

# Enhancing Specific Methane Yield via Mesophilic Anaerobic Digestion: Ferric Chloride Catalysis in the Co-Digestion of Organic Fraction of Municipal Waste (OFMSW) and Waste Activated Sludge (WAS)

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**Abstract**—The mesophilic anaerobic co-digestion of Organic Fraction of Municipal Solid Waste (OFMSW) and Waste Activated Sludge (WAS) was conducted to evaluate the effects of alkaline pretreatment using calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ) and catalytic enhancement using ferric chloride ( $\text{FeCl}_3$ ) towards Specific Methane Yield (SMY). Eight setups (A–H) were done to investigate varying combinations of pretreatment and catalytic dosing. Setups A–C served as controls, while setups D–H were pretreated with  $\text{Ca}(\text{OH})_2$  at 2.3 g/L for OFMSW and 1.48 g/L for WAS, with  $\text{FeCl}_3$  catalyst added at varying dosages ranging from 0 to 0.30 g/L. Among all reactors, setup G (0.20 g/L  $\text{FeCl}_3$ ) exhibited the highest cumulative methane yield (175.31 mL/gVS). Three sigmoidal functions were used to estimate kinetic parameters, with the modified Gompertz function providing the best fit ( $R^2 > 0.99$ ) for methane production behavior. Pearson correlation analyses revealed significant relationships ( $p < 0.05$ ) between  $\text{FeCl}_3$  dosing and SMY, indicating that catalytic supplementation beyond 0.20 g/L did not yield additional improvements in methane production. T-test analysis of pH, BOD, and COD confirmed statistically significant differences ( $p < 0.05$ ) in methane yields between catalyst-enhanced setups and controls. These results suggest that  $\text{Ca}(\text{OH})_2$  pretreatment and  $\text{FeCl}_3$  catalysis at optimal concentrations significantly enhance methane production from the co-digestion of OFMSW and WAS.

**Keywords**—anaerobic co-digestion, methane yield enhancement, alkaline pretreatment, ferric chloride catalysis

## I. INTRODUCTION

The increasing global energy demand and the environmental impacts of fossil fuels have spurred the search for alternative, sustainable energy sources. One of the promising options is biogas, a renewable and versatile fuel that can be produced from various organic wastes by Anaerobic Digestion (AD) [1]. Biogas mainly consists of Methane ( $\text{CH}_4$ ) and carbon dioxide ( $\text{CO}_2$ ), and can be used for heat, electricity, or transportation [2]. However, the biogas production potential of different organic wastes depends on their chemical composition, physical properties, and biodegradability [3]. Therefore, pretreatment methods are often applied to enhance the AD process and increase the biogas yield. Among the various organic wastes, the Organic Fraction of Municipal Solid Waste (OFMSW) is one of the most abundant and widely available sources, comprising yard waste, kitchen waste, wood, and paper [4]. Its management and proper disposal have become a significant global concern, leading to increased risks to public health and environmental pollution [1]. For this study, the OFMSW was prepared from

scratch using a combination of food scraps commonly found in households, ensuring a representative composition of organic waste. In addition, they mixed OFMSW with Waste-Activated Sludge (WAS), a by-product of wastewater treatment. These organic wastes were utilized as the primary samples for this study.

The pretreatment of organic waste is an important step to enhance biogas production by increasing the solubilization and hydrolysis of complex organic matter. The selection of the pretreatment method depends on the type and characteristics of the organic waste, as well as the cost, energy consumption, and environmental impact. Since OFMSW contains high levels of lignocellulosic material, which is resistant to microbial degradation and limits biogas production [5], an alkaline pretreatment was used. This type of pretreatment is used when lignocellulosic biomass is present, as it is readily available, uses reduced temperature and pressure, and yields a higher amount of Methane [6]. In addition, Waste-Activated Sludge (WAS) was added to enhance the breakdown of organic matter in OFMSW, thereby improving digestion efficiency. Furthermore, cow manure, a material with high organic content and a nutrient-rich composition, was used as an inoculum or pretreatment agent to improve the anaerobic digestion performance of OFMSW. By integrating cow manure into the WAS-OFMSW mixture, the substrate properties were optimized, increasing biogas production and improving the overall efficiency of anaerobic digestion.

