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A generalized kinetic model for anaerobic co-digestion of agro-industrial biomass mixtures improved through pretreatments or additives

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ABSTRACT

Anaerobic co-digestion is increasingly recognised as a strategy to enhance biogas production from agro-industrial biomass by exploiting synergistic interactions among multiple organic substrates. However, most existing kinetic models remain case-specific and fail to provide reliable predictions when substrate ratios, pretreatments, or additives are modified, limiting their practical applicability. This study proposes a generalised combinatorial kinetic framework for n-substrate anaerobic co-digestion. The model integrates established formulations, including the Modified Gompertz and first-order models, into a structured interaction-based approach capable of explicitly representing multi-order substrate interactions without repetition. Interaction coefficients are weighted according to substrate contributions, ensuring consistency between mono-digestion and higher-order co-digestion systems. The framework further incorporates pretreatments and nanoparticle additives through multiplicative interaction terms, allowing performance enhancements to be modelled explicitly rather than indirectly absorbed into kinetic parameters. Although the combinatorial structure can manage up to 108 interaction parameters in a three-substrate Gompertz-based system, the methodology reduces the minimum experimental requirement to only 8 BMP tests by sequentially constructing higher-order interactions from lower-order combinations. This significantly decreases experimental effort while preserving scalability. While the model currently simplifies additive concentration effects and potential inhibitory phenomena, it provides a flexible and extensible structure capable of evolving with emerging research trends. Overall, the proposed framework addresses a critical gap in anaerobic co-digestion modelling and supports a more systematic design of agro-industrial biomass co-digestion systems for bioenergy production within circular bioeconomy strategies.

1. Introduction

Anaerobic co-digestion (AcoD) refers to the simultaneous digestion of various substrates within one reaction vessel under anaerobic conditions. This approach can mitigate the limitations of anaerobic mono-digestion (AD) through the synergies and interactions between the substrates. This has been a growing field of study in the past decade as a strategy for the valorisation of agro-industrial biomass and other organic feedstocks, while also producing biogas and digestate for agricultural use (Karki et al., 2021). Compared to AD, AcoD often achieves higher methane yields, improved substrate biodegradability and more stable process performance as a result of a balanced nutrient and water content (Ferdeș et al., 2023) and (Ma et al., 2020). Additives have also emerged as an effective strategy to further enhance AD performance in recent years as reviewed by Abbas et al. (2021), Li et al. (2019) and Yun et al. (2023). The use of low-cost accelerants and mineral-based additives has demonstrated improvements both in nutrient recovery and methane production as well as digestate quality, evidencing their potential for large-scale applications (Xu et al., 2020), (Zhang et al., 2018).

Regarding its potential applications, the European Union considers biomethane a crucial renewable energy vector for climate neutrality and energy security (Calero et al., 2023) with agro-industrial residues and other organic feedstocks representing relevant AcoD substrates. Recent estimates suggest the EU could produce 64.2 billion m^3 of biomethane by 2050 using AD and thus displacing natural gas in the European energy mix (Brémond et al., 2021). A national assessment indicated Spain could generate up to 163 TWh annually from currently available biomass yet current production is only 2.74 TWh (Calero et al., 2023).

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Co-digestion projects can integrate heterogeneous feedstocks, increase biogas output, and support more efficient biomass valorisation. Indeed, Spanish and EU initiatives are incentivizing new biomethane plants, with the National Biogas Plan targeting a total generation of 20 TWh by 2030 (Ministerio para la Transición Ecológica y el Reto Demográfico, 2024), (Ministerio para la Transición Ecológica y el Reto Demográfico, 2022).

Despite the operational success and scaling potential of AcoD, most anaerobic digestion modelling efforts have traditionally focused on specific feedstocks or well-defined substrates, resulting in models tailored to those particular systems (Elniski et al., 2019). When multiple substrates are combined, the digestion dynamics can change through synergistic or antagonistic effects, challenging conventional models (Xie et al., 2016). Many AcoD studies still rely on case-specific empirical models which fit kinetic curves (first-order decay functions) separately for each tested mixture (Karki et al., 2022), or modify complex models with substrate-specific parameters (Arnell et al., 2016). As such, these may be rendered invalid if the feedstock ratio or type is altered, limiting the model's predictive value for other feedstock combinations (Mudzanani et al., 2023). Designing a new co-digestion reactor requires several biochemical methane potential (BMP) tests, followed by a series of pilot tests in lab-scale reactors prior to a full industrial implementation. The literature highlights this gap as inconsistent modelling approaches and non-standardized methodologies are major issues in this field of research (Yang et al., 2025).

Developing a general AcoD model is challenging because each substrate contributes distinct macronutrients, degradation pathways, and interaction effects, as different substrates introduce different macronutrients which, degrade along different biochemical pathways, with potential interactions between them. Simple models cannot capture these interactions, while comprehensive models (such as ADM1) require extensive tailoring for each feedstock combination used (Mudzanani et al., 2023) in order to ensure reliability and applicability. Thus, there is a clear need for a generalized AcoD model that provides broadly applicable predictions. In turn, this would greatly assist in selecting feedstock ratios and pretreatment selection for various waste combinations.

This clear need is further exacerbated by the emerging of newer lines of research such as pretreatment of feedstocks prior to their digestion (Rabii et al., 2021), (Morales-Polo et al., 2021) and the use of additives to modify digestion performance, with a growing focus on the use of nanomaterials (Barrena et al., 2022) such as nano Zero Valent Iron (Li et al., 2024), nano nickel (Zaidi et al., 2021), graphene (Tian et al., 2017), carbon nanotubes (Ziganshina and Ziganshin, 2023), etc.

