdot c^2 \cdot e^{\left[-e^{-ct+b}\right]} \cdot \left(e^{-ct+b}\right)$$

$$\frac{d^2X}{dt^2} = a \cdot c^2 \cdot e^{\left[-e^{-ct+b}\right]} \cdot \left(e^{-ct+b}\right) \cdot \left[\left(e^{-ct+b}\right) - 1\right]$$

$$\frac{d^2X}{dt^2} = a \cdot c^2 \cdot e^{\left[-e^{-ct_m+b}\right]} \cdot \left(e^{-ct_m+b}\right) \cdot \left[\left(e^{-ct_m+b}\right) - 1\right] = 0$$

$$-ct_m + b = 0$$

$$t_m = \frac{b}{c}$$

The concentration of cells where the maximum reproduction speed occurs is calculated by entering the value of t_m in equation (7), and it is shown that the growth rate where the reproduction speed is maximum equals c.

$$X = a \cdot e^{\left[-e^{-ct_m + b}\right]} = a \cdot e^{\left[-e^{-c\frac{b}{c} + b}\right]} = \frac{a}{e}$$

$$\mu_m = c \cdot \ln(a/(a/e) = c$$

The maximum reproduction speed value is obtained by substituting t_m in equation (8):

$$v_{\max} = \frac{dX_{tm}}{dt} = a \cdot c \cdot e^{\left[-e^{-ct_m + b}\right]} \cdot e^{-ct + b} = a \cdot c \cdot e^{\left[-e^{-c\frac{b}{c} + b}\right]} \cdot e^{-c\frac{b}{c} + b} = \frac{a \cdot c}{e}$$

According to the previous thing, the curve tangent X in the point of inflection t_m has the form.

$$X = \frac{a \cdot c}{e} t + k$$

Given the $t = t_m = \frac{b}{c}$ y $X_{tm} = \frac{a}{e}$ so:

$$\frac{a}{e} = \frac{a \cdot c}{e} \cdot \frac{b}{c} + k \quad \rightarrow k = \frac{a}{e} - \frac{a \cdot b}{e} = \frac{a}{e}(1 - b)$$

$$X = \frac{a \cdot c}{e} t + \frac{a}{e}(1 - b) = \frac{a}{e} \cdot (ct + (1 - b))$$

If we define the latency time, t_{lag}, as the time in which the tangent line at the curve inflection point (point that coincides with maximum velocity) cuts the axis of the abscissa, we have that the latency time is in $X = 0$:

$$0 = ct_{lag} + (1 - b)$$

$$t_{lag} = \frac{(b - 1)}{c}$$

From this equation b can also be expressed as:

$$b = c \cdot t_{lag} + 1$$

And $v_{\max} = \frac{a \cdot c}{e}$, the result

$$b = \frac{v_{\max} \cdot e}{a} \cdot t_{lag} + 1$$

Obtaining the Gomperzt equation as equation (9). This equation has become popularized as the *modified Gomperzt equation*.

$$X = a \cdot e^{\left[-e^{\frac{v_{\max} \cdot e}{a} \cdot (t_{lag} - t) + 1} \right]} \tag{9}$$

This equation has been used in current research, such as Bah et al. [36] , Capson-Tojo et al. [3], Bayrakdar et al. [4], Mancini et al. [8], Martín Juárez et al. [9] and Li et al. [7]. To experimentally obtain the maximum reproduction speed and the latency time, *X is measured* as well as the reactor time. Next by defining the value of *a* as the maximum cell concentration obtainable, equation (9) then can be linearized.

$$\ln\left(\ln \frac{X}{a} \right) = -\frac{v_{\max} \cdot e}{a} t + \left(1 + \frac{v_{\max} \cdot e}{a} t_{lag} \right)$$

The latency time and the maximum speed of cellular reproduction will be characteristic of the microbial group in certain conditions.

Cumulative production curve of methane applying Gompertz.

If we consider the product / biomass yield, we have:

$$Y_{p/x} = \frac{P_1 - P_0}{X_1 - X_0} = \frac{dM}{dX}$$

$$\frac{dM}{dt} = Y_{p/x} \frac{dX}{dt} \tag{10}$$

$$\frac{dM}{dt} = Y_{p/x} \cdot a \cdot c \cdot e^{\left[-e^{-ct+b} \right]} \cdot e^{-ct+b}$$

$$\frac{dM}{dt} = Y_{p/x} \cdot a \cdot c \cdot e^{\left[-e^{-\frac{v_{\max} \cdot e}{a} t + \frac{v_{\max} \cdot e}{a} \cdot t_{lag} + 1} \right]} \cdot e^{-\frac{v_{\max} \cdot e}{a} t + \frac{v_{\max} \cdot e}{a} \cdot t_{lag} + 1}$$

$$\frac{dM}{dt} = Y_{p/x} \cdot a \cdot c \cdot e^{\left[-e^{\frac{v_{\max} \cdot e}{a} (t_{lag} - t) + 1} \right]} \cdot e^{\frac{v_{\max} \cdot e}{a} (t_{lag} - t) + 1}$$

$$M = \int_0^t Y_{p/x} \cdot a \cdot c \cdot e^{\left[-e^{\frac{v_{\max} \cdot e}{a}\left(t_{lag} - t\right)+1} \right]} \cdot e^{\frac{v_{\max} \cdot e}{a}\left(t_{lag} - t\right)+1} dt$$

From equation (10) we obtain the cumulative methane production equation (11)

$$M = Y_{p/x} \cdot a \cdot e^{\left[-e^{\frac{v_{\max} \cdot e}{a} \cdot (t_{lag} - t)+1} \right]} \qquad (11)$$

Taking limit when the time tends to infinity, it is shown that the methane potential produced is: $Y_{p/x} \cdot a$.

$$\lim_{t \to 0} M = Y_{p/x} \cdot a \cdot e^{-B} = Y_{p/x} \cdot a \cdot e^{\ln \frac{X_1}{a}} = Y_{p/x} \cdot X_0$$

$$\lim_{t \to \infty} M = Y_{p/x} \cdot a$$

If we calculate the second derivative of the methane production curve and we equate to zero, then a maximum methane speed production point occurs.

$$\frac{d^2 M}{dt^2} = 0$$

$$Y_{p/x} \cdot a \cdot c \cdot \left(e^{\left[-e^{\frac{v_{\max} \cdot e}{a}\left(t_{lag} - t_{CH4m}\right)+1} \right]} \left(-e^{\frac{v_{\max} \cdot e}{a}\left(t_{lag} - t_{CH4m}\right)+1} \right) \cdot \left(-\frac{v_{\max} \cdot e}{a} \right) \cdot e^{\frac{v_{\max} \cdot e}{a}\left(t_{lag} - t_{CH4m}\right)+1} + \right.$$
$$\left. + \cdot e^{\left[-e^{\frac{v_{\max} \cdot e}{a}\left(t_{lag} - t_{CH4m}\right)+1} \right]} \cdot \left(-\frac{v_{\max} \cdot e}{a} \right) \cdot e^{\frac{v_{\max} \cdot e}{a}\left(t_{lag} - t_{CH4m}\right)+1} \right) = 0$$

$$Y_{p/x} \cdot a \cdot c \cdot e^{\left[-e^{\frac{v_{\max} \cdot e}{a}\left(t_{lag} - t\right)+1} \right]} \cdot \left(-\frac{v_{\max} \cdot e}{a} \right) \cdot e^{\frac{v_{\max} \cdot e}{a}\left(t_{lag} - t\right)+1} \left(\left(-e^{\frac{v_{\max} \cdot e}{a}\left(t_{lag} - t\right)+1} \right) + 1 \right) = 0$$

$$\frac{v_{\max} \cdot e}{a}\left(t_{lag} - t\right)+1 = 0$$

$$t = \frac{a}{v_{\max} \cdot e} + t_{lag} = \frac{b}{c}$$

The maximum methane production rate is $v_{CH4\max}$

$$v_{M\max} = Y_{p/x} \frac{a \cdot c}{e}$$

Lay et al. [37] proposed to modify the Gompertz equation (9) by applying the potential of producible methane $M_e = Y_{p/x} \cdot a$, Expressed as equation (12)

$$M = M_e \cdot e^{\left[-e^{\frac{v_{M\max} \cdot e}{M_e} \cdot (t_{lag} - t) + 1} \right]} \quad (12)$$

Table 1 shows the values obtained from the methane potential in various co-digestion studies. All of them were carried out in mesophilic conditions, between 30 and 37ºC. It can be observed that the production of methane in most cases ranges between 0.15 and 0.65 m^3 kg^{-1}VS. Based on this calculation we could classify the digestion processes into three groups: a) low production processes: the amount of methane produced is between 0.15 and 0.30 m^3 kg^{-1}VS; b) medium production processes: the amount of methane produced is between 0.300 and 0.45 m^3 kg^{-1}VS; c) high production processes: the amount of methane produced is greater than 0.45 m^3 kg^{-1}VS.
These types of productions and their energy equivalence mean that anaerobic digestion processes are considered more as a waste management and treatment process with a complementary energy product, than as an alternative energy source to the problems derived from the limitation of fossil fuels.

Conclusions of the Gompertz model

The Gompertz model provides an equation that describes cell concentration over time in a fermentation process.
To define this equation, it is necessary to obtain the value of three constants: *a* is the maximum cellular concentration; *b* is a constant that depends on the initial concentration of cells and *a*; and *c* is the value of the cell growth rate where the growth velocity is maximum, that is, at the inflection point of the curve.
The Gomperzt model implies that there is no maximum cell growth rate.

2.2 Kinetic models

The complexity of the Gomperzt model and the problems that exists when applying the derivatives of the Monod and Contois equation, have led some researchers to suggest models that do not focus on the growth rate, but on the kinetoca of substrate degradation or product formation. Brulé et al. [38] classify the kinetic models into four groups:
a) Reaction in a single step with first order kinetics
b) Two-step reaction with first-order kinetics

c) Reaction in two speeds of a single step with first order kinetics
d) Reaction in two speeds of two steps with first order kinetics.

One-step reaction with first order kinetics

This model shows reaction rate is proportional to the amount of reagent, in this case substrate. So:

$$\frac{dS}{dt} = k \cdot S \;\rightarrow\; S = S_0 \cdot e^{-k \cdot t}$$

Where *S* is the amount of substrate at a time *t*, S_0 is the initial substrate amount, and *k* is the kinetic constant.
As the mass in the reaction is conserved the mass of product *M* (methane) is calculated as

$$M = S_0 \cdot (1 - e^{-k \cdot t})$$

Angelidaki et al. [39] he used this kinetic type, relating the concentration of methane that is generated in a reactor with the maximum potential through the following equation:

$$\ln\left(\frac{M_e - M}{M_e}\right) = -k \cdot t$$

$$M = M_e \cdot (1 - e^{-k \cdot t})$$

Where *M* is the methane produced at a given time *t*, M_e is the value of the final methane production and *k* is the constant of the hydrolysis rate.
Díaz et al. [40] evaluated the digestion of cellulose with manure by comparing the first-order equation, including in the equation the latency time (13), and the modified Gompertz equation. They concluded that both models did not offer significant differences in the coefficient of determination obtained in the models (R^2), neither in the methane potential predicted *Me*. Nor between the constant kinetics *k* and $v_{M\,\max}$. However, it shows that the first-order kinetic model provides a longer latency time. The maximum methane potential *Me* was between 0.30 and 0.33 m^3/kg VS.

$$M = M_e \cdot \left(1 - e^{-k \cdot (t - t_{lag})}\right) \qquad (13)$$

Zang et al. [41] he also compared the modified Gompertz equation and the first-order kinetic model according to equation (13). Zang confirms that the first-order kinetic model provides longer latency times and methane potentials than Gopmpertz. However, it provides slightly lower coefficients of determination.

Two-step reaction with first order kinetics

Shin y Song [42] they considered anaerobic digestion as a two-step process that could work at different speeds. Although this comprises a complex hydrolytic, acetogenic and methanogenic process, a more suitable kinetic model than the previous one would consist in first considering the formation of volatile fatty acids (*VFA*) from the substrate *Se*; and subsequently the conversion of these acids into methane (*M*).

The formation of volatile fatty acids depends on the substrate concentration, following first-order kinetics; where k_1 is the kinetic constant of transformation of the substrate to *VFA, S* is the substrate concentration and S_{VFA} the concentration of acid grades.

$$\frac{dS_{VFA}}{dt} = k_1 \cdot S$$

Given the $S = S_0 \cdot e^{-k_1 \cdot t}$ you have the equation:

$$\frac{dS_{VFA}}{dt} = k_1 \cdot S_0 \cdot e^{-k_1 \cdot t}$$

On the other hand, the elimination of the fatty acids will depend on the concentration of the same, also following a first order kinetics, being k_2 the kinetic constant of transformation of the *VFA* to *M*.
According to the mass balance in the formation of the *VFA*, a differential equation of constant coefficients of first order (14) is obtained:

$$\frac{dS_{VFA}}{dt} = k_1 \cdot S_0 \cdot e^{-k_1 \cdot t} - k_2 \cdot S_{VFA}$$

$$\frac{dS_{VFA}}{dt} + k_2 \cdot S_{VFA} = k_1 \cdot S_0 \cdot e^{-k_1 \cdot t} \qquad (14)$$

Such as

$$y' + a(x) \cdot y = b(x)$$

$$y = e^{-\int a(x)dx} \cdot \int b(x) \cdot e^{\int a(x)dx} dx + C \cdot e^{-\int a(x)dx}$$

The solution to equation (14) results:

$$S_{VFA} = k_1 \cdot S_0 \cdot \frac{e^{-k_2 \cdot t} - e^{-k_1 \cdot t}}{k_2 - k_1}$$

From this equation the accumulated methane production is obtained as:

$$\frac{dM}{dt} = k_2 \cdot S_{VFA}$$

$$\frac{dM}{dt} = k_2 \cdot k_1 \cdot S_0 \cdot \frac{e^{-k_2 \cdot t} - e^{-k_1 \cdot t}}{k_2 - k_1}$$

$$M = S_0 \cdot \left(1 - \frac{k_1 e^{-k_2 \cdot t} - k_2 e^{-k_1 \cdot t}}{k_1 - k_2} \right)$$

Reaction in two speeds of a single step with first order kinetics

The chemical composition of the substrates is generally heterogeneous and can be constituted by several fractions with different hydrolysis rates. This implies that we can consider the process as two parallel but independent mechanisms that occur

simultaneously. If we define as α the relation between the amount of rapidly degradable substrate and the total a; k_F as the first-order kinetic constant for degradation of rapidly degradable substrate; and k_L as the first order kinetic constant for the degradation of slowly degradable substrate; the amount of methane produced can be defined with the model used by Kusch et al. [43] or Luna del Risco [44].

$$M = S_e \cdot (1 - \alpha \cdot e^{-k_F \cdot t} - (1 - \alpha) \cdot e^{-k_L \cdot t})$$

Dennehy et al. [15] compared three different kinetic models to determine the most suitable to describe the kinetics of the discontinuous co-digestion of food waste and pig manure at 37ºC; (1) first order, (2) Gompertz, and (3) two-speed one-step reaction with first-order kinetics. They showed that the three models provide similar determination coefficients, however, the RMSE (root of the mean of the squares of the errors) is significantly reduced when the two-speed digestion is considered. The worst RMSE was for the Gomperzt model. The first-order kinetic model reduced the RMSE by 39%, and the first order kinetic model but with two speeds reduced the RMSE by 80%. The highest methane yields they obtained was 0.521 ± 29 m^3 CH_4 kg^{-1} VS.

Reaction in two speeds of two steps with first order kinetics

If we consider two steps in each of the fractions of which the substrate is composed, both for the rapidly degradable substrate fraction and for the slowly degradable substrate fraction, we can obtain the following equation:

$$M = S_e \cdot \left[\alpha \cdot \left(1 - \frac{k_{HF} e^{-k_{MF} \cdot t} - k_{MF} e^{-k_{HF} \cdot t}}{k_{HF} - k_{MF}} \right) + (1 - \alpha) \cdot \left(1 - \frac{k_{HL} e^{-k_{ML} \cdot t} - k_{ML} e^{-k_{HL} \cdot t}}{k_{HL} - k_{ML}} \right) \right]$$

Brulé et al. [38] evaluated the four kinetic models described, concluding that the models that consider an easy speed in both a step and two steps yield a reasonable estimate. In contrast, the model that considers two speeds with a single step produces overestimates. Therefore, it is considered inadequate. This overestimation is corrected by applying the two-step model at two speeds but complicates its application.

2.2.1 Model based on the transfer function.

Several studies, such as Ghufran and Charles [45], Li et al. [46] or Zahan et al. [13] have used a function derived from the first-order kinetic model but which substitutes the kinetic constant for the ratio between the maximum and the methane velocity.

$$M = M_e \cdot \left(1 - e^{-k \cdot (t - t_{lag})} \right)$$

$$M = M_e \cdot \left(1 - e^{-\frac{v_{\max} M}{M_e} \cdot (t - t_{lag})} \right)$$

2.2.2 Cone model

On the other hand, researchers such as Pitt et al. [47], El-Mashad [48], Li et al. [46] and Zahan et al. [13], analysed the cone model. This model describes the fermentation according to equation (15):

$$M = \frac{M_e}{1+(k\cdot t)^{-n}} \tag{15}$$

2.2.3 Comparison of models

For the evaluation of the models, most researchers usually use two statistics; a) coefficient of determination of the fit (R^2), and b) root of the mean of the squares of the errors (RMSE) calculated by equation (16), where M_{model} is the value of methane predicted by the model at an instant *t*, and *Mob* is the value of methane observed experimentally.

$$RMSE = \sqrt{\frac{\sum(M_{\text{model}} - M_{\text{ob}})^2}{n}} \tag{16}$$

Pitt et al. [47], Ghufran and Charles [45], El-Mashad [48], Li et al. [46] and Zahan et al. [13] they compared the modified Gompertz model, the first order kinetic model, the transfer function model and the cone model, for different types of substrates and combinations in codigestion.
Comparing the values of R^2, RMSE and latency time provided by analysis of variance, the results shown in **Figures 2** and **3** were obtained.

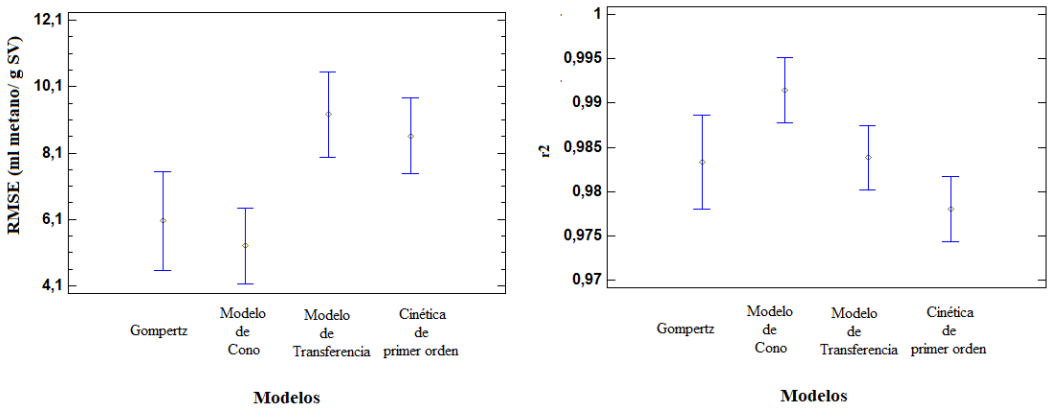

Figure 2. LSD intervals of the analysis of variance at 95% confidence level for the comparison of the RMSE, the R^2 of the different models applied to the fermentation of different substances and combinations in co-digestion.

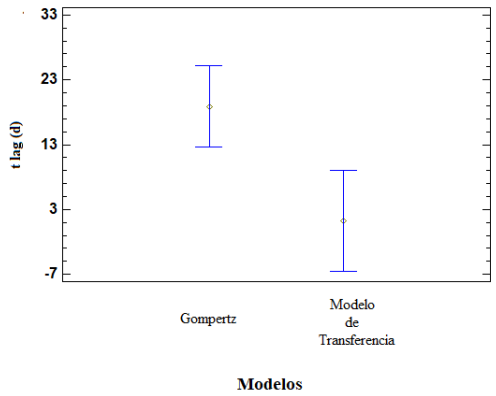

Figure 3. LSD intervals of the analysis of variance at 95% confidence level for the comparison of the latency time of the different models applied to the fermentation of different substances and combinations in co-digestion.

As you can see all the models provide high coefficients of determination and there are few differences between them. The transfer model and the first-order kinetic model generally produce higher RMSE, so the modified Gomperzt model and the cone model make more accurate estimates. However, the Gomperzt model estimates higher latency periods.

Conclusion

In this research work, the most important kinetic models used to describe anaerobic fermentation have been developed. The comparison between them is a subject currently studied as demonstrated in recent publications. All of them provide high coefficients of determination, however, they present significant differences in the RMSE.
The production of methane in most cases ranges between 0.15 and 0.65 m^3 kg^{-1} VS, under mesophilic conditions (30-37ºC). However, digestion processes can be classified into three groups according to the methane production potential:
a) low production processes, when the amount of methane produced is between 0.15 and 0.30 0.65 m^3 kg^{-1} VS;
b) medium production processes, when the amount of methane produced is between 0.30 and 0.45 0.65 m^3 kg^{-1} VS;
c) high production processes, when the amount of methane produced is greater than 0.45 0.65 m^3 kg^{-1} VS.
The average latency time is 14 days.
The mean of the first-order kinetic constant is 0.11 d^{-1}.

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OBJECTIVES

1. General objective

The general objective of this Doctoral Thesis was to systematically quantify the production of biogas and the biochemical transformation of Andean animal manure residues with lignocellulosic residues of difficult gradation.

2. Specific objectives

1. Carry out a physicochemical characterization of the raw materials used and determine their theoretical methane production.
2. Evaluate the influence of the mixing ratio between substrate and inoculum on methane production from monodigestion and co-digestion.
3. Evaluate the synergistic and antagonistic effects on the co-digestion of lignocellulosic residues and animal manure residues by varying the substrate/co-substrate ratio.
4. Compare the experimental results with those obtained through the modified Gompertz kinetic models, transfer, logistic function, cone and modified

CHAPTERS

Chapter I

Biochemical potential of methane (BMP) of camelid waste and the Andean region agricultural crops.

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ABSTRACT

This study analyses agricultural waste, manure and slaughterhouse residues anaerobic digestion processes from typical species found in the Andes region such as llama, vicuna and guinea pig; quinoa, amaranth and wheat straw, not only with the purpose of using them as renewable energy source but also to boost rural development in the region. The raw materials were incubated in batch digesters at 38 °C. The concentration of the samples in the digesters was determined as a function of the amount of volatile solids (VS) that each substrate contained. All the trials were inoculated with mesophilic sludge from the digester of a wastewater treatment plant (WWTP). Methane production was evaluated through the effect of the substrate and inoculum ratio (SIR) for both livestock excrement and agricultural waste. The highest cumulative maximum methane production rate was achieved from llama manure residues and quinoa straw for SIR 1:2 yielding 376.08 ml CH_4/g VS and 377.02 ml CH_4/g VS production, respectively. In these materials, the tests with an SIR of 1:2 improved methane production by 22.56% and 37.54% compared to the tests carried out with an SIR of 1:1. Furthermore, kinetic analysis showed that the modified Gompertz model best adjusts to performance, revealing a steady difference of 7.06% between experimental and predicted values. On the other hand, the modified Gompertz model was adjusted to the experimental results with an R^2 of 0.998 and a root mean square error (RMSE) of 4.09 ml/g VS to 17.12 ml/g VS.

Keywords: agricultural waste, anaerobic digestion, biogas, biomass, kinetic model, methane, waste management.

1. Introduction

In recent years, waste management and renewable fuels demand are important challenges that need study and analysis [1,2]. Manure and slaughterhouse livestock residues, as well as agricultural residue from various crops generate large amounts of waste with enormous energy potential if treated through anaerobic digestion as a methane production source [3].

