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Original Article

Kinetic evaluation of the anaerobic co-digestion of thermochemically pretreated pig manure and Napier grass

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Abstract

This study investigates the anaerobic co-digestion of thermochemically pretreated pig manure and Napier grass under mesophilic conditions, focusing on kinetic analysis and the effects of total solids concentration and PM/NG ratio on methane production. PM was treated with 5% $Ca(OH)_2$ at 70 °C for seven days, while NG was immersed in 0.6% NaOH at 90 °C for two hours. A series of 30-day batch experiments was conducted using a laboratory-scale setup to evaluate cumulative methane yield (CMY) as the principal performance metric. Experimental conditions included five PM/NG mixing ratios (1:0, 3:1, 1:1, 1:3, 0:1) and three TS levels (3%, 5%, and 7%). Methane production exhibited a typical sigmoidal profile comprising a lag phase (3–10 days), an exponential production phase, and a plateau phase. The average methane content was 64.27%. The highest daily methane production rate (29.27 mL/g VS/day) and maximum CMY (210.47 mL/g VS) were recorded at a 1:1 PM/NG ratio and 3% TS. Four kinetic models (Modified Gompertz, Cone, Logistic, and Richards) were applied to evaluate predictive performance. Among them, the Modified Gompertz and Cone models provided the most accurate fits ($R^2 > 0.995$; lowest RMSE), effectively capturing the sigmoidal nature of methane production.

Keywords: Methane production, Anaerobic co-digestion, Kinetic model, Napier grass, Pig manure

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Introduction

In recent decades, global concerns over energy security, environmental degradation, climate change, and the depletion of fossil fuel reserves have intensified. The growing reliance on non-renewable fossil fuels has significantly contributed to greenhouse gas emissions, resulting in adverse impacts on climate stability and ecosystems worldwide. As a result, there is an increasing international consensus on the urgent need for sustainable and renewable energy solutions that can meet rising energy demands while minimizing environmental harm and supporting the achievement of Sustainable Development Goals (UNECE, 2024). Among renewable energy alternatives, anaerobic digestion (AD) technology is highly promising, offering significant environmental benefits by converting organic waste into biogas, a clean energy source primarily consisting of methane which can be utilized for heat, electricity, or fuel (Bist et al., 2024). Co-anaerobic digestion (co-AD), a process involving the simultaneous digestion of two or more complementary substrates, has attracted attention due to its ability to enhance biogas production through improved nutritional balance and synergistic microbial interactions (Chan et., 2021; Leitão et al., 2022; Essalhi et al., 2025). Pig manure (PM), a common agricultural waste, is recognized as an ideal co-substrate due to its high microbial diversity and buffering capacity, which support digestion stability and methane production efficiency (Astals et al., 2014). Meanwhile, Napier grass (NG, Pennisetum purpureum) is identified as an abundant lignocellulosic biomass, notable for its rapid growth rate, high biomass productivity, and carbon sequestration capabilities, thus presenting itself as a sustainable feedstock for bioenergy production. However, Lignocellulosic biomass, such as NG, is characterized by a high degree of structural recalcitrance arising from the rigid and intricate associations among cellulose, hemicellulose, and lignin, which collectively hinder microbial degradation during anaerobic digestion processes (Taherzadeh and Karimi, 2008). To overcome this limitation, thermochemical pretreatment using alkaline agents such as NaOH and $Ca(OH)_2$ has been extensively employed. This approach significantly enhances the digestibility of lignocellulosic substrates by disrupting their complex matrix, thereby increasing the solubility and bioavailability of organic matter, facilitating microbial access, and accelerating hydrolysis, the rate-limiting step in AD (Carrere et al., 2016). Alkaline thermochemical pretreatment has proven particularly effective in the context of co-AD, especially for improving the biodegradability of recalcitrant biomass. NaOH, for instance, has demonstrated notable success in solubilizing organic waste. Pretreatment at 90°C for 2 h with a NaOH concentration of 15g/L was found to improve the solubilization of chemical oxygen demand and methane yield, while decreasing hydraulic retention time in one study (Junoh et al., 2015). A weaker alternative is offered in the form of $Ca(OH)_2$ which may be appropriate when high levels of sodium are also present, as it might reduce microbial activity (Dumlu et al., 2021). Experimental results have reported the increase in methane production by 56% and hydrolysis by 81.9%, when the NaOH pretreatment is done on agricultural waste co-AD with sewage sludge. However, thermochemical pretreatment is effective for depolymerization and solubilization of lignin, which although requires optimization of operational parameters, including chemical concentration, temperature, and treatment duration, to avoid the excessive use of reagents. The pretreatment of sugarcane leaves with NaOH prior to co-AD with PM is an effective way to enhance the release of soluble organic matter, improving microbial decomposition and further elevating biogas production (Luo et al., 2018). Additionally, alternative thermochemical treatments like steam explosion and acid or alkaline hydrolysis have been applied to enhance the accessibility of cellulose and hemicellulose (Kral et al., 2016; Şenol, 2021). These techniques not only increase degradability of substrate but also remove inhibitory compounds such as sulfides, ammonia, volatile fatty acids and phenolics, resulting in enabling a more stable and efficient AD process (Sathyan et al., 2023). Researcher reported that pretreatment of NG with 0.6% NaOH at 90 °C has significantly enhanced methane yield by increasing biomass solubilization and microbial accessibility (Rekha and Aniruddha, 2013). Similarly, the treatments of manure with $Ca(OH)_2$ too have resulted in notable improvements in methane production by disrupting the lignocellulosic substrates (Rafique et al., 2010). Furthermore, thermochemical pretreatment has significantly reduced the lag phase and increased in biogas yield during co-AD (Deepanraj et al., 2017). An important tool for enhancing co-AD, kinetic modeling provides a mathematical basis for predicting biogas production, substrate degradation and system

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performance(Aneeq et al., 2021). The complexity of co-AD that involves the simultaneous processing of multiple organic substrates, each with distinct biodegradation characteristics, kinetic models such as the modified Gompertz, first-order, and logistic equations, are used to identify rate-limiting steps and estimate key parameters including methane production rates, lag phase duration and maximum biogas yield. These models have been significant into the early stages of reactor development and operation. Substrate characteristics significantly affect hydrolysis and biogas yields, with the logistic model providing the best fit (Leite et al., 2024). Additionally, kinetic modeling has become a tool for evaluating the effectiveness of various pretreatment methods and feedstock combinations. The modified first-order and Cone models have demonstrated improved methane yields from alkaline-pretreated lignocellulosic biomass. Moreover, the modified Gompertz equation has shown particularly strong predictive performance, with R^2 exceeding 0.99 in AD of lignocellulosic substrates (Matobole et al., 2024). Incorporating an adjustable exponent parameter that enables it to adjust to the varying hydrolysis rates of heterogeneous substrates, the Cone model is effective in representing non-linear degradation kinetics in AD process (Polastri et al., 2024). While the logistic model is well-suited to representing biological growth particularly the initial exponential phase of microbial activity, it has exhibited limitations in accurately capturing the lag phase, thereby reducing its overall predictive reliability in co-AD processes (Zahan et al., 2018; Kafle and Chen, 2016). The Richards model, on the other hand, adds more form factors for more adaptability; nonetheless, this complexity could lead to overfitting, which would reduce its predictive power and generalizability across other systems (Kythreotou et al., 2014; Zhang et al., 2019).