Pretreatments are often applied to feedstocks with a low grade of biodegradability, typically due to a high concentration of lignocellulosic compounds (Čater et al., 2014). While pretreatments boost methane yields and accelerate digestion, they introduce further variability into a model which must account for such changes to correctly fit the observed data.

Similarly, use of trace additives, especially metallic or carbon-based nanoparticles, has recently gained attention to enhance AD. These can facilitate direct interspecies electron transfer (DIET) between microbes, thereby accelerating metabolism and methane production (Hassanein et al., 2021). NP-assisted AD and the use of conductive carbon-based materials have shown significant potential to enhance methane production and degradation kinetics. These materials facilitate direct interspecies electron transfer (DIET) and improve microbial interactions within the system (Ke et al., 2024), (Li et al., 2021), (Liu et al., 2023).

Several studies report significant methane boosts with nano-additives; adding nanoporous Al2O3 to a dry AD of agricultural waste increased biogas output by 72%, while nano Fe2O3 gave a 34% increase in contrast to nano ZnO which inhibited methane production (Zhao et al., 2023).

NP-assisted AD and the use of conductive carbon-based materials have shown significant potential to enhance methane production and degradation kinetics. These materials facilitate direct interspecies electron transfer (DIET) and improve microbial interactions within the system [Ref1, Ref4, Ref8].

Most conventional models do not explicitly include terms for additives or pretreatment as their impact is indirectly captured by adjusted kinetic. This approach fails when expanding beyond conditions for which those parameters were fitted. Therefore, a generalised AcoD model must be flexible enough to manage pretreatment and additive scenarios, ideally by combining the base kinetics of various substrate components with modulatory factors for enhancements. Such a model would allow the prediction of biogas yields for a given mixture of agro-industrial biomass and other organic feedstocks, with or without pretreatment or additives, using a common framework and thereby expediting the design of new co-digestion projects.

2. Methodology

Mathematical models are essential for understanding and predicting AD and AcoD performance because they transform empirical observations, such as biogas production and biomethane yield, into quantifiable kinetic or mechanistic parameters that describe the process kinetically or mechanistically. For anaerobic co-digestion, several kinetic models have commonly been applied, including first-order decay, modified Gompertz, logistic, and tailored models.

On the mechanistic side, the Anaerobic Digestion Model No.1 (ADM1) is considered the benchmark benchmark comprehensive model, as it contains detailed biochemical reactions for each of its defined steps (Processes, 2005). However, ADM1 and its variants require extensive parameterization and are not readily adaptable to arbitrary co-substrate combinations without modification.

Thus, this section aims to review empirical models and ADM1 adaptations, with an emphasis on their mathematical formulations and fitting behaviour under different scenarios and discuss how pretreatments or additives may be incorporated into each model's framework.

2.1. First-order kinetic model

The first-order kinetic model is one of the simplest representations of batch AD. The model is based on the premise that the rate of biogas or biomethane production is proportional to the remaining biodegradable substrate. Its typical formulation is shown in Eq. 1 (Karki et al., 2022):

$$P_K(t) = H_K * [1 - \exp(-\lambda_K * t)] \quad (1)$$

Where H_K represents the total accumulated methane production potential, whose units vary depending on the variable selected to model the substrate (Maleki et al., 2018), and λ_K is the first-order rate constant (with units being day^{-1}) reflecting the speed of biodegradation. The model curve rises quickly initially in the early stages of digestion, prior to plateauing. It must also be noted that it does not explicitly account for any lag phase before gas production starts. Therefore, if there is significant microbial adaptation or hydrolysis lags within the reactor, the first-order model will begin rising and potentially overestimating gas production during the early days of digestion (McCarty and Mosey, 1991).

Despite its simplicity, the first-order model often fits experimental data well when the tested substrate is easily degraded and follows a degradation curve dominated by one rate-limiting steps. Many studies have successfully used first-order kinetics for various agro-industrial residues and co-digestion mixtures (Andrade Cruz et al., 2022), (Kumar et al., 2021) and (Li et al., 2019). A study on thermophilic digestion of aquatic biomass found that a this model fit cumulative methane curves with $R^2 > 0.99$ across several feedstock types (Lower et al., 2025). However, a critical limitation of this model is its inability to account for multi-phase digestion behaviour. AcoD often

involves fast and slow degrading fractions which cannot be correctly modelled with a single λ_K. As a result, the first-order model can misrepresent yields for complex substrate mixtures by predicting an overproduction of biogas in later stages or by underestimating initial lagging. As such, it is the general consensus amongst researchers that the first-order assumption of a constant rate is unrealistic for dynamic AD systems with multiple substrates (Lafratta et al., 2021). When applied to AcoD processes, the approach is modified, creating a multi-component first-order model (Donatelli and Chang, 2024).

2.2. Modified Gompertz model

The Modified Gompertz model is a popular methodology originally used to describe microbial growth. First applied to AD in Zwietering et al. (1990) to model the cumulative biogas production through its S-shaped curve, the formulation can be seen in Eq. 2.

$$P_G(t) = H_G * \exp\left\{ -\exp\left[\frac{R_G}{H_G} * e * (\lambda_G - t) - 1\right]\right\} \quad (2)$$

Where H_G is the asymptotic methane potential, R_G is the maximum methane production rate and λ_G is the lag phase duration. Within its formula, the model utilises a double exponential structure.

