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The importance of substrate formulation on the hydrolysis process in anaerobic digestion: a numerical and experimental study

La importancia de la formulación de sustratos en el proceso de hidrólisis en digestión anaerobia: un estudio numérico y experimental

A. Albalate-Ramírez[1,2], M.M. Alcalá-Rodríguez[1], L.R. Miramontes-Martínez[1,3], A. Estrada-Baltazar[4], U. Galván-Arzola[1,2], B.N. López-Hernández[1,2], J.R. Morones-Ramírez[1,2], P. Rivas-García[1,2]*

[1]*Departamento de Ingenieria Quimica, Facultad de Ciencias Quimicas, Universidad Autónoma de Nuevo León. Av. Universidad S/N, Cd. Universitaria, zip 64451, San Nicolás de los Garza, Nuevo León, Mexico.*

[2]*Centro de Investigacion en Biotecnología y Nanotecnología, Facultad de Ciencias Químicas, Universidad Autónoma de Nuevo León. Parque de Investigación e Innovación Tecnológica, km 10 Highway to the International Airport Mariano Escobedo, zip 66629 Apodaca, Nuevo León, Mexico.*

[3]*Facultad de Agronomía, Universidad Autónoma de Nuevo León, Francisco Villa s/n, Fraccionamiento Ex Hacienda El Canada, CP 66050, Gral. Escobedo, N.L. 4 Departamento de Ingeniería Química, Instituto Tecnológico de Celaya, Av. Tecnológico y A. García Cubas S/N, zip. 38010, Celaya, Guanajuato, Mexico.*

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Abstract

Substrate formulation has been widely studied to ensure optimum methane production in anaerobic digestion. This work demonstrated a synergistic degradation in the hydrolysis of carbohydrates, proteins, and lipids that enhances CH_4 yields through biochemical methane potential (BMP) tests and a co-digestion index (CI), following a D-Optimal experimental design. Additionally, this work proposes and validates a modification to the hydrolysis step in the Anaerobic Digestion Model No.1 (ADM1), capable of representing such synergistic degradation by incorporating the CI as a dynamic variable into the mathematical structure of the model. High CI values were observed at balanced carbohydrate-protein-lipid and protein-lipid ratios, obtaining values of 3.23 and 2.85, respectively. In contrast, low CI values were obtained at higher lipid-carbohydrate ratios (1.21), demonstrating that adding proteins to the substrate mixture promotes synergy. Incorporating the CI as a dynamic variable in the hydrolysis stage of the ADM1 increases its predictive capacity, reducing the root mean square error value by up to 55.7% when modeling the BMP tests compared to the original ADM1 structure. When subjected to mixtures of real substrates, the proposed model precisely adjusts the experimental data. These results prove the validity of the proposed modification to the ADM1 and its functionality with real substrate mixtures. This work allows the numerical representation of the synergistic effects in the degradation of a substrate and the correct generation of feed formulations that increase CH_4 yields.

Keywords: Anaerobic digestion; ADM1; hydrolysis; co-digestion index; carbohydrates, proteins, and lipids degradation; synergistic effects.

Resumen

La formulación de sustratos ha sido ampliamente estudiada para asegurar la óptima producción de CH_4 en la digestión anaerobia. Este trabajo demostró una degradación sinérgica en la hidrólisis de carbohidratos, proteínas y lípidos que mejora los rendimientos de CH_4 a través de pruebas de potencial bioquímico de metano (BMP) y un índice de co-digestión (IC), siguiendo un diseño experimental D-Optimal. Adicionalmente, este trabajo propone y valida una modificación a la etapa de hidrólisis en el *Anaerobic Digestion Model No.1* (ADM1), capaz de representar tal degradación sinérgica incorporando el IC como variable dinámica en la estructura matemática del modelo. Se observaron valores elevados de IC en proporciones balanceadas carbohidrato-proteína-lípido y proteína-lípido, obteniéndose valores de 3.23 y 2.85, respectivamente. Por el contrario, se obtuvieron valores de IC bajos con mayores relaciones lípidos-carbohidratos (1.21), lo que demuestra que la adición de proteínas a la mezcla de sustrato promueve efectos sinérgicos. La incorporación del IC como variable dinámica en la etapa de hidrólisis de la ADM1 aumenta su capacidad predictiva, reduciendo el valor de error cuadrado medio de raíz hasta un 55,7% al modelar las pruebas de BMP en comparación con la estructura ADM1 original. Cuando se somete a mezclas de sustratos reales, el modelo propuesto ajusta con precisión los datos experimentales. Estos resultados demuestran la validez de la modificación propuesta al ADM1 y su funcionalidad con mezclas de sustratos. Este trabajo permite la representación numérica de los efectos sinérgicos en la degradación de un sustrato y la correcta generación de formulaciones de sustratos que aumentan los rendimientos de CH_4.

