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NEW MODEL OF HYDROLYSIS IN THE ANAEROBIC CO-DIGESTION OF BOVINE MANURE WITH VEGETABLE WASTE: MODIFICATION OF ANAEROBIC DIGESTION MODEL No. 1

NUEVO MODELO DE HIDRÓLISIS EN LA CO-DIGESTIÓN DE ESTIÉRCOL DE VACA CON RESIDUOS VEGETALES: UNA MODIFICACIÓN DEL ANAEROBIC DIGESTION MODEL No. 1

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Abstract

The anaerobic digestion processes are a sustainable strategy for manure (M) management. However, due to its structural characteristics, this substrate leads to low biogas yields. A solution strategy is its co-digestion, where mathematical models are a fundamental tool for the selection of suitable co-substrates. In this work, a new model of co-digestion and of the disintegration and hydrolysis stages, following the classical Anaerobic Digestion Model No.1 (ADM1) structure, is presented. In the proposed model, substrate-microorganism relationships are represented using the Contois equation. The model was validated via the anaerobic co-digestion of vegetable (V) and M with variations in the V/M ratio in the feed. The reaction system consisted of a continuously stirred tank reactor with an operating volume of 5L, a hydraulic retention time of 20d, and a constant temperature of 35°C. The highest biogas yield was observed at a feed ratio of 1:1, lower values of this ratio decrease the biogas yield. The model explains the low digestibility of M as a consequence of unfavorable substrate-microorganism relationships, which cause disintegration to become a limiting stage in the process. The biogas production in the new model is primarily associated with carbohydrate degradation, as well as low concentrations and rapid consumption of intermediary metabolites, which do not favor the proliferation of acetanogenic or methanogenic communities.

Keywords: anaerobic digestion, ADM1, co-digestion, hydrolysis, cattle manure.

Resumen

Los procesos de digestión anaerobia son una estrategia sustentable para la gestión del estiércol (M); sin embargo, debido a sus caracteristicas estructurales, este sustrato conduce a bajos rendimientos de biogás. Una estrategia de solución es la co-digestión, donde los modelos matemáticos son una herramienta fundamental para la selección de co-sustratos adecuados. En este trabajo se presenta un nuevo modelo de co-digestión y de las etapas de desintegración e hidrólisis siguiendo la estructura clásica del *Anaerobic Digestion Model No.1* (ADM1). En el modelo propuesto, relaciones de sustrato-microorganismo son representadas usando la ecuación de Contois. El modelo fue validado mediante la co digestión anaerobia de vegetales (V) y M con variaciones en la relación de alimentación V/M. Como sistema de reacción se consideró un reactor tipo tanque agitado con un volumen de operación de 5L, un tiempo de retención hidráulico de 20d y una temperatura constante de 35°C. El mayor rendimiento de biogás se presentó a una relación de alimentación de 1:1, valores más bajos de esta relación decrementan el rendimiento de biogás. El modelo explica la baja digestibilidad de M como una consecuencia de relaciones desfavorables entre sustrato-microorganismo, las cuales provocan que la desintegración sea la etapa limitante en el proceso. La producción de biogás en el nuevo modelo está principlamente asociada a la degradación de los carbohidratos, así como a las bajas concentraciones y rápido consumo de metabolitos intermediarios, los cuales no favorecen la proliferación de comunidades acetogénicas y metanogénicas.

Palabras clave: digestión anaerobia, ADM1, co-digestión, hidrólisis, estiércol de vaca.

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1 Introduction

Anaerobic digestion (AD) is a multistage process in which a consortium of microorganisms acts upon composite organic matter to produce biogas (CH_4, CO_2, H_2, and H_2S). AD is a lucrative waste management technique that can use cattle manure to produce electricity and fertilizers (Maharaj *et al*., 2018; Sanchez-Herrera *et al*., 2018; Flores-Estrella *et al*., 2016). Cattle manure is an abundant agricultural byproduct that consists primarily of lignocellulosic materials, as well as small fractions of non-structural carbohydrates, proteins, fats and minerals (Tufaner and Avşar 2016; McInerney 1988). Biogas production processes that use manure have low yields; these yields depend on both the configuration and operation of the reactor and the composition of the substrate used (Cantrell *et al*., 2008). According to (Rico *et al*., 2007), the high lignocellulosic material content (40-50%) in manure makes it 45-50% biodegradable, a condition that obstructs microbial action (Zhang *et al*., 2013; Boe and Angelidaki 2009). Li *et al*., (2009) found that AD is unstable when manure is used as a mono-substrate because it has a low C/N ratio (Rico *et al*., 2007; Zhang *et al*., 2013; Boe and Angelidaki 2009; Li *et al*., 2009). Previous studies suggest that an efficient AD system should have a C/N ratio between 13.9 and 19.6 (Kumar *et al*., 2010; Zhu 2007). Another factor that affects biogas yield in manure-fed anaerobic digesters is the presence of sedimented and floating phases of non-degraded insoluble materials (Castrillón *et al*., 2011; Bekkering *et al*., 2010; Tafdrup 1995).

To increase biogas productivity, as well as transform and stabilize manure via AD, co-substrates that complement the nutritional needs of microbial communities can be added; this improves the AD process and increases its economic viability (Hagos *et al*., 2017; Tufaner and Avşar 2016; Macias-Corral *et al*., 2008).

Anaerobic digestion and co-digestion of manure result in a heterogeneous reactive system catalyzed by a diverse community of microorganisms, which transform insoluble, complex substrates into simple molecules such as CH_4, CO_2 and H_2 through a cascade of reactions and intermediates; this phenomenon can be represented by mathematical models. Anaerobic Digestion Model No.1 (ADM1) is the most widely used and complete model of anaerobic digestion and considers substrate-microorganism-product kinetic expressions in sequential stages of degradation: disintegration, hydrolysis, acidogenesis, acetogenesis and methanogenesis. Physicochemical and interfacial mass transfer equations are utilized within this model (Batstone *et al*., 2002). The implementation of ADM1 is not a simple task; it requires the specification of 33 variables, 15 of which can be determined through the characterization of the fed substrate (Klimiuk *et al*., 2015).