This model's strength is its flexibility with three biologically meaningful parameters which, allow it to be fitted to dynamic digestion systems (Andrade Cruz et al., 2022). It has become one of the most widely used models in recent anaerobic digestion studies due to its ability to fit a broad range of substrates (Kafle and Chen, 2016). The study previously referred to, found in Zhao et al. (2023) reported a 3-day lag time and an overall fitting of $R^2 > 0.98$ for all treatments. In another study, found in Li et al. (2019) found the modified Gompertz model to provide an excellent fit $R^2 > 0.986 - 0.998$ for five different vegetable crop residues, outperforming the first-order model.

The Gompertz model is purely empirical – it does not derive from AD reaction mechanisms but is valued for its curve-fitting ability (Wang and Guo, 2024). This stems from the fact that, although R_G and λ_G correlate to microbial activity and acclimation, they are not directly computed from feedstock or microbial kinetics (Velázquez-Martí et al., 2018). Some authors then sum two Gompertz curves to capture multi-phase digestion (Matobole et al., 2024).

Despite these drawbacks, the Modified Gompertz remains a commonplace modelling technique for BMP data. In other fields of research, this model has been further developed through the use of flexible hazard functions to obtain probability distributions which better fit human mortality data (Debelu and Goshu, 2024). In summary, the Gompertz model is best for batch systems with a defined lag and single peak of production.

2.3. Logistic model

The Logistic model also displays a S-shaped growth function used for cumulative biogas production (Moharir et al., 2020). It has a form similar to the Gompertz but is symmetric about the inflection point, where the production rate is maxima, while a Modified Gompertz model is asymmetric (Chu, 2020). The Logistic model is shown in Eq. 3 (H. Zhang et al., 2021a), (Murunga and Were, 2020):

$$P_L(t) = \frac{H_L}{1 + \exp(\frac{4 * R_L}{H_L} * (\lambda_L - t) + 2)} \quad (3)$$

In which H_L is the maximum methane yield; R_L is the maximum production rate and λ_L is the logistic growth constant. Its formulation may vary across different publications.

This model's behaviour is qualitatively similar to Gompertz modelled with lag: initial production is minimal, followed by an increase in production rate as t reaches λ_L, at which point the production peaks and cumulative production totals 50%. Once this inflexion point is reached, production rate decreases until it reaches the asymptote H_L. The logistic model has been used nearly as widely as Gompertz in evaluating BMP test results (H. Zhang et al., 2021a), (Moharir et al., 2020). In many cases the modified logistic and modified Gompertz give comparably good fits to cumulative biogas data (Ghatak, P., 2014), (Kafle and Chen, 2016). These found that both models obtain a similar accuracy and outperformed a simple first-order. Recent studies continue to use logistic modelling such as Sanae et al. (2022) which applied this model to raw and thermally pretreated pulp-paper sludge. This paper reported that the model captured the enhanced kinetics due to pretreatment, with a marginally better fit for some datasets. Similarly, Zhang et al. (2021) in an AcoD study of food waste, cattle manure and corn straw found that the logistic model reflected the synergistic effects of co-digestion.

The choice between logistic and Gompertz is often one of convenience (Ghatak, P, 2014), as both models present 3 ° of freedom (DOF) that must be determined through a non-linear regression. If the data to be fitted exhibits a perfect symmetry around the inflection point, the logistic model may be a choice, whereas the modified Gompertz model can handle cases in which the data set is skewed due to slow-degrading substrates (Moharir et al., 2020), (H. Zhang et al., 2021a).

In summary, the logistic model performs best in batch systems with a single dominant digestion phase and excels in scenarios in which the production curve is symmetric. Its use in co-digestion kinetic modelling is well established, alongside the modified Gompertz model for cross-verification (Zahan et al., 2018).

2.4. Tailored and specific models

Beyond the standard kinetic models described above, many researchers have developed tailored models to better represent specific co-digestion systems. These can be grouped into multi-component empirical models and comprehensive mechanistic models (Xie et al., 2016).

Multi-component kinetic models are based on the premise that different substrates degrade at different rates. The most common approach is to use a sum of two or more of the described models in order to represent a rapidly degrading component and a slowly degrading component (Paranhos et al., 2020). Similarly, the Cone model (López-Aguilar et al., 2025), introduces a shape factor n into the first-order equation, as seen in Eq. 4.

$$P_C(t) = H_C * [1 - \exp(-(\lambda_K * t)^n)] \quad (4)$$

The proposed model reduces to a simple first-order model when $n = 1$; however, it can be used in AcoD applications by altering the value of n, which allows a better fit with empirical data.

Other types of models are those that can be classed as mechanistic due to their complex modelling through expensive parameters of natural processes. Among these, the most used is the ADM1 Model. The ADM1 utilises a variety of parameters to precisely model various biochemical steps, microbial populations and inhibitory effects. This permits its use in AcoD by determining the substrate mixture and characterising it through its lipid, carbohydrate and protein contents. Despite its complexity, the model does not contemplate certain key aspects of AcoD, such as the implementation of synergies or the effects of additives. This led to the development of the Modified ADM1, proposed in Mo et al. (2023) to better accommodate the co-digestion of sewage sludge paired with other organic substrates. Similarly, a generalized ADM1 framework for co-digestion was proposed in García-Gen et al. (2013) by introducing new soluble substrate pathways, allowing for a better simulation of concurrent degradations. However, studies have shown that ADM1's performance is sensitive to parameter uncertainty – slight misestimation can lead to large prediction errors (Mudzanani et al., 2023).

2.5. Incorporating pretreatments and additives to models

Pretreatments and additives can significantly alter AcoD kinetics. Incorporating these into models can be done in two ways: implicitly through parameter changes or explicitly through structural modifications to the model.