The Andean mountainous area of Latin America covers Colombia, Ecuador, Peru and Bolivia, eminently comprising a large cattle and agricultural area. In this zone, livestock production is made up of autochthonous animals such as vicuñas and guanacos (whose management is not barn), and llamas, bobbins and guinea pigs that are managed on farms. Despite livestock production being supplemented with amaranth, quinoa and wheat crops, the area lacks an efficient energy supply. That is the reason why anaerobic digestion seems to be one of the most promising bioenergy technologies for rural development [4].

Anaerobic digestion (AD) is a process that involves the transformation of organic matter into biogas (60-70% methane and 30-40% carbon dioxide) [5,6]. Despite the fact that this process has been known for a long time, nowadays, there are still new raw materials, susceptible to fermentation, that need to be evaluated through the biochemical potential of methane (BMP) to obtain methane [7,8].

The present study addresses the AD through the BMP tests of three agricultural residues that are produced in the Andean area: amaranth straw (AS), quinoa straw (QS) and wheat straw (WS); and four livestock residues: manure of llama (LM), vicuña (VM) and guinea pig (GPM), and slaughterhouse waste (SW). These resources are easily accessible in this area. The excrements of the stable's animals were periodically removed from the farms for sanitary reasons. They usually pile up in free areas where compost is formed. On the other hand, even though vicuñas are not established and live free in the Andean area, they deposit their droppings in specific well-located areas where farmers can easily pick them up with shovels or even mechanical means. However, despite their availability, the fermentative potential of these resources as a source of energy has not been sufficiently evaluated.

Since there are more than 3 million camelid heads spread throughout the Andean region, mainly LM (domesticated species) and VM (wild species) [9] its manure becomes a potential source for AD. Besides, camelid has traditionally served as energy source in the Andean countries, being used for cooking instead of firewood. Further, in the Bolivian antiplane 89% of its inhabitants use manure as fuel, of which 92% is llama manure [10], easily collected since camelids defecate in established identifiable places [11]. It should be noted that the AD of cattle manure depends greatly on the type and ratio of materials added to the biodigester [12]. Hence, a specific analysis is required when new raw materials are explored.

Similarly, SW is of great interest as raw material for AD because most meat industries generate large amounts 45-53% live animal's weight, organic by-products considered industrial organic waste [13]. Comparable, SW residues are characterized by having a high animal protein and fat content [14] becoming an attractive raw material for AD, because of its methane high yield [15].

Although, AS, QS and WS crops are abundant in the Andean regions, only a portion of WS wheat waste is used as animal feed, many AS and QS traces of straw are burned or unused causing a tremendous loss of energy potential.

Given that very few studies have addressed methane's biochemical potential (BMP) from residues obtained under appropriate conditions as those from the Andes, this study aimed to characterize agricultural and livestock residues fermentation process in the Andean

region through the addition of a sewage sludge microbial inoculum in batch biodigesters. The amount of methane obtained the degree of biodegradability of the substrates and the modelling of the kinetics of the process were measured.

2. Materials and methods

2.1 Origin of substrates and inoculum

Livestock waste samples were collected from three different areas within proximity. First, llama manure was primarily collected from farms surrounding the capital city of Guaranda in Bolivar Province whereas VM manure was collected at the Chimborazo province—both located in central Ecuador—volcano pastures and plains, where the animals live freely and wildly. Alternatively, GPM manure was obtained from farms at "Bolivar State University". Finally, SW was collected from the Guaranda Municipal slaughterhouse. The latter was extracted from cattle stomachs as it is a complex substrate composed of manure remains contained inside the intestines like blood, rumen and grass detritus not being completely degraded. Such samples were collected in polyethylene bags obtaining significative manure samples produced on farms or waste sites. Subsequently, they were stored in the laboratory at 6°C for 72 hours before being added to the biodigesters.

On the other hand, AS, QS and WS straw were obtained from plots at the Bolivar State University. This waste was collected from the stubble produced during the summer months (August and September). Once the straw was collected it was stored in the laboratory and dried at room temperature before performing a mechanical pretreatment in two stages. The mechanical pretreatment was aimed at increasing the surface area of the material to improve the reaction rate, accelerating the hydrolysis stage, increasing the biogas yield in the AD according to the studies by Ariunbaatar et al. [16]. First, the particle size was reduced to approximately 3 and 4 cm by a mass mill. Then a second milling was carried out with a smaller mill to reduce its size to a diameter of less than 1 mm to obtain a better homogenization of the size. According to Sharma et al. [17], the particle size of agricultural and forestry residues that produces the maximum amount of biogas is between 0.088 and 0.40 mm.

The inoculum used in all the tests comes from the urban wastewater treatment station (WWTP) in the city of San Miguel de Ibarra (Ecuador). It is extracted from the primary sludge of the anaerobic digester that worked in mesophilic conditions (temperature between 35-37°C approximately).

2.2 Characterization of raw materials and biogas

The materials were characterized by proximal analysis and elemental analysis.

The total solids (TS) of the substrates were determined by the methodology proposed by the standards UNE-EN 18134-1: 2016, UNE-EN 18134-2: 2017 and UNE-EN ISO 18134-3, 2016 [18,19,20]. Volatile solids percentage (VS) with respect to total solids was determined following the procedure proposed by the standard UNE-EN ISO 18123: 2016 [21], while the ashes were determined according to the standard UNE-EN ISO 18122: 2016 [22].

Similarly, for the proximal analysis of the inoculum, whose composition was mostly liquid, a more proper methodology of wastewater proposed by the American Public Health Association (APHA) [23] sections 2540A-2540G was used, determining the TS, VS and ashes.

The elementary analysis from which the percentages of N, C, O, H, S and C/N ratio of the substrates and the inoculum are obtained were determined through the VARIOUS MACRO CUBE elemental analyzer, following the guidelines proposed by the standard UNE ISO16948 15104. The pH was determined at room temperature using a HACH HQ 40D digital multimeter meter potentiometer.
The biogas production was calculated from the pressure exerted by the biogas inside the biodigester. The pressure was measured daily by the manometer (Delta OHM HD 2124.2) equipped with a sensor (Delta TP 704 with a capacity of 100 bar). After the daily pressure measurement, the biogas accumulated in the upper space of the biodigester was completely released; this caused the pressure exerted by the biodigester to be reduced to a pressure close to atmospheric pressure. After releasing the biogas, the pressure in the head space of the biodigester was again measured as an initial condition for the next day measurement. The biogas components (CH_4, H_2S, CO_2 and O_2) were determined with the Geotech BIOGAS GA-5000 analyzer. The biogas estimate was evaluated daily from each biodigester by daily extraction of all the generated biogas.

2.3 Experimental methodology

BMP tests were performed on a laboratory scale and in batch digesters, through which the maximum CH_4 production of different substrates was determined. All BMP tests for the test were performed in glass digesters of 310 ml of total volume (V_T) sealed tightly throughout the digestion process. The reactors were filled occupying a useful volume (V_U) of 60% of the total volume, while the gas or head volume (V_G) was set at 40%. All batch tests were performed in triplicate. Biodigesters were kept at 38°C within a 40-day retention time. Finally, the measurement was carried out daily until the accumulated biogas production stabilized. During data collection, all biodigesters were shaken with an orbital shaker for a period of 120 seconds at 100 rev/min before taking biogas volume and pressure measurements generated in the biodigester.
BMP evaluation was performed with two substrate/inoculum ratios (SIR): 1:1 g/g VS and 1:2 g/g VS. To obtain these proportions, the amount of inoculum in each test was kept constant at 18g VS/l, while the amount of substrate varied according to the respective SIR value. Only sewage sludge was used as inoculum in these experiments. The process was evaluated at mesophilic temperature, with a C/N ratio determined by the elementary analysis of the combination of raw materials. The influence of the inoculum was evaluated by subtracting between the total accumulated volume of the substrate and the total accumulated volume of the inoculum; for this, the volume of biogas and methane produced solely by the inoculum was determined and at the end of the experiment mathematically the total inoculum production was subtracted from the total production of the substrates. Finally, biogas volume in each of the tests was expressed in ml/g VS and normalized under standard conditions (P = 1atm, T = 25 °C) through **(Eq. 1)**.

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

where,

V_{BIOGAS} (STP)	total methane volume under standard conditions
ΔP	represents the difference between the daily pressure exerted by the biogas in the biodigester and the pressure after the gas was released the day before (atm)
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	volume of the digester head space (0.124 l)

2.4 Kinetic modelling

To describing the AD process, the different kinetic models were adjusted to the observed values and thus managed to predict methane production, as a function of time, in the different BMP tests. Once the kinetic parameters were obtained by parameterizing the kinetic equations, they were compared with each other to see their relationship with the observed values. In total, five different kinetic models were evaluated (Mc 1- Mc 5): Mc 1 - modified Gompertz model [24] **(Eq. 2)**, Mc 2 - transfer function model [25] **(Eq. 3)**, Mc 3 - logistic function model [25] **(Eq. 4)**, Mc 4 - cone model [26] **(Eq. 5)**, Mc 5 -model of Richards [27] **(Eq. 6)**.

Mc 1
$$M = M_e . exp\left\{-exp\left[\frac{\nu_{max} * exp}{M_e}(t_{lag} - t) + 1\right]\right\} \quad (2)$$

Mc 2
$$M = M_e\left\{1 - exp\left[-\frac{\nu_{max}}{M_e}(t - t_{lag})\right]\right\} \quad (3)$$

Mc 3
$$M = \frac{M_e}{1 + exp\left[\frac{4\nu_{max}(t_{lag} - t)}{M_e} + 2\right]} \quad (4)$$

Mc 4
$$M = \frac{M_e}{1 + (k.t)^{-n}} \quad \text{Ec. 5}$$

Mc 5
$$M = M_e\left\{1 + d.exp(1 + d)exp\left[\frac{\nu_{max} * exp}{M_e}(1 + d)\left(1 + \frac{1}{d}\right)(t_{lag} - 1)\right]\right\}^{\frac{1}{d}} \quad \text{Ec. 6}$$

where,

M	accumulated specific methane yield over time t (ml CH_4. g^{-1} VS)
M_e	maximum methane yield (ml CH_4. g^{-1} VS)
t	digestion time (d)
k	first order decomposition constant (d^{-1})
ν_{max}	maximum specific rate of methane production (ml CH_4. g^{-1} VS. d^{-1})
t_{lag}	lag phase parameter (d)
d	dimensionless factor
n	factor order
exp	2.71828

Methane production was modeled by adjusting the data from the five kinetic models by nonlinear regression, using the STATISTICA 10 tool. To evaluate the efficiency of the models, the coefficient of determination (R^2) and the mean square error (RMSE) were used. The RMSE reveals the average error in the cross-validation method or set of predictions. The model is considered good when there is a greater correlation between the

experimental values of the BMP test and the predicted values, that is, when the RMSE values are 0 and the coefficient R^2 is as close as possible to 1 [28].

2.5 Calculation of theoretical performance and biodegradability

The maximum theoretical methane yield (TMY) was estimated based on the elemental compositions of organic elements, such as C, H, O and N, based on the Buswell equation [29] **(Eq. 7)**.

$$C_aH_bO_cN_d + \left(\frac{4a-b-2c+3d}{4}\right)H_2O \rightarrow \left(\frac{4a+b-2c-3d}{8}\right)CH_4 + \left(\frac{4a+b+2c+3d}{8}\right)CO_2 + dNH_3 \quad (7)$$

where,

a, b, c and d are the stoichiometric coefficients of biodegradable molecules.

However, all the analyzed substrates had ammonia and H_2S, so the considerations of using the Boyle equation [30] **(Eq. 8)**.

$$TMY = \frac{22\,400 * (4a+b-2c-3d-2e)}{(12a+b+16c+14d+32e) * 8} \quad (8)$$

According to Sobotka et al. [31], the biological efficiency of the anaerobic process is defined as the relationship between experimental and theoretical performance. In this way, knowing the values of experimental yield $\gamma_{(exp)}$ and theoretical $\gamma_{(th)}$, the biological efficiency was estimated from **(Eq. 9)** [24].

$$\varepsilon = \frac{\gamma_{(exp)}}{\gamma_{(th)}} \quad (9)$$

Results and discussion

3.1 Characterization of substrates and inoculums

Table 1 demonstrates proximal and elementary analyzes results from the different raw materials studied. Livestock waste substrates (LM, GPM, VM and SW) have several important differences in TS and VS content. Particularly, SW and GPM VS content is remarkably much higher than VM and LM residues. Nonetheless, LM residues have a very competitive VS content compared to other studies. For instance, LM residues has 61.58% of VS compared to the results obtained by other authors [[32],[33],[34],[9]] hat is 74.4%; 70.9%; 70.3 and 66.1% VS respectively. The fact that the LM and VM residues has a low VS content is justified, to a large extent, by the high ash content of 25.51 and 27.6 %% respectively. On the other hand, the SW and the GPM have a high moisture content of 90.44% and 66.10% respectively. However, despite the greater dilution of the SW and GPM residues used in the trials, they have a high VS content on a dry basis (70.74% and 72.63% respectively).

Agricultural residues (WS, QS and AS) had a high TS content, around 87-93%. This is because they are stubble residues collected in summer. However, they presented significant differences in the percentage of volatile (VS) solids with respect to total solids. The substrates with the highest amount of VS are the residues of QS and WS (77.26%

and 74.79% respectively) residues due to a high protein and lipid content. AS residues have a significantly lower volatile content with 58.37% on average and contain a high ash content (8.4%). Volatile solids obtained in quinoa are lower than those reported by [9], which indicated an average of 95.3% of VS compared to total solids. That there are differences between the characteristics of two equal materials may reflect the different cultivation systems used in each of them.

Regarding inoculum, WWTP sludge was used in all the trials, in accordance with the recommendations of many studies in the literature [35-39]. The inoculum used was the same for all the tests performed and was degassed by incubation for 4 days. The TS were 3.9%; while the wet-based VS were 2.3%, which makes it possible to have an VS/TS ratio of 0.59 typical of WWTP sludge. The VS/TS ratio is an indirect measure of the activity of microorganisms in biomass [40]. In this case, the value obtained from the VS/TS was much higher than the values obtained by Shen et al. and Liu et al. [41,40], who obtained values of 0.49 and 0.38, respectively. The VS obtained were consistent with the results of [42-45] who obtained 38.01%; 44.89%; 45.5% and 65.5% of VS respectively. The C/N ratio of the substrates (LM, VM and GPM) and the residues of (AS and QS) is around 12-17.41, which is low, since for a better methanogenic activity, the C/N ratio should be around 20 -30. The low C/N ratio expects some type of inhibition of microorganisms, due to an excess accumulation of ammonia due to protein degradation [46].

Table 1. Characterization of substrates and inoculums

Parameters	Units	LM	VM	GPM	SW	AS	QS	WS	IN
TS	%	50.6 (1.0)	57.4 (0.5)	33.9 (1.7)	9.6 (1.3)	88.2 (0.1)	87.0 (0.1)	92.6 (0.1)	3.9 (0.1)
VS (% ST)	%	61.6 (0.4)	41.2 (1.6)	72.6 (1.1)	70.7 (0.1)	74.8 (0.3)	58.4 (1.5)	77.2 (0.9)	58.5 (0.5)
Ashes	%	25.5 (0.3)	27.6 (1.8)	13.1 (0.1)	12.8 (0.2)	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)	2.3 (1.0)	0.4 (0.1)	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)	39.5 (1.2)	42.2 (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)	4.6 (0.5	6.3 (0.9)	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)	39.7 (1.2)	38.3 (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)	0.4 (0.0)	0.0 (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)	15.3 (0.8)	101.9 (0.9)	12.9 (0.8)	12.0 (0.9)	29.6 (0.8)	7.5 (0.7)

NOTE: LM (Llama manure), VM (Vicuña manure), GPM (Guinea pig manure), SW (Slaughterhouse waste), WS (Wheat straw), AS (Amaranth straw), QS (Quinoa straw) and IN (inoculum, WWTP sludge). The data in brackets are the standard deviations.

3.2 Cumulative production of biogas and methane from agricultural and livestock waste

The total biogas and methane production accumulated in the digesters was obtained by adding the daily biogas production throughout the experimental period. **Figure 1** shows the total accumulation during the 40 days of AD of all monodigestion residues, with the average yield of all trials. In general, all cumulative performance curves have a similar behavior, which implies that the test material is easily biodegradable. In this sense, according to **Figure 1a**, biogas is produced immediately once the biodegradation process has begun, which makes the initial lag phase very fast and the biogas yield curve stabilizes quickly [47]. When the amount of inoculum varies from 50% to 66.67% **(Figure 1b)**, the biogas production of WS, QS, LM and SW residues increases by 2.19; 23.61; 20.65 and 17.01% respectively. This evidences that the WS residues did not undergo major changes as the amount of inoculum increased. However, with the increase in inoculum, they showed a greater production of biogas in the first days and a quick stabilization in the

days after its biodegradation. On the other hand, the residues of QS, LM and AS did undergo significant changes as the amount of inoculum increased. According to Bouallagui et al. [48], there is a direct relationship between soluble organic matter and hydrolysis, since, at a higher content of soluble organic matter, times for substrate formation are reduced and cumulative production is increased. In this sense, when the amount of inoculum is increased, the lag phase is decreased, and the hydrolysis process is accelerated during the first days of AD.

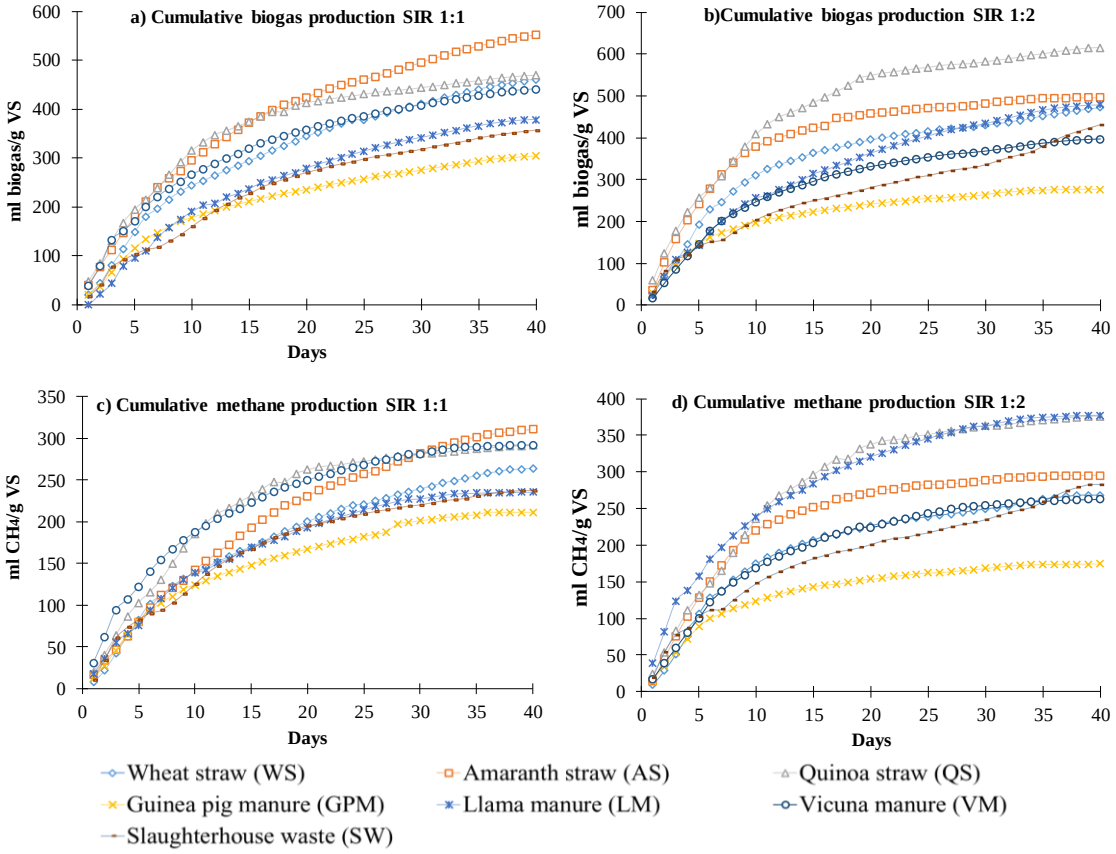

Figure 1. Daily production of biogas and methane from agricultural and livestock waste.

Regarding methane production, its yields are in **Figure 1c** and **Figure 1d**. When the inoculum amount was increased from 50% to 66.67%, the digesters with residues of WS, QS, LM and SW increased by 1.54; 22.56; 37.54 and 16.32% respectively. On the contrary, with the increase in inoculum, digesters containing residues of AS, GPM and VM decreased their total methane production by 5.05; 17.43 and 9.93% respectively. The fact that some residues decreased their production with the increase of inoculum agree with other fermentation studies by Zhou et al. and Boulanger et al. [49,50], who experimented with bean residues. They found that SIR greater than two can negatively affect the AD process, especially methane yield and substrate biodegradability. In this sense, Raposo et al. [51] considers that the decrease in methane yield with the increase of the inoculum is associated with the inhibition of anaerobic microorganism activity due to the accumulation of volatile fatty acids (VFA). The increase in methane in the QS, LM and SW residues was very significant unlike the WS residues, in which it was not very effective. In this sense, for the residues of QS, LM and SW as the inoculum increases, there is a greater adaptation of the microorganisms to the substrate, which means that the delay phase is deduced.

The highest amount of methane was obtained when the amount of inoculum was increased from 50% to 66.67% for the residues of LM and QS, with results of 377.02 and 376.08 ml of CH_4/g VS. Along the same lines, WS and SW residues also improved their methane production with productions of 268 and 283 ml CH_4/g VS. However, AS, GPM and VM residues reduced their methane production by 5%, 17% and 9%, respectively. On the other hand, all the residues with the highest percentages of CH_4 were those of animal origin, that is, the residues of LM and VM (78.76% and 66.34% respectively). These results suggest that increases in the inoculum to camelid residues stimulate bacterial activity, increasing biodegradability and the production of biogas and methane.
Many authors [30,52,54] conclude that one of the most important parameters affecting BMP tests is the inoculum, both the source, and the amount of inoculum added. It is clearly demonstrated that SIR can affect not only biodegradability but also the production rate of CH_4 [30]
If the residue with the highest yield obtained in both biogas and methane (LM) is compared, with other previous studies in the literature the production obtained is in the same ranges. The amount of biogas from the LM residue obtained in this investigation is 379.89 ml of biogas/g VS with a methane percentage of 78.76%. For their part Alvarez et al. [32], obtained results between 20 and 550 ml of biogas/g VS with 50-57% of CH_4, Alvarez and Lidén [9] achieved an average between 150 and 450 ml of biogas g VS with 50-60% of CH_4, Alvarez and Lidén [34] generated 10 and 690 ml of biogas/g VS with 27-55% of CH_4, finally Alvarez and Lidén [53] obtained averages between 30 and 480 ml of biogas/g VS with 47-55% CH_4. It should be noted that the studies carried out by these authors were carried out in continuous flow stirred-tank reactors (CFSTR), with volumes between 1.8 to 9.3 ml and temperature ranges between 11 and 25°C.