The ADM1 has been modified to include several phenomena, some of them are the incorporation of phosphorus, sulfur, and iron (Flores-Alsina *et al*. 2016), as well as phenolic compounds (Fezzani *et al*., 2009); precipitation/dissolution (Maharaj *et al*. 2018); sulfates reduction (Fedorovich *et al*., 2003); dark fermentation (Gadhamshetty *et al*. 2010); and the co-digestion process. The latter being one of its main structural deficiencies. The original ADM1 considers the fractions of the components in the feed fixed in order to support continuous or semi-continuous processes. This shortcoming was addressed by Zaher *et al*., (2009), who proposed an update to the composition of the material fed to the digester by mixing substrate and co-substrate equations. Another notable limitation of the ADM1 is that the stages of particulate matter disintegration and hydrolysis are not associated with the action of the microorganisms present. The ADM1 assumes that the hydrolysis stage is independent of the colonization by hydrolytic bacteria, whose presence is considered to be in excess (Mottet *et al*., 2013). This obviously represents an oversimplification since, according to Rotter *et al*., (2008), biomass does not always have direct access to the substrate.

For both the disintegration and hydrolysis processes, ADM1 proposes first order degradation kinetics. Eq. (1) represents the disintegration of the compounds (X_c), insoluble particles composed of fractions of carbohydrate macromolecules proteins, lipids and inert materials ($f_{Xi,Xc}$), in the presence of microorganisms. The Eq. (1) is not specific to the chemical nature of the organic matter nutrients; the disintegration constant k_{dis} is associated with its average biodegradability (Hagos *et al*., 2017). Eq. (2) represents the hydrolysis phenomena for each macromolecule species released during the disintegration (X_i, i: carbohydrate, protein and lipid polymers), where $k_{hyd,Xi}$ is the hydrolysis constant for each species. As a result of these reactions, bioavailable molecular groupings are created for the microorganisms in the form of soluble monomers such

as monosaccharides, amino acids and fatty acids.

$$\frac{dX_c}{dt} = -k_{dis}X_C \tag{1}$$

$$\frac{dX_i}{dt} = f_{X_i,X_C}k_{dis}X_C - k_{hyd,X_i}X_i \tag{2}$$

In ADM1, disintegration and hydrolysis trigger acidogenic, acetogenesis and methanogenesis reactions and are represented by 19 differential equations -one per species present in the liquid matrix- mediated by 7 groups of microorganisms that can perform specific actions. (Batstone *et al*., 2002) performed a sensitivity analysis and demonstrated that the rate constants for disintegration and hydrolysis are the most sensitive. Vavilin *et al*., (1996) used the Contois model (Eq. (3)) to represent the hydrolysis of suspended biodegradable macromolecules X, carried out by active microorganisms X_{hyd} that colonize the surface of the particles, where k_m is the maximum specific rate of hydrolysis and K_S is the half saturation constant. The authors did not consider the disintegration stage in their study.

$$\frac{dX}{dt} = -k_m X_{hyd}\frac{X/X_{hyd}}{K_S + X/X_{hyd}} \tag{3}$$

Using Eq. (3), Mottet *et al*., (2013) proposed to modify the original structure of the ADM1, substituting Eqs. (1)-(2) for (4)-(5); they also assumed that the solids X_c are divided into fast and slow degradation materials, X_{cr} and X_{cs}, respectively. These authors introduced 5 new groups of microorganisms: 2 associated with the disintegration of each fraction of organic matter (j) and 3 associated with the hydrolysis of proteins, lipids and carbohydrates (i). In their proposal, Mottet *et al*., (2013) did not specify the nature of those microorganisms with the ability to facilitate disintegration and hydrolysis. These assumptions make the ADM1 model more complex by introducing 7 dynamic variables and 10 new kinetic parameters.

$$\frac{dX_{C_j}}{dt} = -k_{m,X_{cj}}X_{dis,X_{cj}}\frac{X_{C_j}/X_{dis,X_{Cj}}}{K_{SX_{Cj}} + X_{C_j}/X_{dis,X_{Cj}}} \tag{4}$$

$$\frac{dX_i}{dt} = f_{X_iX_C}\sum_{j=1}^{2}\left(-\frac{dX_{C_j}}{dt}\right) - k_{m,X_i}X_{hyd,X_i}\frac{X_i/X_{hyd,X_i}}{K_{S,X_i} + X_i/X_{hyd,X_i}} \tag{5}$$

The present study proposes a new model of disintegration, hydrolysis, and co-digestion for ADM1 which considers the following phenomena: i) the association between specific hydrolytic enzymes and the concentration of microbial populations present in the digester, and ii) the constitutive fractions of organic matter (carbohydrates, proteins, lipids, and inert) as dynamics variables.

2 Materials and methods

2.1 Mathematical modeling

Figure 1 presents an idealized summary of the phenomena that occur during AD, as proposed by Batstone *et al*., (2002). In the original ADM1 (black lines), disintegration and hydrolysis are assumed to be independent of the hydrolytic bacterial colonization, and the biomass responsible for producing enzymes is available in excess for immediate and spontaneous contact with the particles. The proposed modification to ADM1 (represented in red color) considers that microorganisms that degrade carbohydrates X_{su} participate in disintegration since the composites (vegetable and manure) are mainly composed of carbohydrate polymer chains. Later, carbohydrates, proteins and lipids macromolecules (X_{ch}, X_{pr} and X_{li}, respectively) are hydrolyzed by microorganisms that degrade their monomers: monosaccharides (X_{su}), amino acids (X_{aa}) and long chain fatty acids (X_{fa}), respectively. The kinetic models describing these modifications are presented in Equations 6-9, which are based on the Contois equation.

$$\frac{dX_C}{dt} = -k_{m,X_C}X_{su}\frac{X_C/X_{su}}{K_{S,X_C} + X_C/X_{su}} \tag{6}$$

$$\frac{dX_{ch}}{dt} = f_{X_{ch},X_C}\left(-\frac{dX_C}{dt}\right) - k_{m,X_{ch}}X_{su}\frac{X_{ch}/X_{su}}{K_{S,X_{ch}} + X_{ch}/X_{su}} \tag{7}$$

$$\frac{dX_{II}}{dt} = f_{X_{li},X_C}\left(-\frac{dX_C}{dt}\right) - k_{m,X_{li}}X_{fa}\frac{X_{li}/X_{fa}}{K_{S,X_{li}} + X_{li}/X_{fa}} \tag{8}$$