Parameter adjustment methods are the most common approach. For example, thermal pretreatment is often modelled by increasing their first-order rate constant and the maximum yield. As an example, an 80% reduction in lag time and a tripling of the rate was observed in Sanae et al. (2022). Similarly, additives that enhance microbial activity can be modelled through a modified Gompertz, as seen in Zhao et al. (2023).

A mechanistic inclusion would be the use of an explicit modelling method based on the effects of a selected pretreatment, splitting the substrate into two fractions. As an example, substrate with a high lignocellulosic content could be modelled with two first-order terms. The first of these could represent the easily digestible substrate, such as carbohydrates, paired with slower equation which accounts for the lignin-rich substrate. The impact of the selected pretreatment would then be modelled by modifying the weighting terms, increasing the percentage of easily digestible substrate and reducing the percentage of the lignin-rich fraction, as seen in Bolado-Rodríguez et al. (2016) in which VS were used as the fragmentation base. These two fractions would therefore present different parameters, with the treated fraction presenting an overall improved digestion.

For the effects of additives on existing models, this approach requires further adjustments when used on mechanistic models, as the effect of pretreatments on the DIET process can be sometimes unclear. In ADM1, conductive additives could be introduced in the model by adding an alternative pathway for hydrogen consumption to simulate the DIET process or by increasing the maximum uptake rate of acetate by methanogens, if the additive improves the methanogenesis stage of digestion. When utilising a modified Gompertz, some researchers have introduced an enhancement factor, such as in Kumar et al. (2021), where it was used to account for the faster electron transfer facilitated by nanoparticles. This factor was calibrated so that the model matched the higher methane production observed with nanoparticles.

3. Model structure and example

3.1. Simplified model structure

As previously presented within this paper, most AcoD models focus on a selected substrate and inoculum combination to develop their respective studies, resulting in a lack of published research on generalised models for AcoD. These generalised models require a flexible structure in order to accommodate various substrates within their formulation and maintain a sufficient level of detail. This duality would ensure that substrates can be studied jointly to determine the synergistic effects of co-digestion and independently to identify their respective AD processes, as stated in Xie et al. (2016).

The proposed model is based on the most widely accepted AcoD kinetic models, the modified Gompertz Model, shown in Eq. 2, and the first order kinetic model, shown in Eq. 1. These models are widely used in the reviewed bibliography, although the modified Gompertz was identified as the more suitable of the two for AcoD applications. This paper develops the generalised formulation for both models.

In order for the model to be applicable within an AcoD reactor with multiple substrates, the generalised formulation was developed based on an independent set of n-tuple distinct substrates (food waste, wastewater treatment plant sludge, cow manure, etc), described in Eq. 5. As the set is of an unknown size, it was decided that a combinatorics-based approach was more suitable to simplify the formulation. However, a matrix-based approach was also deemed viable, although said option was discarded due to the complexity required to operate within n dimension vectors when applying the model. It must also be added that the total number of elements (substrates) within set will set the total number of dimensions. Additionally, this would also need to consider the number of parameters required within a given model, shown in Eq. 6.

$$\mathrm{Re} = \{WWTP\ Sludge,\quad Food\ Waste,\quad \ldots,\quad n\} \tag{5}$$

$$M = \{\lambda_m,\quad H_m,\quad \ldots,\quad m\} \tag{6}$$

The combinatorics approach was utilised in order to correctly identify all the possible interactions between all the substrates found within the described set as well as higher order interactions in cases with more than two substrates, with no possible repetitions. As a result, the total number of combinations a given substrate i can be calculated as the addition of all possible combinations through all the dimensions, which corresponds to the total value of the $(n-1)$ row of the Pascal triangle, shown in Eq. 7, and the total number of interactions (It_{Re}) per parameter is shown in Eq. 8 and the overall number of interactions It_{Tot} in Eq. 9.

$$\sum_{j=1}^{n} C(n,i) = \sum_{i=1}^{n} \frac{n!}{(n-i)!\cdot i!} = 2^{n-1};\ i\epsilon\mathrm{Re} \tag{7}$$

$$It_{\mathrm{Re}} = n\cdot 2^{n-1} \tag{8}$$

$$It_{Tot} = m\cdot n\cdot 2^{n-1} \tag{9}$$

Using this approach, a different unordered set of interactions of interactions ($K'_{C(n-1,i)}$) can be derived for each individual substrate S_i, shown in Eq. 10. The interactions are derived in Eq. 11

$$C(S_i) = \left\{S_iS_1\ ,\ S_iS_2\ ,\ S_iS_3\ ,\ \ldots\ ,\ S_iS_n\ ,\ S_iS_{12}\ ,\ S_iS_{13}\ ,\ \ldots\ ,\ S_iS_{C(n,i)}\right\}\quad \forall\quad i\epsilon\mathrm{Re} \tag{10}$$

$$K'(S_i) = \left\{K'_{S_1},\quad K'_{S_2},\quad K'_{S_3},\quad \ldots,\quad K'_{S_n},\quad K'_{S_{12}},\quad K'_{S_{13}},\quad \ldots,\quad K'_{S_{C(n-1,i)}}\right\}\forall\quad i\,\epsilon\,\mathrm{Re} \tag{11}$$

As the model is based on the total number of interactions of a specific substrate with all the substrates found within the set Re, this model permits its application to mono AD studies by reducing the set to a single element, the substrate being studied. Through the application of the identified interactions to the modified Gompertz and the first-order formulations and their *DOF*, utilising a weighted average approach to normalise the obtained coefficients based on a significant parameter within the reactor.