Accurate assessment of reaction rates and rate-limiting processes in biogas generation relies heavily on kinetic modeling. In co-AD systems, an imbalance between microbial activity and the structural resistance of NG may prevent methane generation (Sawanon et al., 2017). While thermochemical pretreatment using agents like NaOH and $Ca(OH)_2$ improves substrate bioavailability which in turn increases hydrolysis efficiency and methane production (Madhawan et al., 2019), most current models fail to take for these enhancements, reducing their accuracy and practical value of these models in predicting biogas yields. Moreover, existing models often assume single substrate, overlooking the interactive effects between co-substrates interactions particularly differences in hydrolysis rates and microbial compatibility, which may disrupt microbial balance. To overcome the limitations, kinetic models were developed for complex and pretreated co-AD process. These models would improve prediction, enhance operational control, and support the sustainable scale-up of biogas technology. This study aims to develop and validate kinetic models used for the co-AD of thermochemical pretreated PM and NG. The investigation was conducted under mesophilic conditions using laboratory-scale batch. A co-AD experiment was performed with varying PM/NG mixing ratios (1:0, 3:1, 1:1, 1:3, and 0:1) and total solids concentrations of 3%, 5%, and 7%. Cumulative methane yield (CMY) was employed as the primary measurement for evaluating digestion performance. The mathematical models of the Modified Gompertz, Cone, Logistic and Richards functions were used to experimental data to simulate the methane production kinetics. The prediction accuracy and applicability of these models under various substrate compositions and solids concentrations were evaluated. This study aimed to quantify methane production from PM-NG mixtures, identify the suitable kinetic model, and evaluate thermochemical pretreatment for improving substrate degradability and methane yield.

Material and Methods

Substrate preparation

In this research, Napier grass (Pakchong 1) was employed as a lignocellulosic substrate due to its high productivity and importance in sustainable agriculture because it is widely utilized as animal feed and contributes significantly to soil conservation. Stems and leaves of NG aged around 45 to 60 days were used in this study. The harvested NG was initially reduced in size using a hammer mill, producing particles within the 0.2 to 0.5 cm range. Following size reduction, the NG was subjected to alkaline thermochemical treatment by immersing it in a 0.6% (w/v) NaOH solution at a temperature of 90 °C for a period of 2 h (Rekha and Aniruddha, 2013). PM was sourced from a university-affiliated livestock facility and subjected to alkaline pretreatment using 5% $Ca(OH)_2$ to enhance its biodegradability. Although NaOH is widely recognized for its strong alkaline properties and efficacy in lignocellulosic disruption, $Ca(OH)_2$ at a 5% concentration is frequently preferred in the treatment

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of PM due to its lower cost, reduced environmental footprint, and comparatively safer handling characteristics. Unlike NaOH, $Ca(OH)_2$ exhibits lower causticity, rendering it more appropriate for large-scale or decentralized operations where operator safety and chemical stability are of paramount importance(Meléndez-Hernández et al., 2021). The PM-$Ca(OH)_2$ mixture was thoroughly agitated, thermally treated at 70 °C, and maintained under controlled conditions for a duration of seven days. To preserve sample stability and inhibit microbial activity prior to anaerobic digestion, the pretreated PM was subsequently stored at 4 °C.

Anaerobic sludge obtained from an actively operating biogas reactor was utilized as an inoculum. Prior to its application, the sludge was washed with a nutrient solution and subsequently incubated at 35 ± 2 °C for a period of seven days under strictly anaerobic conditions. This pretreatment aimed to minimize residual biogas production and to stabilize microbial activity, thereby ensuring consistency in subsequent experimental procedures (Angelidaki et al., 2009).

Characterization of substrates

Total solids (TS) and volatile solids (VS) were quantified following Eaton and Franson (Eaton and Franson, 2005). Samples were oven-dried at 103–105 °C until constant mass, cooled, and weighed to calculate TS. The dried residues were ashed at 550 °C for 1–2 h, and the corresponding mass reduction was recorded as VS. Moisture content was derived from the weight difference between wet and dried samples. Total carbon and nitrogen were measured with an elemental analyzer (multi N/C 2100s, Analytik Jena). The pH of slurry samples was determined using a LAQUAtwin-PH-11 portable meter (Horiba). All determinations were performed in triplicate to ensure reproducibility. The key properties of PM, NG and the inoculum are listed in Table 1.

Table-1. Characterization of substrates and inoculum.

Parameter	Napier Grass	Pig manure	Inoculum
TS (g/kg)	223.624 ± 0.527	262.254 ± 0.468	118.012 ± 0.132
VS (g/kg)	190.238 ± 1.004	201.147 ± 1.028	83.356 ± 0.164
C/N	45.8 ± 2.62	11.6 ± 0.85	25.27 ± 0.74
Moisture (%)	71.96 ± 0.2	58.78 ± 0.41	86.18 ± 0.16
pH	7.2 ± 0.1	6.3 ± 0.1	7.0 ± 0.1

Experimental design and operating procedure

The experimental setup employed 2,000 mL plastic reactors, each with an effective working volume of approximately 1,000 mL, for batch anaerobic digestion tests. Five different substrate mixing ratios of thermochemically pretreated PM to NG were investigated on a VS basis: 1:0, 3:1, 1:1, 1:3, and 0:1, as detailed in Table 2. The reactors were incubated in a temperature-regulated chamber set within the range of 33 to 37 °C for a duration of 30 days. Throughout the digestion process, each reactor was manually agitated once daily to promote uniform digestion and gas release.

The co-AD process was conducted with a total substrate load of 1,000 g. An inoculum, sourced from an operational anaerobic digester, was incorporated at a rate of 10% based on the total weight of the mixture. Biogas output was recorded daily using the water displacement technique under mesophilic temperature conditions. Methane content was analyzed under steady-state conditions, with measurements taken each day throughout the experimental period. The daily methane yield was determined by linking the biogas volume produced to the corresponding methane concentration at each sampling point (Zhong et al., 2012), as illustrated in Figure 1.

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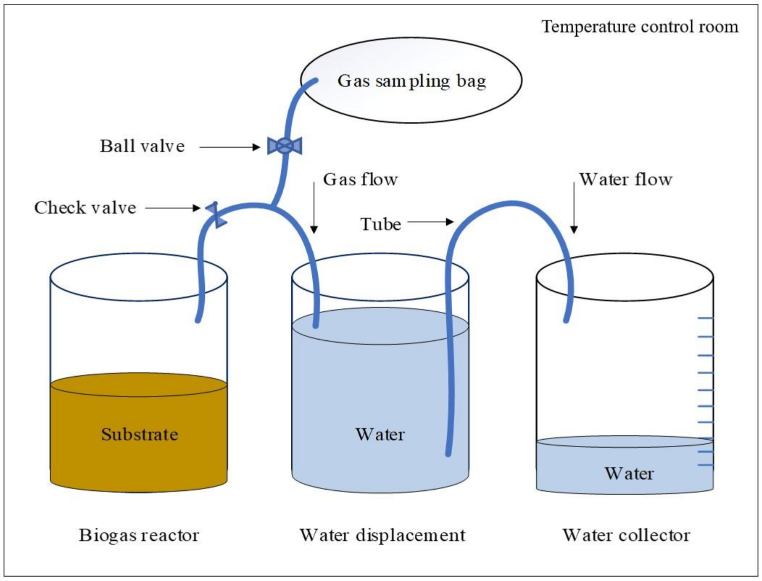


Figure-1. Laboratory-scale biogas production setup.

Table-2. Experimental conditions setup.

Run order	PM/NG ratio	TS (%)
1	1:0	3
2	3:1	3
3	1:1	3
4	1:3	3
5	0:1	3
6	1:0	5
7	3:1	5
8	1:1	5
9	1:3	5
10	0:1	5
11	1:0	7
12	3:1	7
13	1:1	7
14	1:3	7
15	0:1	7

Analytical methods

Water displacement was used to measure the volume of biogas, and a Shimadzu GC-2014 gas chromatograph coupled to gas-tight Tedlar® sampling bags was used to determine its composition. Measurements were carried out in triplicate to attain statistical reliability.