3.3 Kinetic model analysis

Table 2 summarizes the results of the kinetic study using the different models. As you can see, all the models fit well with the experimental data. The kinetic constants were calculated during 40 days of digestion, which was the time necessary to obtain more than 95% biogas. All kinetic models adequately described the cumulative biogas production of the biodigesters. Kinetics is a very sensitive process, which makes biogas production related to bacterial growth. [55]. In general, the kinetic parameters determined from modeling provided additional valuable information on the results of BMP tests on the biodegradation patterns of the substrates.
The lag phase (t_{lag}) yielded mostly negative values for both biogas and methane production. GPM and QS residues decrease as inoculum amount is increased. In contrast, SW residues increase their t_{lag} when SIR increases. The negative periods of the lag phase of some substrates indicates the high bioavailability of organic compounds within the substrates [27]. On the other hand, the residues of AS, QS and VM have positive values for the transfer model, which suggests that these residues present a more complex degradation of fats in their initial process. However, the model that presents more irregular values with respect to the other models is the transfer. In this sense, it is worth noting that this irregularity of the transfer model overestimated the t_{lag} to a higher degree than the rest of the models.
The amount of M_e predicted by the 5 models evaluated have the same trend in their behavior. For its part, the cone model is the one that overestimates this parameter and moves it away from the rest of the models. Thus, for example, when the amount of inoculum is increased in both methane and biogas, the SW residue has values of 716.77 ml CH_4/g VS and 2136.53 ml biogas/g VS respectively, causing it to overestimate me by

more than 200%. The performance values modelled with the Gompertz, logistics and Richards models are the ones that have more similarity to each other and at the same time are the ones that have less error difference with the experimental performance. In principle, the Richards model is a generalization of the logistic model, since it introduces a fourth parameter d, which allows some flexibility in the shape of the curve. For d = 0 and 1, the Richards model is reduced to the Gompertz and logistic model respectively [56]. In this sense, the three and four parameter sigmoidal kinetic models better describe the methane production kinetics. On the other hand, the data of the Richards model are more like those of Gompertz, since in all the tests carried out the parameter d tends more to 0 than to 1, with which Richard's model has the tendency to be reduced to the model from Gompertz. The asymptotes calculated for the model that best adjusts the specific performance (Gompertz) causes them to vary by no more than 7.06% with respect to the experimental data. On the other hand, the best adjustments are in the residues of GPM (-0.01%) and LL (-0.15%). According to these results, Zahan et al. and Raposo et al. [57,58], low deviations reached between predicted and measured values (almost equal to or less than 10%) in LM and GPM residues suggest that the proposed model predicts digesters role more accurately.

The maximum rate of methane production (ν_{max}) shows that the highest peaks are obtained with the transfer model, especially in the residues of QS (30.52 ml/g VS day) and VM (26.74 ml/g VS day) in the SIR (1:1), and in the residues of AS (41.23 ml/g VS day) and QS (38.59 ml/g VS day) in the SIR (1:2). With regard to biogas production, the highest results were obtained in the residues of QS (50.40 ml/g VS day) and VM (35.96 ml/g VS day) for SIR (1:1), and in WS (50.74 ml/g VS day) and AS (72.61 ml/g VS day) for SIR (1:2). The highest values of ν_{max} were obtained in the exponential phases and when the amount of inoculum to the tests was increased since a better dissolution of the organic matter was obtained.

3.4 Evaluation of the different kinetic models

For the evaluation of the models, two statistics have been used **(Table 3)**; a) the coefficient of determination of the adjustment R^2 and b) the root of the mean of the squares of the errors (RMSE). In the **Table 2**, it is observed that the highest values of R^2 were recorded in the cone and transfer models for both methane and biogas measurements in their different SIR. Thus, the cone model had the best fit of R^2 = 0.999 for residues of VM (CH_4 SIR of 1:1 and 1:2), AS (CH_4 SIR 1:1 and 1:2), VM (biogas SIR 1:1 and 1:2) and AS (biogas SIR 1:1). At the same time, the transfer model recorded values of R^2 = 0.996-0.999 for residues of VM (CH_4 SIR of 1:1 and 1:2), AS (CH_4 SIR 1:1 and 1:2), VM (biogas SIR 1:1 and 1:2) and AS (biogas SIR 1:1 and 1:2). Similarly, the Gompertz model and the Richards model provide the same results of R^2 since the parameter d, of the Richards model, tends to 0. However, the Gompertz model comprises R^2 values between 0.969 and 0.998 for GPM residues (biogas SIR 1:2), LM and VM (CH4 SIR 1:2). Regarding the logistics model, R^2 values range from 0.954 to 0.990 for GPM (biogas SIR 1: 2) and QS (CH4 SIR1:1,1:2) waste. Regarding the logistics model, R^2 values range from 0.954 to 0.990 for GPM (biogas SIR 1: 2) and QS (CH_4 SIR1:1,1:2) waste.

Table 2. Kinetic parameters of methane and biogas for different models.

SIR	Models	Parameters	Units	METHANE							BIOGAS						
				WS	AS	QS	GPM	LM	VM	SW	WS	AS	QS	GPM	LM	VM	SW
(1:1)	GOMPERTZ	M_e	ml CH_4/g VS	262.500	317.470	286.540	211.050	238.240	290.560	235.360	456.384	542.385	454.540	295.771	384.279	432.137	358.203
		ν_{max}	ml CH_4/g VS day	10.600	11.960	17.820	8.970	10.790	14.150	10.630	17.605	22.153	27.561	12.737	14.663	19.321	13.884
		t_{lag}	days	-2.090	-1.400	-0.460	-3.020	-2.150	-3.210	-1.890	-2.714	-2.727	-1.646	-3.175	-2.642	-3.456	-1.640
	TRANSFER	M_e	ml CH_4/g VS	235.360	358.380	297.510	221.400	251.570	300.130	250.320	490.490	580.579	467.855	309.615	410.357	449.964	400.147
		ν_{max}	ml CH_4/g VS day	10.630	18.580	30.520	16.400	18.890	26.740	18.160	30.480	38.288	50.401	23.488	30.259	35.963	21.811
		t_{lag}	days	-1.890	0.130	0.640	-0.420	-0.140	-0.740	-0.080	-0.277	-0.458	0.016	-0.509	-0.488	-0.830	-0.082
	LOGISTIC	M_e	ml CH_4/g VS	255.450	304.860	282.320	206.890	233.260	286.200	229.440	443.666	527.776	448.545	290.084	372.658	424.206	344.246
		ν_{max}	ml CH_4/g VS day	9.740	11.460	16.610	7.990	9.830	12.500	9.940	16.113	20.340	24.960	11.298	13.553	17.183	13.362
		t_{lag}	days	-2.710	-1.480	-0.660	-4.060	-2.800	-4.250	-2.230	-3.448	-3.399	-2.190	-4.288	-3.241	-4.506	-1.676
	CONE	M_e	ml CH_4/g VS	361.620	454.470	318.930	284.220	303.430	366.300	304.650	662.804	760.744	521.633	399.074	563.041	576.185	513.611
		k	1/day	0.060	0.050	0.120	0.070	0.080	0.100	0.080	0.055	0.063	0.136	0.076	0.053	0.085	0.053
		n	dimensionless	1.090	1.140	1.550	1.020	1.140	1.020	1.140	1.005	1.016	1.287	0.998	1.007	0.968	1.104
	RICHARDS	M_e	ml CH_4/g VS	263.390	317.410	286.640	211.440	239.080	290.540	235.470	457.486	542.281	454.630	*	386.659	433.024	358.336
		d	dimensionless	0.000	0.010	0.000	0.000	0.030	0.010	0.010	0.005	0.005	0.006	*	0.005	0.006	0.007
		ν_{max}	ml CH_4/g VS day	9.990	13.550	20.950	10.760	12.750	17.220	12.490	20.710	26.060	33.200	*	18.860	23.380	15.820
		t_{lag}	days	-2.230	-1.420	-0.510	-3.110	-2.280	-3.230	-1.920	-2.757	-2.717	-1.663	*	-2.801	-3.576	-1.669
(1:2)	GOMPERTZ	M_e	ml CH_4/g VS	254.654	287.603	370.254	168.703	376.477	258.092	282.461	440.958	480.989	598.180	266.590	484.336	380.589	459.354
		ν_{max}	ml CH_4/g VS day	16.157	23.192	22.571	10.984	17.666	14.807	8.580	27.910	38.870	35.023	16.106	18.212	21.240	10.598
		t_{lag}	days	-0.801	-0.236	-0.492	-2.070	-3.459	-1.393	-5.962	-1.238	-0.891	-1.847	-3.003	-2.919	-1.435	-7.536
	TRANSFER	M_e	ml CH_4/g VS	263.161	293.954	384.972	171.942	389.468	266.949	307.942	454.409	489.269	615.680	264.220	524.671	394.615	541.916
		ν_{max}	ml CH_4/g VS day	28.866	41.232	38.593	21.402	33.387	26.796	15.009	50.743	72.611	32.391	30.979	30.979	38.423	16.878
		t_{lag}	days	0.657	0.765	0.633	-9.137	-0.877	0.305	-2.420	0.353	0.405	-7.996	-0.554	-0.554	0.348	-3.844
	LOGISTIC	M_e	ml CH_4/g VS	251.166	284.803	364.602	167.125	370.543	254.503	272.158	435.098	476.940	590.336	264.222	469.446	374.846	431.519
		ν_{max}	ml CH_4/g VS day	14.678	21.339	21.050	9.560	15.610	13.320	7.815	25.224	34.949	31.567	13.745	16.830	19.129	10.005
		t_{lag}	days	-1.289	-0.497	-0.692	-2.994	-4.532	-2.022	-7.164	-1.799	-1.347	-2.451	-4.253	-3.544	-2.083	-8.328
	CONE	M_e	ml CH_4/g VS	287.830	308.304	414.295	191.515	485.454	297.860	716.768	502.322	520.264	693.638	311.057	729.282	446.560	2136.533
		k	1/day	0.130	0.167	0.121	0.164	0.098	0.122	0.012	0.137	0.187	0.132	0.164	0.050	0.115	0.002
		n	dimensionless	1.431	1.670	1.530	1.230	0.985	1.328	0.655	1.347	1.494	1.247	1.084	0.968	1.291	0.607
	RICHARDS	M_e	ml CH_4/g VS	254.782	287.577	370.212	168.674	376.392	257.997	283.035	441.212	481.655	598.086	266.598	483.751	380.657	459.302
		d	dimensionless	0.002	0.004	0.006	0.004	0.004	0.005	0.004	0.002	0.003	0.005	0.003	0.006	0.005	0.009
		ν_{max}	ml CH_4/g VS day	19.260	27.670	26.520	13.530	21.500	17.720	10.130	33.510	47.240	31.930	19.620	21.300	25.420	12.090
		t_{lag}	days	-0.843	-0.243	-0.497	-2.067	-3.475	-1.407	-6.132	-1.276	-1.001	-1.854	-3.033	-2.900	-1.455	-7.556

NOTE: LM (Llama manure), VM (Vicuña manure), GPM (Guinea pig manure), SW (Slaughterhouse waste), WS (Wheat straw), AS (Amaranth straw), QS (Quinoa straw) and IN (inoculum, WWTP sludge). (*) Predicted data does not converge with that observed in this model.

Table 3. Evaluation of the kinetic models for methane and biogas.

SIR	Parameters	Feedstock	R^2					RMSE				
			Gompertz	Transfer	Logistic	Cone	Richards	Gompertz	Transfer	Logistic	Cone	Richards
(1:1)	Methane	Wheat straw (WS)	0.981	0.992	0.968	0.996	0.981	9.7	17.08	12.52	4.23	9.72
		Amaranth straw (AS)	0.994	0.999	0.986	0.999	0.994	6.53	1.96	10.19	2.04	6.56
		Quinoa straw (QS)	0.997	0.997	0.99	0.997	0.997	4.09	4.06	7.49	4.24	4.11
		Guinea pig manure (GPM)	0.977	0.991	0.963	0.995	0.977	8.15	5.02	10.21	3.65	8.15
		Llama manure (LM)	0.991	0.998	0.982	0.998	0.991	5.75	2.83	8.25	3.06	5.87
		Vicuña manure (VM)	0.989	0.997	0.979	0.999	0.989	7.33	3.5	10	2.36	7.35
		Slaughterhouse waste (SW)	0.992	0.996	0.985	0.995	0.992	5.56	3.76	7.57	4.17	5.57
	Biogas	Wheat straw (WS)	0.979	0.993	0.983	0.996	0.979	16.99	9.78	21.6	7.28	17.03
		Amaranth straw (AS)	0.987	0.997	0.976	0.999	0.987	15.93	7.64	21.66	4.25	15.97
		Quinoa straw (QS)	0.992	0.998	0.983	0.998	0.992	9.89	4.84	14.56	4.91	9.92
		Guinea pig manure (GPM)	0.973	0.989	0.958	0.995	-	12.16	7.73	15.1	5.22	-
		Llama manure (LM)	0.987	-	0.976	0.998	0.987	11.36	-	15.28	3.85	11.4
		Vicuña manure (VM)	0.984	0.995	0.973	0.999	0.984	13.15	7.20	17.16	3.19	13.19
		Slaughterhouse waste (SW)	0.993	0.997	0.987	0.996	0.993	7.8	5.65	11.02	6.02	7.82
(1:2)	Methane	Wheat straw (WS)	0.977	0.993	0.961	0.996	0.977	10.15	5.66	13.27	0.61	10.16
		Amaranth straw (AS)	0.991	0.998	0.979	0.999	0.991	7.07	3.02	10.6	4.3	7.09
		Quinoa straw (QS)	0.997	0.997	0.99	0.997	0.997	5.47	5.34	9.73	1.67	5.5
		Guinea pig manure (GPM)	0.975	0.991	0.96	0.997	0.975	6.25	3.74	7.92	5.74	6.26
		Llama manure (LM)	0.988	0.997	0.979	0.998	0.988	9.67	4.99	12.96	1.98	9.69
		Vicuña manure (VM)	0.988	0.998	0.976	0.999	0.988	7.25	2.79	10.37	3.46	7.27
		Slaughterhouse waste (SW)	0.969	0.982	0.957	0.991	0.969	11.39	8.78	13.35	1.89	11.4
	Biogas	Wheat straw (WS)	0.977	0.992	0.962	0.996	0.977	16.91	10.19	21.84	7.07	16.93
		Amaranth straw (AS)	0.986	0.997	0.974	0.999	0.986	13.3	6.2	18.38	3.36	13.34
		Quinoa straw (QS)	0.993	0.998	0.985	0.997	0.993	12.23	6.25	18.01	7.9	12.26
		Guinea pig manure (GPM)	0.969	0.997	0.954	0.996	0.969	10.42	7.02	12.74	3.51	10.43
		Llama manure (LM)	0.987	0.997	0.977	0.999	0.987	14.06	7.03	18.78	4.69	14.09
		Vicuña manure (VM)	0.982	0.996	0.968	0.998	0.982	13.29	6.32	17.83	4.02	13.31
		Slaughterhouse waste (SW)	0.969	0.98	0.959	0.988	0.969	17.12	13.83	19.59	10.68	17.15

Regarding RMSE statistic performance, it is observed that the behavior of this statistic is much lower in the cone and transfer models. Thus, the transfer model ranges its RMSE between 1.96 ml CH_4/g VS (SIR 1:1) and 17.18 ml biogas/g VS (SIR 1:2) for AS and WS residues, respectively. On the other hand, in the cone model RMSE was recorded between 0.61ml CH_4/g VS (SIR 1:1) and 10.68 ml biogas/g VS (SIR 1:2) for WS and SW residues, respectively. In the RMSE analysis for the Gompertz model, values between 4.09 ml CH_4/g VS (SIR 1:1) and 17.12 ml biogas/g VS (SIR 1:2) were obtained for the residues of QS and SW, respectively. Finally, the logistic model is the one that had the greatest difference between the observed and estimated values. In this model, RMSE values of 7.49 ml CH_4/g VS (SIR 1:1) and 21.84 ml biogas/g VS (SIR 1:2) were recorded for the residues of QS and WS, respectively. In general, for all models the residues that best approximated the observed data were those of QS and AS and those that were least adjusted were those of WS and SW.

The best estimates of R^2 and RMSE were obtained for cone and transfer models, however, these models have more extreme values in the calculation of Me with error differences of 20.55 (transfer model) and 79.85% (cone model). In this sense, in these models the value of Me is overestimated with respect to sigmoidal model models. In addition, the transfer model is the only one that registered positive values on the lag phase. On the other hand, in the cone model the lag phase cannot be compared with the other models since this model does not provide this parameter.

The Gompertz and Richards models adjusted better since they did not oversize the estimated M_e of the performance observed in the digesters. In addition, these models presented a high coefficient of determination and a low value in the RMSE for all the analysed residues. This showed that these two proposed models can accurately describe the variation of methane and biogas yield curves. On the other hand, the low t_{lag} value observed for methane and biogas in these models demonstrated the low inhibition of AD and the high biodegradability of the residues. According to the results obtained, it shows that these models, in particular the Gompertz, are the most used kinetic model in the literature due to their good adjustments [59].

The fact that sigmoidal models do not overestimate M_e as the cone model does is because all models are based on functions that increase monotonously (that is, the function always assumes that the growth rate increases and is never the same to zero or decrease) [60]. However, sigmoidal models have a turning point, where the sign of the curvature changes from concave to convex or vice versa, that is, ν_{max} [61]. Thus, for example, the logistic and Gompertz functions have fixed inflection points. On the one hand, the logistic function is symmetric with respect to its inflection point that exists when growth reaches half of its final growth (maximum asymptote) [60,61]. While Gompertz's function is asymmetric about its inflection point that occurs at a much earlier point than that of the logistic model, approximately 1/e of its final growth (maximum asymptote) [62].

3.5 Biodegradability and theoretical yield

The biological efficiency was calculated considering the experimental performance of the biodigesters and the theoretical performance of the elemental analysis of the substrates. The results estimated a biodegradability between 44% and 70% for all the substrates tested. This is because the theoretical yield was much higher than the experimental one. In general, the reactions that take place in AD are not completely terminated during the assay process, which makes the experimental performance have discrepancies with the theoretical performances. The fact that reactions do not occur completely in experimental trials is due to the presence of toxins, insufficient mixing, establishment of the microbial

population, lignin complexity and other effects of the process condition (pH, temperature and redox) [63]. The theoretical values are very optimistic and do not coincide with the experimental ones since in practice there is no complete reaction and there is no 100% decomposition of the cellulosic materials. On the other hand, the theoretical performance does not consider the non-degradable material or the energy demand of microorganisms. Thus, the equations of Buswell and Müller (1952) and Boyle (1976) imply a complete conversion of biomass, which results in an overestimation of methane yields. The determination of the elemental composition is relatively rapid for all compounds, although this equation does not differentiate between biodegradable and non-biodegradable matter, and part of the biodegradable organic matter used by bacteria to grow does not contribute to the theoretical value of BMP [64,65].

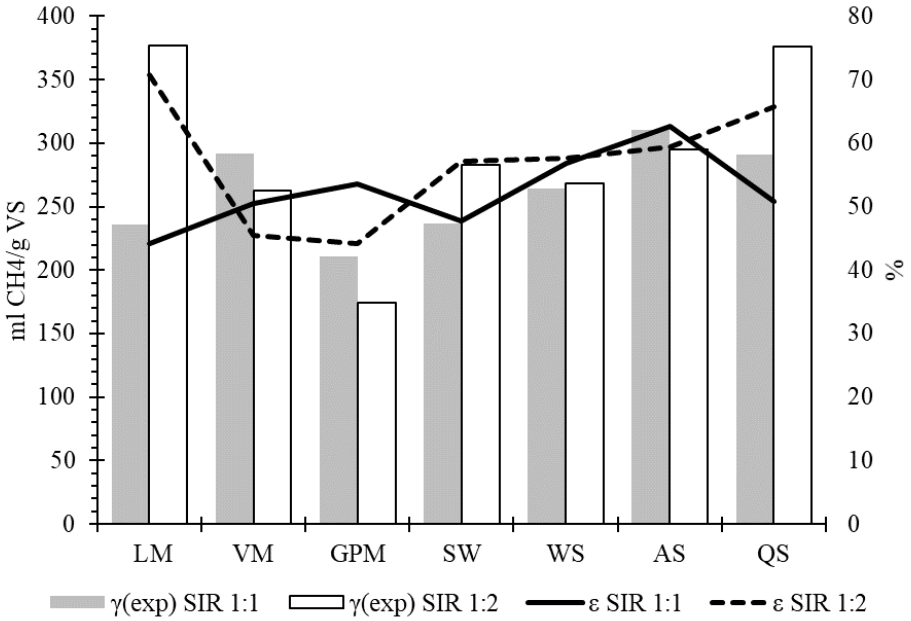

Figure 2. Production of experimental methane and biodegradability of the substrates used for SIR 1:1 and 1:2.
NOTE: LM (Llama manure), VM (Vicuña manure), GPM (Guinea pig manure), SW (Slaughterhouse waste), WS (Wheat straw), AS (Amaranth straw), QS (Quinoa straw.

Figure 2 shows that of all the residues analyzed, the SW and GPM residues have the lowest ε; this decrease is due to the fact that these substrates contain a greater presence of hydrogen and nitrogen, which makes it possible to produce a toxic concentration of ammonia and hydrogen sulfide [66]**.** On the contrary, it is observed that the productivity of CH_4 increases with the increase of the C/N ratio to 30 as in the case of the residue of WS (C/N=29.61). In this regard, some researchers have suggested that the C/N ratio for optimal digestion performance is in the range of 20-30, while many have shown that digestion can be performed successfully using a wider range of the ratio C/N [67,68].
When the amount of inoculum was increased from 50% to 66.67%, the LM and QS residues had a biological efficiency (ε) of 70.74 and 65.65%, respectively, followed by AS, SW and RM (59.41; 57.68 and 57.13%), and the lowest ε was obtained for GPM and VM (44.17 and 45.54%). In addition, with the addition of inoculum, the LM, SW, WS and QS residues increased the values of ε, while the residues of VM, GPM and AS experienced a slight decrease in their ε. A possible reason for the increase in ε after the addition of more inoculum is that it is possible to avoid further inhibition of high VFA concentration and acidic pH in methane production [69]. On the other hand,

lignocellulosic residues such as those of QS and WS increased ε with the increase of the inoculum since it is achieved that the mixture of cellulose and hemicellulose has a better balance of nutrients and facilitates the optimal growth of the microorganisms responsible for the process of AD [24].

Conclusions

This study investigated the impact on the addition of inoculum in agricultural and livestock waste treatments. Overall, findings showed that the addition of an inoculum to treatments can reinforce degradation performance. More specifically, an SIR 1:2 yielded the highest methane in the residues of QS and LM providing 376.08 ml CH4/g VS and 377.02 ml CH_4/g VS, respectively. Althoguh an adequate amount of mud is required for efficient operation, higher SIR improves methane production except AS, GPM and VM residues which exhibited a yield decrease by 5.32%; 21.12% and 11.02%.
With respect to prediction models, sigmoidal models with three and four parameters are the ones that best estimate BMP. Thus, the asymptotes calculated with the Gompertz model adjust very precisely specific performance which causes them to vary by no more than 7.06% with respect to the experimental data. Also, the best adjustments are in the GPM and LM residues whose yield varied by 0.01% and 0.15% with respect to those observed. In short, he modified Gompertz model was better adjusted to the experimental results than the rest of the kinetic models with the highest R^2 (0.998) and RMSE of 4.09 ml/g VS and 17.12 ml/g VS.
Finally, theoretical yield proved to be higher than experimental values for both SIR 1:1 and 1:2 although the highest efficiency and the greatest biodegradability was obtained in the LM and QS residues with 70.74 % 65.65%.

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Chapter II

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

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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 modelling, 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.

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

1. Introduction

The Andean area of South America is an economically depressed area where the energy supply is deficient [1]. Furthermore, in the last year the supply of gas and fuel has become more expensive. This has led to the need to efficiently take advantage of all available resources 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 as the main means of subsistence [5]. The main camelid grazing 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]. Camelids resist 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 camelid constitute an important source of economic income for the livestock sector, since 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, 2,048 and 3,149 hectares 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. Waste that could provide energy (in the form of biogas), avoiding the use of local biomass (deforestation) [11]. The use of camelid 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 in the production of methane [12]. Similarly, vicuña manure (VM) is complementary to other camelid manure since 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 since it serves as a substitute for firewood [11]. Camelid manure is generally easy to manage. Domestic camelids carry out their defecations in stables, and wild camelid, despite living free, carry out defecations 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 camelid manure. Many agricultural residues in rural communities are not properly managed, since they are burned after each harvest [16]. The transformation of agricultural waste into biogas would not only provide energy benefits; 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 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 addresses the energetic study of totally new materials, making this study serve as a precedent for future research; above all, for the commissioning of continuous reactors on a pilot and 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 camelid manure and agricultural residues of AS, QS and WS. In addition, the optimal relation between the main substrate and the co-substrate is analysed. Finally, the kinetics, the synergistic and antagonistic effects, and the relation between theoretical and experimental performance are determined.