To suppress the assumption made in the ADM1 that the constitutive fractions (f_{X_i,X_c}) of the composites in the digester and in the feed are equal and constant, a dynamic mass balance was proposed to evaluate these fractions and compositions as a function of the daily feed (which can change during de AD process) and the composition of the reactive medium within the digester. The proposed model is represented by Eqs. (10)-(12),

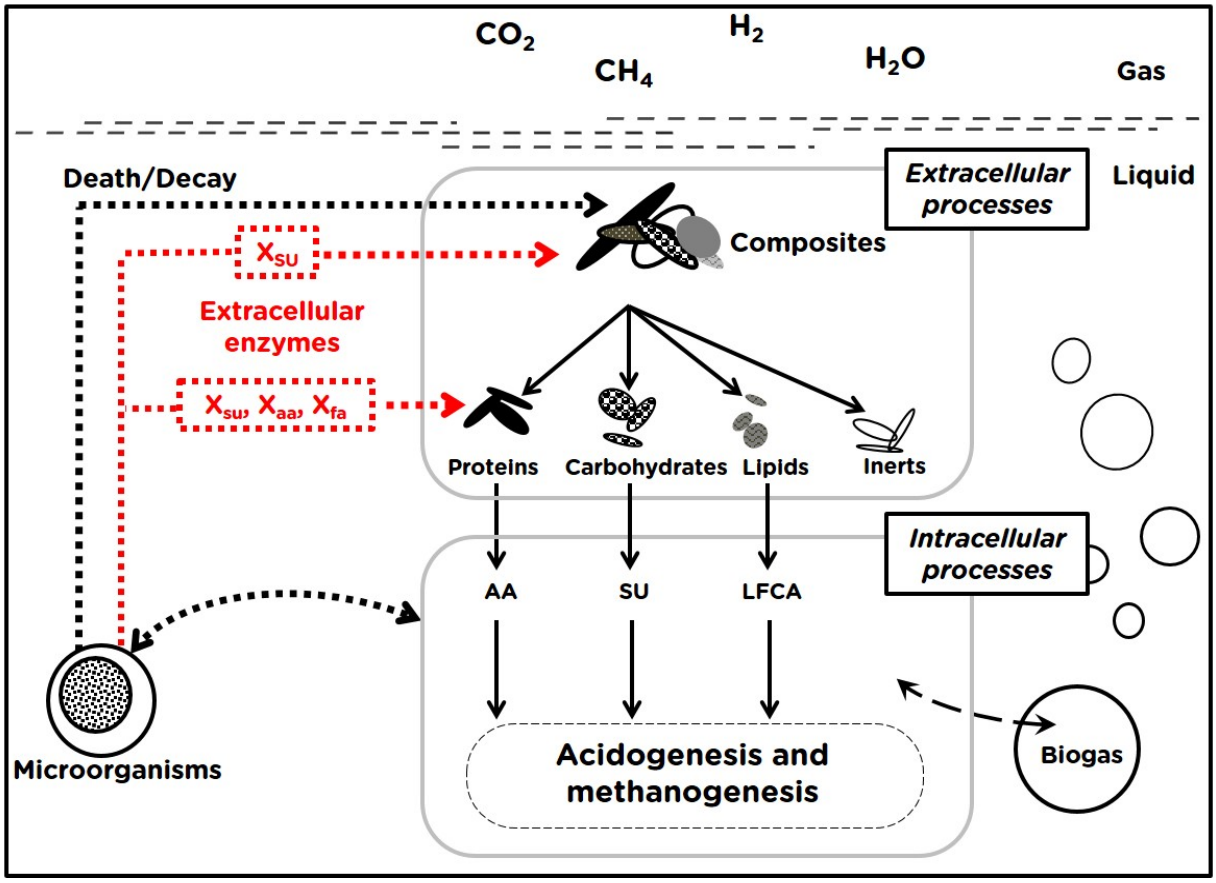

Fig. 1. Idealized, simplified model of anaerobic digestion. Black lines: ADM1 Batstone *et al*., (2002). Red lines: proposed modification.

$$\frac{df_{X_i,X_C}}{dt} = \frac{q_{in}}{V_L}\left[\frac{X_{C(in,k)}}{X_C} f_{X_i,X_C(in,k)} - f_{X_i,X_C}\right] - \frac{f_{X_i,X_C}}{X_C}\frac{dX_C}{dt} \quad (9)$$

$$X_{C(in,k)} = r_{s/Co} X_{C,S} + (1 - r_{S/Co}) X_{C,Co} \quad (10)$$

$$f_{X_i,X_C(in,k)} = \frac{X_{C,S} F_{X_i,X_C(S)} + X_{C,co} f_{X_i,X_C(Co)}}{X_{C(in,k)}} \quad (11)$$

where X_C is the composite concentration in the digester (evaluated by Eq. (6)); $X_{C(in,k)}$ and $f_{X_i,X_c(in,k)}$ are the concentrations of the composite feeds and their respective constitutive fractions in each feed period k (a description of the feeding periods is presented in section 2.2); $r_{S/Co}$ is the ratio between substrate and co-substrate in the feed (such as is shown in second column of Table 1); $X_{C,S}$, $X_{C,Co}$, $f_{X_i,Xc(S)}$, and $f_{Xi,Xc(Co)}$ are the concentrations of the composites in substrate and co-substrate and their respective constitutive fractions; and q_{in} and VL are the feed input flow and the volume of the digester, respectively.

2.2 *The anaerobic co-digestion system*

Co-digestion of domestic vegetable waste and bovine manure was studied in a 7 L stirred tank reactor (Applikon). The operating volume was 5 L; the stirring speed was 200 rpm, produced using Rushton stirrers; and the temperature was maintained at 35°C through hot water recirculation. The reactor was originally charged with 4.5 L of sewage sludge from a local pig farm plus 0.5 L of slurry of ground vegetables. After 7 days of stabilization, the experiment began with a daily feed of 0.25 L; the same volume was withdrawn as product and sample (hydraulic retention time of 20 days). The feed formulation was modified weekly according to Table 1. The vegetable waste was obtained from a local market and consisted primarily of tomatoes, onions, potatoes, carrots and lettuce. The manure was obtained from a local dairy with Holstein cows. Both substrates were separately ground in a domestic blender and filtered through a No. 20 mesh.