Kinetic model analysis

The Modified Gompertz model was selected to predict CMY, incorporating a specific parameter to account for the maximum methane production rate. Modification of Gompertz improves the model's capacity to accurately capture the temporal dynamics of biogas production. The mathematical expression of the Modified Gompertz model is presented in Equation (1) (Pan et al., 2024):

$$\mathbf{M_t = M_{max} \cdot exp\left[-exp\left(\frac{R_{max}\cdot(\lambda-t)}{M_{max}}+1\right)\right]} \quad (1)$$

Commonly utilized to explain population dynamics and growth-limited processes, Logistic model was also used to simulate methane production during AD of organic substrates (Moharir et al., 2020). This model is suitable for characterizing systems that initially exhibit exponential development before plateauing because of limiting variables. The mathematical form of the Logistic model is given in Equation (2):

$$\mathbf{M(t) = \frac{M_{max}}{\{1+exp[4R_{max}\frac{(\lambda-t)}{M_{max}}+2]\}}} \quad (2)$$

By introducing more shape and rate parameters to the logistic growth equation, the Richards model improves its flexibility in fitting empirical data across diverse fields such as ecology, biology, and bioenergy (Wang et al., 2012). Due to ability to capture a broader range of sigmoidal behaviors, it is particularly suitable for modeling methane production in complex biological systems. The model is expressed in Equation (3) (Matobole et al., 2024):

$$\mathbf{M_t = M_{max}\left[1+\upsilon . exp\left(-k(t-\lambda)\right)\right]^{-1/\upsilon}} \quad (3)$$

Another known model to simulate the kinetics of organic material biodegradation is the Cone model. Although originally intended to represent hierarchical data structures, it has been effectively modified for biogas research, providing reliable estimations of methane yield and degradation efficiency. Its ability to simulate non-linear and time-dependent behaviors observed in anaerobic digestion contributes to its importance (Venkateshkumar et al., 2022). The Cone model is described mathematically in Equation (4):

$$\mathbf{M_t = \frac{M_{max}}{1+(Ht)^{-n}}} \quad (4)$$

Where, *M(t)* (mL g^{-1} VS) denotes the cumulative methane production at time *t* (days); M_{max} corresponds to the maximum methane production potential (mL g^{-1} VS); R_{max} represents the maximal production rate (mL g^{-1} VSd^{-1}); λ is the lag phase duration(days); and *exp* is the base of the natural logarithm (approximately 2.718) ; k is the specific methane production rate (day^{-1}), υ is the coefficient of curve's shape; H is the hydrolysis rate constant (day^{-1}); and n represents a shape factor without units.

The model's goodness of fit was assessed by comparing it with other kinetic models using statistical metrics such as the coefficient of determination (R^2), as defined in Equation 5, along with the root mean square error (RMSE).

$$\mathbf{R^2 = 1 - \frac{SS_E}{SS_T}} \quad (5)$$

Where, SS_E and SS_T represent the residual and total sum of squares, respectively. RMSE measures the variation between the recorded and predicted biogas yield. A good model fit is indicated by a minimal RMSE value. The calculation of RMSE were performed using the following formula:

$$\mathbf{RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(M_{p,i}-M_{n,i}\right)^2}} \quad (6)$$

Here, M_p denotes the predicted biogas output, M_n refers to the experimentally observed value, and N indicates the total number of data points. These parameters are subsequently used in non-linear regression analysis.

The mean absolute error (MAE), shown in Equation (7), is a widely adopted indicator for assessing the performance of regression models. It calculates the average size of the absolute differences between predicted and actual values, offering a clear and simple evaluation of a model's precision. A smaller MAE suggests greater accuracy, reflecting strong agreement between predicted outputs and observed experimental results.

$$\mathbf{MAE = \frac{1}{N}\sum_{i=1}^{N}\left|M_{p,i}-M_{n,i}\right|} \quad (7)$$

Where, M_p refers to the estimated biogas yield, M_n indicates the observed yield from experiments, and N represents the overall number of recorded data points.

In order to accurately represent biogas, the most appropriate kinetic models were used to estimate M_{max}, R_{max}, λ, and H under various experimental conditions. To evaluate the combined impact of TS content and PM/NG ratio on these kinetic parameters, they were subsequently treated as dependent response variables within the experimental.

Results and Discussion

Biogas yields

The trend of CMY throughout the co-AD process is illustrated in Figure 2. Because microbial communities acclimated to the substrates, a gradual increase in CMY during the initial phase was observed, indicating a lag phase (Ahmad et al., 2019). This was followed by a period of relatively stable methane production. As the process progressed, microbial activity increased, leading to an accelerated phase characterized by a significant rise in methane production (Mutungwazi et al., 2020). However, producing methane began to decrease after day 20, due to the accumulation of inhibitory compounds within the reactor, attributable to high nitrogen that caused ammonia toxicity and an absence of micronutrients that inhibited microbial activity (Anacleto et al., 2024). Therefore, to achieve methane production, it is important to maintain the correct C/N ratio and make sure that trace elements are supplemented enough (Chow et al., 2020).

In the biogas produced, the average methane concentration was 64.27%. Methanogenesis was initiated relatively early across most experimental runs; in run orders 1, 6, 7, 13, and 9, biogas production was seen as early as day 2. In contrast, delayed onset was recorded on day 4 in run orders 11, 2, 12, 3, 8, 4 and 5; on day 6 in run orders 10 and 15; and on day 7 in run order 14. This relatively rapid initiation of biogas production is likely attributable to the thermochemical pretreatment applied to the substrates. This effectively disrupts the structural integrity of cellulose, hemicellulose and lignin to improve microbial and enzymatic accessibility and to enhance hydrolytic efficiency (Hendriks and Zeeman, 2009). This pretreatment strategy also contributed to a reduction in the overall digestion time, thereby expediting bioenergy generation (Kumar and Sharma, 2017). The highest daily methane production was recorded at 29.27 mL g^{-1} VS d^{-1}, while the maximum cumulative methane yield of 210.47 mL g^{-1} VS was achieved with a 1:1 PM/NG mixing ratio at a TS concentration of 3% (run order 3). In contrast, the lowest cumulative methane yield, 38.23 mL g^{-1} VS, was observed in the 1:0 PM/NG mixture at a TS concentration of 7% (run order 15).

The co-AD of PM and NG at a 1:1 mixing ratio has demonstrated superior CMY compared to other ratios such as 1:0, 3:1, 1:3, and 0:1. The main cause of this improved performance is an optimized C/N ratio, which falls within the range of 20–30 for effective methanogenic activity. A balanced substrate that promotes microbial activity and methane production is provided by PM which is rich in nitrogen and NG, high in carbon (Mata-Alvarez et al., 2014). In contrast, mono digestion or unbalanced mixtures often lead to C/N imbalances, frequently causing ammonia inhibition or poor hydrolysis. Additionally, the 1:1 ratio also enhances biodegradability and system's buffering capacity. PM contributes active microbial populations and buffering agents (Zhang et al., 2011), while NG offers a fibrous carbon source that supports prolonged digestion. Where either higher PM or higher NG, imbalanced ratios may disrupt the process due to excessive nitrogen or lignocellulosic resistance. In contrast, the balanced composition enhances hydrolysis, acidogenesis, acetogenesis and methanogenesis that all is important stages of AD. Additionally, the 1:1 ratio also promotes syntrophic interactions among key microbial groups, increasing community diversity and stability preventing the accumulation of volatile fatty acids (Gu et al., 2014). This result can inhibit methane production. Furthermore, operational benefits of the 1:1 ratio simplifies feedstock handling, reduces chemical input needs and enhances economic feasibility.

A decline in cumulative methane yield is often observed as TS concentration increases from 3% to 7%, primarily due to inhibited microbial activity caused by elevated substrate levels. Higher TS leads to increased viscosity, which impairs mass transfer and limits nutrient diffusion. High TS conditions also promote the accumulation of volatile fatty acids and ammonia, particularly when nitrogen-rich pig manure is co-digested with NG. Excess ammonia from protein degradation can inhibit methanogenic archaea, while volatile fatty acids may accumulate if methane-forming microbes are suppressed, resulting in reduced gas yield and metabolic imbalance. Additionally, elevated TS affects reactor performance by hindering mixing, causing stratification, and leading to uneven microbial activity and partial digestion. These conditions reduce methane conversion efficiency and weaken the system's buffering capacity, increasing

susceptibility to pH instability and process failure. Maintaining an optimal TS range is therefore critical to sustain microbial synergy, ensuring process stability, and maximizing methane yield in co-digestion systems (Kriswantoro et al., 2023).
Despite thermochemical pretreatment, methane production from co-AD of PM and NG was quite modest, reflecting challenges commonly reported in lignocellulosic biomass digestion. Gentle settling conditions can fail to substantially break the lignin and crystalline cellulose structure while agents like $Ca(OH)_2$ can release ammonia from PM and generate inhibitory by-products such as phenolics and furfurals from NG. Long pretreatment and washings may also decrease volatile solids responsible for available carbon for methane production. Operational conditions such as 30-day batch periods, higher TS levels and limited control of organic loading and dilution could have hampered full synergy between PM and NG making the C/N balance and inhibitor management crucial. In general, the modest yields emphasize the intricate relationship between pretreatment chemistry and bioprocessing tolerance.