2. Materials and methods

2.1 Substrates, co-substrates and inoculum used.

Pre-treatment and conservation of materials

The evaluated materials were divided into substrates and co-substrates. Thus, as substrate, llama manure (LM) and vicuña manure (VM) were used; while as co-substrate, lignocellulosic residues of Andean character were used, such as: amaranth straw (AS), quinoa (QS) and wheat (WS). The LM was collected from the rural communities of Guaranda, Ecuador; while 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 ºC 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 contents, and their elemental composition. The contents of total solids (TS) and volatile solids (VS) 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 analyser. 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.

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)

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

2.2 Experimental methodology

BMP Assays of Anaerobic Digestion

In this study, BMP (Biochemical Methane Potential) 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]. Since 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 N1-N4 represent the individual fractions of each factor, whereas the mixtures M5-M23 and N5-N23 represent the binary combinations. The design allows to evaluate the synergistic or antagonistic interactions according to the individual or mixed fractions supplied in each digester.

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

Note: % 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.

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 through (**Eq. 1**). The biogas was normalized to standard conditions (25°C and 1 atm) and expressed as ml/g VS.

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

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)

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

2.3 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 ºC and 1 atm, respectively. The γ_{th} was estimated through its elemental composition and the stoichiometry of the degradation reaction (**Eq. 2**), considering Buswell's formula and Boyle's equation (**Eq. 3**) [31-33]

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

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

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

2.4 Biodegradability and synergistic and antagonistic effects of substrates.

The biological efficiency (ε) of the anaerobic process was determined by the following equation (**Eq. 4**) [35].

$$\varepsilon = \frac{\gamma_{(exp)}}{\gamma_{(teo)}}.100\% \quad (5)$$

The synergistic and antagonistic effects can be obtained as the relationship between the experimental performance (γ_{exp}) and the weighted performance (γ_{pond}) (**Eq. 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].

$$\alpha = \frac{\gamma_{exp}}{\gamma_{pond}} \quad (4)$$

The result of α indicates:

α > 1; the mixture has a synergistic effect on the final production.

α = 1; Substrates function independently of the mixture of substrate and co-substrate.

α <1; The mixture presents antagonistic or competitive effects in the final production.

The (γ_{pond}) can be estimated using (**Eq. 6**)

$$\gamma_{pond} = \frac{\gamma_{sp}.\lambda + \gamma_{cs}.\beta}{\lambda + \beta} \quad (6)$$

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 correspond to the VS fractions added by the main substrates and the co-substrates.

2.5 Kinetic fit models

Methane production was modelled 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 equations. (**Eq. 7**) - (**Eq. 11**), respectively.

$$M = M_e.\exp\left\{-\exp\left[\frac{\nu_{max} * e}{M_e}(t_{lag} - t) + 1\right]\right\} \quad (7)$$

$$M = M_e\left\{1 - \exp\left[-\frac{\nu_{max}}{M_e}(t - t_{lag})\right]\right\} \quad (8)$$

$$M = \frac{M_e}{1 + \exp\left[\frac{4\nu_{max}(t_{lag} - t)}{M_e} + 2\right]} \quad (9)$$

$$M = \frac{M_e}{1 + (k.t)^{-n}} \quad (10)$$

$$M = M_e\left\{1 + d.\exp(1 + d)\exp\left[\frac{\nu_{max} * e}{M_e}(1 + d)\left(1 + \frac{1}{d}\right)(t_{lag} - 1)\right]\right\}^{\frac{1}{d}} \quad (11)$$

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}), ν_{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.

3. Results

3.1 Characterization of the raw material

3.1.1 Main substrates used.

The characterization data of the llama and vicuña manure were analysed with respect to the VS/TS and C/N ratio and are presented in **Table 3**. The camelid manure 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 efficiently balance the carbon and nitrogen of the raw material 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 since their average VS/TS ratio is 80.37%, 74.75% and 62.19% respectively. Also, to a lesser extent, other authors have considered that llama manure has enormous potential in biogas production [47, 11, 12, 50]; since 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, 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].

3.1.2 Co-substrates used.

The organic fraction of the co-substrates presented very favourable values for AD. The tabulated data of the 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 of 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.

3.2 Generation and methane potential from camelid manure

3.2.1 Comparison of SIR from BMP tests

Figures 2 and **3** 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 (**Figure 1)**. 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.

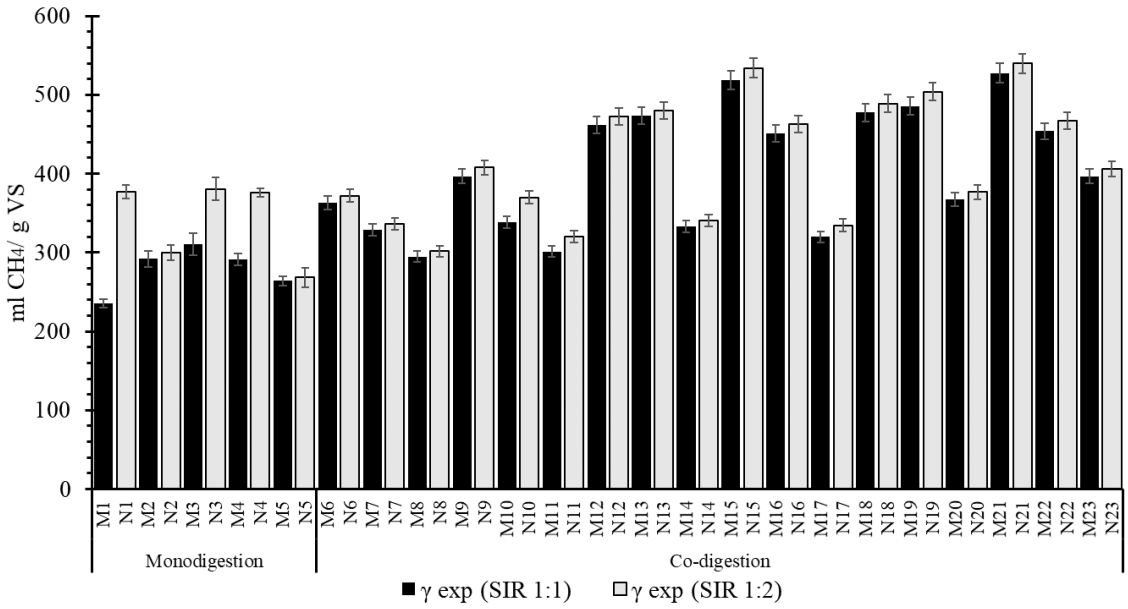

Figure 1. Influence of inoculum on methane production by comparing two SIRs (1: 1 and 1: 2).
Note: The left part of the figure shows the variability of methane for monodigestion and the right part shows the variability of methane in co-digestion**.**

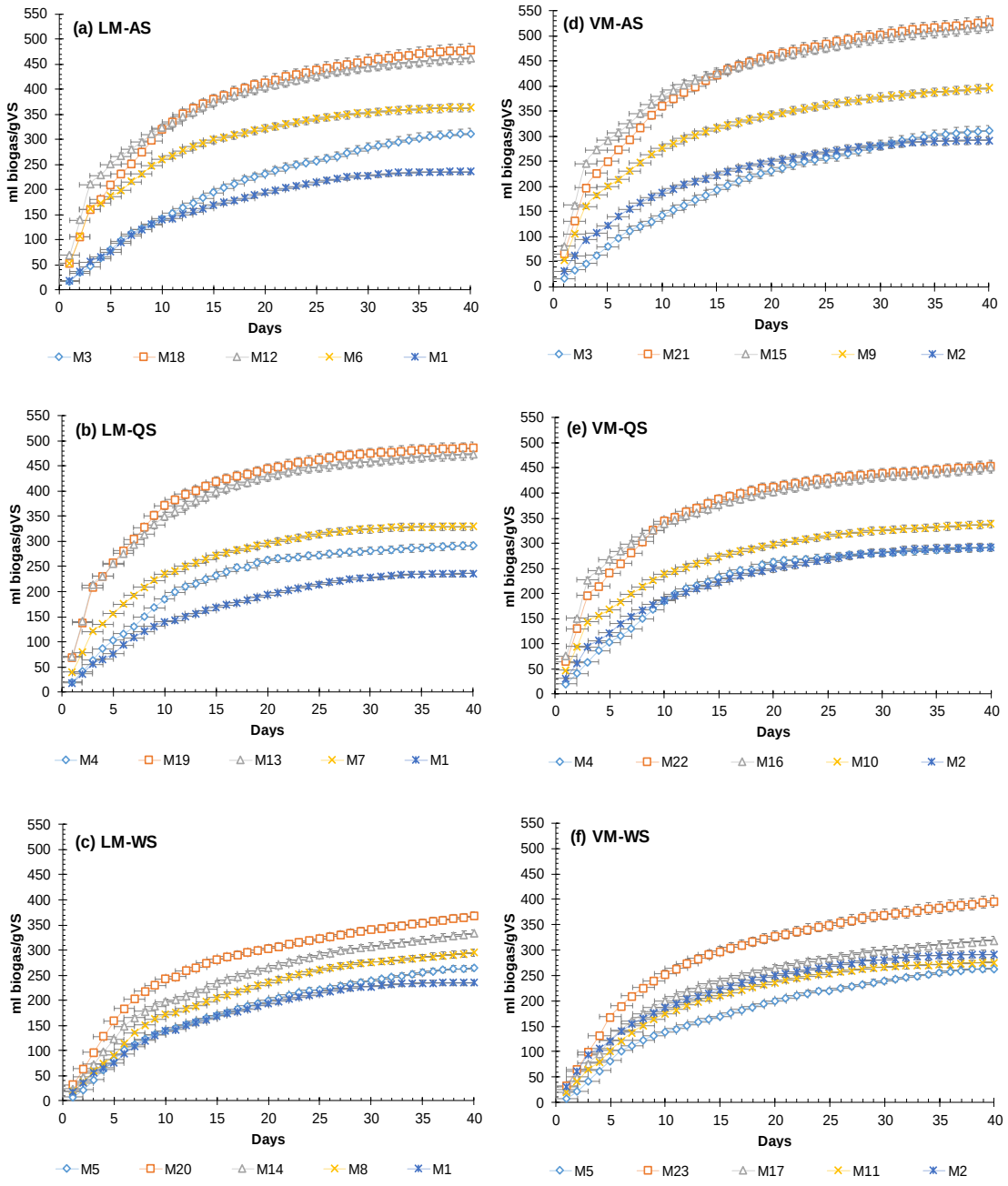

Figure 2. Cumulative profiles of CH_4 production as a function of time, for SIR1:1 assay.
Note: LM = llama manure; VM = vicuña manure; WS = wheat straw; AS = amaranth straw; QS = quinoa straw. The tests M1-M5 represent the biodigesters of monodigestion and the tests M6-M23 represent the biodigesters of co-digestion.

The results of this study revealed that the individual fractions of the main substrates of LM and VM are influenced by 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 behaviour 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, N4 showed significant differences ($p < 0.05$). Also, the co-digestion tests, with a SIR1:2, also increased methane production with respect to the SIR1:1.

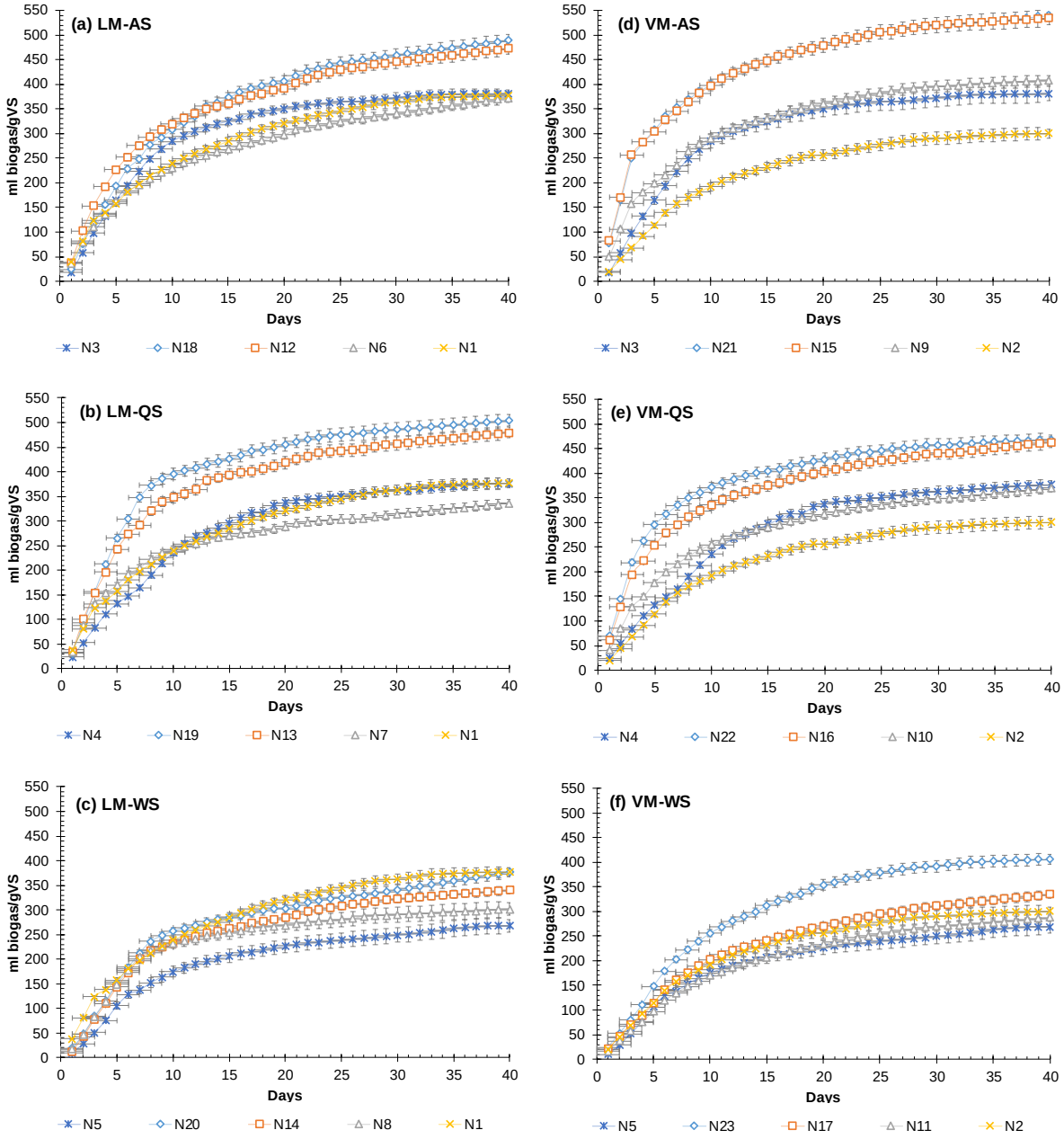

Figure 3. Cumulative profiles of CH_4 production as a function of time, for SIR1:2 assays. Note: LM = llama manure; VM = vicuña manure; WS = wheat straw; AS = amaranth straw; QS = quinoa straw. The tests N1-N5 represent the biodigesters of monodigestion and the tests N6-N23 represent the biodigesters of co-digestion.

The 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 the values 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] investigated 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. In addition, the data from this study have been contrasted with others 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.

3.2.2 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 a wide range of mixtures and, more importantly, 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 (**Figure 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. Also, mixtures M19 (486 ml CH_4/g VS) and M18 (477 ml CH_4/g VS) 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 behaviour 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 of the co-digestion mixtures with respect to the digestion of individual LM and VM is since anaerobic co-digestion can increase the efficiency of the process due to a healthier balance of nutrients and carbon [77,78,75]The fact that the mixtures increase the production of methane with the increase in the co-substrate concentration may be since an increase in manure leads to an eventual accumulation of volatile fatty acids (VFA), producing an acidification in the composition of the digesters [31].

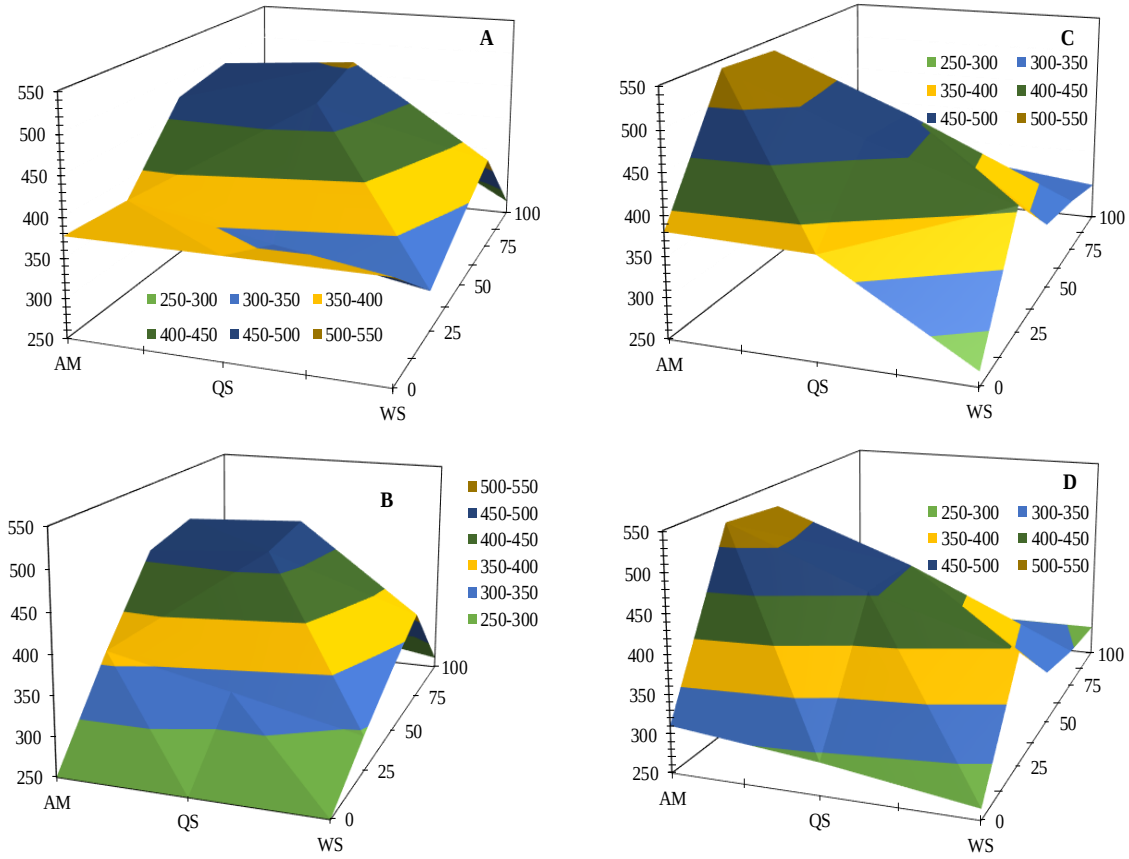

Figure 4. Mixing of substrates and methane potential through co-digestion of llama manure (LM) and vicuña manure (VM) with different agricultural co-substrates
Note: A = LM with SIR 1:2, B = LM with SIR 1:1, C = VM with SIR1:2, D = VM with SIR 1:1

3.2.3 Biodegradability and synergistic effects

Figure 4 presents the results of the synergistic effects, biodegradability and % methane potential of the different mixtures. In **Figure 4A** it is observed that, for a SIR1:1, all the mixtures show synergistic effects ($\alpha > 1$) on 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 that correspond to the digesters that had 50% and 75% AS. Similarly, biodegradability (ε) follows the same behaviour 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 **(Figure 4B)**, not all mixtures exhibited synergistic effects; mainly, the mixes of the LM configuration. According to Nielfa et al. [79], 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, N18-N20 did not

generate more methane than the individual composition of LM, which caused antagonistic effects (α <1) to occur in the test. 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. In addition, the increase in inoculum 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.

3.3 Kinetics

3.3.1 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 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, whose content has a high content of organic matter for energy production [80]. In this sense, the introduction of sufficient active microorganisms in the digesters led to a direct initiation of methanogenesis without a measurable latency period. According to 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}, since it would give values close to 0 and possibly negative. In this case, the methane production curves with the least amount of inoculum experienced a more sigmoid behaviour, compared to the curves with more inoculum that presented more oval curves.

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

The cone model was used to observe the behaviour 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 al. [81], if there are no inhibitory effects during digestion, the cumulative yields of methane or biogas generation usually follow a first-order accumulation pattern. In **Tables 4** and **5** it is observed that many digesters experienced an improvement in the constant k with increasing the 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 [82].
The values obtained for k were quite heterogeneous in all the trials, whose values ranged in SIR1:1 between 0.08 d^{-1} (M15) and 0.21 d^{-1} (M12), and in SIR 1:2 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. [82], who reported results of 0.09-0.18 d^{-1}. Furthermore, the results of k were superior to the studies by Pitt et al. [84], 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 [85]. 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 while 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 VFA, so the hydrolysis rate cannot be determined precisely from methane yields. Initial concentrations of VFA are very common in manure [86]. 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 [87].
Regarding ν_{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 the SIR1:1, values of 64.34 ml/g VS day were recorded in the M19 digesters, and 62.23 ml/g VS day for the 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 ν_{max} decreased in all the digesters with all the models tested.

3.3.3 Effects on maximum methane yield (M_e)

When the maximum methane yield is analysed, it is observed that the experimental values followed the same trend as the theoretical models.
However, it can be found that the cone model overestimates the performance M_e. This would be related to the high initial concentrations of VFA in substrate mixtures [88]. Since most of the methane was generated during the first five 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, Logístico and Richards model 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%.

3.3.4 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 data of co-digestion. According to **Table 4** and **5**, the models that best fit are the transfer model and the cone model. For its part, in the SIR1:1 the transfer model ranged its value of R^2 between 0.991 and 0.999; while, 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 well the cumulative biogas and methane production curve. 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 ν_{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 hours.

At time t = 0 days, all models exhibited positive values for all digesters, including the transfer model, since 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.

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

Mixture	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

Note: 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

Mixture	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

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

4. Discussion

In this study, two scenarios were analysed: the influence of the inoculum by comparing two SIR (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 SIR did not present significant differences in methane production, except for the LM, AS and QS that improved with the increase in inoculum by 60, 22 and 29% respectively. Owen et al. [89] have considered that a SIR1:1 is adequate, but Chynoweth et al. [90] state that an increase in SIR may be necessary for some type 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 highly influenced by the inherent characteristics of substrates [91], which suggests that organic materials require specific studies on the effect of SIR [92]. In addition, to correctly evaluate the effect of the inoculum, it is necessary to know the type, incubation time and origin of the inoculum used [93]. In this study, the little influence of the SIR on the methane yield may be since 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 [92]. 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 [94,95]. The observed improvements in methane production can be attributed to the synergistic effects of agricultural crop residues [64]. Effects that have improved the load of the biodegradable substrate, the hygienic stabilization and the increase in the speed of the digestion process [96]. The increase in methane from co-digestion occurred mainly in the mixtures with AS and QS. The optimal amounts of mixing between LM, VM and agricultural residues were in a 50:50 ratio of VS. 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 since some chemical characteristics (fiber, sugars, fats, proteins) of SA straw are similar to those of corn straw [97]. Many researchers have considered corn straw one of the main agricultural residues to obtain high methane yields [98,99]. 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. [100], carried out an investigation in which they reported the improvements in the co-digestion of pig manure, bobbin and poultry manure residues; 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. [101] 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 increasing from SIR1:1 to SIR1:2 did not generate significant figures in methane production in most LM and VM mixtures. 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) that 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 the predicted and predicted values very well, especially the transfer and cone model ($R^2 >$ 99%, RMSE < 2 ml CH_4 /g VS).