Some studies have shown that using cow manure as an inoculum for the AD of OFMSW and WAS increases methane yield. However, there are no specific studies that made use of ferric chloride as a catalyst for the co-digestion of the OFMSW/WAS mixture with cow manure. Generally, research shows that among the various chemical activation agents that can be introduced into AD, ferric chloride can improve nutrient removal and augment the production of high-quality biomethane [7]. Moreover, the pretreatment methods and parameters used in the literature vary widely, making it difficult to compare and generalize the results [7]. Therefore, there is a need for further investigation into the effects of alkaline pretreatment on the mixture of OFMSW, WAS, and cow manure on biogas production and quality [7]. This study filled this gap by conducting a series of experiments with different ratios and analyzing biogas yield, composition, and kinetics, as well as the physicochemical and

microbiological characteristics of substrates and digestates.

The physicochemical properties of the substrates and the final digestate were evaluated solely by measuring pH, Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Total Solids (TS), and Total Volatile Solids (TVS). Trace amounts of secondary gases such as ammonia, hydrogen, hydrogen sulfide, etc., were assumed to be negligible. The daily methane generation from the digester with the highest biogas yield was determined.

## II. LITERATURE REVIEW

The effective management of Municipal Solid Waste (MSW) has emerged as a prominent global issue, driven by expanding urbanization and the resulting increase in waste production. Currently, the annual global generation of MSW stands at approximately 1.3 billion tons, with projections anticipating an increase to around 2.2 billion tons per year by 2025 [8]. One practical and cost-effective approach is to extract energy from the Organic Fraction of Municipal Solid Waste (OFMSW), commonly known as bio-waste, which typically accounts for approximately 46% of MSW [9].

Tyagi *et al.* [10] described Anaerobic Digestion (AD) as one of the most practical methods for harnessing energy from MSW. It converts organic matter into biogas and digestate. Over 560 AD plants globally generate a capacity exceeding 7.3 terawatt-hours (TWh) per year [11]. OFMSW yields up to 200 m<sup>3</sup> of biogas (approximately 400 kilowatt-hours of power) per ton or up to 330 liters of Methane per kilogram of total volatile solids (TVS) [3, 12]. Energy derived from biogas reduces CO<sub>2</sub> emissions by 200–300 kg per ton of biowaste.

This research focuses on enhancing the efficiency of anaerobic digestion, which is influenced by three major factors: temperature, pH, and biomass composition. Temperature influences microbial growth rate, enzymatic activity, and substrate characteristics, but elevated temperatures can hinder microbial activity and shift the dominant methanogen types [13]. On the other hand, pH is vital for biogas production and should be maintained within the range of 6.8–7.4 to enhance the conversion efficiency of volatile acids [14, 15]. Lastly, biomass composition, especially the Carbon-to-Nitrogen Ratio (C/N), determines the nutrient balance required for microbial growth and activity. A ratio of 20–30:1 is ideal for maximizing methane production and maintaining process stability [16].

Due to various challenges, traditional anaerobic digestion systems have long struggled to maintain consistent performance, particularly because of the inherent variability in feedstock composition. Different organic materials provide varying levels of essential nutrients and microbial support, making it challenging to optimize digestion. Co-digestion addresses this issue by processing multiple substrates simultaneously, offering several advantages over mono-digestion—such as balanced nutrient profiles, reduced inhibitory effects, and improved process stability. This approach has led to substantial research on optimizing microbial consortia, abiotic conditions, alkalinity, and inhibitor management throughout the process [17]. To ensure efficiency, it is also important to consider key operational factors, such as temperature and mixing ratios. Although

studies have shown that thermophilic regimes can enhance biogas yields [18–22], they also increase the risk of ammonia inhibition and microbial imbalance, which may suppress methane-producing organisms. In the Philippines, mesophilic conditions are more commonly adopted due to their manageability and energy efficiency. Among co-digestion strategies, an optimal OFMSW-to-sewage sludge ratio of 40:60 by volume has been found to yield up to 69.5% methane, attributed to enhanced hydrolysis via acetoclastic pathways [23, 24].