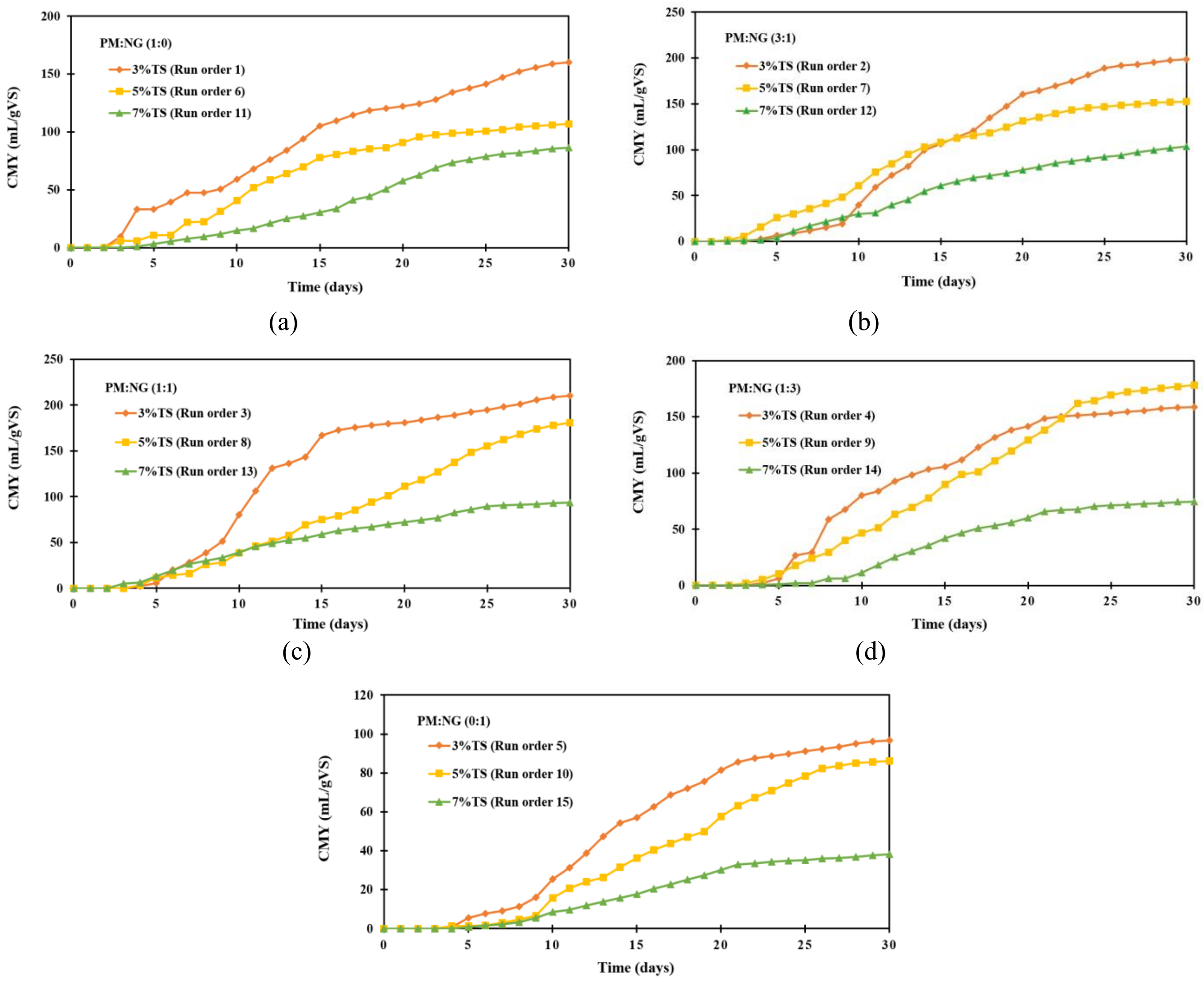


Figure-2. Daily CMY recorded throughout the experimental period for each condition.

Predictive performance of alternative kinetic equations

In this study, the kinetic behavior of Co-AD system was analyzed using four mathematical models: Modified Gompertz model, Logistic model, Richards model, and Cone model. These models were employed to predict methane production and were compared against the experimental data. Model performance was evaluated based on three statistical indicators: R^2, RMSE, and MAE. The results of the model fitting analysis are summarized in Table 3.

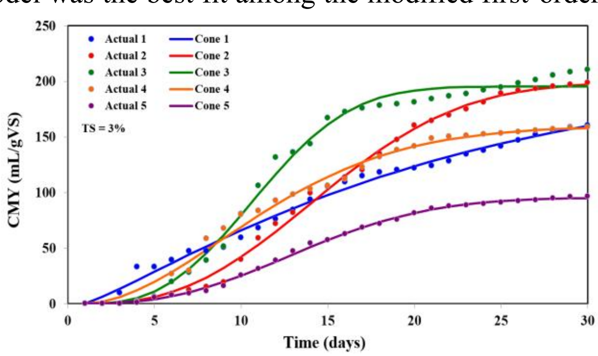
Table-3. Model performance evaluation.

Run order	Model											
	Modified Gompertz			Logistics			Cone			Richards		
	R^2	RMSE	MAE	R^2	RMSE	MAE	R^2	RMSE	MAE	R^2	RMSE	MAE
1	0.9881	5.1345	4.2751	0.9827	6.1845	4.7936	0.9904	4.5997	3.8071	0.9747	7.4898	5.7257
2	0.9972	3.8621	2.734	0.9927	6.2786	5.4034	0.9962	4.492	3.2525	0.985	8.9846	7.8399
3	0.9941	5.7741	4.6185	0.9903	7.4143	6.2229	0.9904	7.3555	5.9933	0.935	19.1868	17.5105
4	0.9881	5.9497	4.4027	0.9768	8.3169	6.4386	0.9889	5.748	4.0785	0.9574	11.2616	8.9347
5	0.9986	1.3178	1.0231	0.9957	2.31	1.9055	0.998	1.567	1.1804	0.9821	4.7183	4.073
6	0.9969	2.0168	1.658	0.9927	3.1089	2.5648	0.9958	2.371	1.9475	0.9683	6.4947	5.8141
7	0.9967	2.8864	2.241	0.9925	4.3276	3.4365	0.9966	2.9109	2.1596	0.9771	7.5729	6.4227
8	0.9972	3.1713	2.7675	0.9936	4.7629	3.8006	0.9981	2.6071	2.318	0.9946	4.3799	3.3136
9	0.9964	3.7395	3.0664	0.9946	4.5646	3.7915	0.9968	3.517	2.7329	0.9948	4.4674	3.6816
10	0.996	1.9725	1.5797	0.9924	2.7188	2.3024	0.9963	1.889	1.4776	0.9925	2.7029	2.2094
11	0.9948	2.2133	1.8713	0.9974	1.5706	1.3395	0.9957	2.0041	1.7157	0.998	1.3817	1.1597
12	0.9966	2.0287	1.7963	0.9906	3.3568	2.9153	0.9968	1.9563	1.644	0.9809	4.7851	3.9734
13	0.9908	2.7987	2.3445	0.9806	4.0534	3.3674	0.9958	1.8817	1.5187	0.9735	4.7455	3.7632
14	0.9987	1.036	0.7701	0.9949	2.0471	1.7079	0.9968	1.6198	1.2437	0.9817	3.8664	3.3884
15	0.9972	0.7397	0.5733	0.9964	0.8389	0.7134	0.9981	0.6057	0.4883	0.9911	1.3297	1.1559
Average	0.9952	2.9761	2.3814	0.9909	4.1236	3.3802	0.9954	3.0083	2.3705	0.9791	6.2245	5.2644