This results in a critical point within the formulation, as the selection of an unsuitable parameter would yield a defective model. In the case of AD and AcoD models, the C/N ratio C/N) could be a suitable parameter, as various studies have demonstrated this to be a characteristic parameter for AD and AcoD processes, as reviewed by Inayat et al. (2021) and Zhou et al. (2021) among others. The model is designed to be flexible and as such, any other parameter can be used to weigh the contribution of the sampled substrates so long as it is deemed relevant for all. The proposed weighting for a given DOF_m of a given model is presented in Eq. 12.

$$DOF_m = \frac{\sum_{i=1}^{n} DOF_{m_i} \cdot C/N_i \cdot \sum_{j=1}^{2^{n-1}} {}^{m}_{S_i}K'_{S_j}}{\sum_{i=1}^{n} C/N_i}; i \epsilon \text{ Re}; m \epsilon M; j \epsilon \ K'(S_i) \tag{12}$$

Where C/N_i represents the C/N ratio supplied by substrate i; ${}^{m}_{S_i}K'_{S_k}$ represents the interaction of substrate i and a given combination of the different substrates, selecting each combination using index j to ensure all possible combinations are included as listed in Eq. 11, for a given degree of freedom m. This modelling also accounts for the interaction of a substrate with itself, as previously mentioned. These coefficients are considered to be equal to 1, to maintain the coherency with mono-digestion models, presented in Eq. 13. The total coefficients per parameter m to be determined is thus reduced by the total number of substrates used, given by the formula found in Eq. 14 and the overall number in Eq. 15.

$${}^{m}_{S_i}K'_{S_i} = 1 \forall \quad i \epsilon \text{ Re} \tag{13}$$

$$It_{\text{Re}} = n \cdot (2^{n-1} - 1) \tag{14}$$

$$It_{Tot} = m \cdot n \cdot (2^{n-1} - 1) \tag{15}$$

3.2. Representation of higher order AcoD

To clarify the proposed structure, an example is developed for the case $n = 4$. This would require a total of 4 DOF_{m_i} estimates and a total of 32 interactions, as each substrate interacts with itself, and all possible combinations of the remaining substrates. As previously explained, the interaction of a substrate with itself must be set to 1, reducing the number of interactions and coefficients to be determined from 36 to 32 for a given DOF_m. First order interactions account for the individual interaction of substrate S_i with the remaining substrates, ordered through j. Terms for the second order pertain to the interaction of substrate i the different combinations of the remaining 3 substrates in pairs. Lastly, third order terms refer to the interaction of substrate i with the three substrates together.

The model utilises a summation to govern the interactions between substrates in higher n-tuple orders, as results obtained for a lower order may be reused and the effects of new interactions be expressed within these greater order terms. The different possible interactions for any given DOF_{m_i} are also illustrated graphically in Fig. 1.

3.3. Additive and pretreatment model expansion

Once substrate interaction was modelled, the effect of additives and pretreatments were considered, utilising a similar formulation and expanding said coefficient to include these. The most notable difference is that in this case it is considered that pretreatments and additives modify the interaction coefficients multiplicatively, displayed in Eq. 16. This modelling permits a further development and tailoring to a specific scenario, as these can be further expanded to include various pretreatments or additives.

$$\sum_{j=1}^{2^{n-1}} {}^{m}_{S_i}\text{K}_{S_j} = \sum_{j=1}^{2^{n-1}} {}^{m}_{S_i}K'_{S_j} \cdot {}^{m}_{S_i}\text{AD}_{S_j} \cdot {}^{m}_{S_i}\text{PT}_{S_j}; i \epsilon \text{ Re}; m \epsilon M; j \epsilon \ K'(S_i) \tag{16}$$

Similar to the previous formulation, K'_{S_j} maintains its formulation and is thus unaffected by the added terms, prior to the effect of additives or pretreatments. ${}^{m}_{S_i}\text{AD}_{S_j}$ represents the impact of the use of additives on substrate i and the substrate combinations given by index j, as with the original formulation. ${}^{m}_{S_i}\text{PT}_{S_j}$ indicates the interaction caused by the pretreatments on substrate i and the substrate combinations given by index j. As an explanatory note, the use of simple multiplicative coefficients does not capture the complexity these interactions except for the used empirical data set. Ultimately, a function could be developed provided

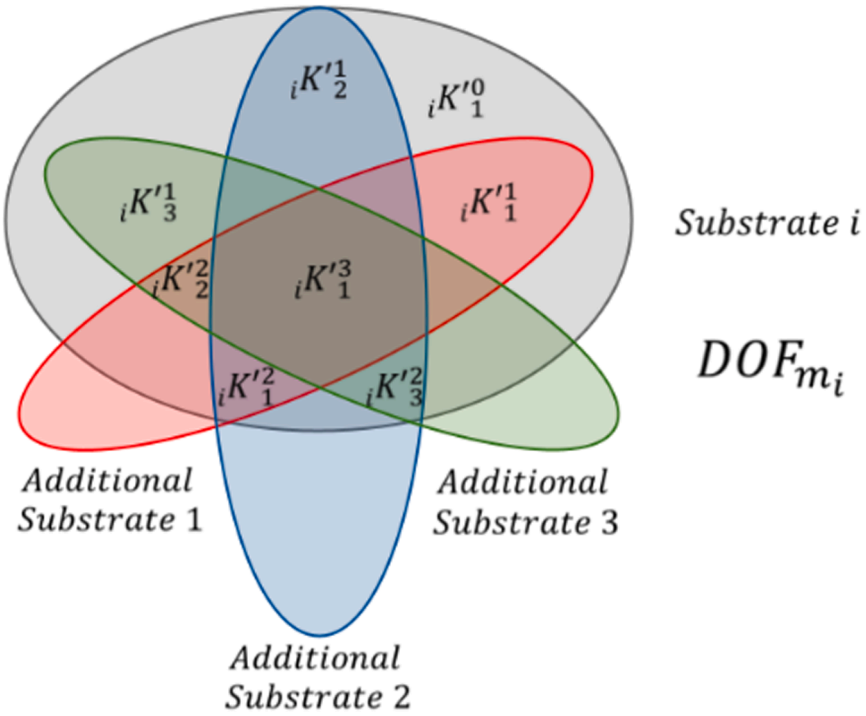

Fig. 1. Graphical representation of substrate combinations for i = 4.

that there is enough data to determine the governing function, which would depend on the substrate mixture, substrate combinations, as well as the additive concentration among others.