Furthermore, lower Total Solids (TS) percentages have been shown to boost biogas production [18, 21, 22, 25–27], which can be achieved through feed slurry dilution methods [28]. However, in some cases, even after dilution, specific substrates remain resistant to biodegradation, necessitating chemical pretreatment to alter their structure and enhance digestibility [29, 30]. Alkaline pretreatment—particularly using Ca(OH)<sub>2</sub>—has proven effective due to its ability to increase methane yield under milder reaction conditions [17, 31].

The substrates OFMSW and WAS are both rich in micro- and macronutrients, particularly from the sludge component [32]. However, while these nutrients are essential, their supplementation must be carefully controlled. A study by [33] showed that the addition of trace metals and vitamins unexpectedly suppressed digestion by increasing volatile fatty acid accumulation, thereby inhibiting the process. This highlights the delicate balance required when managing nutrient supplementation. Furthermore, beyond its nutritional role, FeCl<sub>3</sub> is also widely used in wastewater treatment as a flocculant due to its chemical properties and cost-effectiveness. It promotes the removal of pollutants and suspended particles by forming flocs through coagulation and adsorption mechanisms [34, 35]. Recent studies have demonstrated that FeCl<sub>3</sub>, when used in conjunction with acid or alkaline pretreatments, enhances the digestibility of lignocellulosic biomass by disrupting cellulose crystallinity [36]. This improves enzymatic access, thereby increasing glucose yield and improving fermentation efficiency [37]. In hydrothermal treatment, FeCl<sub>3</sub> enhances the breakdown of Extracellular Polymeric Substances (EPS), improves hydrophobicity, and reduces the bioavailability of toxins [38]. These effects collectively enhance protein solubility and increase Soluble Chemical Oxygen Demand (SCOD), helping overcome the hydrolysis step—a common rate-limiting step in AD [39].

Overall, enhancing the anaerobic digestion of OFMSW and WAS requires a complex approach that balances various factors, including operating conditions, substrate composition, and chemical augmentation. Strategies such as co-digestion, alkaline pretreatment, and the catalytic use of FeCl<sub>3</sub> have shown promise in improving hydrolysis and methane yield. However, enhancing these interventions to mitigate inhibitory effects remains a crucial area for continued research, especially once they are applied in a full-scale setup.

## III. MATERIALS AND METHODS

### A. Sample Sourcing

The substrates, the Organic Fraction of Municipal Solid

Waste (OFMSW) and Waste-Activated Sludge (WAS), were collected from domestic households and the Maynilad Pasay Water Reclamation Facility, respectively. On the other hand, the inoculum, cow manure, was collected from Samahang Manggagatas ng Batangas Cooperative (SAMABACO) in Tanauan, Batangas. Samples are sealed in HDPE containers and stored at 4 °C prior to experimentation.

## B. Sample Preparation

### 1) Incubation and degassing

The inoculum was incubated for one month in an environment that promotes both microbial growth and gas release at a moderate temperature of 34 °C [40]. This was done in an improvised water bath with manual agitation once a day to keep the manure moving constantly. Fig. 1 shows the actual incubation setup of cow manure. This continuous movement is necessary for effective biogas collection. This incubation and degassing process is performed before digestion to prevent the inoculum's productivity from interfering with the evaluation of the actual biogas produced by anaerobic digestion of substrates. The inoculum is only designed to initiate the digester's biological processes by introducing active microorganisms. This was then diluted to achieve 5% total solids before being combined with the substrates.



Fig. 1. Actual incubation of inoculum.

The Inoculum-to-Substrate (ISR) was maintained at approximately 2:1 (based on total solids) to ensure sufficient microbial seeding. No blank reactors were run; however, inoculum was pre-incubated and degassed to minimize background methane production.