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Chapter III

Anaerobic co-digestion of slaughter residues with agricultural waste of amaranth, quinoa and wheat

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ABSTRACT

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

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

1. Introduction

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

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

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

At present there is a diversity of slaughterhouses, which depends on the type, quantity and variety of animals treated. The Guaranda slaughterhouse processes cattle and pigs. Most of the research in the literature addresses the anaerobic digestion of previously pre-treated RM, in which the contaminant load has been reduced. This makes the waste generated, as raw material in slaughterhouses, diverse and depends on the type of

slaughterhouse to be treated. In this sense, this research addresses the anaerobic co-digestion of mixed RM not pre-treated with agricultural residues of AM, QU and TR. Furthermore, the effect of inoculum (sewage sludge) on methane yield is evaluated. The research process was carried out under mesophilic conditions and on a laboratory scale.

2. Materials and methods

2.1 Substrates, co-substrates and inoculum used.

RM and residues of lignocellulosic materials

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

Characterization of substrates, co-substrates and inoculum.

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

2.2 Theoretical methane production

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

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

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

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

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

where γ_{CODt} is the theoretical production, R is the gas constant (R = 0.082 atm l/mol K), T is the biodigester temperature (298 K), P is the atmospheric pressure (1atm), VS added (g) are the volatile solids of the substrate and n_{CH4} is the amount of molecular methane (mol).
The value of n_{CH4} has been determined from **Equation 4** [19]**.**

$$n_{CH4} = \frac{CODt}{64\left(\frac{g}{mol}\right)} \quad (4)$$

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

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

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

2.3 Biodegradability of anaerobic co-digestion

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

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

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

$$\alpha = \frac{\gamma_{exp}}{\gamma_{pond}} \quad (8)$$

Where γ_{exp} refers to the experimental performance obtained by the BMP and γ_{pond} corresponds to the weighted experimental performance.
γ_{pon} is determined by **Equation 9** [22].

$$\gamma_{pond} = \frac{\gamma_{sp}.\lambda + \gamma_{cs}.\beta}{\lambda + \beta} \quad (9)$$

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

2.4 Experimental setup and procedure

Initial conditions of co-digestion

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

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

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

Anaerobic Co-digestion Biochemical Methane Potential (BMP) Assays

BMP experiments were used to determine the influence of co-substrates and inoculum on methane yield during anaerobic co-digestion of RM. All BMP experiments were performed in triplicate, in 311ml glass biodigesters filled with 60% working volume. The proportions of the substrates and co-substrates before being put into the biodigester were mixed with a kitchen blender to ensure that the experimental samples are uniform. Once the co-digestion mixtures had been made, the batch biodigesters were closed with rubber septa and aluminium lids to guarantee anaerobic conditions inside. The experiments were carried out during 40 days and 37 °C. Distilled water was added to obtain a final working volume of 60% of the volume of the biodigesters when necessary. As controls, three blank biodigesters containing only inoculum and distilled water were also incubated under the same conditions as the rest of the biodigesters. The biogas yield from these blank biodigesters was used to correct for the biogas produced solely by the inoculum.
The volume of biogas produced in each biodigester was calculated daily by measuring the pressure in the headspace of each biodigester using a portable pressure gauge (Delta OHM HD 2124.2) (**Figure 1**). The pressure in the head space of the biodigester was

measured after the insertion of a syringe needle through the rubber stopper. The composition of the biogas (content of CH_4, O_2, CO_2, H_2S) was measured using the BIOGAS GA-5000 meter from Geotech. In this way, using a 200 ml hermetic syringe, biogas samples were taken from the headspace of each biodigester after releasing the gas. Before measuring the biogas composition in the headspace, the reactors were shaken for two minutes at 100 rev/min. The composition of the biogas was measured once a day until the end of the fermentation.
The maximum methane yield was expressed as the maximum volumetric yield of methane per gram of initial substrate VS added (ml CH_4/g VS). Each trial was performed in triplicate, and the results were obtained as the average of these.

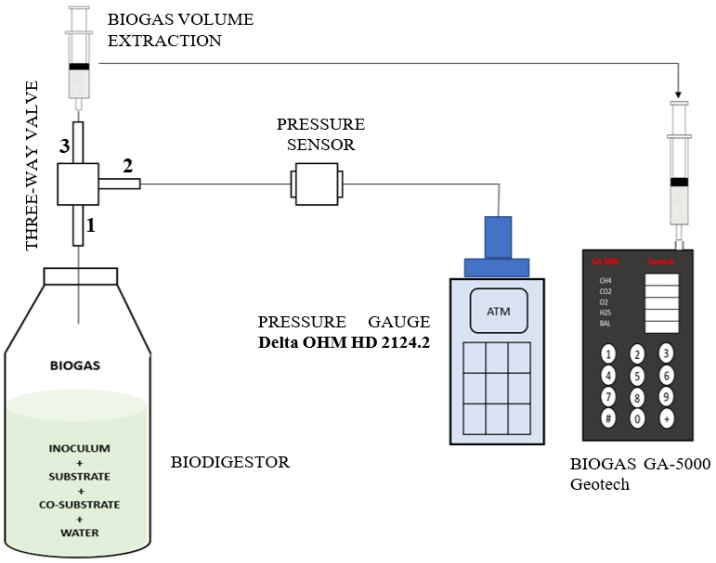

Figure 2. Manometric determination of the BMP of the co-digestion of slaughterhouse residues (RM) with lignocellulosic residues of agricultural origin.

2.5 Experimental modelling of the data to estimate the BMP.

Five kinetic models were selected, that is, the modified Gompertz kinetic model (**Equation (10)**), the transfer model (**Equation (11)**), the logistic function model (**Equation (12)**), the cone model (**Equation (13)**), and the modified Richards model (**Equation (14)**) to fit the cumulative methane production obtained from the experimental data.
The most suitable kinetic model was selected not only to predict the efficiency of the biodigesters used, but also to correctly analyse the metabolic pathways and the mechanisms involved during AD of the co-digestion of slaughterhouse waste with lignocellulosic waste [23]. However, all five kinetic models have individual specific benefits. The cone model is the simplest model and provides information on the degradation of substrates during the hydrolysis phase through the hydrolysis rate coefficient (k; d^{-1}) [24]. The modified Gompertz, logistic, transfer and Richards model are more sophisticated, since they take into account the phenomenon of the latency phase (t_{lag}; d) and the maximum specific methane production rate (ν_{max}) [25]. Therefore, the five kinetic models were used in this study to determine the cumulative biogas production potential, the hydrolysis kinetics, the lag phase duration, and the maximum methane production. All the parameters of the kinetic models were determined by fitting between the experimental and estimated data through the statistical tool STATISTISCA 10. To evaluate the performance of the models, the coefficient of determination (R^2) and the

percentage of squared error were used medium (RMSE; %). These coefficients were calculated to provide additional information on the goodness of fit of the different models. If the model accurately predicts the kinetic coefficient, R^2 should be close to 1 and the RMSE should be as close to 0.
Modified Gompertz model [26]:

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

Transfer model [27]:

$$M = M_e\left\{1 - exp\left[-\frac{\nu_{max}}{M_e}(t - t_{lag})\right]\right\} \quad (11)$$

Logistics function model [27]:

$$M = \frac{M_e}{1 + exp\left[\frac{4\nu_{max}(t_{lag} - t)}{M_e} + 2\right]} \quad (12)$$

Cone model [28]:

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

Modified Richard model [28]:

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

Where,
M is the amount of methane (ml/g VS_{added}) with respect to time t (days),
M_e is the maximum methane potential of the substrate (ml/g VS_{added}),
k is the hydrolysis rate constant (d^{-1}),
t is the digestion time (days),
ν_{max} is the maximum biogas production rate (ml/g VS_{added} .d),
t_{lag} is the time of the lag phase (days),
e is the Euler function equal to 2.7183.

3. Results

3.1 Characteristics of the raw material

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

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

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

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

The RM and TR residues were characterized by presenting the highest C/N contents, 101.9 and 29.6, respectively, while the QU (12,9) and AM residues showed a lower and similar C/N ratio. Thus, the high C/N ratio of the RM and TR residues could compensate for the low C/N ratios of the QU and AM residues through the co-digestion process. The mixture of different residues allows an optimal digestion process between the different substrates and co-substrates tested. On the other hand, having a fairly high C/N value as is the case of RM (101,9) does not significantly affect the efficiency of digestion [33], since not all the carbon and nitrogen in the matter raw are available for anaerobic digestion [29]. In this sense, the biodegradable C/N ratios are lower than the total C/N ratios of the substrates and co-substrates [34].
Even though the inoculum (IN) presented a low solids content (3.9% and 2.3% in TS and VS, respectively). The IN values were like those presented by Sun et al. [30], who reported TS, VS and VS/TS of 5.9%; 3.19% and 0.58, respectively. Similarly, IN results were comparable to those of Pellera and Gidarakos [15], who reported TS, VS and VS/TS of 2.7%; 1.7% and 0.62, respectively.

3.2 Potential methane production

Daily and cumulative methane production

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

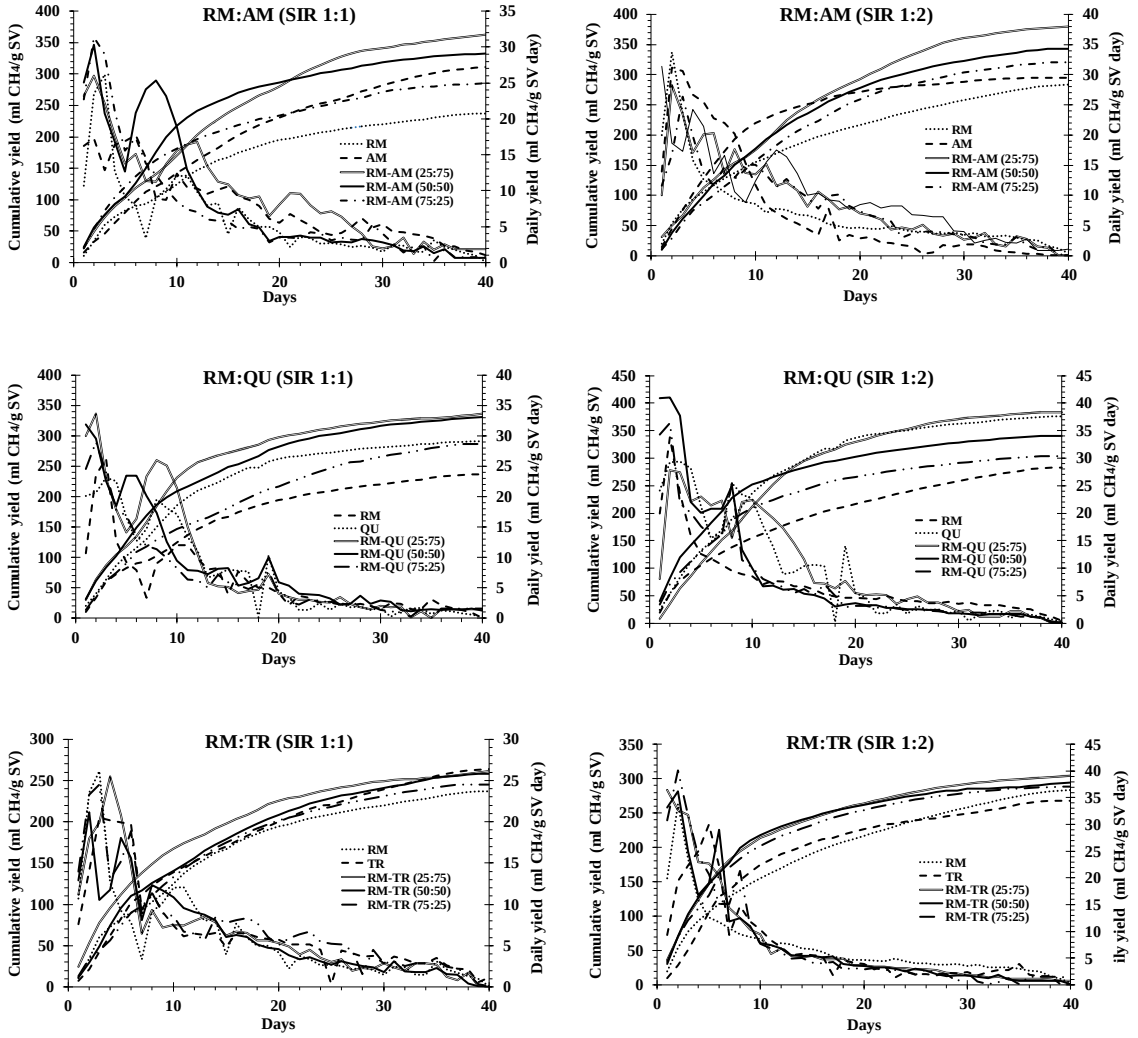

Figure 3. Daily and cumulative methane production for RM co-digestion for both SIR 1:1 and 1:2.

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

the RM-AM (25:75), RM-QU (50:50) mixtures experienced the highest methane peaks (34.46 ml CH_4/g VS and 41.11 ml CH_4/g VS) for a SIR1:1 and a SIR1:2, respectively.
The highest cumulative methane yields were found in trials using a SIR1:2, especially in the RM and QU mixtures. Thus, the mixtures RM-QU (25:75) and RM-QU (25:75) generated results of 406.86 and 391.45 ml CH_4/g VS, respectively. Similarly, the RM-AM mixture (25:75) generated high amounts of methane (379.38 ml CH_4/g VS). The percentages of improvement in methane production, when increasing the inoculum from a SIR1:1 to a SIR1:2, were 0.6-23%; however, the individual substrate of RM decreased by 5% with increasing inoculum. Co-digestion also enhanced methane production from individual RM substrates. For a SIR1:1 co-digestion increased methane production by 1-14%; and for a SIR1:2 production increased by 0.5-22%.
The results obtained in this study are similar to those of other authors in the literature [36-39], who carried out the co-digestion of RM with various crops (straw and fruit and vegetable waste) and obtained methane productions from 461, 499, 208 and 380 ml CH_4/g VS, respectively. Similarly, the RM yields are in the same line with the results obtained by Cuentos et al. [40], who obtained yields of 400 ml CH_4/g VS when they co-digested liquid waste from poultry slaughterhouses and solid urban waste. Furthermore, the RM results obtained are much higher than those obtained by Álvarez and Lidén [29], who reported that the co-digestion of pig slaughterhouse waste with pig manure produces specific methane yields of 260 ml CH_4/g VS. The results obtained were also greater than the results reported by Rosenwinkel and Meyer [41], who obtained 230 ml CH_4/g VS when they co-digested slaughterhouse waste (stomach content of pigs and cows) with sewage sludge. However, the results were somewhat lower than those reported by Luste and Luostarinen [4], who obtained results of 430 ml CH_4/g VS when they worked on the co-digestion of livestock waste (pig slaughterhouse) with sewage sludge.

Synergistic effects of agricultural co-substrates.

Agricultural residues from AM, QU and TR had a significant influence on methane production. The synergistic effects of agricultural residues are reflected in the improvement of the methane yield of the individual mixtures of the RM. It was shown that mixtures with a higher amount of agricultural residues increase methane yield regardless of the type of SIR used. However, the highest productions were obtained when 25% RM and 75% AM, QU and TR residues were used. Thus, for the SIR1:1 the mixtures RM-AM (25:75), RM-QU (25:75) and RM-TR (25:75) generated 363.17; 335.94 and 301.61 CH_4/g VS, respectively. Similarly, for a SIR1:2 the mixtures RM-AM (25:75), RM-QU (25:75) and RM-TR (25:75) generated 379.78; 406.86 and 303.71 CH_4/g VS, respectively (**Figure 3**).
The average methane content of the biogas produced in all the reactors varied between 54.31% and 68.74% for the SIR1:1 and between 54.42% and 76.55% for the SIR1:2. However, the increase in inoculum increased methane production in most of the biodigesters, except in the RM-AM (75:25), RM-AM (50:50) and RM-TR (75:25) mixtures in which decreased by 1.4; 0.46 and 0.54%. The percentages of methane obtained in this study were very similar to those reported by other authors in the literature. Thus, for example, Borowski [42] found methane content in biogas between 55% and 60% for the monodigestion of municipal solid waste and between 58% and 66% for the co-digestion of municipal solid waste and sewage sludge. Regarding fruit and vegetable residues, Bouallagui et al. [43] reported a methane content in biogas of 64%, while Scano et al. [44] reported average methane content of 75%. Lin et al. [45] reported percentages

of methane between 53.7% and 63.8% on the co-digestion of fruit and vegetable residues, and food waste.

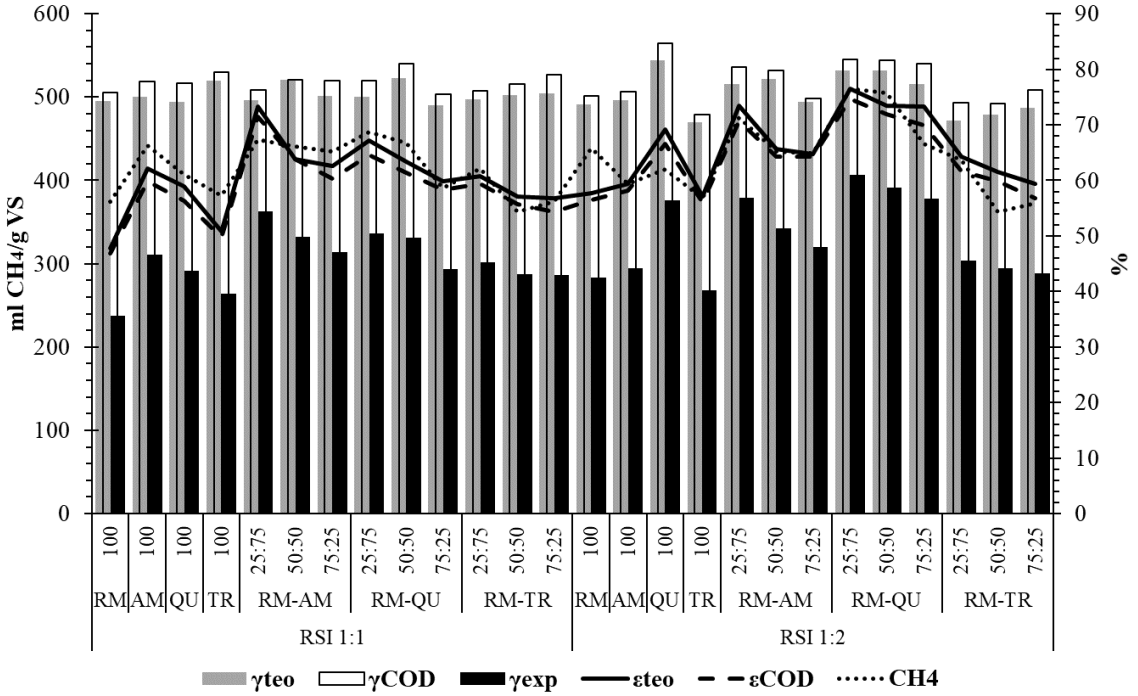

Figure 3. γ_{teo}: Theoretical maximum methane yield based on elementary analysis, **γ_{COD}**: Theoretical maximum methane yield based on CODt, **ε_{teo}**: biodegradability based on γ_{teo}, **ε_{COD}**: biodegradability based on CODt, CH_4: Percentage of methane from the biogas obtained.

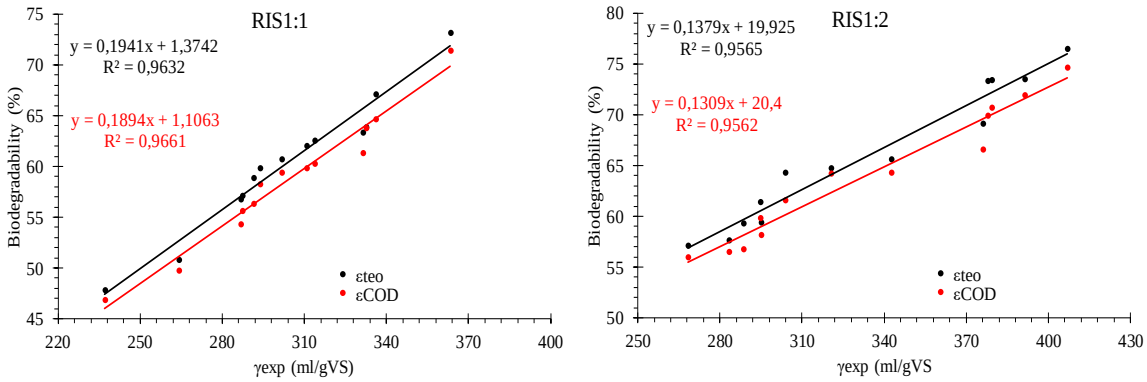

Figure 4. Effect of experimental performance γexp on biodegradability: εteo: biodegradability based on γteo, εCOD: biodegradability based on CODt.

In addition, **Figure 3** shows the biodegradability (ε_{teo} and ε_{COD}) for all the mixtures used. The results ranged from 46-73% for the SIR1:1 and between 56 and 77% for the SIR1:2. Thus, an increase in the amount of inoculum increased the biodegradability in a range of 0.20-18%. The data showed considerable concordance between ε_{teo} and ε_{COD}, showing that the theoretical methane production values obtained by Buswell's stoichiometric method (γt_{eo}) and elemental analysis of CODt (ε_{COD}) were similar (**Figure 4**).
Biodegradability values were correlated with experimental methane production. This agreement resulted in a coefficient of determination greater than 95% being obtained for both the SIR1:1 and the SIR1:2.

3.3 Kinetic study of the anaerobic digestion of slaughterhouse waste

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

Maximum specified rate of methane production

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

Specific Maximum Methane Production

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

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

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

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

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

Delay phase time

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

First order constant

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

4. Discussion

In this research, the daily methane production remained constant during the first three days, subsequently it decreased continuously and remained at very low levels. The early onset of microbial activity caused the mixtures to generate more than 70% methane during the first 10 days. Zhang et al. [56] consider that around 80% of the methane can be obtained during the first ten days of digestion. Furthermore, many authors in the literature suggest that some of the BMP trials require short treatment periods [57]. A possible reason why a high generation of methane has been obtained during the first days is because the inoculum and the methanogenic microorganisms immediately acclimatized to the mixtures used in the tests [58,59]. The methane accumulation curves also reflected a rapid adaptation of the microorganisms, since it caused very small and even zero lag periods (tlag) to be shown. In general, the accumulation curves showed a rapid exponential growth during the start of digestion. According to Remigi and Buckley [60], the rapid growth of the methane accumulation curves is due to three factors: use of easily biodegradable materials, immediate production of methane when starting the AD process, and the presence of a stationary phase as the biodegradable material is depleted.
The use of straw residues from amaranth, quinoa and wheat increased methane production from slaughterhouse residues. According to Vivekanand et al. [61]a mixture has a synergistic effect if more methane is produced relative to an estimate based on methane yields from single substrate digestions. In this case, the simultaneous presence of RMs with various co-substrates (AM, QU and TR) improved the co-digestion process, due to the synergistic interactions of the mixtures [62]. In this way, a mixture of different substrate fractions with different characteristics can provide all the nutrients and trace elements that microorganisms need [37]. This fact is justified, since the catalytic centers of the enzymes involved in the methanogenic pathways depend to a great extent on the

micronutrients [63]. In addition, the synergistic effects of mixtures can contribute trace elements, nutrients, enzymes, or any other amendment that a substrate alone may lack [64]. In short, the mixture of many heterogeneous substrates increases the activity of microorganisms and, therefore, stimulates AD. In this study, the most relevant findings were the following: a higher concentration of VS of the co-substrates (AM, QU and TR) in the mixtures caused the production of methane to increase up to 22% in the individual mixtures of the RM; in addition, the co-digestion of the RM-QU and RM-AM mixtures generated the highest methane productions regardless of their SIR, and finally, the concentrations of 50-75% of AM and QU were optimal to improve methane production.
In the characterization of the raw materials, the VS of the slaughterhouse RM were 6.8 while the VS of the straw waste of AM, QU and TR were higher with 66%, 51% and 72% respectively. In this case, the use of agricultural residues helped to balance the physicochemical properties of the RM by improving the biodegradability of the VS of the mixtures [65-67]. In this way, the addition of agricultural residues provided a better substrate for methanogenic bacteria, causing them to accelerate the fermentation process and increase methane production [68,69].
For a SIR1:2, the co-digestion of the RM-QU and RM-AM mixtures generated the highest amount of methane with ranges of 378-407 and 320-380 ml/g VS, respectively. However, the RM-QU (25:75) mixtures generated 7% more than the RM-AM (25:75) mixtures. Similarly, the RM-QU (50:50) mixtures generated 13% more than the RM-AM (50:50) mixtures. These results were very similar to other studies in the scientific literature. Thus, in the co-digestion of urban solid waste, Mojapelo et al. [70] and Kubaska et al. [71] reported 386 ml/g VS and 385 ml/g VS, respectively. Salminen et al. [72], by fermenting solid waste from poultry slaughterhouses, they obtained 550 to 670 ml/g VS. Li, et al. [73], presented yields of 300 ml/g VS for the AD of lignocellulosic biomass of agricultural residues. Similarly, Mussgnug et al. [74], reported methane productions for the anaerobic digestion of 6 different microalgae between 218 and 387 ml/g VS. Although the reported results were comparable with other previous studies, the methane yields were of medium production. According to Velázquez et al. [75] digestion processes can be classified into three groups according to methane production potential: low production processes (150 and 300 ml/g VS), medium production processes (300 and 450 ml/g VS) and processes high production (more than 450 ml/g VS).
According to Raposo et al. [76] the experimental methane yield can be used to calculate the level of anaerobic biodegradability under the defined test conditions compared to its theoretical value. In this study, theoretical calculations provided a rough first estimate of methane production. However, it was found that the theoretical yield was much higher than the experimental one. According to Herrmann and Rath [77],the theoretical estimates are usually much higher than the experimental yield because in the theoretical analysis all biomass is biodegradable. On the other hand, in obtaining experimental methane, the suitability of fermentation decreases with the lignification of the substrate, since lignin is not degraded in the fermenter and makes the degradation of other components of the cell wall difficult [78]. Furthermore, in experimental trials there is a wide variety of substances that can inhibit anaerobic processes [79]. In short, the conversion of organic substances into methane, in the experimental tests, is lower than in the theoretical estimates since the ideal conditions cannot be met [80]. The tests of this research showed that the data for obtaining biodegradability are adequate, since the results of biodegradability and experimental performance showed a concordance of more than 95% in their coefficient of determination (R^2) (**Figure 4**). This concordance between biodegradability and experimental performance was superior to the tests performed by Labatut et al. [64] on digestion of complex substrates.