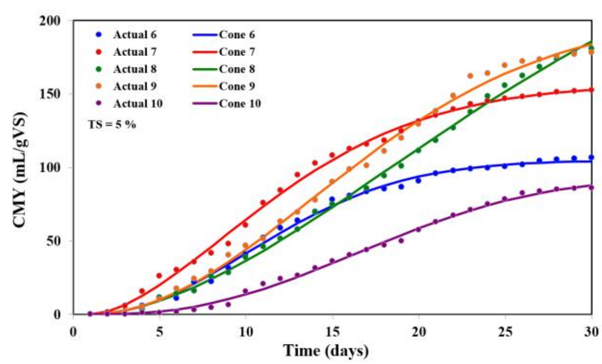
As presented in Table 3 and Figures 3–6, the predicted and observed values of CMY across all experiments exhibited a strong correlation, indicating high accuracy in all four kinetic models. Among them, the Cone model demonstrated the best performance, with $R^2 \geq 0.9889$, RMSE ≤ 7.3555, and MAE ≤ 5.9933. It averaged these three metrics at 0.9954, 3.0083, and 2.3705, respectively. The Modified Gompertz model came in second with comparable average values of 0.9952, 2.9761, and 2.3814, respectively, indicating high predictive accuracy as well. This similarity in R^2 and RMSE between both models is attributable to the fact that both are capable of depicting sigmoidal (S-shaped) growth which is commonly found in most biological activities including that of biogas production and microbial growth (Tjørve and Tjørve, 2017). Further insight is provided about these results by the curve shapes shown in Figures 3–6. Indeed, it was curves from the Cone model (Figure 3) and Modified Gompertz model (Figure 4) that were of sigmoid shape and fit well with the experimental data. In contrast, produced by the Logistic model (Figure 5) and the Richards model (Figure 6), the sigmoid curves demonstrated a relatively worse fit. Significantly, at run orders 3 and 7 of the co-AD process, both the Logistic and Richards models showed greater deviations in accurately representing the data, hence indicating their limited prediction performance under these conditions.

The Modified Gompertz model is most preferred for describing the AD kinetics since it briefly defines major biological phases as adaptation, exponential growth and saturation (Pardilhó et al., 2022). In contrast, the Cone model excels in its flexibility, particularly through variation of the exponent parameter to fit non-linear or complicated degradation dynamics of organic matter (Polastri et al., 2024). This adaptability agrees with some results obtained in thermochemically pretreated lignocellulosic biomass like NG since pretreatment improves microbial

activity in reactor by breaking the cellulose, hemicellulose and lignin structure (Deepanraj et al., 2017). Effective pretreatment of substrates ensured streamlined experimental data that aligned with the expected results of both the Modified Gompertz and Cone models. Despite structural differences in their equations, both models possess adaptable parameterization capabilities, rendering them useful for capturing the sigmoid-shaped biogas production curves (Mohammadianroshanfekr et al., 2024). Moreover, the low MAE values obtained from both models suggest that both models provided accurate fits, further confirming that they can be reliably used to model systems with pretreated substrates that exhibit uniform degradation behavior(Li et al., 2015).
This finding is consistent with previous studies demonstrating the Cone model's superiority relative to other kinetic models used in AD processes. In the co-AD of sewage sludge and food waste, several studies found that the Cone model performed better than the Gompertz and two-substrate models. In one study, the Cone model was found to outperform others in predictive accuracy and correlation with experimental results (Pan et al., 2019). Also, in the modeling of the co-AD of sewage sludge and Egeria densa, the Cone model was the best fit among the modified first-order and modified Gompertz models (Zhen et al., 2015). The Cone model's reliability was further confirmed in a kinetic study of AD of bovine manure where it bested modified first-order, MG, and double-pool kinetic model's predictive capabilities (Zhang et al., 2019). Furthermore, in the AD of residual sludge with the addition of iron nanoparticles, the Cone model once again demonstrated better fitting accuracy than modified first order and function transfer models.
Although the Logistic and Richards models yield statistical indicators that were within acceptable limits, they performed considerably worse than the Modified Gompertz and Cone models. The Logistic model, for instance, is far less accurate when it comes to predicting the early phases of methane production, leading to increasingly inaccurate forecasts of biogas production and diminished accuracy in the forecasted values (Zahan and Othman, 2019). The Richards model demonstrated the lowest overall performance, which is mostly because of its intricate and high-parameter framework that leads to overfitting in accuracy and reduces its capacity to generalize to new data (Zhang et al., 2019).



(a)



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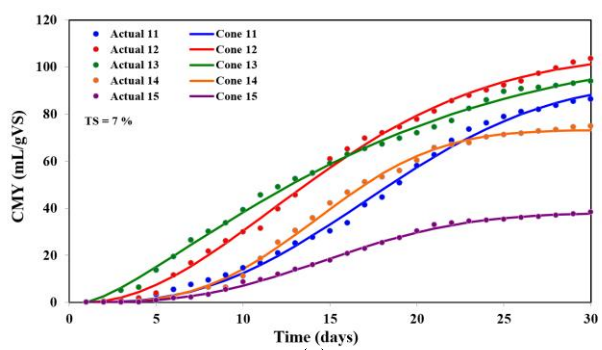

(c)

Figure-3. Experimental data compared with Cone model predictions for (a) run order 1-5, (b) run order 6-10, (c) run order 11-15.

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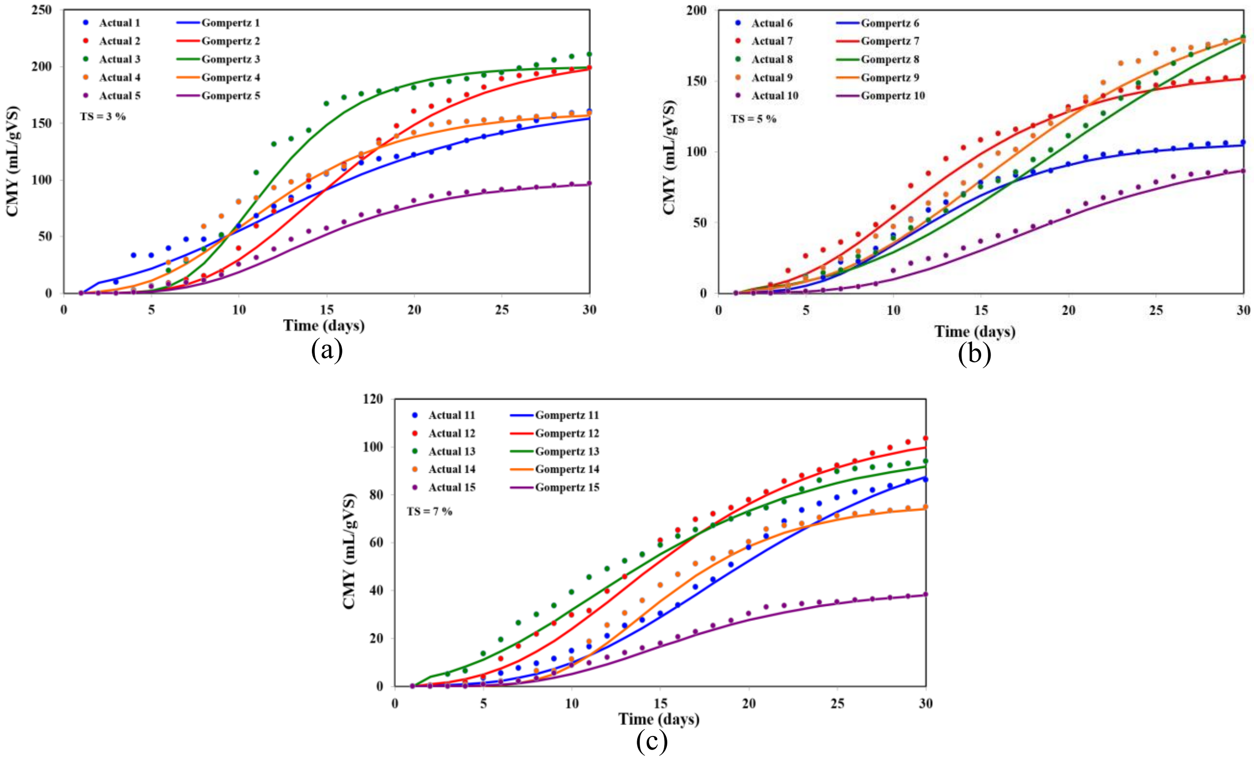

Figure-4. Experimental data compared with Modified Gompertz model predictions for (a) run order 1-5, (b) run order 6-10, (c) run order 11-15.