Palabras clave: Digestión anaerobia; ADM1; hidrolisis; índice de co-digestión; degradación de carbohidratos, proteínas y lípidos; efectos sinérgicos.

*Corresponding author. *E-mail*: pasiano.rivasgr@uanl.edu.mx
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1 Introduction

Anaerobic digestion (AD) is a biological process in which diverse groups of microorganisms decompose organic matter to produce biogas (in different compositions of CH_4, CO_2, H_2, and other minoritarian gases). AD can be used as an organic solid waste management process that obtains economic and environmental benefits from producing clean electricity and biofertilizer (Flores-Estrella it et al., 2016; Maharaj it et al., 2018; Rivas-Garcia it et al., 2015; Sanchez-Herrera it et al., 2018). Implementing this technology closes the nutrient cycle by substituting conventional energy and chemical fertilizers, fostering a circular economy (EBA, 2020).

AD usually operates at low-profit schemes due to frequent problems derived from inhibitory effects on the CH_4 yield (Galván-Arzola it et al., 2022) causing the process to have a waste management approach, and is not seen as an energy and biofertilizer production system. Complete monitoring of intermediate metabolites is requested to enhance the AD process performance. These metabolites are responsible for process inhibitions such as high ammonium concentration, accumulation of volatile fatty acids (VFA), and long-chain fatty acids (Leng it et al., 2018; Wu it et al., 2019).

Inhibition by accumulating intermediate metabolites in AD processes manifests an ongoing failure; e.g., VFA inhibition is the most common problem, among 60 - 70% of industrial-scale anaerobic digesters in Latin-American manifest failures by VFA accumulation (Galván-Arzola it et al., 2022; Miramontes-Martínez it et al., 2020; Rivas-García it et al., 2019). The solution strategies are usually corrective, entailing high costs derived from the addition of chemical agents that counteract low pH levels or co-digestion. In any of these cases, it is unavoidable that the stability and profitability of the processes might be compromised. In developing regions, such as Latin America, it is complicated to solve inhibitions problems once they manifest, since AD processes lack adequate agitation, monitoring, and control systems (Galván-Arzola it et al., 2022).

Other authors recommend studying the influence of substrate formulation fed into the anaerobic reactors regarding the relation between carbohydrates: proteins: lipids (C:P:L) on the AD performance. C:P:L proportion and its influence on CH_4 yields of batch AD processes have been studied by Xue it et al. (2020) in batch anaerobic digesters at 37 °C, concluding that a proportion of 22.6:14.1:63 propitiates higher CH_4 yields, with a maximum of 595 mL CH_4 g VS^{-1} and optimum values of C/N between 25 and 30. Astals it et al. (2014) determined a similar optimum relation of 17:17:66 using cellulose, casein, and olive oil as representative substrates of each macromolecule. (Miramontes-Martínez it et al. (2021) found that a 26:40:34 relation is optimal when using co-digestion of slaughterhouse wastes and fruit and vegetable wastes. In said studies, the authors point out that an apparent balance of C:P:L diminishes inhibition phenomena such as acidification, associated with a high carbohydrate content in VS fed. A correct proportion between these macromolecules can foster synergistic interactions (Ebner it et al., 2016), increasing the degradation rate of the substrate (Astals it et al., 2014), and diminishing inhibition phenomena permitting an optimal CH_4 production (Charnier it et al., 2018; Jacobi it et al., 2011; Reed it et al., 2013). In recent studies, synergistic interactions have been proven to not only improve CH_4 yields, but also benefit the environmental profile of the AD processes (Albalate-Ramírez it et al., 2022).

Studying the influence of C:P:L proportion on anaerobic degradation requires quantification of intermediate and final metabolites and sophisticated and expensive experimental techniques, which is difficult at the lab- and industrial scales, especially in developing regions (Galván-Arzola it et al., 2022). A partial solution is the implementation of mathematical models. The Anaerobic Digestion Model No.1 (ADM1) (Batstone it et al., 2002) has a complete structure, and considers the anaerobic organic degradation employing biochemical dynamics through not structured kinetic models, a complete inhibition model, physicochemical relationships, and mass transfer phenomena. However, Zaher it et al. (2009), Mottet it et al. (2013), and Rivas-García it et al. (2020) observed a substantial deficiency; the ADM1 interprets the hydrolysis steps through first-order kinetic, just dependent on the individual concentration of carbohydrates, proteins, and lipids. This strategy restricts the visualization of synergistic effects in carbohydrates, proteins, and lipids hydrolyzation.