Table 1. Digester feed regime.

Week (k)	% Vegetables-Manure	OLR (g VS L^{-1} d^{-1})
Stabilization	100-0	1.41
I	100-0	3.3
II	75-25	3.15
III	50-50	2.95
IV	25-75	2.8
V	0-100	2.65

OLR: Organic Load Rate

The total solids (TS) concentration in the suspensions was adjusted to approximately 40 g/L. The experimental run was performed in duplicate. In accordance with the literature, it is not necessary to add additives and trace elements to the reactor in co-digestion processes with manure, as this substrate contains many of the micronutrients necessary by the anaerobic biomass (Zhang *et al*., 2007; Pobeheim *et al*., 2010; Raposo *et al*., 2012; Labatut *et al*., 2011; Lisboa *et al*., 2013).

2.3 Experimental determinations

The biogas produced was measured using a water column displacement system. The reactor substrate and effluent samples were characterized by conventional physicochemical tests as follows: moisture and TS, NMX-F-083-1986; fixed solids and volatile solids (VS), NMX-AA-034-SCFI-2001; protein, NMX-F-068-1980; fats, NMX-AA-005-SCFI-2013; raw fiber, NMX-F-090-S-1978; and acidity and alkalinity, NMX-AA-036-SCFI-2001. Gravimetric techniques were employed to determine the amounts of soluble and insoluble solids, using Whatman No. 1 paper as a filter medium.

To determine the short chain volatile fatty acids (VFAs), 20 mL reactor effluent samples were centrifuged at 10,000 rpm for 5 min and then filtered through 0.45 μm Millipore cellulose acetate membranes. VFA concentrations were determined using a gas chromatograph with a PerkinElmer® Clarus® 500 GC-FID flame ionization detector. A PerkinElmer® Elite-5 capillary column with a length of 30 m, an internal diameter of 0.53 mm and a film thickness of 5 μm was used. The temperature at the injection port and in the detector was 250 oC. The following temperature program was implemented in the furnace: 100 ºC for 2 minutes and a ramp of 10 ºC per minute up to 150 ºC. Standard solutions of acetic, propionic, butyric and valeric acid (Sigma-Aldrich) were used in concentrations of 0-3000 mg L^{-1} to estimate VFA levels.

2.4 Determination of kinetic parameters

The kinetic parameter values for the new disintegration and hydrolysis model are presented in Table 2. Parameters for the disintegration process were proposed by this study; parameters for the hydrolysis step were taken from the literature. The value of remaining kinetic parameters -catabolic and cellular performance- as well as those corresponding to the mass transfer, physicochemical and inhibition models that correspond to the post-disintegration and hydrolysis processes, were fixed as in the original ADM1 (Batstone *et al*., 2002). No adjustments were made to the kinetic parameters in the present study; with the purpose to study the model's ability to explain experimentally observed phenomenology.

2.5 Numerical integration

The differential equations that represent the substrate consumption rates, the metabolite production, and the growth of the seven microbial groups included in the ADM1 structure were solved through a fourth order Runge-Kutta method considering a time integration step of 1E-06 days. The model for the determination of pH and the species in chemical equilibria was solved using the bisection method. In order to numerically validate the results, a dynamic global mass balance was implemented alongside the model. The FORTRAN 90 programming language and the Compaq Visual Fortran compiler were used to implement and solve the numerical structure. The simulation results were sampled at intervals of 0.5 days and were transferred to a Microsoft Excel spreadsheet. This solution method was taken from Rivas-García *et al*., (2013).

Table 2. Parameter values utilized in the hydrolysis model.

Parameter	Value
$k_{m,Xc}$	3.0^a
$K_{s,Xc}$	30.0^a
$k_{m,Xch}$	10.0^b
$K_{s,Xch}$	0.5^b
$k_{m,Xpr}$	10.0^b
$K_{s,Xpr}$	0.5^b
$k_{m,Xli}$	10.0^b
$K_{s,Xli}$	0.5^b

Units: $k_{m,esp}$=kg COD_S (kg COD_X d)$^{-1}$; $K_{s,esp}$= kg COD_S m^{-3}

a: this study; b: Mottet *et al*., (2013).

3 Results and discussion

Table 3 describes the inoculum, vegetables and manure suspensions used to formulate the feed for the digester. The inoculum contained a high percentage of primarily insoluble fixed solids (80.44% of total solids) as a result of its extraction from the bottom of a porcine farm drainage. The vegetables were a rich source of nonstructural carbohydrates, such as starches, fructans, and simple sugars, while the manure was primarily comprised of fibrous materials.

The initial conditions and factors $f_{Xi,Xc}$ of each substrate are shown in Table 4. These data were obtained by analyzing Table 3 and reviewing the literature.

Figure 2 shows the experimental data for daily biogas production during the different feed periods k identified in Table 1. The yields during these periods were 148, 302, 311, 284 and 60 mL of biogas g^{-1} of VS; the specific production rates were 0.325, 0.698, 0.731, 0.609 and 0.210 m^3 m^{-3} d^{-1} for k = I – V, respectively. Both experiments performed best in terms of yield and rate of production during week III (combination 50% vegetables and 50% manure). In manure fed lagoon-type digesters, average biogas production rates are reported at 0.1822 and fall within a range of 0.011 to 0.61 m^3 m^{-3} d^{-1}; the retention time for these digesters is typically 2 months (Safley and Westerman 1988, 1992; Park and Craggs 2007). In manure fed agitated reactors operated semi-continuously, production rates range from 1.04 to 1.45 m^3 m^{-3} d^{-1} for retention times of 10-20 d [33] and 0.78 m^3 m^{-3} d^{-1} for retention times of 21 d (Pain *et al*., 1984).