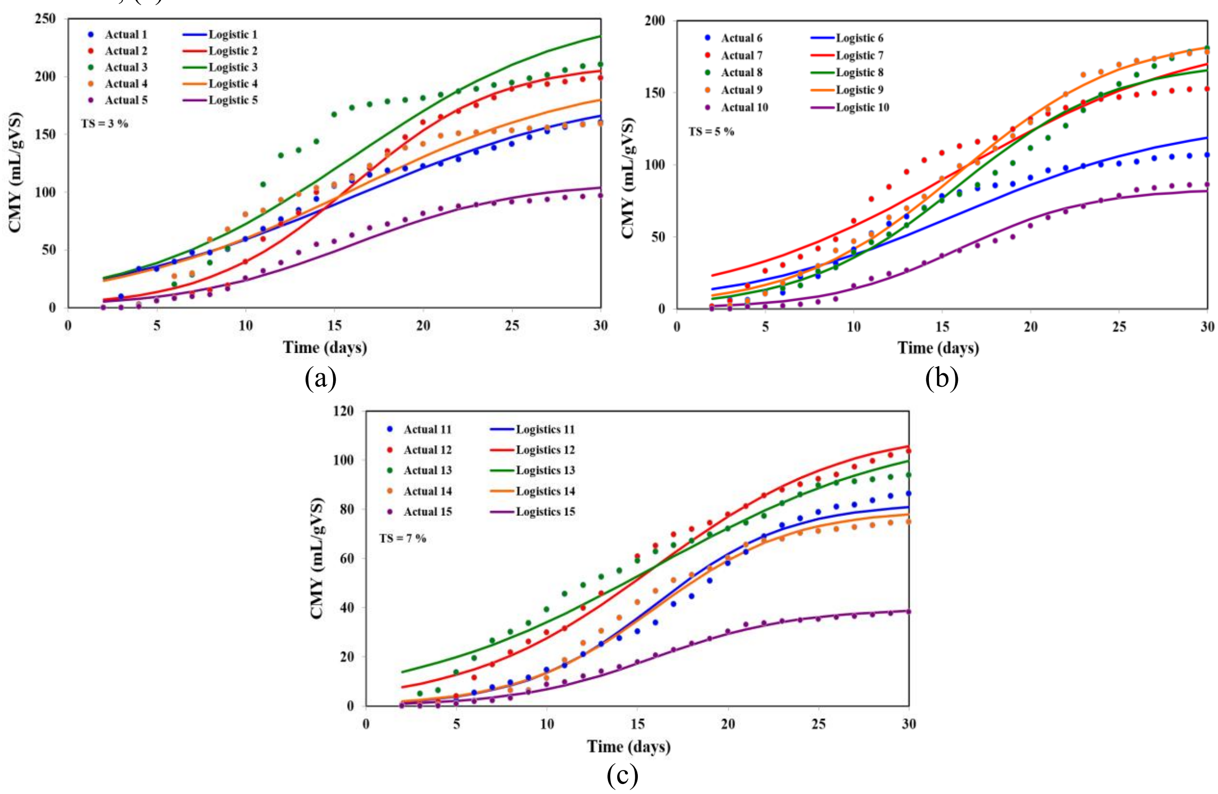

Figure-5. Experimental data compared with Logistic model predictions for (a) run order 1-5, (b) run order 6-10, (c) run order 11-15.

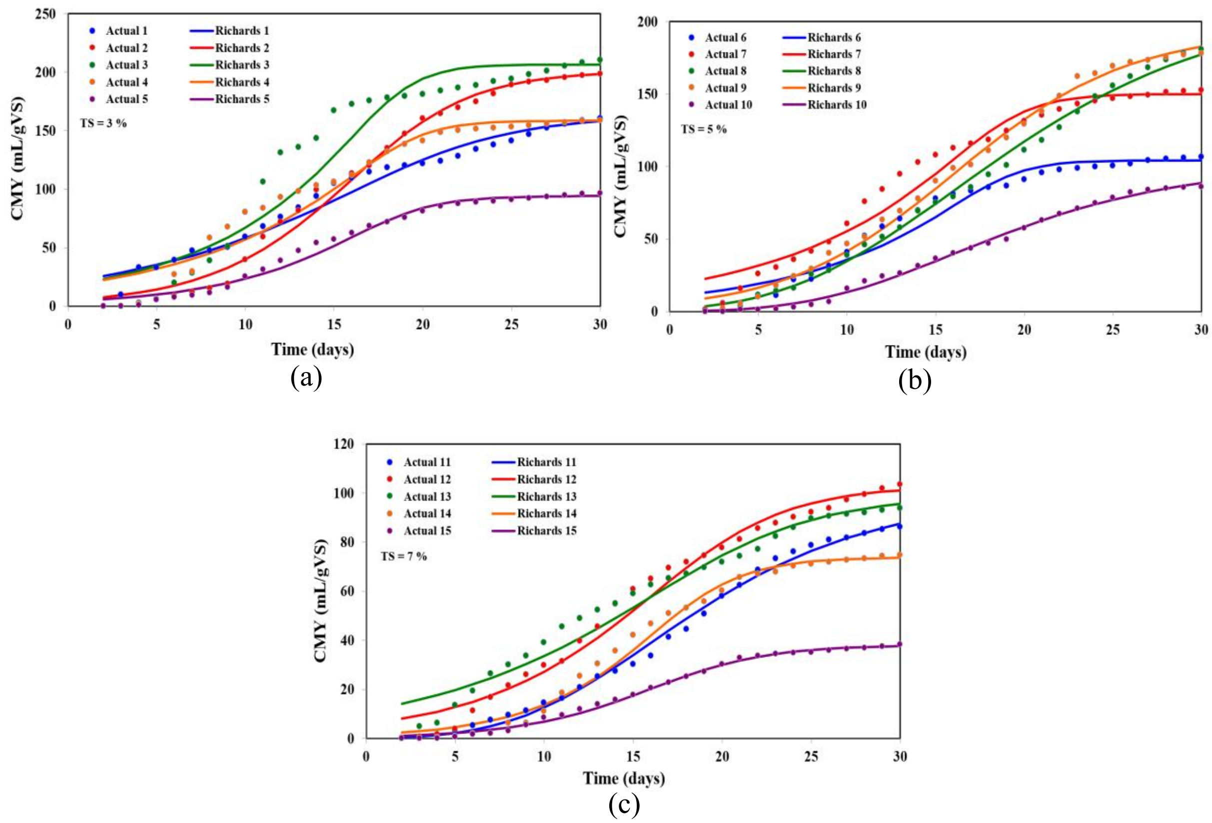

Figure-6. Experimental data compared with Richards model predictions for (a) run order 1-5, (b) run order 6-10, (c) run order 11-15.

The Modified Gompertz, Logistic, Richards, and Cone models were employed to estimate the kinetic parameters of methane production from the co-AD of PM and NG to better understand the impact of the co-AD process. Based on the most accurate model fitting, the values for essential parameters (M_{max}, H, R_{max}, λ, and n) were calculated. The corresponding results are presented in Table 4.

With all of the kinetic models used, M_{max} at TS concentration of 3% increased with the proportion of PM in the substrate up to a PM: NG mixture ratio of 1:1. However, a further increase in the PM proportion from 1:1 to 3:1 resulted in a decline in M_{max}, indicating an optimal PM:NG ratio at 1:1 under these conditions. In contrast, at 7% TS, M_{max} consistently increased with higher PM proportions, suggesting enhanced synergistic effects at elevated PM levels. At 5% TS, however, M_{max} exhibited no discernible trend in response to changes in the PM:NG ratio. Overall, an increase in TS concentration was generally associated with a reduction in M_{max} across all co-AD scenarios. According to the predictions of the Modified Gompertz and Logistic kinetic models, R_{max} at TS concentration of 3% followed a trend like that observed for M_{max}. In contrast, at TS concentrations of 5% and 7%, R_{max} exhibited no consistent pattern in response to variations in the substrate composition. Among the kinetic models evaluated, the Logistic model produced the highest estimates of M_{max}, followed sequentially by the Richards, Modified Gompertz, and Cone models. The duration of the lag phase remained relatively consistent across varying TS concentrations and PM/NG ratios, indicating minimal sensitivity of this parameter to changes in substrate composition. Specifically, the lag phase ranged from 1.74 to 2.45 days as predicted by the Modified Gompertz model, and from 11.96 to 18.10 days according to the Logistic model.