Substrates that are commonly destined for AD usually contain complex macromolecules that require deep hydrolysis. The hydrolysis process is the "gateway" of the AD process, if this step is not favorable the later stages of the AD process can be affected, leading to low CH_4 yields. To aboard the hydrolysis step deficiencies in the ADM1, Zaher it et al. (2009) developed a model which, through the optimization of the kinetic parameters of carbohydrates, proteins, and lipids hydrolyzation, improves the CH_4 yield prediction. Mottet it et al. (2013) proposed a model based on the bioaccessibility concept of substrates through the Contois equation, splitting the organic matter into slowly and rapidly hydrolyzable fractions. These authors obtained an acceptable prediction degree in the CH_4 production, but through a model that requires more additional parameters than those of the original ADM1, complicating the task of a correct initialization. Rivas-García it et al. (2020) proposed a model incorporating substrate-microorganism relationships using the Contois equation. The model improved the prediction of several results compared to the original ADM1. There are several efforts to improve the representation of the hydrolysis step through the ADM1; however, the existing proposals do not consider together the importance of a balanced feed in terms of the C:P:L ratios and the possible synergistic effects in the hydrolytic degradation.

This study evaluates the synergistic interactions during the hydrolysis steps in the anaerobic degradation of carbohydrates, proteins, and lipids and their influence on CH_4 yield. The present research proposes and validates a modification to the ADM1 capable of representing such phenomena in anaerobic digestion of solid wastes. This contribution could be helpful in early avoiding possible problems in large-scale anaerobic digesters by an adequate formulation in feed substrates.

2 Materials and methods

2.1 Experimental design

Based on a literature review, the minimum and maximum limits of carbohydrates, proteins, and lipids in the VS of the several common residues used in AD as substrates were defined (Table 1). A D-Optimal Design (Cornell, 2011) was defined using an interaction model framed in these limits (Equation 1), the experimental design and the analysis results were carried out using the Design-Expert 7.1.5 software (Stat-Ease, Inc.). The resulting experimental design is shown in Table 2.

$$Y_{CH_4} = \beta_1 C + \beta_2 P + \beta_3 L + \beta_4 CP + \beta_5 CL + \beta_6 PL \quad (1)$$

In the equation, Y_{CH_4} is the cumulative CH_4 yield (mL CH_4 g VS^{-1}); β_{1-6} are the statistical coefficients in the interaction model; and C, P, and L are the percentual fractions of carbohydrates, proteins, and lipids (%VS), respectively.

2.2 Inoculum selection, characterization, and feed formulation

The inoculum was obtained from the sludge of a mesophilic anaerobic digester fed with wastewater from a chocolate industry. As strategy of demethanization, the inoculum was kept in a 6 L Applikon® bioreactor under isothermal conditions and continuous stirring at 200 rpm. The demethanization process stopped when no appreciable biogas production was observed (~25 days), through a biogas volumetric quantifier (Prendo MVG-10). The inoculum characterization was conducted through the evaluation of the parameters showed in Table 3. Considering the work done by Astals it et al. (Astals it et al., 2014), cellulose (SIGMA-ALDRICH®), casein (Becton Dickinson®), and olive oil (Great Value®) were used as substrates to represent carbohydrates, proteins, and lipids, respectively.

Table 1. Carbohydrates, proteins, and lipids fraction in VS for common waste substrates used in anaerobic digestion.

Fraction %VS	FW	FVW	CM	SHW	
Carbohydrates	63.2	87.0	69.0	8.50	2.10
Proteins	3.70	17.0	14.0	2.20	72.7
Lipids	1.60	7.0	1.00	89.3	25.1
Reference	Galí it et al., (2009)	Holliger it et al., (2016)	Ebner it et al., (2016)	Palatsi it et al., (2009)	Astals it et al., (2014)

FW: Food waste. FVW: Fruit and vegetable waste. CM: Cattle manure. SHW: Slaughterhouse waste.

Table 2. D-Optimal experimental design.

Experiment	%Carbohydrates	%Proteins	%Lipids
E1	6	70	24
E2	48.5	1	50.5
E3	29.7	70	0.3
E4	6	5	89
E5	87	12.7	0.3
E6	20	40	40
E7	58.4	41.4	0.3
E8	87	1	12
E9	6	37.5	56.5
E10	10	1	89

Table 3. Inoculum characterization parameters.

Parameter	Value	Reference
Moisture (%)	94.85 ± 0.09	NMX-AA-034-SCFI-2015
Total solids (%)	5.15 ± 0.09	NMX-AA-034-SCFI-2015
Volatile solids (% TS)	60.03 ± 0.26	NMX-AA-034-SCFI-2015
Ash (% TS)	39.97 ± 0.26	NMX-AA-034-SCFI-2015
Alkalinity (g $CaCO_3$ L^{-1})	5.01 ± 0.27	NMX-AA-034-SCFI-2015
Volatile fatty acids (g L^{-1})	0.28 ± 0.06	NMX-AA-036-SCFI-2001
pH	7.34 ± 0.12	NMX-AA-25-1984

NMX: Mexican standard; TS: Total solids.