The literature indicates that AD results in different biogas yields when manure and vegetables are digested separately compared to when they are co-digested. Yan *et al*., (2017) have studied biogas yields for a large variety of plants and identified productivity ranging from 65 to 241 mL g^{-1} of VS; these values are generally lower than those reported for manure digestion in other studies: 363, 258 and 133 mL g^{-1} of VS (El-Mashad and Zhang 2010; Lehtomäki *et al*., 2007; Macias-Corral *et al*., 2008). However, when manure is co-digested with other substrates, productivity improves substantially. For example, one study found production rates of 221 and 366 mL g^{-1} of VS for manure and manure-beet 50%-50%, respectively (Lehtomäki *et al*., 2007); another study found production rates of 466 and 553 mL g^{-1} of VS for manure and manure-organic municipal waste, respectively (Hartmann and Ahring 2005). This increase in biogas productivity may be due to the release of NH+4 during the degradation of amino acids from the hydrolysis of proteins present in the manure, which improves the buffer capacity of the medium and the C/N when combined with VFAs (Zhang *et al*., 2013; Kumar *et al*., 2010).

Figure 3 presents the results of this study's original ADM1 simulations, including the biogas production rates from the anaerobic digestion of vegetables and manure with different k_{dis} disintegration constants. As k_{dis} increases, the biogas production rates increase and then this increment is more slowly. The data indicate that maximum biogas productivity occurs near the feed period k = III.

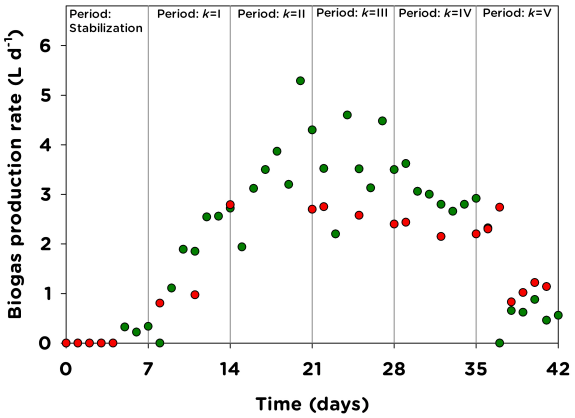

Fig. 2. Biogas production rates during co-digestion of vegetable-manure mixtures. The green circles correspond to experiment 1; the red circles correspond to experiment 2.

Table 3. Characterization of inoculum and substrates fed to the digester.

Parameter	Units	Inoculum	Manure	Vegetables
Total solids	g L^{-1}	29.39	38.81	51.67
Fixed solids	g L^{-1}	21	9.1	3
Volatile solids	g L^{-1}	8.34	30.99	49.07
Dissolved solids	g L^{-1}	0.19	0.1	0.54
Carbohydrate	g L^{-1}	3.51	4.44	42.92
Protein	g L^{-1}	0.71	5.73	2.38
Lipid	g L^{-1}	0.96	2.1	2.41
Fiber	g L^{-1}	3.12	18.52	3.3
pH	-	7.2	7.47	5.12
Alkalinity	mg $CaCO_3$ L^{-1}	6649	6800	1956

Table 4. Initialization parameters for ADM1.

	Symbol	Units	Inoculum	Manure	Vegetables
Species					
Composite	X_c	gCOD L^{-1}	12.8363	53.161	66.591
Monosaccharides	S_{su}	gCOD L^{-1}	0.1685	0.06	0.553
Amino acids	S_{aa}	gCOD L^{-1}	0.0344	0.04	0.031
Inorganic carbon	S_{IC}	mol L^{-1}	0.1526[a]	0.0150[a]	2.36E-4[b]
Inorganic nitrogen	S_{IN}	mol L^{-1}	0.1302[a]	0.0195[a]	2.36E-4[b]
Disintegration factors f					
Carbohydrate fraction of X_c	$f_{Xch,Xc}$	-	0.3253	0.1889	0.767
Protein fraction of X_c	$f_{Xpr,Xc}$	-	0.0785	0.1531	0.0508
Lipids fraction of X_c	$f_{Xli,Xc}$	-	0.2168	0.1146	0.105
Inert fraction of X_c	$f_{Xi,Xc}$	-	0.3791	0.5435	0.0773

a: Rosen and Jeppsson (2006); b: USDA (2017)

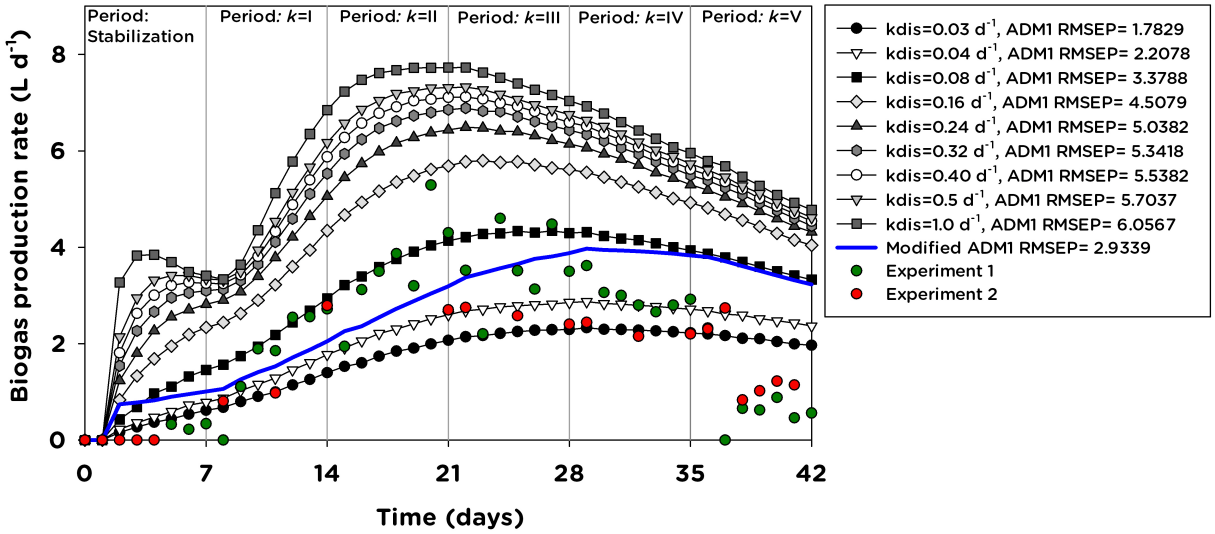

Fig. 3. Biogas production rates during co-digestion of vegetable-manure waste.