Similarly to cases of mono-digestion, if no additives or pretreatments are used, the model simplifies to the previously presented formulation as the additional terms are considered to have no impact and are thus equal to 1. The final formulation for the interactions within a given *DOF* is displayed in Eq. 17. The total number of interactions (Int_{Tot}) with different pretreatments and/or additives (*Int*) to be modelled would also increase with the complexity of the model, given by the formula in Eq. 18.

$$DOF_m = \sum_{i=1}^{n} DOF_{m_i} \cdot C/N_i * \sum_{j=1}^{2^{n-1}} \left({}^{m}_{S_i}K'_{S_j} \cdot \frac{{}^{m}_{S_i}\mathrm{AD}_{S_j} \cdot {}^{m}_{S_i}\mathrm{PT}_{S_j}}{\sum_{i=1}^{n} C/N_i} \right); \quad i \in \mathrm{Re}; m \in M; j \in K'(S_i) \tag{17}$$

$$Int_{Tot} = m \cdot Int \cdot n \cdot 2^{n-1} \tag{18}$$

3.4. Numerical example

A numerical model was also constructed, for the case $i = 3$. This solution was modelled based on the modified Gompertz parameters (thus setting the number of model parameters to $m = 3$). The raw data used for this analysis was procured from Morales-Polo et al. (2023). This example uses 3 different substrates: Vegetables (*V*), Meat (*C*) and Fish (*P*). The dataset contained only samples for individual AD BMP tests, noted as C, V and P, as well as an AcoD BMP in which all 3 substrates were in equal proportion (V3C3P3, 33% of the volume). In all cases the inoculum used was sludge obtained from an agro-industrial wate water treatment plant.

Prior to applying the model, each sample was fitted with a modified Gompertz curve. The obtained results are shown in Table 1.

As the used data does not contain information on BMP test for dual mixtures (C-V, C-P and V-P), the model cannot be applied to it, and these parameters will need to be estimated in the regression. This was performed to compare the results with those of the simple Gompertz Model and to obtain approximate functions for the missing combinations. The formulations for the various *DOFs* of dual AcoD tests are provided in Eq. 19 through to 21.

$$DOF_{CV} = DOF_C \cdot \frac{C}{N_C} \cdot \left(1 + \frac{{}^{m}_{C}K'_V) + DOF_V \cdot \frac{C}{N_V} \cdot (1 + {}^{m}_{V}K'_C)}{C/N_C + C/N_V}\right. \tag{19}$$

$$DOF_{CP} = DOF_C \cdot C/N_C \cdot \left(1 + \frac{{}^{m}_{C}K'_P) + DOF_P \cdot C/N_P \cdot (1 + {}^{m}_{P}K'_C)}{C/N_C + C/N_P}\right. \tag{20}$$

$$DOF_{VP} = DOF_V \cdot C/N_V \cdot \left(1 + \frac{{}^{m}_{V}K'_P) + DOF_P \cdot C/N_P \cdot (1 + {}^{m}_{P}K'_V)}{C/N_V + C/N_P}\right. \tag{21}$$

In addition, the overall interaction for AcoD of the three simultaneous substrates is shown in Eqs. 22, 23, 24 and 25.

$$Int_C = 1 + {}^{m}_{C}K'_V + {}^{m}_{C}K'_P + {}^{m}_{C}K'_{VP} \tag{22}$$

$$Int_V = 1 + {}^{m}_{V}K'_C + {}^{m}_{V}K'_P + {}^{m}_{V}K'_{CP} \tag{23}$$

$$Int_P = 1 + {}^{m}_{P}K'_V + {}^{m}_{P}K'_C + {}^{m}_{P}K'_{CV} \tag{24}$$

Table 1
Modified Gompertz parameters for V, C, P and V3C3P3 samples.

Substrate	C/N(-)	H_G (NmL)	R_G (NmL/day)	λ_G (days)	R^2 (-)
V	8.3397	1551.7	256.29	0.7531	0.9853
C	12.9520	1844.7	685.53	0.9831	0.98616
P	4.3927	1091.6	414.2	1.0247	0.96973
V3C3P3	7.6148	1475.9	451.09	0.8580	0.92426

Table 2
Estimated parameters for CV, CP, VP and V3C3P3 using the proposed model.

Substrate	C/N(-)	H_G (NmL)	R_G (NmL/day)	λ_G (days)	R^2 (-)
CV	10.646	2617.2	784.37	1.3561	-
CP	8.6724	2500.3	936.03	1.5071	-
VP	6.3662	2112.5	461.26	1.2882	-
V3C3P3	7.6148	1475.9	451.09	0.8580	0.92426

$$DOF_m = \frac{DOF_C \cdot C/N_C \cdot \mathrm{Int_C} + DOF_V \cdot C/N_V \cdot \mathrm{Int_V} + DOF_P \cdot C/N_P * \mathrm{Int_P}}{C/N_C + C/N_V + C/N_p} \tag{25}$$

This was then applied to the data sets for used to estimate the parameters shown in Table 2, along with its R^2 to validate the fit of the fully composed sample V3C3P3 and compare it with the initial Gompertz. It must be mentioned that in this example, as the data set does not contain all the previous combinations, the proposed model yields estimated parameters for the dual AcoD tests CP, CV and VP (assuming equal weights of each).