2.3 Biochemical methane potential test and data analysis

Biochemical methane potential (BMP) tests were carried out following the experimental design in Table 2. The volume of each bioreactor was 120 mL with an operating volume of 60 mL, containing 30 mL of inoculum and 30 mL of substrate mixture (40 g VS L^{-1}) -inoculum to substrate ratio (ISR) = 0.76. The BMP tests were conducted without the addition of macro/micro - nutrient solutions. Industrial grade N_2 was used to displace the air inside the reactors and then closed with a septum and aluminum airtight seal to ensure anaerobic conditions. The operational conditions of the BMP tests were 35 °C with manual agitation each 24 h during 25 d. The operating time was set at 25 d because, in AD processes, the greatest synergistic effects and microbial activity are observed during the first 20 d, as demonstrated by Miramontes-Martínez it et al. (2021).

CH_4 production was calculated using the biogas density method proposed by (Justesen it et al. (2019). A scheme of the experimental set-up can be found on Supplementary Materials (Figure S1). All BMP tests were done by triplicate, including blanks using the inoculum and instead of substrate distilled water. The effluents from each bioreactor were characterized in the parameters of volatile solids (APHA/AWWA/WEF. 2012), alkalinity and volatile fatty acids, and pH (AOAC-1980).

The CH_4 yields obtained in the BMPs and their respective proportion of C:P:L in the feed were analyzed through a response surface fitted to the interaction model described in Section 2.1 (Equation 1). To quantify the synergistic interactions between C, P, and L, a derivation of the co-digestion index (CI) proposed by Ebner it et al. (2016) was used:

$$CI_i = \frac{B_i}{\sum_{j=1}^{3} B_j(f_{VS\,ji})} \forall i \tag{2}$$

where B_i is the CH_4 yield (ml CH_4 g VS^{-1}) in the experiment i (Table 2); B_j is the CH_4 yield of each macromolecule individually (i.e., mono-digestion of C, P, or L); and $f_{VS\,ji}$ is the VS fraction of the macromolecule j in the experiment i.

For Equation 2, CI > 1 indicates synergistic biodegradation between carbohydrates, proteins, and lipids fractions, since the system generates a greater amount of CH_4 than the average production of each macromolecule C, P, and L (denominator of Equation 2). Values of CI < 1 denote antagonistic effects.

2.4 Mathematical modelling of the BMP

ADM1 (Batstone it et al., 2002) was used to simulate the anaerobic digestion of substrate formulations (Table 2) through the BMP tests. Equation 3 shows the differential equation for the hydrolysis of macromolecules as an example of the mathematical structure of the model; where j is the macromolecule (carbohydrates, proteins or lipids), $X_{j,Xc}$ is the concentration of the macromolecule j, X_c is the concentration of the composite in the mixture, $f_{j,Xc}$ is the fraction of the macromolecule in the composite (X_c), $k_{dis,j}$ is the disintegration constant of the composite, $k_{hyd,j}$ is the hydrolysis constant for the macromolecule j, and X_j is the concentration of the microorganisms in charge of $X_{j,Xc}$ hydrolysis. The system was modeled as an isothermal stirred tank reactor in batch mode with no reaction volume change. The numerical integration of the model was as recommended by Rivas-García it et al. (2013), where the differential equations representing the consumption rates of the substrate, metabolite production, and growth of microbial groups were solved with a fourth-order Runge Kutta implemented in a FORTRAN code with an integration step of 10^{-6} d. The physicochemical model, consisting of the determination of the pH, was solved using a bisection method. The numerical validation of the model was carried out with a dynamic global mass balance alongside the model.

$$\frac{dX_{j,X_c}}{dt} = f_{j,X_c} k_{dis,X_c} X_c + k_{hyd,j} X_j \tag{3}$$

The assumption of the original ADM1, where carbohydrates, proteins, and lipids are degraded by independent process and have constant hydrolysis coefficients, is an oversimplification of the phenomenon. This fact does not consider the evidence reported in the scientific literature on the synergistic effects on the hydrolysis of these compounds (Astals it et al., 2014; Ebner it et al., 2016; Miramontes-Martínez it et al., 2021; Xue it et al., 2020). The proposed modification for ADM1 assumed that the hydrolysis coefficients k_{hyd_Xj} vary dynamically. This variation is considered proportional to the daily co-digestion index in each experiment (Equation 4), which bonds the hydrolysis rates of carbohydrates, proteins, and lipids by incorporating synergistic effects into the model (Equation 5).

$$CI_i(t) = \frac{B_i(t)}{\sum_{j=1}^{3} B_j(t)(f_{VS\,ji})} \tag{4}$$

$$k_{hyd,X_j}(t) = k_{hyd,X_j} CI_i(t) \tag{5}$$

The experiments described in Section 2.1 were modeled using the original and modified ADM1. Additionally, the initial hydrolysis coefficients were adjusted to increase the

Table 4. Adjusted hydrolysis coefficients.