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

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

Conclusions

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

values was the cone model with an R^2 of 0.982 to 0.999 and RMSE of 0.61 to 6.92 CH_4/g VS.

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Chapter IV

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

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ABSTRACT

The objective of this research was to evaluate the anaerobic co-digestion of guinea pig manure (GP) with Andean agricultural residues such as amaranth (AM), quinoa (QU) and wheat (TR) in batch biodigesters under mesophilic conditions (37ºC) for 40 days. As microbial inoculum, sewage treatment sludge was used in two inoculum/substrate ratios (ISR of 1 and 2). In terms of methane production, the best results occurred in treatments containing AM and QU as co-substrate and an ISR of 2. Thus, the highest methane production occurred in the GP:AM biodigesters (25:75) and GP:QU (25:75) with 341.86 mlCH4/g VS added and 341.05 mlCH4/g VS added, respectively. On the other hand, the results showed that methane production with an ISR of 2 generated higher yields for guinea pig waste, where the methane fraction of the biogas generated was in a range of 57% and 69%. Methane production kinetics from these raw materials was studied using five kinetic models: modified Gompertz, logistic equation, transfer, cone, and Richards. The cone model adjusted best to the experimental values with those observed with an R^2 of 0.999 and an RMSE of 1.16 mlCH4/g VS added. Finally, the highest biodegradability (experimental yield/theoretical yield) was obtained in the GP-AM biodigesters (25:75) with 67.92%.

Keywords: anaerobic digestion; lignocellulosic waste; biogas; co-substrate; synergy; inoculum; kinetic model.

1. Introduction

This work has been carried out in order to analyse applicable technologies in Andean areas of South America where the conventional energy supply is deficient, both in electricity and gas, often non-existent [1]. 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 [2]. Optimizing performance techniques are necessary under the conditions of economic, social and environmental sustainability, since it has to be integrated into a traditional way of life being socially accepted by users [3]. Increasing access to "*technified*" rural energy is essential to counteract the problems of these depressed areas and offer development possibilities [4]. In the same way, deforestation and the reduction of greenhouse gas emissions would be avoided [5,6].

Most of the Andean communities base their economy mainly on self-sufficient agriculture and family farming [7-9]. Their agricultural activities from agropastoral nature and are developed in semi-arid areas at high altitude where there is a great variety of microclimatic areas as well as ecosystems [10]. In the higher areas, the raising of guinea pigs (*Cavia porcellus*) constitutes one of the main agricultural activities. The guinea pig (GP) is one of the most common animals in rural communities in the Andes [11,12]. They are found in Peru, Ecuador, Bolivia and Colombia, having been domesticated between 2,500 and 3,600 years ago [13,14]. In this respect the production and use of guinea pigs represents significant interest for the sustainability of the area, associated with its traditional and ethnic/regional character [10,15]. At present, GP manure has been little explored in terms of energy purposes, undervaluing these resources [11,16]. Bioenergy conversion of this waste is of special interest in this scenario. One way to address the energy needs of the Andean communities is through the production of biogas agricultural and livestock waste made possible by anaerobic digestion (AD).

The application of anaerobic digestion to guinea pig manures has been little studied. However, Garfí et al. [11] 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 GP's high manure nutrient-content (P-P_2O_5, K-K_2O, N-NH_4), functions as potential waste with multiple benefits, especially in biogas and organic fertilizer production [17]. 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) [18]. 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 GP manure into biogas [19].

In the literature there is little information on the use of guinea pig manure as a raw material for biogas production. Garfí et al. [11] investigated the digestion of GP manure, to produce biogas, under psychrophilic conditions and with continuous digesters at high altitude. Additionally, GP manure co-digestion with cow manure was analysed with no additional inoculum in tubular digesters thus assessing the effects of high-altitude temperature.

The work presented here expands on Garfí's work comparing simple anaerobic digestion GP manure processes to guinea pig anaerobic digestion with inoculum from sewage sludge. Also, guinea pig co-digestion with lignocellulosic materials typical from Andean agriculture, accessible in these rural areas, such as quinoa straw (QU), wheat (TR) and amaranth (AM). Thus, the high carbon content residues from crops and the rich nitrogen content of animal manure make for an optimal and balanced C/N ratio [20]. In the same way, the use of an inoculum in AD can have an effect on the speed of the process [21-23]

affecting not only biodegradability but also the CH_4 production rate [24,25]. Therefore, it is necessary to investigate GP manure digestion performance with other co-substrates and inoculum to observe the effects on biogas production synergy. Eventually, it is intended to investigate the effects of the substrate/inoculum ratio to improve the anaerobic co-digestion system with lignocellulosic materials.

2. Materials and methods

2.1 Substrates and inoculum

In this study, the GP manure, collected from the farms of the Bolívar State University, was analysed in co-digestion with three co-substrates: AM, QU and TR straw residues. As soon as the samples were collected, they were stored at 4°C in polyethylene bags, for conservation purposes. Before co-digestion, 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 and mixed with a kitchen blender to ensure that the experimental samples were 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 five 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 [26] 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) (**Figure 1**). First, a 100-bar pressure sensor (Delta TP 704) was used, which remained connected to the portable pressure gauge. The measurement process consisted of setting up a system, in which three devices were connected: the biodigester, the portable pressure gauge and a syringe for the extraction of the biogas. This connection system was set up with a three-way valve and simultaneously. At the beginning of each extraction, the pressure generated in the head space of each biodigester was measured. Biogas extraction was completed when the pressure inside the biodigester equaled atmospheric pressure. Next, biogas volume of each biodigester was calculated through

(**Equation 1**). Finally, the cumulative biogas and methane yields (ml/g VS added) 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 [27,28]. The volume of biogas was measured daily after shaking the biodigesters.

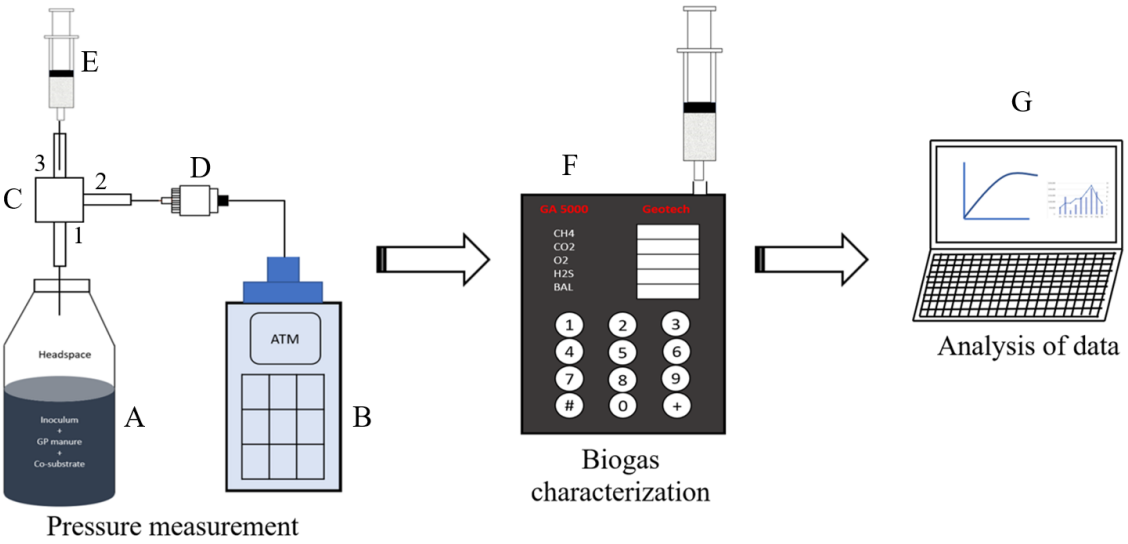

Figure 1. Obtaining and characterizing biogas. A (biodigester), B (Delta OHM HD 2124.2 portable pressure gauge), C (Three-way valve), D (Delta TP 704 100 bar pressure sensor), E (200 ml syringe), F (GA-5000 BIOGAS meter from Geotech) and G (computer to process the data).

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

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 (CH_4, O_2, CO_2, H_2S 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

Total solids (TS) and volatile solids (VS) residues were measured in triplicate according to UNE-EN 18134 and UNE-EN ISO 18123 standards. While TS and VS inoculum content was determined according to 2540A-2540G the American Public Health Association methods [29]. A portable digital multi-meter potentiometer (HACH HQ 40D) was used to

obtain biodigesters pH samples. Elemental analysis (C, H, N, O and S) was performed using 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 (CODt), 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 calculated from the amount of material and the CODt concentration, assuming its validity for any type of substrate [30,31].

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

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

The value of n_{CH4} has been determined from the CODt (**Equation 3**) [32]. CODt for methane is 64 g of oxygen per methane mole while 1 mole of methane per 64 CODt grams is, therefore, the maximum amount of methane that can be obtained if all CODt is converted to methane [33].

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

The CODt of all substrates and co-substrates was estimated through their elemental composition and the stoichiometry oxidation reaction (**Equation 4**), using the equation (Equation 5) [34]. The calculation of the CODt based on the atomic composition provides an attractive and easy alternative to obtain organic resistance of some solid substrates [25].

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

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

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

Another way to determine the theoretical yield (γ_{teo}) is through the reaction of **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 [34-36].

$$C_aH_bO_cN_d + \left(\frac{4a\text{-}b\text{-}2c + 3d + 2e}{4}\right)H_2O \rightarrow \left(\frac{4a + b\text{-}2c\text{-}3d\text{-}2c}{8}\right)CH_4 + \left(\frac{4a + b + 2c + 3d + 2e}{8}\right)CO_2 + dNH_3 + eH_2S \quad (6)$$

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

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** [37].

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

Mixing a substrate with one or more substrates, through co-digestion, causes three types of internal component reactions: methane greater production (synergistic effects), less methane production (antagonistic effects) or simply neither an increase nor a decrease production in terms of a substrate or co-substrate individual production (independence of waste from the co-digestion). To evaluate the synergy, antagonism and independence that occurs in the biodegradation process, **Equation 9** was used [38].

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

γ_{exp} refers to the experimental performance obtained by the BMP. γ_{pond} corresponds to the weighted average yield using **Equation 10** [39]. 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.

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

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 substrates kinetics biodegradation processes. Thus, experimental performance, digestion time and biodegradation kinetics can help predict methane production from a specific substrate [40]. In this work, co-digested mixtures methane potential was predicted using five mathematical models applied to BMP experimental tests. The following models were used: modified Gompertz (**Equation 11**) [41-44] transfer model (**Equation 12**) [45,46] logistic equation (**Equation 13**) [46-48] cone models (**Equation 14**) [43,49,50]. and modified Richards model (**Equation 15**) [48,49].

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

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

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

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

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

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

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 statistical calculations such as the determination of the kinetic parameters were carried out with the STATISTICA 10 package.

3. Results

3.1 Characterization of the physicochemical properties of the raw material

Analysis results of the substrate, co-substrate and inoculum physicochemical characteristics are presented in Table 1. GP manure TS and VS content were 33.9% and 24.6%, respectively. These results were lower than TS and VS content compared to other studies reported in the literature, which varied between 68.51% and 27.82%, respectively [51]. Variations in the composition of TS and VS can be attributed to possible changes in nutrition and age of the animals, as well as changes in manure management, storage conditions, and sampling time [52].

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. [53] who obtained TS, VS and VS/TS values of 74.1%; 62.9% and 0.84, respectively. The co-substrate of AM presented similar VS characteristics (88.2%), TS (65.9%) and VS/TS (0.75) to those of TR. Furthermore, the AM results were superior to those obtained by Seppälä et al. [54], who reported TS and VS values of 18.0% and 14.4%, respectively. Finally, QU co-substrate presented a high TS value (87.0%) and low VS values (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 [55], who obtained values of 95.3%; 91.9% and 0.88, respectively.

The high VS content indicated that the raw materials contained a large amount of organic matter. The VS/TS ratio of the substrates and co-substrates ranged from 0.58-0.57, which indicated that the raw materials are potential energy waste [56]. Similarly, the C/N ratio of GP manure was very similar to animal manure values previously analysed in other studies (5-30) [57,58]. However, the AM and QU co-substrates had a lower C/N ratio than most lignocellulosic residues, which is usually greater than 50 [59]. This indicates that these co-substrates need to be investigated to clarify their true energy potential.

Finally, the inoculum (IN) had TS of 3.9%; VS of 2.3% and a VS/TS ratio of 0.59. The IN values were similar to those used by Sun et al. [53], who reported TS, VS and VS/TS of 5.9%; 3.19% and 0.58. Likewise, the IN results were comparable to those of Pellera and Gidarakos [34], reporting 2.7%; 1.7% and 0.62 TS, VS and VS/TS, respectively

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

Parameters	Units	Substrates	Co-substrates			Inoculum
		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)

3.2 Effects of inoculum on biogas production

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

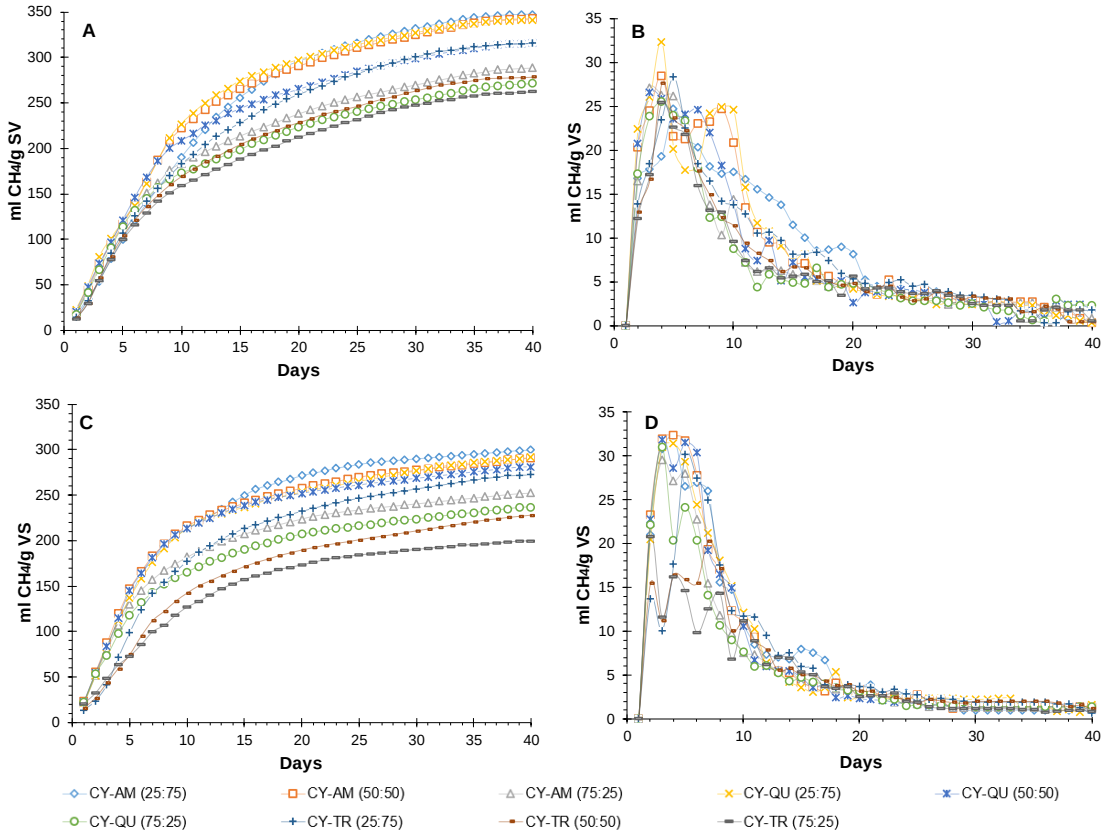

Figure 2. Daily and cumulative profiles of CH_4 production as a function of time, for trials with different IRS.

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

Maximum accumulated methane production was obtained after 960h of digestion (40 days) as daily methane productions were 1% of the total accumulated production [60]. The results showed that an increase in the amount of inoculum contributed to the samples increasing their methane production. When comparing ISR of 1 methane production with ISR of 2, trials showed significant differences (P> 0.05) according to the Tukey test; except for the GP-QU (50:50) mixture that did not present significant figures (P <0.05). A considerable improvement from 9 to 31%. in ISR of 2 methane rates took place compared to ISR of 1. These results suggested that a high ISR>1 ratio favour methane production in manure co-digestion of GP 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 [61]. 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 produced the highest methane rate were those with the highest concentration of co-substrate (AM, QU and TR). Thus, all tests with 75% co-substrate significantly improved compared to those mixtures containing 25 and 50% co-substrate. The highest amount of methane was obtained in GP-AM (25:75), GP-AM (50:50), GP-QU (25:75), GP-QU (50:50) and GP-TR (25:75) mixtures: 341.86; 333.91; 341.05; 315.24 and 315.92 ml/g VS, respectively. However, results revealed that when using 75% or 50% of co-substrate in the mixtures, methane production rates did not present significant figures (P<0.05). In addition, it was proved that by increasing the amount of co-substrate from 25% to 75%, mixtures increased methane production between 20 and 26%. Likewise, when the amount of co-substrate increased from 25 to 50%, methane production in the biodigesters rose between 16 and 20%. It was also found that all lignocellulosic residues were valuable substrates optimizing GP manure digestion. Such tests are justified since all were performed with 50 and 75% co-substrate concentration and did not present significant figures (P<0.05) in methane generation.

Anaerobic co-digestion synergistic effects

In **Figure 3**, methane production results, mono-digestion synergy, CY co-digestion manure residues, including AM, QU and TR agricultural residues are presented. Mono-digestion data incorporated in this article has already been calculated in another article [62] in which the same methodology of this research has been followed. This allows the individual performance of GP manure to be compared with the performance of substrate and co-substrate mixtures.

The value of the synergistic effect (α) in the mono-digestion of the manure of GP 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 GP (ISR of 1) and GP (ISR of 2) were 211.07 and 174.27 ml/g VS added, respectively. Co-digestion mixtures improved mono-digestion methane production regardless of the ISR used, increasing from 8 to 42% in ISR of 1 and between 50 and 96% in ISR of 2. All comparisons between mono-digestion and co-digestion data showed significant differences (P>0.05), except for ISR of 1 GP-TR (25:75) mixture that did not show significant differences (P<0.05).

Figure 3 shows that α values of the ISR of 1, for GP-AM (75:25), GP-QU (75:25), GP-TR (50:50) and GP-TR (75:25) mixtures ranged from 0.957 to 0.988, suggesting that the co-digestion of these mixtures is independent on the substrate and co-substrates since the

value of α was close to 1. However, GP-AM and GP-QU mixtures containing of 50 and 75% co-substrate, had synergistic effects (α>1) with values between 1.11 and 1.17. In the ISR of 2, the synergistic effects were much more promising since their values ranged between 1.13 and 1.50; except for the GP-QU (25:75) mixture which α was 0.975. In any event, in the last mixture there were no synergistic effects, nor were completely antagonistic effects, since α was close to 1.

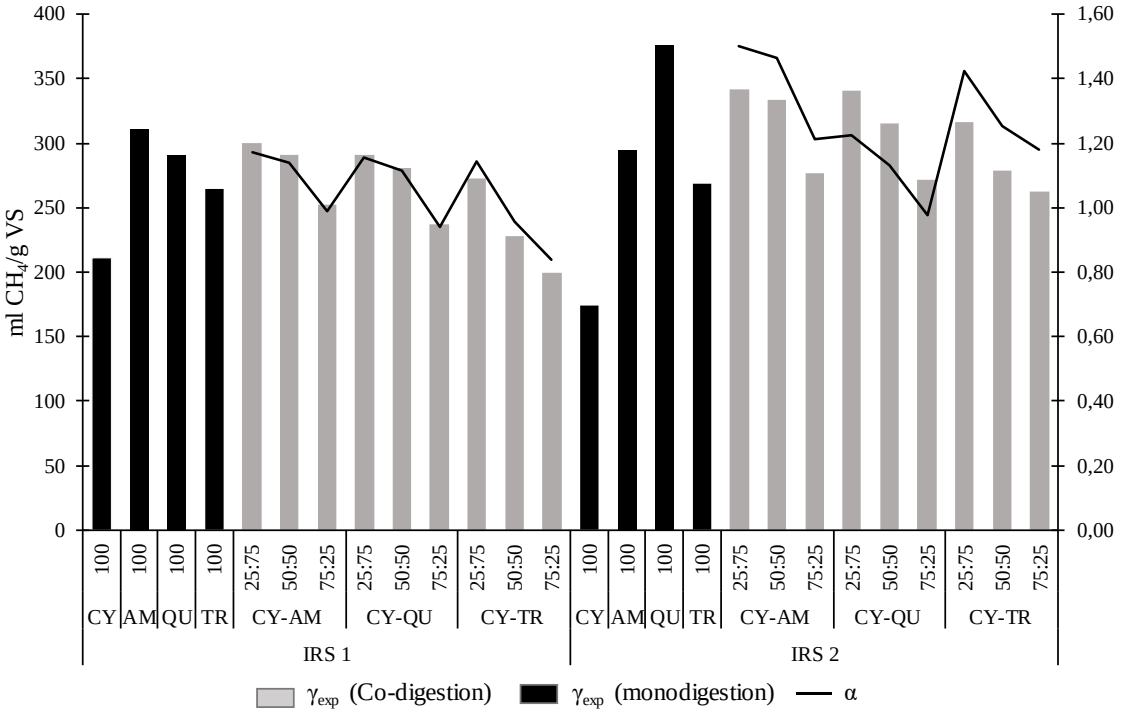

Figure 3. Synergy index of the co-digestion of guinea pig manure with different lignocellulosic co-substrates. α>1 indicates synergistic effect and α<1 indicates antagonistic effect.
(Source: Adapted from Meneses et al. [62]).