### 2) Substrate pretreatment

The OFMSW was subjected to physical pretreatment to reduce its particle size to approximately 10 mm, thereby increasing the substrate surface area in the digester and promoting enhanced microorganism activity. The initial total solids, volatile solids, and pH will be measured for both substrates to adjust their total solids to 8% TS for OFMSW and 1% TS for WAS before proceeding to alkaline pretreatment. This chemical treatment will use calcium hydroxide as its reagent through cited doses of 62 mEq/L and 40 mEq/L, respectively, via direct combination. These mixtures will then be set overnight at room temperature. The

final substrates' pH will be measured and adjusted with HCl, if necessary, to achieve a starting pH of 6.82 or a pH within the range of 6–8.

## C. Experimental Design

The substrate and inoculum were combined at a 2:1 ratio, with a 40:60 substrate composition (OFMSW: WAS) [20], and digested anaerobically at 34 °C in a 1-L Erlenmeyer Flask for 30 days. The reactor used a 650 mL working volume composed of 173.33 mL OFMSW, 260 mL WAS, and 216.67 mL inoculum. The digester mixture was purged with nitrogen gas for 5 min to ensure the system remained anoxic. These setups were manually agitated once a day to ensure homogeneity and release of trapped gas between particles.

The reactor setup employed a positive-displacement method combined with CO<sub>2</sub> and H<sub>2</sub>S absorbers to eliminate harmful by-products, especially H<sub>2</sub>S, and to assume that the collected gas consists of methane and trace amounts of nitrogen. Due to equipment limitations, direct methane composition analysis was not conducted; therefore, methane volume was inferred from the gas displacement following CO<sub>2</sub> removal. This approach is commonly used in batch BMP tests when gas chromatography is unavailable.

Each treatment was performed in triplicate ( $n = 3$ ) to ensure the statistical reliability of the results. Reported values in tables and figures represent the mean values. One-way ANOVA was conducted to examine the effect of FeCl<sub>3</sub> dosing on methane yield. At the same time, paired t-tests were used to evaluate pre- and post-digestion differences in pH, BOD, and COD at the  $p < 0.05$  significance level.

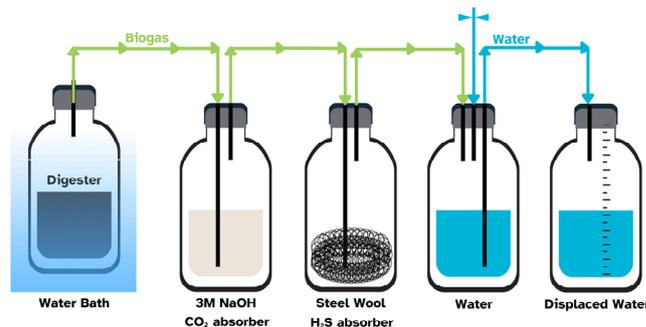


Fig. 2. Physical set-up for anaerobic digestion.

The biogas directly produced by the digester will be redirected to pass through the CO<sub>2</sub> absorption unit containing 3 M of NaOH placed in a 1-L HDPE bottle followed by the H<sub>2</sub>S absorber where a steel wool is placed in a 1-L HDPE bottle to induce corrosion before passing the gas to another 1-L HDPE bottle that contains distilled water, which is to be displaced and read volumetrically per day. The design of the reactor setup is illustrated in Fig. 2, with the overall setup shown in Fig. 3, and the actual digestion setup depicted in Fig. 4. All sub-units of this figure are enclosed using rubber cork, rubber, and glass tubing.

The experimental setup depicted in Table 1 comprises eight reactor setups: three control groups and eight catalytic setups. The Ca(OH)<sub>2</sub> pretreatment dosages are from 62.0 mEq/L, equivalent to 2.3 g/L for OFMSW and 40 mEq/L, equivalent to 1.48 g/L for WAS [41]. The ferric chloride dose was optimized based on the study by Qin, which determined a Qin equivalent of 200 mg/L FeCl<sub>3</sub> for WAS [42].