Parameter	Description	Value [d^{-1}]
k_{hyd_X1}	Hydrolysis coefficient of carbohydrates	0.093
k_{hyd_X2}	Hydrolysis coefficient of proteins	0.210
k_{hyd_X3}	Hydrolysis coefficient of lipids	0.035

model prediction, shown in Table 4. The root mean square error of prediction (RMSEP) test was used to evaluate the predictive capacity of both models.

3 Results and discussion

3.1 Biochemical methane potential tests

The CH_4 yields and the respective C:P:L ratio fed to the BMP tests are shown in Figure 1A. In this study, the lowest yield was 272.2 ± 8.51 mL CH_4 gVS^{-1}, corresponding to experiment E10 with a ratio of 10:1:89 in the fed VS. CH_4 yields of 143 to 325 mL CH_4 g VS^{-1} for lipid-rich residues have been reported in the literature (Davidsson it et al., 2008; Kabouris it et al., 2008; Long it et al., 2012). Lipids have relatively low hydrolysis rates compared to carbohydrates and proteins in anaerobic environments (Batstone it et al., 2002; Esposito it et al., 2012), so in the short term and as shown in Figure 1, they have yields of lower CH_4 compared to other processes. Lipid components and their degradation products are barely soluble in aqueous solutions, forming aggregates on the surface of the reagent medium and limiting bioaccessibility to microorganisms (Cuetos it et al., 2010; Long it et al., 2012). The highest yields were obtained at a high protein ratio. Specifically, the highest yield was 495.8 mL CH_4 g VS^{-1}, corresponding to experiment E3. Comparable CH_4 yield results for high-protein substrates were reported by Astals it et al. (2014), 431 ± 6 mL CH_4 g VS^{-1}, using casein as a representative protein substrate. Dominguillo-Ramírez it et al., (2023) obtained similar results the authors demonstrated that the proteins are the balancing component of substrate mixtures. According to Hassan it et al. (2017), one of the causes that influence the AD processes is the C/N ratio because this ratio is responsible for regulating the nutrient balance in the methanogenic biomass. A low C/N ratio will possibly inhibit the AD process due to the abundance of ammonia nitrogen from the degradation of substrates with high protein content (Hassan it et al., 2017; Wang it et al., 2012), while a high C/N ratio leads to an increased production of carboxylic acids, which inhibits AD due to low pH levels and in some cases too high nitrogen competition with microorganisms (Njuguna Matheri it et al., 2018).

Various studies have reported values considered optimal for C/N: 20 to 30 (Wang it et al., 2012), 20 (Hassan it et al., 2017), and 15 to 30 (Tufaner & Avşar, 2016). In this study, high C/N ratios are associated with low CH_4 yields, as shown in Figure 1B, which may be associated with a limiting nitrogen condition in the substrate (the lowest protein content correspond to E2, E4, E8, and E10, Table 2) fostering possible inhibition by intermediate metabolites (accumulation of VFA) since the pH values of these experiments oscillate between 6.3 - 6.6. These results are confirmed in Figure 1C, where low pH values for those experiments with limited nitrogen condition (lower pH) foster low CH_4 yields. These results allow us to associate the behavior of BMP to synergistic/antagonist effects in the hydrolysis stage, as is addressed in the next section.