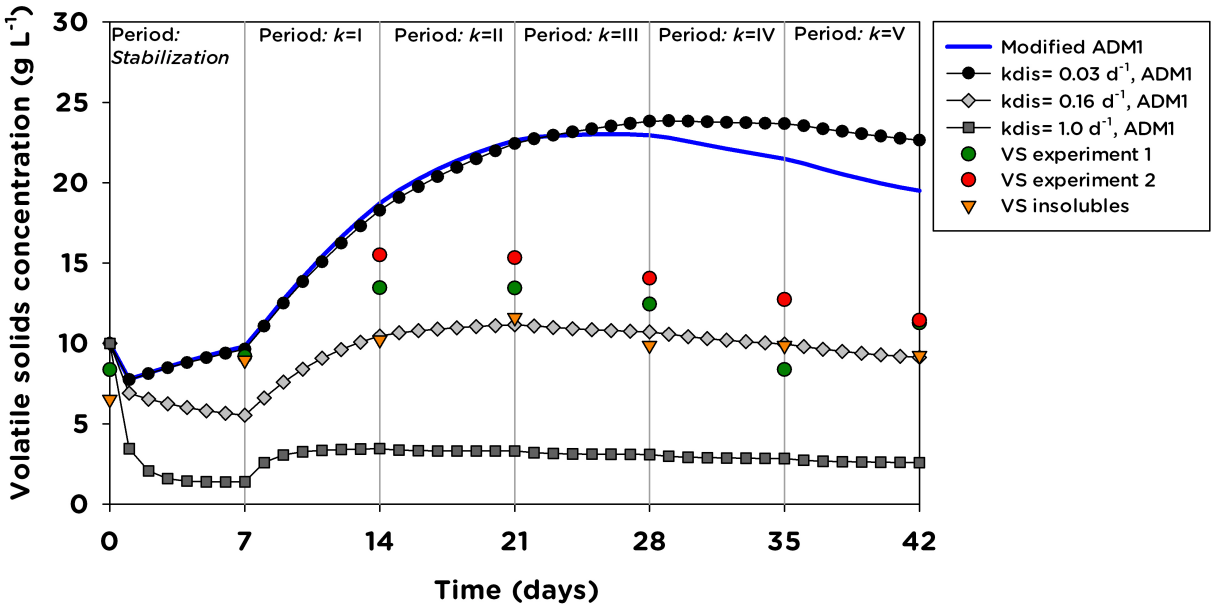

Fig. 4. Concentration of volatile solids (VS) and insoluble VS during the co-digestion of vegetable-manure mixtures; disintegration stage.

These results support a recommendation for the feed composition that will achieve the highest biogas production in this study system. The experimental data fall within the simulation curves corresponding to the interval k_{dis} = 0.03-0.16 d^{-1}.

The modified model shown in Figure 4 describes low rates of disintegration and predicts that, in experiments 1 and 2, the composites are maintained as insoluble particles. In Figure 3, the biogas production curve generated by the modified model regularly represents the experimental data, as supported by its location between the k_{dis} 0.04 and 0.08 d^{-1} simulation curves for the original ADM1. The accuracy of models prediction was assessed by the root mean square error of prediction (RMSEP), the values of these parameters are shown in Figure 3. Both models (ADM1 and modified ADM1) exhibit a lack of adjustment to the experimental data in the first period as well as the last two periods. The first period is lag phase, with no significant biogas production. The stages of disintegration and hydrolysis, represented by first order equations in the ADM1 and by expressions derived from the equations of Monod and Michaelis-Menten (Schügerl 1985) in the modified ADM1, do not consider this period. In the periods $k = IV$ and V, there is a greater abundance of manure in the feed, as well as a decrease in the biogas production, both experimentally and in the simulations. Observations of the fed suspension compositions (Tables 1, 3, and 4) suggest that biogas production is associated with the amount of VS fed daily. For example, in the periods from 100% vegetable ($k = I$) to 100% manure ($K = V$), the following values were obtained: 85.05, 76.91, 68.77, 60.63 and 52.50 g of VS; however, when compared with biogas production rates, no proportional relationship could be found.

The VS and insoluble VS concentrations identified during the digestion experiments are presented in Figure 4. The majority of the VS exist as insoluble composites in the reactor. VS decrease when feeding begins because this substrate contains less of these materials, causing gradual decreases in the OLR (Table 1). Different k_{dis} values in the original ADM1 represent VS in different biodegradable forms, which assumes that organic matter is susceptible to degradation by microbial processes (Madsen *et al*., 2001). When k_{dis} = 0.16 d^{-1}, the AMD1 adequately represents the experimental data; however, this parameter is arbitrary and is always adjusted without any satisfactory physical interpretation. In the case of the modified model, its description adjusts to the experimental trend but does not have a good fit; however, in this case, the disintegration is associated with the dynamics of the microbial communities, in particular those microorganisms that degrade carbohydrates, X_{su}. In Eq. (6), the rate of disintegration is a function of the substrate/microorganism ratio X_c/X_{su}.