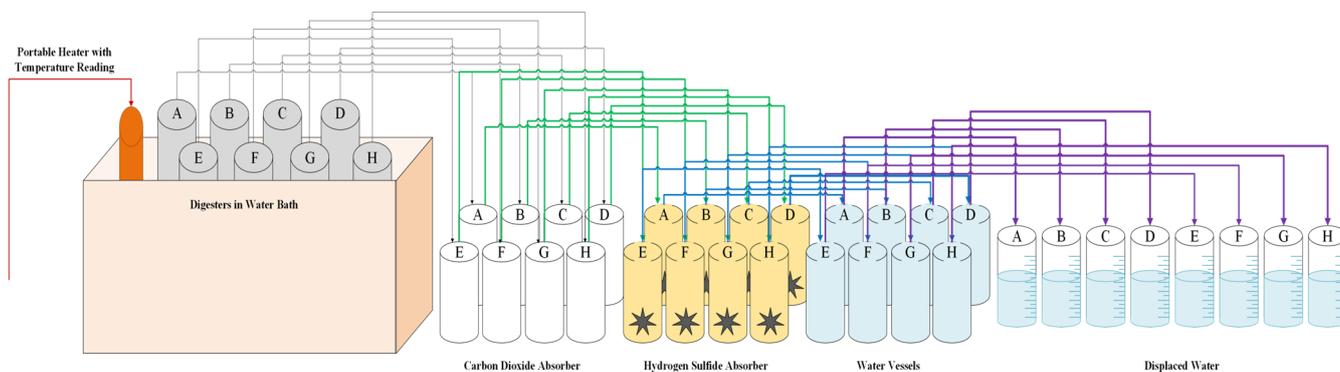


Fig. 3. Overall experimental set-up.



Fig. 4. Actual digestion set-up.

 Table 1. Anaerobic co-digestion experimental matrix incorporating dosages for  $\text{Ca}(\text{OH})_2$  pretreatment and  $\text{FeCl}_3$  catalytic enhancement

Parameter	Set-up	$\text{Ca}(\text{OH})_2$ Pretreatment		$\text{FeCl}_3$ Catalyst
		OFMSW	WAS	
Control	A	×	×	×
	B	2.3 g/L	1.48 g/L	×
	C	×	×	0.20 g/L
	D	2.3 g/L	1.48 g/L	0.01 g/L
	E	2.3 g/L	1.48 g/L	0.05 g/L
Catalytic Effects	F	2.3 g/L	1.48 g/L	0.10 g/L
	G	2.3 g/L	1.48 g/L	0.20 g/L
	H	2.3 g/L	1.48 g/L	0.30 g/L

#### D. Statistical Analysis of Data

The effects of the pretreatment method and the catalytic effect of ferric chloride were assessed using 4 major parameters, which are the Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), pH, total volatile solids (TVS) and Total Solids (TS). These were measured before and after the experiments are conducted to determine which setup is the best.

##### 1) Feed characterization, BOD5 and COD

Digester samples from the working mixture (one sample) and post-digestion (setups A-H) were evaluated for Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) using the APHA Standard Methods. Initial and final pH levels were measured using an Eutech pH 2700 m to maintain optimal alkalinity for digestion stability and reduce the risk of inhibition.

##### 2) TS and VS

The APHA Standard Method 2540B and APHA 2540G were used to determine the samples' Total Solids (TS) and Volatile Solids (VS), respectively [43]. A sample aliquot is to be placed in a crucible and dried at 105 °C for one-hour intervals until the weight achieves a constant 4% of the previous weight.

$$TS = \frac{W_{total} - W_{dish}}{W_{sample} - W_{dish}} \times 100\% \quad (1)$$

$$VS = \frac{W_{total} - W_{volatile}}{W_{total} - W_{dish}} \times 100\% \quad (2)$$

#### 3) Kinetics study

The kinetics study was conducted by measuring and recording the volume of water displaced every 24 h. On the other hand, the cumulative  $\text{CH}_4$  production (M) over time was fitted to three sigmoidal growth functions: the Modified Gompertz Model (MGM), the Modified Richards Model (MRM), and the Logistic Function (LF), as shown in Eqs. (3), (4), and (5), respectively.