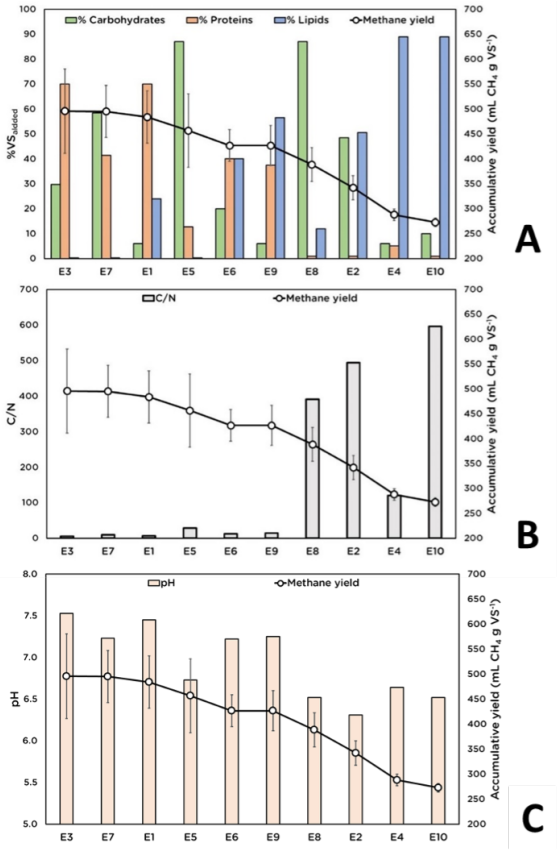

Figure 1. A) Proportion of carbohydrates, proteins and lipids in the feed and their relationship with CH_4 yields. The experiments are sorted from higher to lower in terms of the CH_4 yields. B) C/N ratio in the feed and its relationship with CH_4 yields. The experiments are sorted from higher to lower in terms of the CH_4 yields. C) Final pH of each experiments and its relationship with CH_4 yields. The experiments are sorted from higher to lower in terms of the CH_4 yields.

3.2 Statistical analysis and substrate synergy

Figure 2 shows the results of the response surface analysis adjusted to an interaction model, in which a correlation coefficient (R^2) of 0.99 was obtained. Table 5 shows the values obtained for the coefficients of the proposed interaction model.

The predictive capacity of the proposed model was evaluated by employing a BMP test in triplicate with a proportion of carbohydrates, proteins, and lipids of 20%, 20%, and 60%, respectively. The experimental yield was 339.3 ± 30.4 mL CH_4 g VS^{-1}, and the interaction model was 336.0 mL CH_4 g VS^{-1}, obtaining an error less than 1%.

In Figures 1A and 2, the highest CH_4 yield corresponds to an interaction between carbohydrates and proteins, and in minor proportion, the region of proteins with a lower proportion of lipids. These results aim to possible synergistic interactions between C-P and P-L, increasing the anaerobic biodegradability. The results of the CI index (used as a parameter to determine the degradative synergy between substrates) are shown in Figure 3.

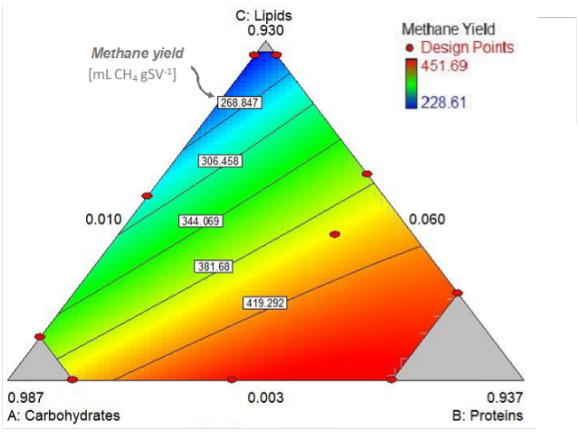

Figure 2. Cumulative CH_4 yield response surface from the experiments framed in Table 2.

Table 5. Regression model coefficients (Equation 1).

Coefficients	Value
β_1	376.87 ± 14.03
β_2	434.49 ± 24.70
β_3	224.66 ± 9.95
β_4	186.98 ± 83.49
β_5	-21.79 ± 63.6
β_6	283.78 ± 71.37

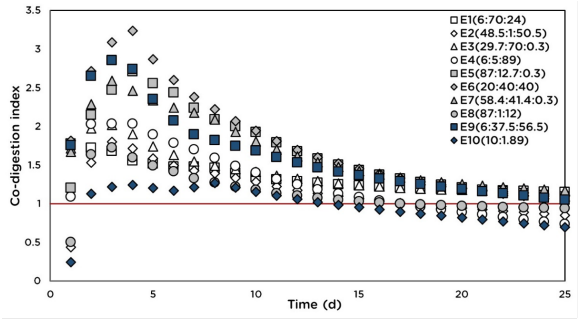

Figure 3. Co-digestion index in biochemical CH_4 potential (BMP) tests. Values in parenthesis represent the C:P:L proportion in %VS of the substrate.

The processes with a balanced condition between proteins with carbohydrates and lipids have the highest values in the CI. During the first seven days of operation, the maximum CI is associated with experiments E6 and E9 (3.23 and 2.85, respectively), corresponding to a low C/N ratio (Figure 1B) and a more equilibrated proportion between proteins with carbohydrates and lipids. This can be due to the balancing effect of the proteins in the substrate mixture (Dominguillo-Ramírez it et al., 2023), as the addition of proteins promotes synergistic interactions and assists the effective degradation of a substrate with high fractions of non-lignocellulosic carbohydrates and lipids. CI values of 2.1 and 1.4, like those obtained in experiments E4 and E8, are reported in the literature for co-digestion of carbohydrate- and lipid-rich residues, respectively (Ebner it et al., 2016; Miramontes-Martínez it et al., 2021).