Lastly, the estimated parameters for each interaction are provided in Table 3 for H_G, R_G and λ_G.

4. Results and discussion

As stated in the previous section, this model requires a large number of samples in order to model complex AcoD scenarios and their interaction parameters. Thus, it can be broadly classed as a multiple component model, although its use of explicit parameters to model substrate, additive & pretreatment interaction differs from what was traditionally used, namely summation of various, independent degradation curves for different substrates. Unless the studied system uses several substrates, the number of coefficients required can be contained within reasonable limits based on the sample sizes used during testing. These coefficients, along with the exclusion of biologically based mechanisms also differentiates it from complex mechanistic models such as the ADM1. The scaling of these is shown in Fig. 2 for a modified Gompertz model, which has 3 total *DOFs*.

Models with a higher number of parameters would present an ever-increasing number of coefficients. This can be addressed either by experimentally modelling substrate combinations or by using empirical data to solve the complete nonlinear system.

Analysing the proposed model from an experimental design perspective, the total number of data points collected will depend on the BMP test duration (T_R). Otherwise, the total data points collected (T_S) will be smaller, as the samples may not be accessible during weekends. Therefore, the minimum number of datapoints collected for an experimental run would depend on the *n* substrates evaluated and the *m DOFs* found within the model, yielding Eq. 26, which accounts for the considered interactions (*Int*) within the model. This can be further

Table 3
Estimated Interaction Coefficients for H_G.

Interaction	H_G (-)	R_G (-)	λ_G (-)
C (K'_C)	1.00	1.00	1.00
V (K'_V)	1.00	1.00	1.00
P (K'_P)	1.00	1.00	1.00
C with V (K'_V)	−0.24461	−0.23801	−0.2419
C with P (K'_P)	−0.24461	−0.23801	−0.2419
V with P (K'_P)	−0.24161	−0.25862	−0.23824
V with C (K'_C)	−0.24161	−0.25862	−0.23824
P with C (K'_C)	−0.24198	−0.25698	0.24084
P with V (K'_V)	−0.24198	−0.25698	−0.24084
V with CP (K'_{CP})	−0.24161	−0.25862	−0.23824
C with VP (K'_{CVP})	−0.24461	−0.23801	−0.2419
P with CV (K'_{CV})	−0.24198	−0.25698	−0.24804

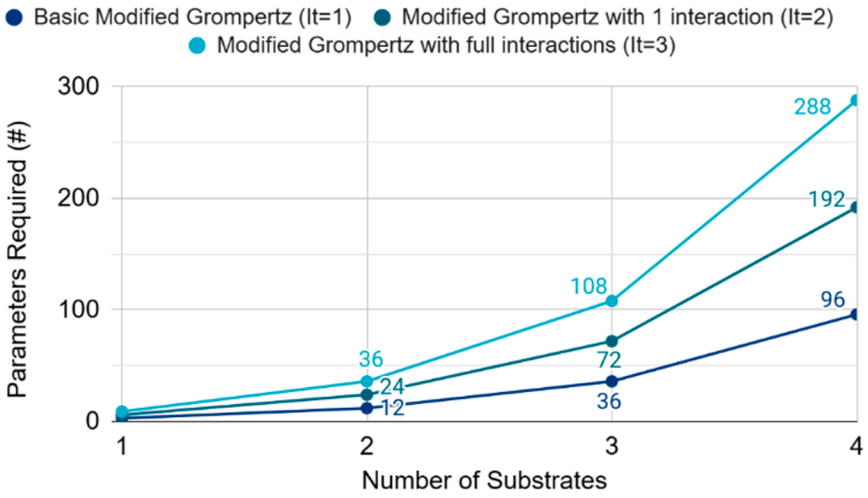

Fig. 2. Total number of parameters required for a modified Gompertz Model based on interactions.

Table 4
Minimum sample size for a $T_S = 15$ in different scenarios of interactions.

Number of Substrates(n)	Basic Modified Gompertz ($It = 1$)	Modified Gompertz with 1 interaction (AD or $PT, It = 2$)	Modified Gompertz with full interactions ($It = 3$)
1	1	1	1
2	1	2	3
3	3	5	8
4	7	13	20
5	16	32	48

specified by selecting a $T_R = 21$, which yields a $T_S = 15$ once weekends are discounted. The increase in $S_{\min}$ is shown for this example in Table 4.

$$S_{\min} = \lceil \frac{m * n * Int}{T_S} \rceil \tag{26}$$

As seen in Fig. 2, the use of 3 substrates requires a maximum of 108 parameters under a modified Gompertz. With a relatively short T_R of only 21 days, the minimum sample size is 8, which would yield a total of 120 data points. As a result, this methodology simplifies the need for large sample sizes when studying higher order co-digestions. The sample size can also be affected by experimental design, as the model is meant to build upon previous experiments, thus some parameters from lower order interactions may be known beforehand, further reducing sample size and optimising the overall procedure through segmented or stratified runs.

Another benefit afforded by this model is capacity to isolate the impact of each substrate on the overall process as well as determine its individual digestion rate within the AcoD system.