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

3.4 Biogas composition of CY waste

In **Figure 4 and Table 2**, biogas composition of the different combinations between co-substrates and inoculum is shown. Results showed CH_4 and H_2S percentages increased by the rising of inoculum. On the contrary, CO_2 production decreased as the amount of inoculum increased. GP-QU composed mixtures generated the highest percentage of methane regardless of ISR used. In the trials with ISR of 2, the mixtures formed by GP-QU experienced a rise of 2.26-4.52% compared to GP-AM combinations and improvements of 2.68-5.68% compared GP-TR structured biodigesters. In the same way, the biodigesters formed by GP-QU of the ISR of 1, generated higher percentages of CH_4. The difference was 9.89-10.58% and 12-84-14.59% with respect to GP-AM and GP-TR biodigesters, respectively.
In this study, CO_2 was between 29-42%, H_2S was almost negligible with percentages of 0.40-1.70%. On the contrary, CH_4 average percentage was 57-69%. The results obtained

were similar to those of other investigations in the literature. Thus, for example, Garfí et al. [11] in a study on anaerobic digestion guinea pig manure, obtained values 63-65% for CH_4, and 0.19% for H_2S. Similarly, in another study on guinea pig manure, Garfí et al. [11] obtained 59% and 0.15% for CH_4 and H_2S, respectively. Also, Ferrer. [63], recorded values of 60% of the methane fraction in previous bio-methanization studies.

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

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

(Source: Adapted from Meneses et al. [62]).

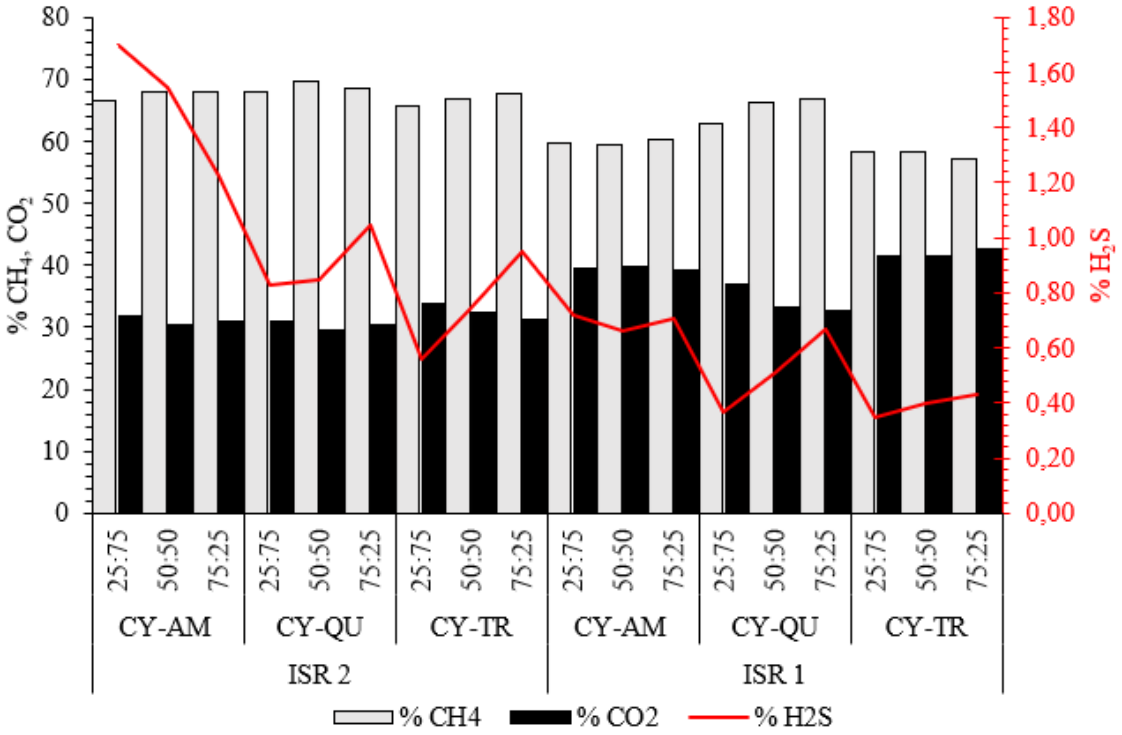

Figure 4. Percentages of CH_4, CO_2 and H_2S from CY manure biogas.

3.5 Kinetic study

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

When analysing the ν_{max} parameter, the modified Gompertz models and the logistic equation correlate the most and have the most similarity, since their ν_{max} values are between 11.98-20.80 $mlCH_4/g$ VS d and between 10.69-19.52 $mlCH_4/g$ VS d, respectively. On the other hand, the methanogenic activity occurred at a faster rate in the transfer model, since, for this model, ν_{max} ranged between 21.86 and 31.31 $mlCH_4/g$ VS d. The model that differs the most from the rest is the Richards model, where the range of ν_{max} was between 2.13 and 13.76 $mlCH_4/g$ VS d. In contrast to other investigations, ν_{max} values in this study are lower than those reported for food residues (28.03 to 174.63 $mlCH_4/g$ VS d) [64] and those reported for manure chicken (19.4 to 48.9 $mlCH_4/g$ VS d) [65]. However, ν_{max} results of this study are similar to those reported for corn stubble (16.3 to 32.1 $mlCH_4/g$ VS d) [65] and higher than pig manure co-digestion with sewage sludge (4.8–14.0 $mlCH_4/g$ VS d) [66].

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 [48], who obtained differences of 0.54 and 27.07%.
Regarding specific experimental methane yield, ISR of 2 results best fits M_e. kinetic parameter. Thus, the mean difference between the observed and predicted values is 0.16-5.53% approximately. (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 digestion process variables and estimating anaerobic dgestion yield and kinetic parameters. On the other hand, in the cone model, the differences between the predicted and observed values were overestimated since they ranged between 5.85-18.95%. The fact that there are discrepancies in the mean differences between the experimental and predicted performance is due to the types of kinetic models used, raw material, conditions used and digestion of more complex residues (co-digestion). However, the average variation obtained between specific performance and M_e were in line with those from Ware and Power [48] 0.54 and 27.07%.
Regarding the latency period (t_{lag}), many of the digesters experienced very short periods, even 0 days, indicating organic compound high bioavailability within substrates [48]. In this context, CY co-digestion experienced zero periods in the latency phase, except for CY-AM digesters (25:75), whose maximum periods were approximately 0.41 days (modified Gompertz); 0.98 days (transfer); 0.71 days (logistics) and 0.42 days (Richards). The fact that there are low latency periods in these trials indicates that there was a rapid microorganism response in their adaptation process to the experiment environment conditions. Furthermore, low t_{lag} values demonstrated the simple nature of 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 [67] and 12.3 days [68], 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 5**, the kinetic model with the highest correlation coefficient R^2 (0.992-0.999) and the lowest RMSE (1.37-10.04 $mlCH_4/g$ VS) is the transfer model. Similarly, the cone model fits the data quite well with R^2 (0.978-0.999) and (1.16-8.85 $mlCH_4/g$ VS) RMSE. While the logistic equation model best adjusts to the values observed with the models, since the value of R^2 and the RMSE range between 0.974-0.997 and 3.58-14.30 $mlCH_4/g$ VS, respectively. Subsequently, modified Gompertz and Richards models are very similar. In the modified Gompertz model the correlation coefficient is in an interval of (0.965 0.999) and the RMSE in an interval of (2.16-11.31 $mlCH_4/g$ VS); while in the Richards model R^2 is between (0.982-0.999) and RMSE between (2.16-11.33 $mlCH_4/g$ VS). The similarity between these models is 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) [69] had a higher RMSE as the sigmoidal growth of curves was described.
Although, the transfer model did not show total convergence between the observed and predicted values when the non-linear regression was performed. The fact that there was no convergence for the entire duration of co-digestion meant that there were no predicted values in the biodigesters tested. In this sense, this model did not provide the necessary information for the correct evaluation nor evaluation of data.
It should be noted that the suitability and precision of models always vary considerably depending on the experimental conditions, operating parameters, as well as inoculum

origin and type of substrate used [70]. In this study, out of every proposed model, the cone model best adjusted to the real evolution of methane production. Similarly, El-Mashad [71] demonstrated that the cone model provides a more realistic experimental methane yields simulation. It is fascinating that the cone model surpasses methane production expectations since many studies have traditionally considered the Gompertz model to be the most suitable [72-74]. On the contrary, other authors [49] have considered that the cone model does not adequately produce methane production. Despite low credibility in the cone model, its high precision may be due to may authors unfamiliarity with this model [70].

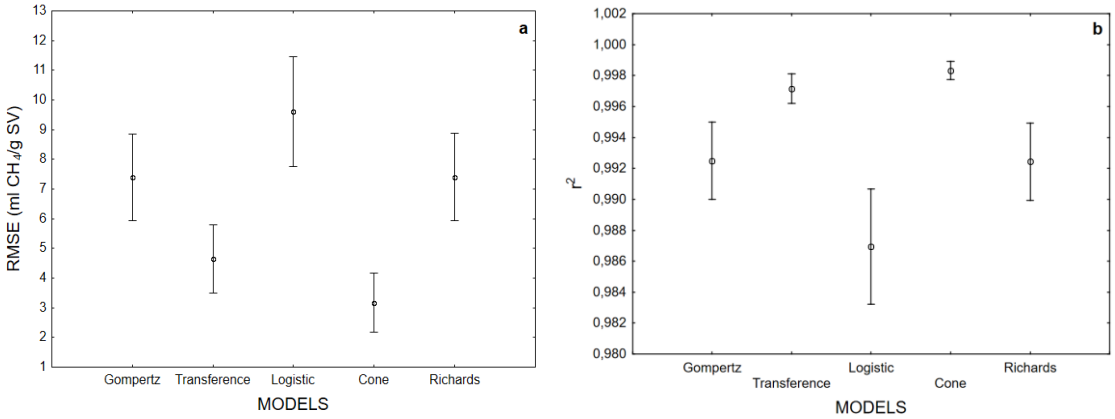

Figure 5. LSD (Least significant difference) intervals of the analysis of variance at the 95% confidence level for the comparison of the RMSE, the R^2 of the different models applied to the co-digestion of CY manure.

Table 3. 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
	ν_{max}	17,13	19,09	13,67	20,8	17,02	13,02	14,86	13,75	11,98	13,27	13,63	10,62	14,67	13,01	8,85	11,02	9,12	7,3
	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
	ν_{max}	25,95	*	25,86	*	31,31	24,74	25,45	24,69	21,86	24,94	26,51	20,63	28,2	24,36	17,38	19,14	15,87	12,96
	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
	ν_{max}	16,83	17,66	12,01	19,52	15,2	11,4	13,7	12,37	10,69	11,79	11,92	9,27	13	11,67	7,66	10,12	8,38	6,68
	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
	ν_{max}	12,14	6,78	2,13	10,75	7,22	4,63	6,72	6,1	1,07	5,98	3,23	0,56	3,7	-35,95	3,95	4,48	3,35	3,91
	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.

4. Discussion

4.1 Effect of ISR on biomethane potential and biodigester stability

In the current study, results showed that methane yields increased at a higher ISR are equivalent to previous studies in which different substrates have been used [20,75-78]. Apparently, an optimal ISR in a biodigester should contain a balanced amount of anaerobic microorganisms for primary and intermediate product digestion [78]. Furthermore, an adequate inoculum can increase degradation rate, improve biogas production, shorten the start-up time, and make the digestion process more stable [79]. However, determining ISR optimal values is not an easy step, especially when the substrates and co-substrates used are relatively unknown. Raposo et al. [80] in a BMP test of corn waste used an ISR range of 3, 2, 1.5, and 1. They concluded that their results presented a slight variation in higher proportions. Caillet et al. [81] in a sugarcane distillery wastewater bio-methanization test determined that ISR of 1 methane production rate was faster and higher than in 2; 2.6 and 3.9 proportions. The use of a high or low ISR can be decisive in BMP tests. While a very high ISR will primarily challenge the experimental setup due to relatively substrate low gas production [81], a low ISR could cause an overload in microbial community, as it has already been shown in previously studies [82,83]. From the literature consulted, it can be concluded that methane production rates are specific to the substrate and the inoculum, so it is not always possible to generalize digestion performance.

In this study, the use of an ISR of 2 notably improved the biodegradability of the materials compared to an ISR of 1. These results are consistent with other solid waste studies, such as those of Zhou et al. [84] and Boulanger et al. [85] , who found that ISR ratios less than or equal to 0.5, negatively affect the anaerobic process for the conditions of this study. This phenomenon is associated with the inhibition of anaerobic microbial consortia due to the accumulation of VGA, since it has been shown that at ISR ratios lower than 0.25, the biodegradability of the substrates begins to decline [86]. Another probable cause of the effect of the ISR ratio on GP manure is hydrolysis; according to Bouallagui et al. [87] there is a direct relationship between soluble organic matter (SOM) and hydrolysis, since the higher the SOM content, the times for the formation of fundamental substrates in anaerobic digestion are reduced and the production of methane increases. In this study, the increase in the ISR ratio implied an increase in the particulate organic matter present in the substrate.

Despite all this, more trials are needed with different ISR ratios (greater than 2 and less than 1) to fully evaluate the influence of the inoculum; especially since the co-digestion tests were carried out from easily degradable material (GP 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

Generally, CY manure co-digestion with lignocellulosic residues repeatedly increased methane production. However, throughout the world traditionally animal manure has been used as a mono-substrate in most bio-methanization tests, co-digestion processes were dynamic [88]. 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 [89,90]. Results in this study were very similar to those of other authors and corroborated previous studies (**Table 4**). Unequalled

results ranged from 300-340 CH_4/g VS added, corresponding to average methane production. According to Velázquez et al. [91] the low methane productions range between 150 and 300 CH_4/g VS added, the average productions between 300 and 450 CH_4/g VS added and the high productions are higher than 450 CH_4/g VS added.

GP manure mono-digestion production was low (around 170-211 CH_4/g VS added) compared to previous studies of cow, pig, and poultry manure that ranged from 238, 271, and 328 ml/g VS added, respectively [92].The low production of methane can be attributed to the quality and management techniques of the organic matter in manure [63]. In rural Andean areas, harsh climatic conditions and frost-tolerant forages result in unconventional animal diets compared to other climates and conditions [93]. The type of animal diet has an effect on GP manure as protein and lipid content may be low therefore, the amount of digesting material increases [94].

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 added). Low efficiencies can be attributed to a higher content of lignin or other recalcitrant carbon in the composition of the biodigester [95]. Ma et al. [96] 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 [97]. Determining the appropriate ratio between substrate and co-substrate is essential to optimize the co-digestion processes [56]; above all because co-substrates volume in co-digestions, vary greatly between different studies [98,99].

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. Methane synergy biochemical potential are directly related to substrate composition [100]. 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 [39]. Additionally, the presence of antagonistic effects in some biodigesters (GP-TR (75:25; GP-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 [101]. Finally, in biodigesters that present antagonism, binary mixtures have not been able to provide all the nutrients and trace elements necessary to that microorganisms have a higher methanogenic activity [102].

The C/N ratio of the GP substrate was 15.3 and that of the AM and QU co-substrates was 12.9 and 12, respectively. According to Li et al. [103] a C/N ratio of 20-30 is optimal for anaerobic digestion. A high C/N ratio would reduce the biodegradation rate, while a low C/N ratio would tend to produce excess ammonia and VFA, which can cause inhibition in anaerobic digestion [104]. However, in this study, a C/N ratio of 12-15 did not influence methane production. In fact, the best results were obtained for the mixtures of GP-AM and GP-QU. Lin et al. [105] used low C/N ratios of 10-20 and obtained good results, they attributed the high methane production to the biodegradability of carbon. Romano and Zhang [106] recommended that the C/N ratio be kept at 15 for the co-digestion of onion juice and digested sludge. Zhu et al. [107] inoculated corn stubble with digested sewage sludge, obtaining excellent results with a C/N of 15 to 18. In another study, Jeung et al. [56], demonstrated that the optimal C/N ratio for sludge-based anaerobic co-digestion is approximately 15-20. In this sense, the optimal C/N ratio varies

with the type of raw material to be digested. In addition, the good results of the present study may be due to the fact that the properties of proteins, sugars, fats and fibre of amaranth are very similar to those of corn, which is an excellent substrate for producing biogas [108].

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 [109].

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	[110]
RS: KW: GW	57:29:14	303,4	[111]
DM:CS:TO	54:33:13	415,4	[112]
PD:WS	70:30	330,1	[113]
PD:MG	50:50	340,1	[113]
ESBP: PM	25:75	212,4	[114]
BS:TPM	50:50	152,3	[20]
BS:CM	50:50	120-125	[20]
GP-AM	25:75	341,9	Data from this study
GP-QU	25:75	341,1	Data from this study
GP-TR	25:75	315,9	Data from this study

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).

5. 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 CH_4/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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GENERAL DISCUSSION

1. GENERAL DISCUSSION

In this chapter, the results of the study are highlighted, discussed, and compared and compared with other more relevant findings from the literature. The discussion focuses on the objectives of the investigation. First, the physicochemical characterization of the raw materials used is analysed, then the influence of the inoculum on both monodigestion and co-digestion is analysed. Next, the synergistic and antagonistic effects of the raw materials used are analysed and finally a comparison is made between the experimental and predictive results using different kinetic models.

1.1 Physicochemical characterization of raw materials and theoretical methane production

Physicochemical characterization

Given that the production and productivity of biogas is highly dependent on the biochemical composition of the organic matter used as a substrate, it is of great importance to know the macromolecular composition of the biomass [1]. Above all, because the raw material used is unknown and has been little investigated for energy purposes.

In all the chapters of the thesis, to produce biogas, four main types of substrates have been used (slaughterhouse waste, llama manure, vicuña manure and guinea pig manure) and three secondary substrates (amaranth straw, wheat straw and quinoa straw). All the substrate/co-substrate configurations were in turn mixed with a common inoculum (sewage sludge from municipal waste).

Table 1 shows the physicochemical characterization of the substrates, co-substrates, which formed the basis for the work in monodigestion and co-digestion experiments. The high VS/TS ratios of animal manure residues with slaughterhouse residues ranged from 70.7 to 75.6%, indicating that these residues could be suitable raw materials for AD [2]. In the same way, the residues of the amaranth, quinoa and wheat straw co-substrates also had a good energy potential, since they had a VS/TS ratio between 58 and 77%. However, the quinoa straw biomass residues were the ones that contributed the least VS; this could be due to the relatively high content of recalcitrant components in the inorganic material in the waste [3]. Likewise, the quinoa straw residues presented a low C/N ratio (12) and a high ash content (30.3%). On the other hand, the maximum VS content (77.2%) was obtained in wheat straw residues, which made them have a low ash content (11.8%) and a high C/N ratio (29. 6). Comparing the substrates used with others in the literature is not easy because the substrates are highly variable in nature. Animal manure, for example, depends on different animal breeds, ages, diets, and management practices [4]. Likewise, the variability of agricultural residues is due to the origin and the different pre-treatment processes. This means that the physicochemical characterization of the materials used to obtain biogas is diverse and presents variabilities with respect to other similar studies.

Table 1. Physicochemical characteristics of substrates used in the co-digestion of organic residues in BMP assays.

Parameters	Units	LM	VM	GPM	SW	AS	QS	WS	IN
TS	%	50.6 (1.0)	57.4 (0.5)	33.9 (1.7)	9.6 (1.3)	88.2 (0.1)	87.0 (0.1)	92.6 (0.1)	3.9 (0.1)
VS (% ST)	%	61.6 (0.4)	41.2 (1.6)	72.6 (1.1)	70.7 (0.1)	74.8 (0.3)	58.4 (1.5)	77.2 (0.9)	58.5 (0.5)
VS/TS		75,6	72,2	72,6	70,7	77,0	75,0	58,0	-
Ashes	%	25.5 (0.3)	27.6 (1.8)	13.1 (0.1)	12.8 (0.2)	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)	2.3 (1.0)	0.4 (0.1)	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)	39.5 (1.2)	42.2 (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)	4.6 (0.5	6.3 (0.9)	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)	39.7 (1.2)	38.3 (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)	0.4 (0.0)	0.0 (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)	15.3 (0.8)	101.9 (0.9)	12.9 (0.8)	12.0 (0.9)	29.6 (0.8)	7.5 (0.7)

NOTE: LM (Llama manure), VM (Vicuña manure), GPM (Guinea pig manure), SW (Slaughterhouse waste), WS (Wheat straw), AS (Amaranth straw), QS (Quinoa straw), IN (inoculum).

Theoretical methane production

In all the chapters of the thesis, an analysis of the theoretical methane potential was carried out to determine the biodegradability of the substrates. In general, the calculation of the theoretical yield is widely recognized to obtain an approximation of the maximum methane production of a specific waste [5,6]. In this study, the exact physicochemical composition of the waste was known, which made it possible to predict methane production by balancing a stoichiometric equation to analyse the total conversion of organic material into CH_4, CO_2 and H_2S. However, the theoretical methane yields were much higher than the experimental yield due to the difficulty of degrading strongly lignocellulosic material [7].

Table 2 shows that in monodigestion the biodegradability of individual substrates and co-substrates increased with increasing inoculum, except for vicuña manure, guinea pig and amaranth residues whose biodegradability decreased by 9%, 17% and 5%, respectively. However, for llama manure and slaughterhouse residues the biodegradability increased by 60% and 20% with the increase in inoculum. Likewise, the biodegradability of co-digestion improved remarkably, especially for llama manure. The anaerobic digestion of llama manure and amaranth represented increases between 55-71%. Likewise, vicuña manure substrates, guinea pig and slaughterhouse residues increased to 20%, 17% and 15% its biodegradability when mixed with amaranth residues. Generally, the biodegradability of the main substrates improved in mixtures with the amaranth co-substrate and to a lesser extent with the quinoa co-substrate. In contrast, the mixtures of the substrates with the wheat straw co-substrate did not improve the biodegradability of the tests with increased inoculum much. The mixtures with wheat straw improved co-digestion by around 1%-14% with the increase in inoculum. The fact that there has been a significant improvement could be due to the relatively high content of recalcitrant components in the wheat waste compared to the waste from the other grains.

Table 2. Theoretical Yield and Biodegradability of Methane.