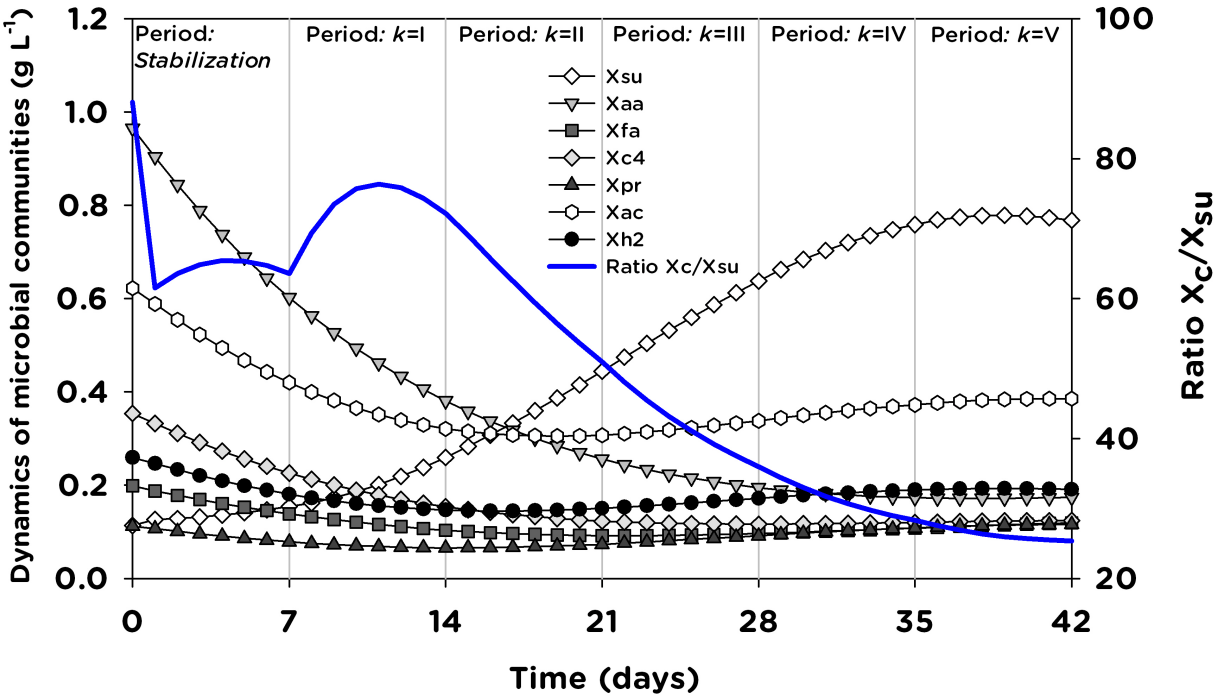

Fig. 5. Microbial population dynamics during co-digestion of vegetable-manure mixtures.

This ratio can be interpreted as the degree of colonization and the activity of microorganisms on the substrate. If this ratio is high with respect to the saturation constant $K_{S,Xc}$, disintegration is primarily a function of the concentration of microorganisms, as is the case of limiting reactants in chemical kinetics. On the other hand, if the ratio X_c/X_{su} is small, the speed of the reaction will decrease due to high competition between microorganisms. Changes in biogas production rates are associated with the amount and biodegradability of the fed substrates, as well as microorganism population dynamics during AD.

Figure 5 illustrates the microbial communities' dynamics. As can be seen, only monosaccharide (X_{su}) degraders proliferated during AD. We primarily used the Monod equation (Schügerl, 1985) in the kinetic models of microorganism growth because microbial growth in this equation is a function of the microorganism and substrate concentrations, as well as their mutual affinity. In ADM1, these models also include terms to represent inhibition phenomena. In the AD process studied in this article, the most abundant substrates were carbohydrates derived primarily from vegetable sources. The fermentation of glucose -as a monosaccharide model in the ADM1- which primarily generates acetic acid, was the metabolic route that generated the most ATP molecules; in other words, it is a route with high metabolic flow (Batstone *et al.*, 2002). In addition, the degraders of amino acids (X_{aa}) and fatty acids ($X_{fa,c4,pr,ac}$) significantly reduced their population; under this condition, the primary source of biogas synthesis is carbohydrate degradation. The decrease in microorganism concentrations was not due to the washing of the reactor; the dilution rate for this experiment was 0.05 d^{-1}, which is significantly lower than the specific growth rates for the microbial communities (6-50 d^{-1}) reported by Batstone *et al.*, (2002).

Figure 5 shows the relationship between X_c/X_{su}; when this ratio is high, the rate of disintegration depends primarily on the concentration of microorganisms, but at low values, it indicates the condition of a limiting substrate. Furthermore, as the proportion of manure in the feed increases, the X_c/X_{su} ratio decreases because of the lower VS input. The average ratio was also estimated for the hydrolysis of carbohydrates, proteins and lipids and was identified as follows: X_{ch}/X_{su} = 0.0591, X_{pr}/X_{aa} = 0.0137 and X_{li}/X_{fa} = 0.02119, respectively. Thus, the limitation of the specific substrate in the secondary stage is clearly more severe. The growth of microorganism communities is limited by the low bioavailability of substrates.

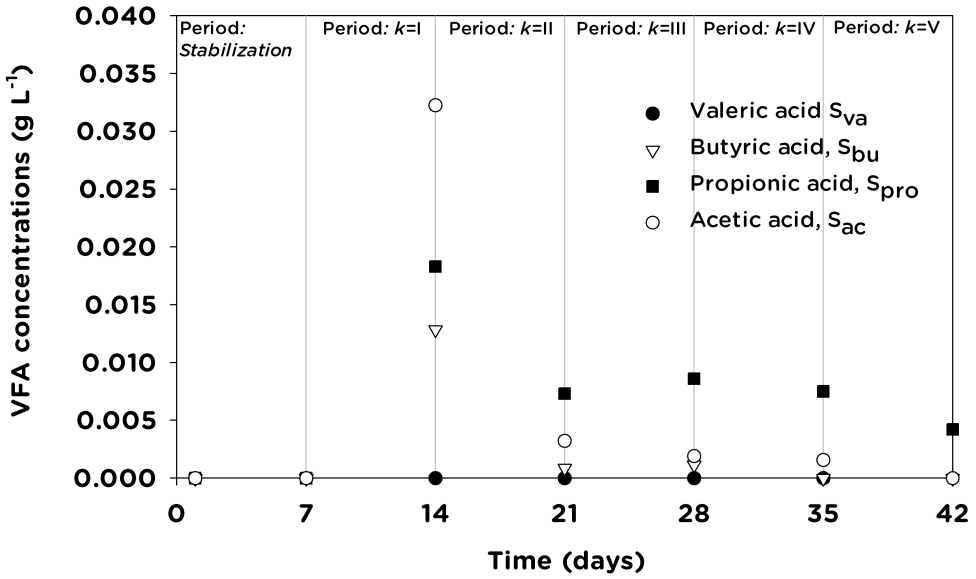

Fig. 6. Production of short chain volatile fatty acids (VFA) during co-digestion of vegetable-manure mixtures.