To comprehensively assess the synergistic effects of TS concentration and PM/NG ratio on the co-AD process, the kinetic parameters derived from the Modified Gompertz model, including M_{max}, R_{max} and λ, are presented in the accompanying table and visually represented in Figures 7–9. Furthermore, as obtained from the Cone model, H is also provided in the table and illustrated in Figure 10.

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Table-4. The kinetic parameters corresponding to the Modified Gompertz, Logistic, Cone, and Richards models.

Run order	Model											
	Modified Gompertz			Logistics			Cone			Richards		
	M_{max}	R_{max}	λ	M_{max}	R_{max}	λ	M_{max}	H	n	M_{max}	k	υ
1	154.13	9.06	1.87	166.14	6.41	14.01	160.02	0.0122	2.43	158.12	0.15	1.22
2	197.50	13.36	2.15	204.83	12.80	15.45	197.06	0.0183	2.24	198.45	0.13	1.34
3	199.06	20.07	1.98	235.04	10.30	14.76	195.43	0.0247	2.40	206.52	0.16	1.19
4	156.80	11.24	2.01	179.89	7.38	16.02	157.97	0.0114	2.41	158.63	0.15	1.26
5	95.74	6.89	1.74	103.75	5.77	17.07	94.87	0.0051	1.47	94.19	0.14	1.30
6	104.55	7.59	1.89	118.77	5.11	18.10	104.23	0.0099	1.58	104.26	0.14	1.27
7	151.65	9.53	2.10	170.09	6.88	17.99	152.85	0.0147	2.67	150.06	0.15	1.24
8	178.23	8.45	2.45	165.68	9.69	16.55	185.78	0.0213	1.56	177.52	0.16	1.23
9	180.89	9.41	2.32	181.35	10.11	15.52	183.83	0.0099	2.33	182.93	0.16	1.21
10	86.69	4.91	2.14	81.84	5.59	14.28	88.01	0.0018	1.69	88.81	0.14	1.32
11	87.46	4.76	2.28	80.81	5.60	13.89	88.15	0.0013	1.79	87.52	0.13	1.33
12	99.74	5.77	2.01	105.75	5.32	13.00	101.17	0.0036	1.26	101.11	0.14	1.29
13	91.68	3.90	1.95	99.71	4.00	12.46	94.75	0.0079	1.58	95.54	0.15	1.25
14	74.05	5.86	2.13	77.97	5.25	12.15	73.11	0.0018	2.24	73.63	0.15	1.23
15	38.03	2.51	2.02	38.63	2.51	11.92	37.69	0.0013	1.08	37.61	0.13	1.35

As illustrated in Figure 7, increasing TS concentration from low to moderate levels slightly reduces M_{max} due to increased viscosity and limited mass transfer, which hinder microbial activity and nutrient diffusion, particularly under mesophilic conditions. Researchers reported that biogas production at 5% TS was 64% higher than at 25%, due to reduced microbial efficiency at higher TS levels (Ahmadi-Pirlou and Mesri Gundoshmian, 2021). While moderate TS improves sludge disintegration and heating efficiency, excessive TS reduces fluidity, hindering disintegration (Gao et al., 2023). Moreover, increasing TS from 2% to 10% improved cell viability and reduced intracellular oxidative stress. However, TS levels beyond 10% led to the accumulation of ammonia nitrogen and volatile fatty acids, suppressing enzymatic activity and shifting the methanogenic community from acetylotrophic to less efficient hydrogenotrophic pathways. Conversely, increasing PM/NG ratio significantly enhances M_{max} due to the complementary biochemical properties of the substrates. PM supplies nitrogen and readily degradable organics, while NG contributes carbon-rich lignocellulosic material, creating a balanced C/N ratio that supports microbial activity and methanogenesis. Higher PM ratios enhance hydrolysis, acidogenesis, and pH stability but risk ammonia toxicity. An optimal PM/NG ratio maximizes methane yield, favoring methanosaeta-dominated communities that efficiently convert acetate to methane under low-ammonia, stable conditions (Chen and He, 2015).

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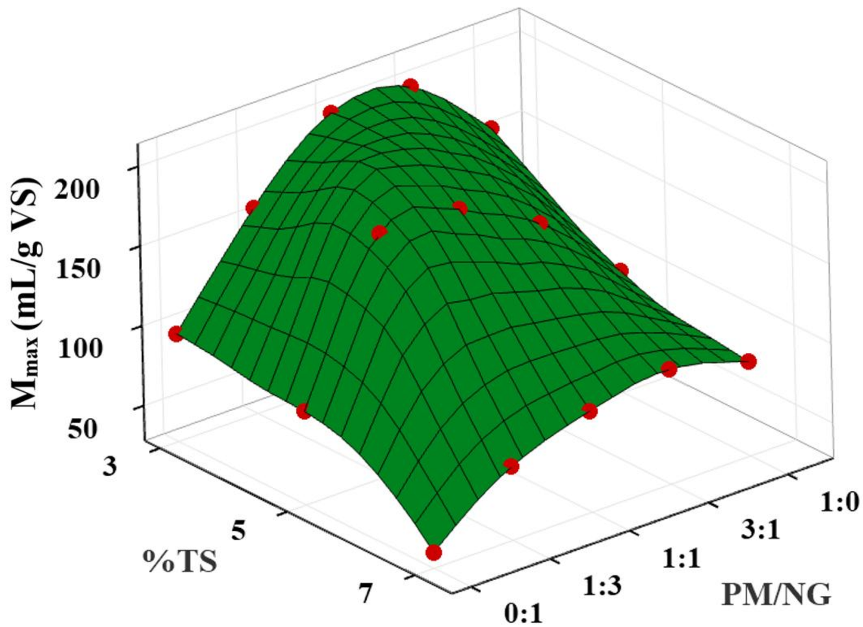

Figure-7. Relationship between PM/NG ratio and TS, emphasizing their impact on $M_{max.}$

As shown in Figure 8, at a TS concentration of 3%, R_{max} increased as the PM/NG ratio rose from 0:1 to 1:1, but declined when PM became dominant (1:0). This trend highlights the importance of a balanced co-AD, where PM provides essential nutrients and buffering capacity, enhancing microbial activity and methane production. These findings are consistent with previous studies (Zhen et al., 2015) reporting improved digestion performance when PM was mixed with other substrates. The initial R_{max} increase is linked to an optimal C/N ratio and enhanced degradation of NG's fibrous content by PM-induced microbial stimulation. However, excessive PM leads to ammonia accumulation and reduced structural carbohydrates, inhibiting methanogenesis. At higher TS levels (5% and 7%), R_{max} remains largely unchanged across PM/NG ratios, likely due to increased viscosity and mass transfer limitations that override the effects of substrate composition.