These equations contain 6 constants - A,  $R_m$ ,  $\lambda$ ,  $e$ ,  $t$ , and  $v$ . These refer to the maximum specific methane production potential (mL  $\text{CH}_4/\text{g VS}_{added}$ ), maximum specific methane production rate (mL  $\text{CH}_4/\text{g VS}_{added} \cdot \text{d}$ ), lag time (d), Euler's number (approx. 2.7183), time, and shape parameter respectively. The researchers assessed the data through the use of MATLAB, where the  $R^2$  and RMSE values were evaluated to identify which model was the most accurate and most suitable to describe the AD process [44].

$$M = A \cdot \exp \left\{ - \exp \left[ \frac{R_m e}{A} (\lambda - t) + 1 \right] \right\} \quad (3)$$

$$M = A \left\{ 1 + v \cdot \exp(1 + v) \cdot \exp \left[ \frac{R_m}{A} \cdot (1 + v)^{1 + \frac{1}{v}} \cdot (\lambda - t) \right] \right\} \quad (4)$$

$$M = \frac{A}{1 + \exp \left[ \frac{4R_m(\lambda - t)}{A} + 2 \right]} \quad (5)$$

## IV. RESULT AND DISCUSSION

The characterization of the raw substrates used in the anaerobic digestion process provides critical insights into their composition and biodegradability potential. The three primary substrates analyzed were waste-activated sludge (WAS), Organic Fraction of Municipal Solid Waste (OFMSW), and Cow Manure (CM). Their Total Solids (TS), Total Volatile Solids (TVS), pH, Biochemical Oxygen demand ( $\text{BOD}_5$ ), and Chemical Oxygen Demand (COD) were measured to assess their suitability for anaerobic digestion (Table 2).

Table 2. Characterization of WAS, OFMSW, and cow manure

Parameter	WAS	OFMSW	Cow Manure
Total Soluble Solids (mg/L)	2497	—	—
Total Solids (mg/L)	0.266	8.915	5.395
Total Volatile Solids (mg/L)	5.698	7.432	3.947
pH	5.76	3.95	7.16
$\text{BOD}_5$ (mg/L)	81.60	110.40	96.00
COD (mg/L)	132.192	231.84	211.20

To ensure consistency with existing literature on optimal TS concentrations for anaerobic digestion, a dilution adjustment was performed (Table 3). The TS levels of the substrates were modified to align with commonly reported TS ratios for OFMSW, WAS, and inoculum mixtures, facilitating a standardized comparison with prior studies. This adjustment ensured that the digestion process matched established research conditions, enabling a more accurate assessment of microbial activity and organic matter degradation under typical operating parameters.

Table 3. Total solids adjustment for cow manure, WAS, and OFMSW

	Total Solids Recorded	Total Solids Required	Dilution Factor, mL H <sub>2</sub> O/L sample
Cow Manure	5.3965	1.000	4,394.86
WAS	0.266	1.000	-
OFMSW	8.915	8.000	114.38

The alkaline pretreatment dosages were also adjusted to align with the optimized Ca(OH)<sub>2</sub> loading rates, ensuring compatibility with the modified TS concentrations (Table 4).

Table 4. Alkaline pretreatment adjustment based on TS Content

Specimen	TS, Basis	TS, Current	Basis Dosage, g/L	Adjusted Dosage, g/L
OFMSW	8.00	8.00	2.30	2.30
WAS	1.00	0.266	1.48	0.394

Table 5 presents the initial characterization of the digestate from each digester setup (A-H) prior to anaerobic digestion. The control group includes setups A, B, and C, while setups D to H represent the experimental groups subjected to various pretreatment and co-digestion conditions.

Table 5. Digester set-up characterization before digestion

	Set-up	pH	BOD <sub>5</sub>	COD
Control	A	6.19	33.60	57.80
	B	6.10	67.20	109.4
	C	6.13	86.40	138.1
Experimental	D	6.36	48.00	77.50
	E	6.32	100.80	160.9
	F	6.24	115.20	188.2
	G	6.28	129.60	202.7
	H	6.27	134.40	215.6

On the other hand, Table 6 presents the characterization of the digestates after undergoing anaerobic digestion. One critical observation was the formation of flocs throughout digestion. In the initial days, fine suspended particles were observed accumulating at the bottom of the digesters, gradually forming larger aggregates as digestion progressed. This floc formation was particularly prominent in FeCl<sub>3</sub>-treated setups. However, flocs were also observed in setup A in the latter days of the digestion process, which may be explained by late microbial extracellular polymeric substances facilitating natural bio-flocculation, leading to the aggregation of organic and inorganic matter, as evidenced in the lag-phase of this set-up.