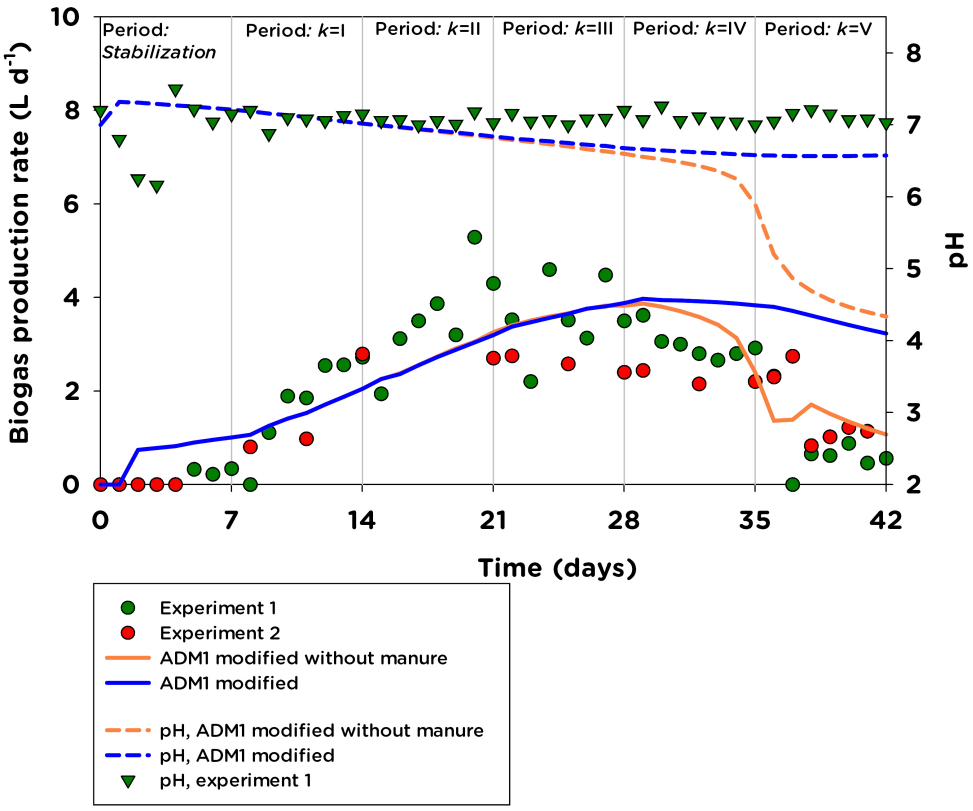

Fig. 7. pH profiles and biogas production rates during co-digestion of vegetable-manure mixtures. Acidification scenario.

To corroborate this argument, short-chain VFAs were sought as intermediates in the acetogenesis and methanogenesis stages. Figure 6 reveals that the VFA concentrations are very low, which implies that their transformations into acetic acid and methane occur rapidly; there is not a significant accumulation of intermediates, which favors the growth of acidogenic and methanogenic bacteria.

Figure 7 shows the simulated biogas production rates and the pH of the system, including an acceptable adjustment compared to the experimental pH. In addition, in the hypothetical case where only vegetable waste is used, the biogas generated comes entirely from this substrate and from the inoculum (orange profiles). After the fourth week, a substantial decrease in the biogas production rate appeared to occur, as reflected in the decrease in the 17% accumulated amount in relation to vegetable-manure co-digestion. This primarily affected the pH, which, in the absence of manure in the feed, dropped drastically. Acidification present in the simulation is explained by the fact that in the absence of manure, the alkalinity from the NH_4^+ released during the fermentation of the amino acids from the hydrolysis of the protein material is lost, as studied by Zhang *et al*., (2013). In addition, manure has a rich flora of microorganisms, with acetic acid (X_{ac}) degrading agents primarily responsible for changes in acidity in the medium (Rivas-García *et al*., 2013).

Conclusions

During the co-digestion of vegetables and manure in a semi-continuous regime, the best yield and productivity of biogas (311 mL of biogas g^{-1} of VS and 0.731 m^3 m^{-3} d^{-1}, respectively) were reached using a 1:1 ratio of vegetable:manure in the feed. The ADM1 and the proposed model have the ability to predict and recommend this combination. The yield and productivity of biogas decreased when manure was the dominant feed. The modified model explains this phenomenon as a function of the relationship between substrate and microorganism concentrations. Disintegration is the control stage of the reactive system with two periods: i) microorganisms were the limiting reagent when vegetables were the dominant feed, and ii) substrates were the limiting reagent when manure was the dominant feed. Biogas production is primarily associated with the degradation of carbohydrates. Intermediate metabolites, such as short chain VFAs, were present in very low concentrations, a condition that limits the proliferation of degradation microorganisms of these acids.

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FBS.

Nomenclature

t	time, d
i	carbohydrate (ch), protein (pr) and lipid (li) polymers
k	feed period
k_{dis}	disintegration constant, d^{-1}
k_{hyd,X_i}	hydrolysis constant of X_i, d^{-1}
k_m	maximum specific rate of hydrolysis, $kgCOD_S$ $kgCOD_X^{-1}$ d^{-1}
K_S	half saturation constant, kgCOD m^{-3}
X_C	insoluble particles, kgCOD m^{-3}
X_i	macromolecule specie i released during the disintegration, kgCOD m^{-3}
X_{hyd}	microorganisms that colonize the surface of the particles, kgCOD m^{-3}
X_{C_j}	microorganisms associated with the disintegration of Xc, kgCOD m^{-3}
$X_{dis,X_{Cj}}$	insoluble particles disintegration for the microorganisms j, kgCOD m^{-3}
X_{su}	microorganisms that degrade carbohydrates, kgCOD m^{-3}
X_{aa}	microorganisms that degrade proteins, kgCOD m^{-3}
f_{X_i,X_C}	fraction of insoluble particles i in the insoluble particulate, $kgCOD_{Xi}$ $kgCOD_{Xc}^{-1}$
q_{in}	input flow, m^3 d^{-1}
V_L	volume of the digester, m^3
$r_{S/Co}$	ratio between substrate (S) and co-substrate (Co) in the feed

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