Lastly, although the model was designed to be used in AcoD studies, it could also be applied backwards to common substrates currently used in AD and AcoD experiments such as the Organic Fraction of Municipal Solid Waste or Animal Byproducts, both of which are composed of different fractions despite them being treated and characterised as a single substrate.

5. Conclusions

The proposed approach, based on combinatorics, ensures that the structure can be replicated in n-tuple substrate experiments by providing a structured framework for agro-industrial biomass co-digestion without being limited by the inclusion of additives or pre-treatments. Importantly, further terms could be added to the model, as the dimension-based, interaction coefficient approach was limited to these two modifications of the AcoD process because of their current relevance in research. As new research lines appear, their effects on digestion could also be included with very little modification and without impacting its overall methodology, ensuring that it remains relevant in future research lines which may diverge from those analysed in this paper (co-digestion, pretreatments and additives).

Furthermore, this model proposes an approach which can provide a more detailed analysis of different AcoD mixtures with a structure that allows for a better integration of synergistic effects, modelled explicitly, improving existing sigmoidal models used today in research without requiring the use of mechanistic models, which are not designed for flexible or generalised applications.

This proposed methodology also suggests a standardised experimental approach to the various empirical tests, such as BMPs, required to fully complete the model in higher-order applications. By modelling the AcoD system as the combination of simpler co-digestions and the addition of terms which govern interactions between newer combinations such as triads or quadruples (with the required lower-order terms). This approach eliminates the need for the brute force approach of testing every possible combination in favour of a systemic methodology which builds on previous collected data and thus save time and resources during the experimental phase.

As per future work and current limitations, although the model simplifies the approach for higher order digestions as well as reduces the need for various experimental runs to obtain enough information, the effect of both nanoparticles and pretreatments could further analyse the impact of varying concentrations and substrates in order to develop the proposed coefficients into equations to be substituted within the proposed formulation, which could also include inhibitory effects or toxicity thresholds. This would thus reduce the model's complexity while further improve its generalised and flexible nature.

Moreover, the model's structure, especially with regards to DOF_m subsets of coefficients for each substrate, would allow for the model to be further advanced into a algebraic-based approach by developing an interaction matrix, either containing functions or coefficients to use in different operations, further generalising the model into m-order columns which would depend on the parameters required by the selected mathematical model.

Lastly, although it has only been lightly covered within this paper, this model can also be used to analyse heterogeneous biomass feedstocks that are modelled as a single type, even though these contain different fractions which digest at different rates. This detailed analysis could then be used to optimise the anaerobic co-digestion of such biomass mixtures.

Abbreviations

λ_G	Lag Phase Duration of the Modified Gompertz Model
λ_{GN}	Lag Phase Duration of the Generalised n-tuple Modified Gompertz Model
λ_K	Lag Phase Duration of the First-Order Kinetic Model
λ_{KN}	Lag Phase Duration of the Generalised n-tuple First-Order Kinetic Model
λ_L	Lag Phase Duration of the Logarithmic Model
μ	Mean of a Normal Distribution
σ	Standard Deviation of a Normal Distribution
AcoD	Anaerobic Co-Digestion
AD	Anaerobic Mono-Digestion
AD_{S_j}	Impact of the Use Additives on Substrate i for Combination j
ADM1	Anaerobic Digestion Model 1
BMP	Biochemical Methane Potential
C/N	Carbon-to-Nitrogen Ratio
C/N_i	Carbon-to-Nitrogen Ratio of Substrate i
$C(S_i)$	Combinations of Substrates for Substrate i
DIET	Direct Interspecies Electron Transfer
DOF	Degree of Freedom
H_C	Maximum Methane Yield of the Components Model
H_G	Maximum Methane Yield of the Modified Gompertz Model
H_{GN}	Maximum Methane Yield of the Generalised n-tuple Modified Gompertz Model
H_K	Maximum Methane Yield of the First-Order Kinetic Model
H_{KN}	Maximum Methane Yield of the Generalised n-tuple First-Order Kinetic Model
H_L	Maximum Methane Yield of the Logarithmic Model
Int	Number of considered additives and/or Pretreatments
Int_{Tot}	Total Number of Interactions, considering Pretreatments and/or Additives
It_{Re}	Total Number of Interactions for a Substrate
It_{Tot}	Total Number of Interactions
Int_i	Individual Digestion of Substrate i
K_{S_j}	Overall Interaction of Substrate i for Combination j, considering Pretreatments and/or Additives
$K'(S_i)$	Interaction Coefficients for Substrate i
K'_{S_j}	Interaction of Substrate i for Combination j
P_C	Methane Yield of the Components Model
P_G	Methane Yield of the Modified Gompertz Model
P_{GN}	Methane Yield of the Generalised n-tuple Modified Gompertz Model
P_K	Methane Yield of the First-Order Kinetic Model
P_{KN}	Methane Yield of the Generalised n-tuple First-Order Kinetic Model
P_L	Methane Yield of the Logarithmic Model
K'_{S_j}	Interaction by the Pretreatments on substrate i for Combination j
R^2	Coefficient of Determination
R_G	Maximum Production Rate of the Modified Gompertz Model
R_{GN}	Maximum Production Rate of the Generalised n-tuple Modified Gompertz Model
R_L	Maximum Production Rate of the Logarithmic Model
$S_{\min}$	Minimum Sample Size
T_R	Hydraulic Retention Time
T_S	Sampled Days
VS	Volatile Solids

CRediT authorship contribution statement

Javier Victoria-Rodríguez: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **María del Mar Cledera-Castro:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Carlos Morales-Polo:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to improve readability and language. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of Competing Interest

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

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Data availability

No data was used for the research described in the article.

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