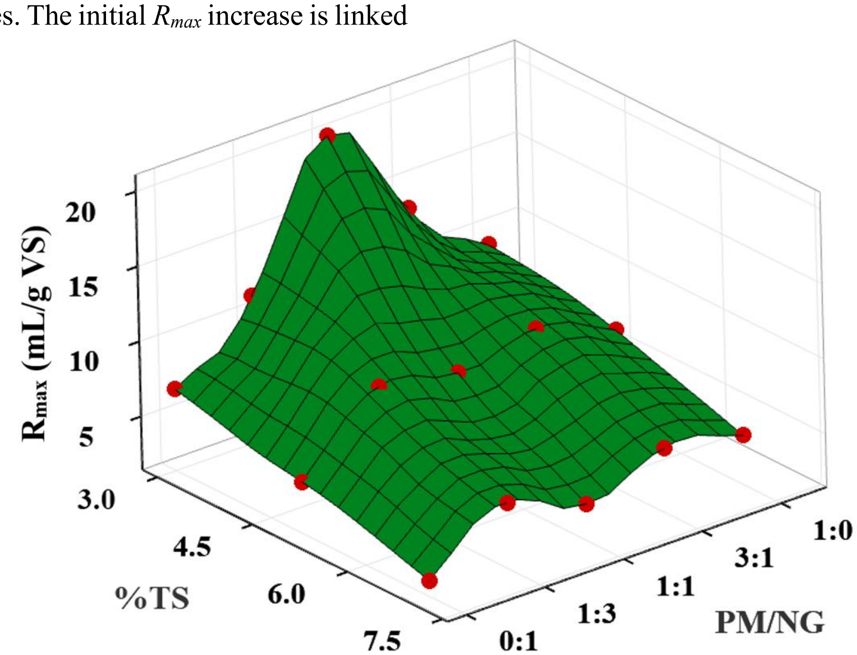

Figure-8. Relationship between PM/NG ratio and TS, emphasizing their impact on R_{max}.

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Figure 9 demonstrates that the lag phase duration, the period before significant methane production, remains relatively stable despite changes in TS concentration and PM/NG ratios. This consistency is attributed to the high adaptability of microbial consortia and the complementary nature of the substrates, with PM supplying readily degradable nitrogen-rich matter and NG providing slowly hydrolyzing lignocellulosic biomass. Modified Gompertz modeling suggests that temperature and inoculum activity have a greater impact on the latency phase than substrate composition. The buffering capacity and nutrient balance between PM and NG also help stabilize pH and limit inhibitory compounds, supporting early microbial activity. Pretreatments such as alkaline and thermochemical processing enhance substrate breakdown without significantly altering microbial acclimatization time, indicating that inoculum characteristics are more influential. While some studies (Zahan et al., 2018) report a shortened latency with higher substrate concentrations, others(Wang et al., 2020) observe the opposite, highlighting the complex interplay between substrate availability and microbial dynamics. These insights are crucial for optimizing co-digestion performance in batch and semi-continuous systems.

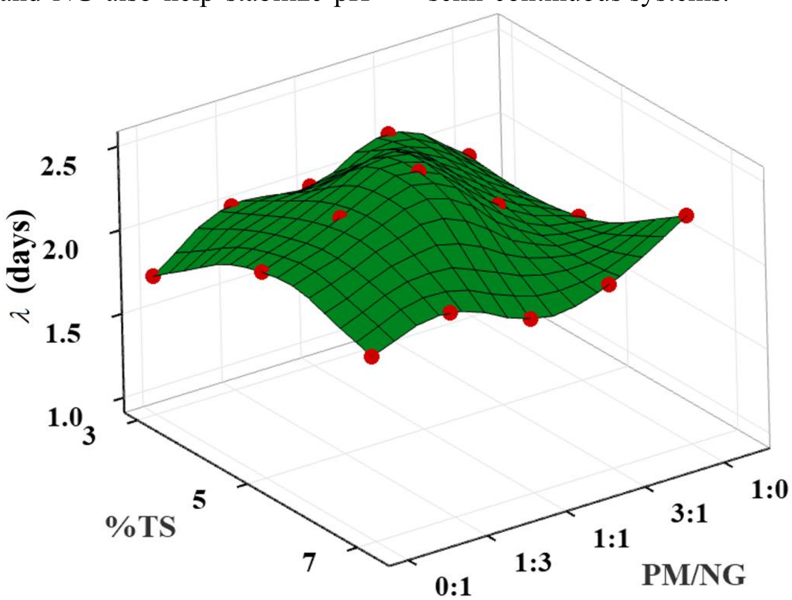

Figure-9. Relationship between PM/NG ratio and TS, emphasizing their impact on λ.

In the Cone model, at TS concentration of 3%, H increased from 0.0051 to 0.0247 day^{-1} as the PM/NG ratio rose from 0:1 to 1:1, indicating that co-digestion of NG and PM enhances the hydrolysis rate. The PM/NG ratio of 1:1 yielded the highest H value of 0.0247 day^{-1}, with the 3:1 ratio producing the second-highest value at 0.0183 day^{-1}. The corresponding kinetic parameters are presented in Table 4 and visually illustrated in Figure 10. H, a key factor in organic matter breakdown, is affected by TS concentration and substrate ratios. While higher TS levels slightly reduce the hydrolysis rate due to increased viscosity and reduced mass transfer, increasing the PM/NG ratio significantly enhances it. This is due to the biochemical synergy between PM, which supplies easily degradable nutrients, and NG, which benefits from enhanced enzymatic activity. The efficiency of hydrolysis and methane production can be improved by adjusting the PM/NG ratio to an optimal level. Additionally, higher PM content improves C/N balance, stabilizing microbial communities and minimizing inhibitors like ammonia and volatile fatty acids. The Modified Gompertz model confirms that substrate ratio has a stronger influence on hydrolysis kinetics than TS concentration, consistent with findings from previous studies (Zhang et al., 2014). However, increasing the PM/NG ratio beyond 1:1 (e.g., 3:1) leads to a decline in H due to ammonia inhibition and reduced structural carbohydrate content. Excess nitrogen from PM generates free ammonia, which inhibits microbial activity and slows the degradation of lignocellulosic material.

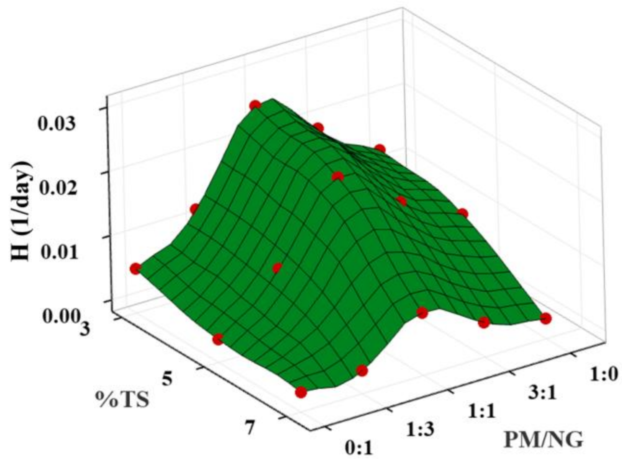

Figure-10. Relationship between PM/NG ratio and TS, emphasizing their impact on *H*.

Conclusion

This study examined the effects of PM/NG mixing ratios and TS concentrations on the co-digestion of thermochemically pretreated PM and NG. A 30-day batch experiment was conducted using PM/NG ratios (1:0 to 0:1) and TS levels (3%, 5%, and 7%). Four kinetic models, Modified Gompertz, Cone, Logistic, and Richards, were applied to determine methane production kinetics, with CMY as the primary performance indicator. Methane production exhibited three distinct phases: initial lag, active generation, and a final decline due to inhibition. The average methane content was 64.27%, with the highest daily yield (29.27 mL/g VS/day) and CMY (210.47 mL/g VS) observed at a 1:1 PM/NG ratio with 3% TS. Methane production began as early as day 2 in some trials, likely due to enhanced microbial access from pretreatment. Among the models, the Modified Gompertz and Cone provided the best predictive accuracy, while the Logistic and Richards models were less effective. The findings support the use of thermochemical pretreatment and appropriate kinetic modeling to optimize methane yield and improve the design of anaerobic digestion systems. Maintaining an optimal C/N ratio and sufficient micronutrient supply is essential for stable, long-term microbial activity and inhibition control.

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Use of Generative AI Tools Statement

Generative AI tools were not employed in experimental design, data collection, or analysis. Their use was limited solely to enhancing the clarity of language and improving the formatting of references and citations.

Contribution of Authors

Artnaseaw A: Processed, analyzed and interpreted data and manuscript write-up.
Santaweesuk A: Conducted the experimental procedures and processed the data and manuscript write-up.
Artnaseaw A: Supervised the overall research activities, coordinated the experimental procedures, and contributed to the writing and critical revision of the manuscript.

All authors read and approved final draft of the manuscript.

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