Raw material	SIR	Parameters	Units	Amaranth straw (AS)					Quinoa straw (QS)					Wheat straw (SW)				
				0%	25%	50%	75%	100%	0%	25%	50%	75%	100%	0%	25%	50%	75%	100%
Llama Manure (LM)	1:1	$\gamma_{THEORETICAL}$	ml/g VS	533,02	506,11	515,37	524,33	496,53	533,02	564,10	554,61	558,50	572,84	533,02	488,46	495,51	544,28	465,05
		ε	%	44,18	50,82	52,22	69,56	62,57	44,18	29,79	34,60	45,82	50,84	44,18	43,33	53,67	59,30	56,79
	1:2	$\gamma_{THEORETICAL}$	ml/g VS	533,02	506,11	515,37	524,33	496,53	533,02	564,10	554,61	558,50	572,84	533,02	488,46	495,51	544,28	465,05
		ε	%	70,74	79,24	89,57	94,33	59,41	70,74	71,95	84,31	86,12	65,65	70,74	78,16	77,21	75,27	57,68
Vicuña Manure (VM)	1:1	$\gamma_{THEORETICAL}$	ml/g VS	577,41	517,33	537,74	557,77	496,53	577,41	573,84	574,92	574,48	572,84	577,41	488,46	514,64	513,24	465,05
		ε	%	50,56	71,07	71,95	84,87	62,57	50,56	58,93	68,22	70,86	50,84	50,56	59,79	62,14	71,01	56,79
	1:2	$\gamma_{THEORETICAL}$	ml/g VS	577,41	517,33	537,74	557,77	496,53	577,41	573,84	574,92	574,48	572,84	577,41	488,46	514,64	513,24	465,05
		ε	%	45,54	73,31	86,44	91,96	59,41	45,54	64,42	78,45	79,11	65,65	45,54	55,94	65,01	81,20	57,68
Guinea pig Manure (GPM)	1:1	$\gamma_{THEORETICAL}$	ml/g VS	394,52	503,33	509,92	516,31	496,53	394,52	536,80	549,86	561,84	572,84	394,52	490,74	505,74	544,14	465,05
		ε	%	53,50	50,55	56,06	58,00	62,57	53,50	44,71	50,01	53,27	50,84	53,50	45,60	48,92	52,02	56,79
	1:2	$\gamma_{THEORETICAL}$	ml/g VS	394,52	503,33	509,92	516,31	496,53	394,52	536,80	549,86	561,84	572,84	394,52	490,74	505,74	544,14	465,05
		ε	%	44,17	55,63	65,48	67,92	59,41	44,17	50,55	57,33	60,70	65,65	44,17	51,82	56,74	58,06	57,68
Slaughterhouse Waste (SW)	1:1	$\gamma_{THEORETICAL}$	ml/g VS	495,65	588,92	693,08	649,87	496,53	495,65	636,24	714,87	815,02	572,84	495,65	544,14	643,71	772,82	465,05
		ε	%	47,81	62,35	68,21	71,67	62,57	47,81	61,33	64,66	68,10	50,84	47,81	57,13	58,22	60,03	56,79
	1:2	$\gamma_{THEORETICAL}$	ml/g VS	495,65	588,92	693,08	649,87	496,53	495,65	636,24	714,87	815,02	572,84	495,65	544,14	643,71	772,82	465,05
		ε	%	57,13	65,65	68,84	75,42	59,41	57,13	68,00	74,36	71,33	65,65	57,13	58,89	63,21	68,67	57,68

NOTE: SIR (Inoculum to substrate ratio).

In all the tests the biodegradability was less than 100% which means that the theoretical yield is higher than the experimental one. In principle, in the calculation of the theoretical yield, it is assumed that all the material of the substrates is biodegradable, an idea that is not true, since each substrate has a series of recalcitrant materials that are not very biodegradable. Many authors use a biodegradability factor, where they consider that only 80% of the raw material is biodegraded for the production of biogas [8]. Likewise, this factor is approximate since each raw material has a different behaviour, which makes the theoretical estimate unrealistic with the actual biogas production. In this sense, creating a model that considers all possible effects and includes all parameters would be complex, especially for small-scale biogas production.
For a wide variety of substrates and co-substrates, theoretical biogas yields based on the Boyle model provide useful information and allow the potential of different materials to be compared based on their composition. This study provides a simple model that does not require many inputs and can be applied to many raw materials if the user has elemental analysis data for the elements carbon, hydrogen, oxygen, nitrogen, and sulphur.

1.2 Effect of inoculum on AD in animal manure materials with lignocellulosic

The inoculum plays a vital role in supplying the initial microbial population in the anaerobic process [9]. The biodegradation rate and the time of the lag phase of organic waste depend on the concentration of microorganisms. The lignocellulosic structure especially of the agricultural cereals of quinoa, wheat and amaranth is the main obstacle in their bacterial degradation [10]. An alternative to minimize these effects is the use of an active inoculum that provides additional methane-producing microorganisms [11]. In this study, sewage sludge was used as an inoculum source to improve the anaerobic biodegradability of raw materials. In all the trials, the effect of the inoculum shortened the lag phase and made the process more stable [12]. In addition, it was evidenced that the micronutrient concentration of the inoculum increased the enzymatic activity and the biogas yield in almost all the trials [13].
In this thesis it was proposed to investigate the influence of the inoculum, especially from the point of view of the variation of the methane yield. According to Li et al. [14], methane production can decrease or even stop without the proper proportions of the SIR. Furthermore, many authors in the literature have reported that SIR relationships affect methane yield [15-22].
Table 3 shows the values of the experimental performance of the monodigestion and co-digestion of all the substrates and co-substrates tested. The results revealed that the increase in inoculum improved methane production in most of the raw materials. In the monodigestion it supposed an increase of methane of the 60%; 19%; 29% and 2% in llama manure, slaughterhouse waste, quinoa waste and wheat straw, respectively. However, with the increase in inoculum, vicuña and guinea pig manure, and amaranth residues decreased by 9%; 17% and 5%, respectively. The combinations of the mixtures in the co-digestion all improved with the increase in inoculum. Thus, for SIR 1: 2, llama manure in combination with amaranth, quinoa and wheat co-substrates improved methane production between 25% and 71%. The lowest values of methane increase were reflected in the combinations of the main substrates with wheat straw.

Table 3. Experimental performance of the different substrates and co-substrates used.

Raw material	SIR	Parameters	Units	Amaranth straw (AS)					Quinoa straw (QS)					Wheat straw (SW)				
				0%	25%	50%	75%	100%	0%	25%	50%	75%	100%	0%	25%	50%	75%	100%
Llama manure (LM)	1:1	γ_{Exp}	ml/g VS	235,49	257,21	269,13	364,72	310,68	235,49	280,87	302,82	311,75	291,23	235,49	211,65	265,94	322,76	264,10
		ε	%	44,18	50,82	52,22	69,56	62,57	44,18	49,79	54,60	55,82	50,84	44,18	43,33	53,67	59,30	56,79
	1:2	γ_{Exp}	ml/g VS	377,06	401,04	461,62	494,60	294,99	377,06	405,87	467,59	480,98	376,07	377,06	381,78	382,58	409,68	268,24
		ε	%	70,74	79,24	89,57	94,33	59,41	70,74	71,95	84,31	86,12	65,65	70,74	78,16	77,21	75,27	57,68
Vicuña manure (VM)	1:1	γ_{Exp}	ml/g VS	291,94	367,67	386,90	473,38	310,68	291,94	338,16	392,21	407,08	291,23	291,94	292,05	319,80	364,45	264,10
		ε	%	50,56	71,07	71,95	84,87	62,57	50,56	58,93	68,22	70,86	50,84	50,56	59,79	62,14	71,01	56,79
	1:2	γ_{Exp}	ml/g VS	262,95	379,25	464,82	512,93	294,99	262,95	369,67	451,02	454,47	376,07	262,95	273,24	334,57	416,75	268,24
		ε	%	45,54	73,31	86,44	91,96	59,41	45,54	64,42	78,45	79,11	65,65	45,54	55,94	65,01	81,20	57,68
Guinea pig manure (GPM)	1:1	γ_{Exp}	ml/g VS	211,07	254,43	285,86	299,46	310,68	211,07	240,00	274,98	299,29	291,23	211,07	223,78	247,41	283,06	264,10
		ε	%	53,50	50,55	56,06	58,00	62,57	53,50	44,71	50,01	53,27	50,84	53,50	45,60	48,92	52,02	56,79
	1:2	γ_{Exp}	ml/g VS	174,26	280,00	333,90	350,68	2309,11	174,26	271,35	315,23	341,04	376,07	174,26	254,30	286,96	315,93	268,24
		ε	%	44,17	55,63	65,48	67,92	465,05	44,17	50,55	57,33	60,70	65,65	44,17	51,82	56,74	58,06	57,68
Slaughterhouse waste (SW)	1:1	γ_{Exp}	ml/g VS	236,97	367,19	472,75	465,76	310,68	236,97	390,21	462,23	555,03	291,23	236,97	310,87	374,77	463,92	264,10
		ε	%	47,81	62,35	68,21	71,67	62,57	47,81	61,33	64,66	68,10	50,84	47,81	57,13	58,22	60,03	56,79
	1:2	γ_{Exp}	ml/g VS	283,16	386,63	477,12	490,13	294,99	283,16	432,64	531,58	581,35	376,07	283,16	320,44	406,89	530,70	268,24
		ε	%	57,13	65,65	68,84	75,42	59,41	57,13	68,00	74,36	71,33	65,65	57,13	58,89	63,21	68,67	57,68

NOTE: SIR (Inoculum to substrate ratio).

In the same way, Liu et al. [16], found that the increase in inoculum improves the methane production of food waste and grass waste. This study showed that increasing the SIR from 1: 1 to 1: 2 improves the biodegradability of substrates, especially in co-digestion. On the other hand, the inverse effect of some substrates that did not increase their performance in the 1: 2 SIR could be due to a low methanogenic activity and/or the number of methanogens, in the digesters, which could result in the accumulation of volatile fatty acids (VFA) produced during the acidogenic stage [23]. Some authors reveal that an increase in SIR decreases biogas and methane yields, since high SIRs increase the concentration of fatty acids and, therefore, reduce the pH [24].
Furthermore, the poor digestion performance of SIR 1:2 can be attributed to the large volume of solid fatty waste contained in slaughterhouse and guinea pig waste compared to activated sludge from wastewater, resulting in accumulation of fatty acids in the process. Since solid waste from slaughterhouses is high in protein nitrogen, ammonia production in the early stages of digestion with high SIR may play an inhibitory role for methanogens [25]. However, more research is needed to discover the exact reasons for the decrease in methane with increasing inoculum of some substrates.
On the other hand, in all the tests the results show that methane started to be generated immediately after incubation. This may indicate a rapid acclimatization of the microorganisms to the raw material. These findings occurred regardless of the type of SIR used, which shows that the inoculum favours the immediate activation of anaerobic digestion, leading to a decrease in the lag phase. Similarly, Boulanger et al. [26], in the anaerobic digestion of urban solid waste also showed that the increase of the inoculum increases the methane yields in shorter retention times, which was related to the reduction of the risk of pH decrease and the increase in the population of methanogenic bacteria.

1.3 Effect of co-digestion of lignocellulosic materials in animal manure: synergy and antagonism

Despite its suitability for anaerobic digestion, the use of manure as a raw material in obtaining methane could inhibit methanogenic archaea due to the ammonia released during the process [27]. Hence, manure has traditionally been used as a fertilizer for agricultural soils due to its high nitrogen and phosphorus content [28]. However, the fermentation of animal manure can be improved and optimized by co-digestion with carbon-rich agricultural residues. Anaerobic co-digestion of residues can be defined as the feeding of two or more substrates of different characteristics and/or origin in the same anaerobic digester [29]. Product of the co-digestion different benefits are obtained that enrich the process of digestion of the raw materials. This section analyses the synergistic benefits of co-digestion by combining livestock waste and Andean cereal straw waste.
In **Figure 1**, the percentage of methane improvement of the mixtures of the main substrates with the secondary co-substrates is represented. The improvement percentages are calculated with respect to the methane production of the individual substrates of llama, vicuña and guinea pig manure, and with respect to the slaughterhouse residues. In all the tests it was evidenced that the highest percentages of methane occurred when the co-substrate mixtures were 50 and 75% volatile solids. However, the highest methane values were obtained when the co-substrate percentage was 75% volatile matter, but these mixtures did not present significant figures with respect to the mixtures in which 50% volatile matter was used. Varsha et al. [30] reported that the greatest synergy in the co-digestion of kitchen waste and sewage sludge is obtained for a 50:50 ratio, since a greater increase in sewage sludge causes an imbalance between the generation of VFA (voltile fatty acids) and its conversion into methane, producing an unstable pH.

The improvement in methane production with co-digestion improved regardless of the amount of inoculum used. Thus, both SIR1:1 and SIR 1:2 improved methane production by increasing the percentage of co-substrate. The biodigesters in which the amaranth and quinoa co-substrate were used notably improved co-digestion, while the biodigesters in which wheat straw was used did not improve the methane production as much. In this case, the biodigesters composed of slaughterhouse waste and quinoa produced methane productions of 581 and 555 ml/g VS. These results were superior, even to those obtained in the fermentation of urban kitchen waste (527.5 ml/g VS) [31]. In addition, it has been shown in the literature that methane production from slaughterhouse residues in co-digestion tends to be highly variable with methane yields ranging between 230 and 700 ml/g VS [32-36].

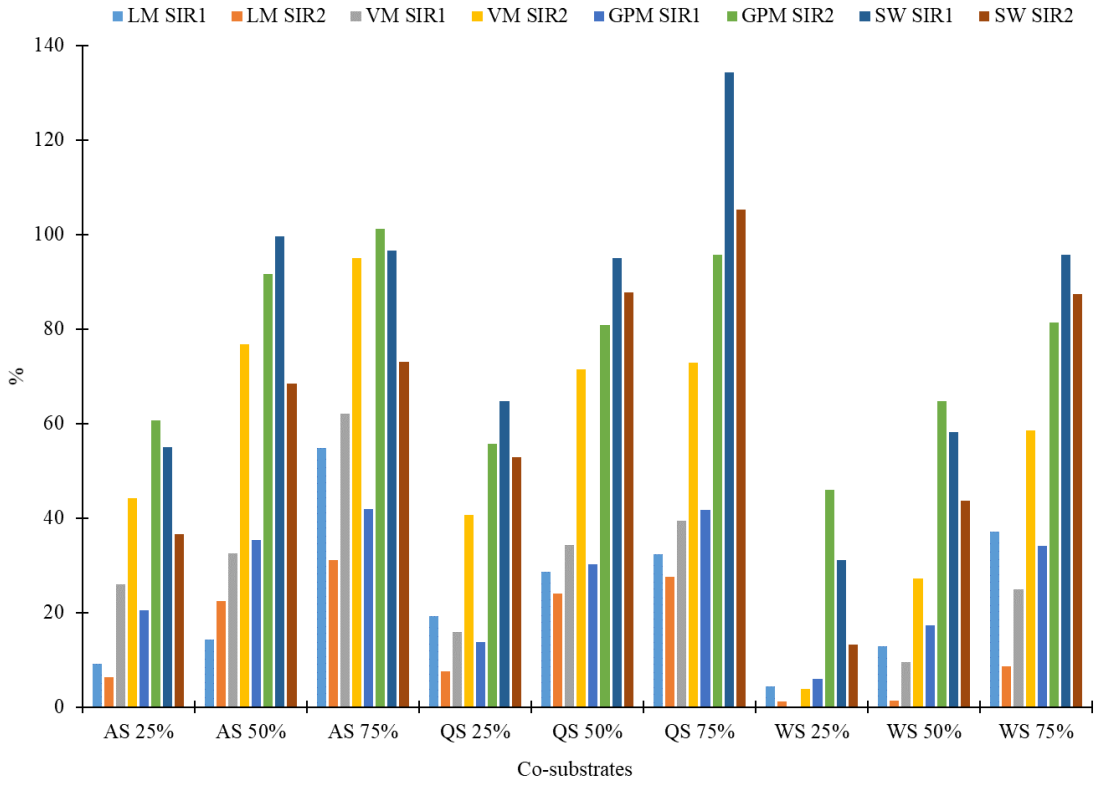

Figure 1. Synergistic effects in the fermentation process.

NOTE: LM SIR1 (Llama manure to SIR 1), LM SIR2 (Llama manure to SIR2), VM SIR1 (Vicuña manure to SIR 1), VM SIR2 (Vicuña manure to SIR2), GPM SIR1 (Guinea pig manure), GPM SIR2 (Guinea pig manure to SIR 2), SW SIR1 (Slaughterhouse waste to SIR 1), SW SIR2 (Slaughterhouse waste to SIR 2).

It should be noted that all major substrates improved methane production by adding a lignocellulosic co-substrate. This improvement in performance has been associated, compared to conventional monodigestion, with better nutrient availability, increased presence of trace elements, dilution of potential inhibitory compounds, and changes in the rheology of the media that improve mass transfer and mixture [37]; the biological process benefits from the optimized structure of the microbial community and the improvement of metabolic intensity [38]. Hence, anaerobic co-digestion has attracted great attention in recent years because it can provide a more balanced nutrient medium for the growth of microorganisms [39]. In this sense, anaerobic co-digestion is a viable solution to overcome ammonia inhibition in livestock waste and improve methane yield [36].

Regarding the mixtures in which very great synergistic effects have not been obtained, mainly in those in which WS has been used as a co-substrate. This could be due to the undesirable characteristics of the sample, which could lead to decomposition and failure of the digester, when mixed with the main substrates [31]. Added to this, the low biogas production can also be attributed to the quality of the organic matter in the manure and its management. In addition, we must take into account that guinea pig manure was pre-treated, that is, it was the only manure that was crushed to facilitate dilution before feeding the digesters, which caused the aerobic decomposition of easily degradable compounds [40].
All the mixtures in which a higher quantity of volatile co-substrate solids was used, both from amaranth, quinoa and wheat residues, considerably increased the biodegradability of the co-digestion. This may be because agricultural residues are often fibrous and low in nitrogen [41].

1.4 Kinetic study of anaerobic digestion

To understand the impacts of the types of agricultural residues on anaerobic co-digestion, it is necessary to study the methane production kinetics of anaerobic co-management with different raw materials [42]. The application of kinetic models to anaerobic digestion processes is important to evaluate the efficiency and the different variables of the monitoring processes. In addition, the evaluation of these curves allows a better understanding of the behaviour of the substrate during the anaerobic digestion process [4].
In this thesis, the models were used to determine the duration of the inoculum adaptation period, estimate the maximum biomethane yield and to observe the conversion rate of the tested substrates. Empirical enzymatic kinetic models (cone model) and microbial growth models (modified Gompertz model, transfer, logistic equation and Richards) were used.
The measured and predicted methane production results from all the trials were closely related. Thus, in the monodigestion all the logistic models the calculated asymptotes were adjusted very precisely for the specific yield (M_e), which made them not vary more than 7.06% with respect to the experimental data. However, the cone model was not as adequate to predict methane production since the differences between experimental methane production and M_e were of the order of 26%. According to Raposo et al. [43], the difference between experimental production and M_e should not be greater than 10%; above this value the kinetic model is considered invalid to predict anaerobic digestion processes. About co-digestion, the results were diverse and varied depending on the model tested. Thus, in the co-digestion of camelids, all the kinetic models adjusted very well the methane production values between the experimental and predicted results, especially the transfer and cone models; above all, because the R^2 values were > 99% and the RMSE values were less than 2 ml/g VS. In the co-digestion of slaughterhouse residues, the SW kinetic study revealed that the differences on methane production between the predicted and observed values, were for the sigmoidal models 0.25-19.48% (modified Gompertz), 0.32 -18.22% (logistic equation) and 0.40-19.42% (Richards). In comparison, the cone model experienced differences between 20% and 36% and the transfer model experienced a difference between 0.85% and 12.69%. Although the cone model generated many differences between the experimental and predicted performance, in this model an R^2 of 0.982 to 0.999 and RMSE of 0.61 to 6.92 CH_4/g VS were obtained. Finally, the co-digestion of guinea pig manure, the results of the kinetic modelling 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 the estimated values, especially in the GPM-AS (50:50) and GPM-QS (50:50) biodigesters. Ultimately, the methane prediction from the kinetic models depended on the raw material used [44].

The lag phase (t_{lag}) of growth functions, which is the time required for bacteria to adapt and begin methane production, were very small in all trials of both monodigestion and co-digestion. In all the mixtures of the sigmoidal models of Gompertz, Richards and the logistic equation, t_{lag} was practically zero. While in the model of the transfer experiment delay phases were 1.16 days. The low t_{lag} values found in this study are in line with the report by Talha et al. [45], who stated that the lowest lag phase depends on the activity of the adapted inoculum and the biodegradability of the organic part of the raw materials.

Most lignocellulosic substrates have cellulose as their main polymeric component [46]. The hydrolysis rate constant (k) of cellulose is normally the limiting step of biomethane production [47,48]. In this study, the k values ranged from 0.01 to 0.21; mostly the highest values were recorded when the relationship between the substrate and the inoculum was increased. In some cases (especially in the co-digestion of SW) the constant k increased for biodigesters composed of SW-QS and decreased for biodigesters composed of SW-WS. According to Dudek et al. [47], the higher k values are positive, as that means a higher bioavailability of cellulose, which results in a faster rate of methane production [49]. In short, the production of biomethane represents the rate of hydrolysis of the bioavailable substrate that decreases with the decrease in VS [50].

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CONCLUSIONS

CONCLUSIONS

The objective of this doctoral study was to develop and apply methods for the systematic quantification of methane production from organic manure residues from Andean livestock and straw residues from agricultural crops. The study was carried out through the physicochemical characterization of the fractions used, evaluating the biochemical potential of methane (BPM) of the raw materials, developing the theoretical quantification of methane from the substrates and cosubstrates and performing the codigestion of the residues to analyze the synergistic effects of the process. In addition, a kinetic study of the process was carried out to identify optimal codigestion and monodigestion scenarios. The main findings and conclusions of this doctoral thesis are summarized below:

1. In the physicochemical characterization it was determined that the VS/TS ratios of the main substrates ranged between 70.7% and 75.6% with a C/N ratio between 15 and 102, which indicated that these wastes are suitable raw materials for production of methane. Similarly, the co-substrates also had good energy potential, since they had a VS/TS ratio between 58% and 77% and a C/N ratio between 12 and 29.6.

2. The biodegradability of monodigestion (going from a 1:1 SIR to a 1:2 SIR) increased between 60% and 20%, except for vicuña manure, guinea pig and amaranth residues which biodegradability decreased by 9%, 17% and 5%, respectively. The biodegradability of co-digestion also improved with increasing inoculum, especially for mixtures of llama manure and amaranth residues. The biodegradability of llama manure and amaranth straw represented increases between 55-71%, while the substrates of vicuña manure, guinea pig and slaughterhouse residues increased its biodegradability up to 20%, 17% and 15%, respectively, when mixed with amaranth residues. The biodegradability of the main substrates improved in the mixtures with the amaranth co-substrate and to a lesser extent with the quinoa co-substrate. On the other hand, the mixtures with wheat straw improved significantly with the increase in inoculum since their improvements were in the 1-14% tone.

3. The improvement in biodegradability was directly related to the improvement in methane production. An increase in inoculum from a SIR 1:1 to a SIR1:2 improved the final methane production of most BMP tests. In the monodigestion it supposed an increase of methane of the 60%, 19%, 29 and 2% in llama manure, slaughterhouse waste, quinoa waste and wheat straw, respectively. However, with the increase in inoculum, vicuña and guinea pig manure, and amaranth residues decreased by 9%, 17% and 5%, respectively. The combinations of the mixtures in the co-digestion all improved with the increase in inoculum. Thus, for SIR 1:2, llama manure in combination with amaranth, quinoa and wheat co-substrates improved methane production between 25% and 71%. However, the lower values of methane increase were reflected in the combinations of the main substrates with wheat straw.

4. All the mixtures of the main substrates with the secondary co-substrates produced synergistic effects. In all the tests it was evidenced that the highest percentages of methane occurred when the co-substrate mixtures were 50% and 75% volatile solids. However, the highest methane values were obtained when the co-substrate percentage was 75% volatile matter, but these mixtures did not present significant figures with respect to the mixtures in which 50% volatile matter was used. Furthermore, the improvement in

methane production with co-digestion improved regardless of the amount of inoculum used. Thus, both SIR1:1 and SIR 1:2 improved methane production by increasing the percentage of co-substrate, especially amaranth and quinoa. Thus, the biodigesters composed of slaughterhouse waste and quinoa produced methane productions between 581 and 555 ml/g VS.

5. In the kinetic study, five models were used, of which the logistic ones were the ones that produced the best results, since the cone model oversized the specific methane production. In the monodigestion, in all the logistic models the calculated asymptotes were adjusted very precisely to the specific yield (Me), which made them not vary by more than 7.06% with respect to the experimental data. The cone model, in comparison, generated differences between the experimental methane production and Me of the order of 26%. Likewise, in co-digestion, the cone model generated large differences (20 and 30%) between the experimental production and Me. Of all the logistic and complex models, the transfer model adjusted the results quite well since in many tests an R^2 greater than 99% and RMSE values less than 2 ml/g VS were obtained. However, the methane prediction from the kinetic models depended on the raw material used, since not all the mixtures had the same behaviour.

6. The preparation of this study contributed to the improvement of the energy situation in rural areas of the Andes, through the energy valuation of their own resources. In addition, it contributed to the improvement of waste management, especially those from agriculture and livestock, to prevent environmental pollution.