3.3 Mathematical validation of the modification proposal to the ADM1

Numerical tests were carried out using the original ADM1 (Batstone it et al., 2002) in order to evaluate if it is able to represent the synergistic effects described in Sections 3.2 and 3.3. Figure 4A shows the CH_4 yield of experiments E3, E8 and E10, which correspond to the processes with a majority of proteins, carbohydrates and lipids in the substrate, respectively (Table 1). The model accurately fits E10 with a RMSEP value of 14.8, while in E3 and E8 the model underestimates the CH_4 yield with RMSEP values of 74.3 and 27.2, respectively. ADM1 cannot correctly represent the synergistic effects between carbohydrates and proteins that are occurring experimentally (explained in section 3.2 and 3.3), this may be due to deficiencies in the hydrolysis model in ADM1.

The results of the proposed hydrolysis model for ADM1 (which considers the effect of synergy through CI) are shown in Figure 4B. A greater fit in the CH_4 yields for the three experiments throughout the reactive process is noticeable. Table 6 shows the results of final performance and RMSEP for both models, being remarkable that the proposed model presents better adjustments the experiments.

The results in Figure 4 show that this modification to the hydrolysis process, without unnecessary complications and that considers the synergistic interactions between substrates, improves the prediction of ADM1. This is results are corroborated by the parity graphs shown in Figures 5 A - B. As an additional test, simulations were performed using a mixture of real substrates. The modelled data was taken from results published by our research group for a real mixture of fruit and vegetable residues (FVW) with cow manure (CM) in a 1:1 ratio (%VS).

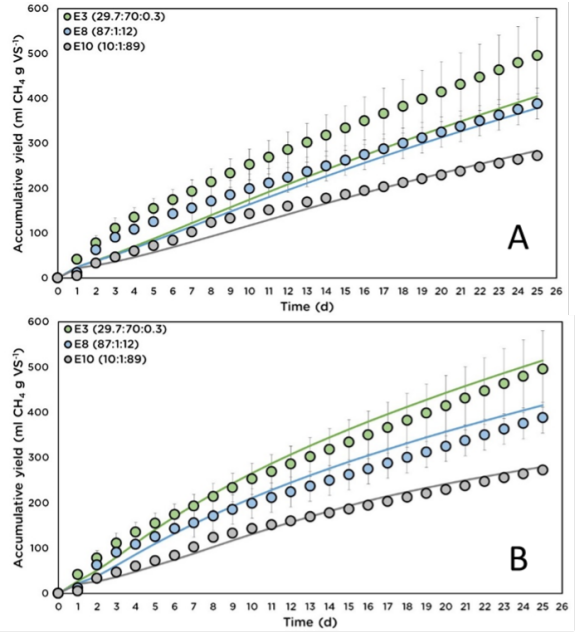

Figure 4. A) Experimental and simulated CH_4 yield with original ADM1. B) Experimental and simulated CH_4 yield with the modified ADM1. Values in parentheses represent the proportion of carbohydrates, protein, and lipids in %VS in the substrate.

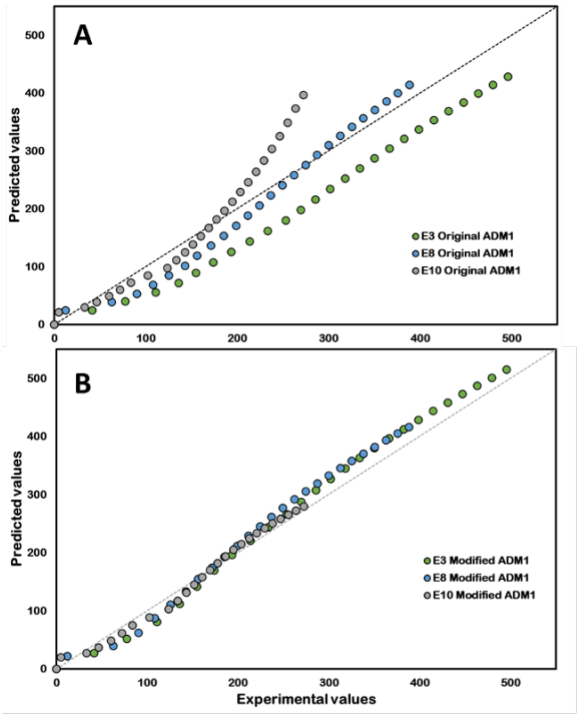

Figure 5. A) Parity plot for the simulation of the original ADM1 for the experiments shown in Figure 4-A. B) Parity plot for the simulation of the modified ADM1 for the experiments shown in Figure 4-B.

Table 6. Comparison of RMSEP test of the original ADM1 model and the modification proposal.

Experiment	RMSEP ADM1 Original	RMSEP ADM1 Modified
E1 (6:70:24)	71.42	9.8
E2 (48.5:1:50.5)	32.4	25.64
E3 (29.7:70:0.3)	74.34	22.7
E4 (6:5:89)	39.11	33.76
E5 (87:12.7:0.3)	118.81	65.01
E6 (20:40:40)	117.33	64.4
E7 (58.4:41.4:0.3)	118.04	52.25
E8(87:1:12)	27.24	24.52
E9 (6:37.5:56.5)	93.09	35
E10 (10:1:89)	14.84	10.98

ADM1: Anaerobic Digestion Model No.1.
RMSEP: root mean square error of prediction

Table 7. Hydrolysis coefficients for real substrate mixture simulation.

Parameter	Description	Value [d^{-1}]
k_{hyd_X1}	Hydrolysis coefficient of carbohydrates	0.25
k_{hyd_X2}	Hydrolysis coefficient of proteins	0.20
k_{hyd_X3}	Hydrolysis coefficient of lipids	0.10

The initialization values of the model are presented in Table 7. The results shown in Figure 6-A present the

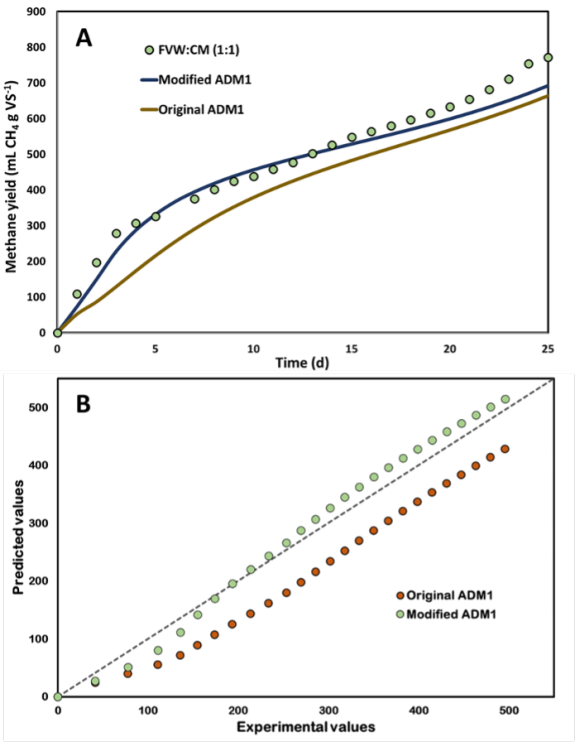

Figure 6. A) ADM1 simulations of cow manure (CM) and fruit and vegetable wastes (FVW) (1:1 %VS). B) Parity plot for the simulations of the real substrate mixture. Experimental results from (Miramontes-Martínez *et al.*, 2021).

adjustment of the original ADM1 model and the model with the proposed modification (Equations 4 - 5). The proposed model precisely adjusts the experimental data compared to the original ADM1 (Figure 6-B). These results prove the validity of the proposed modification to the ADM1.

Conclusions

In this work, the synergistic interactions during the hydrolysis steps in anaerobic digestion of carbohydrates, proteins and lipids and its influence in the CH_4 yield were studied, alongside with the validation of a modification to the ADM1 capable of representing these effects. The most adequate ratio for an anaerobic digestion process was 29.7%, 70.0% and 0.3% of carbohydrates, proteins, and lipids, respectively, showing the highest yield of 428.28 mL CH_4 g VS in this work. It was found that the protein content of the substrate promotes synergistic interactions, reaching a co-digestion index value up to 3.25, assisting an effective degradation of carbohydrates and lipids. The incorporation of the co-digestion index as a dynamic variable in the hydrolysis stage of the ADM1 model increases the predictive capacity of the model, reducing the value of the root mean square error (RMSEP) by up to 55.7%. When subjected to mixtures of real substrates, the proposed model precisely adjusts the experimental data. These results prove the validity of the proposed modification to the ADM1 and its functionality with real substrate mixtures.

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Nomenclature

AD	Anaerobic digestion
VFA	Volatile fatty acids
C:P:L	Carbohydrates:Proteins:Lipids
ADM1	Anaerobic digestion model No. 1
VS	Volatile solids
BMP	Biochemical methane potential
CI	Co-digestion index

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