Table 6. Digester set-up characterization after digestion

	Set-up	pH	BOD <sub>5</sub>	COD
Control	A	6.96	76.80	125.80
	B	6.42	43.20	71.50
	C	6.67	52.80	88.20
Experimental	D	7.20	38.40	61.90
	E	7.38	52.80	85.75
	F	7.37	62.40	102.30
	G	7.30	57.60	91.60
	H	6.81	48.00	75.25

### A. BOD<sub>5</sub> and COD Removal

The characterization of each digester setup before anaerobic digestion revealed a wide range of organic loading, with BOD<sub>5</sub> values spanning from 33.60 to 134.40 mg/L and COD values from 57.80 to 215.60 mg/L (Table 5). These values reflect the initial substrate complexity and concentration, which are critical parameters influencing microbial activity and methane production kinetics.

Notably, setups with higher initial BOD<sub>5</sub> and COD values — particularly Setups G and H — demonstrated the highest organic loading, suggesting greater availability of both readily and slowly biodegradable organic compounds. These results are consistent with increased methane production in those setups, as greater organic availability supports prolonged microbial activity and higher cumulative biogas yields.

Across all setups, the observed increase in pH — from an initial range of 6.10–6.36 to a final range of 6.42–7.38 — supports the interpretation of successful methanogenesis. The shift toward near-neutral to slightly alkaline pH values reflect the consumption of acidic intermediates and stabilization of the digestate, conditions favorable for methanogenic archaea. The pH increase is particularly notable in setups where significant reductions in COD and BOD<sub>5</sub> were achieved, further indicating efficient digestion.

Overall, the variation in BOD<sub>5</sub> and COD removal among the setups emphasizes the importance of proper substrate-to-inoculum balance and loading characteristics. Setups with optimized organic content and buffering capacity favored more complete digestion, as evidenced by lower residual BOD<sub>5</sub>/COD values and stabilized pH. Conversely, poor-performing setups point to underlying process imbalances that may have hindered complete substrate utilization.

### B. pH Observation and Its Impact on Digestion

The pH values before and after digestion provide further insights into process stability. Initially, the experimental setup had a pH of 6.36, which remained within the optimal range for anaerobic digestion. After digestion, pH values across all setups increased slightly. The increase in pH in most setups can be attributed to the Ca(OH)<sub>2</sub> pretreatment's buffering capacity, which neutralizes acids produced during microbial metabolism. The FeCl<sub>3</sub>-treated setups also exhibited relatively stable pH values, as Fe<sup>3+</sup> ions may have precipitated acidic components, thereby maintaining a neutral environment conducive to microbial activity.

The pH stability in FeCl<sub>3</sub>-treated setups further supports the notion that iron-based precipitation mechanisms contributed to controlling acidity fluctuations. However, in Setup A, which exhibited the highest BOD<sub>5</sub> and COD after digestion, the pH increased slightly to 6.96, possibly due to ammonia released from protein degradation.

Furthermore, volatile fatty acid (VFA) profiles and alkalinity ratios were not measured in this study. Therefore, interpretations of microbial pathway shifts and buffering dynamics are based solely on pH.

### C. Kinetics Study

#### 1) Specific methane production

Daily methane production data provide direct insight into the dynamic behavior of each anaerobic digestion setup

throughout the 30-day retention period. These daily volume readings reflect the volume of gas displaced per day and indicate microbial activity levels, substrate availability, and the presence of inhibitory effects.

The daily CH<sub>4</sub> volumes were plotted for each experimental setup to illustrate temporal patterns and differences in methane production behavior. Fig. 5a presents the daily Methane displaced volumes, while Fig. 5b illustrates the cumulative Methane displaced per setup.

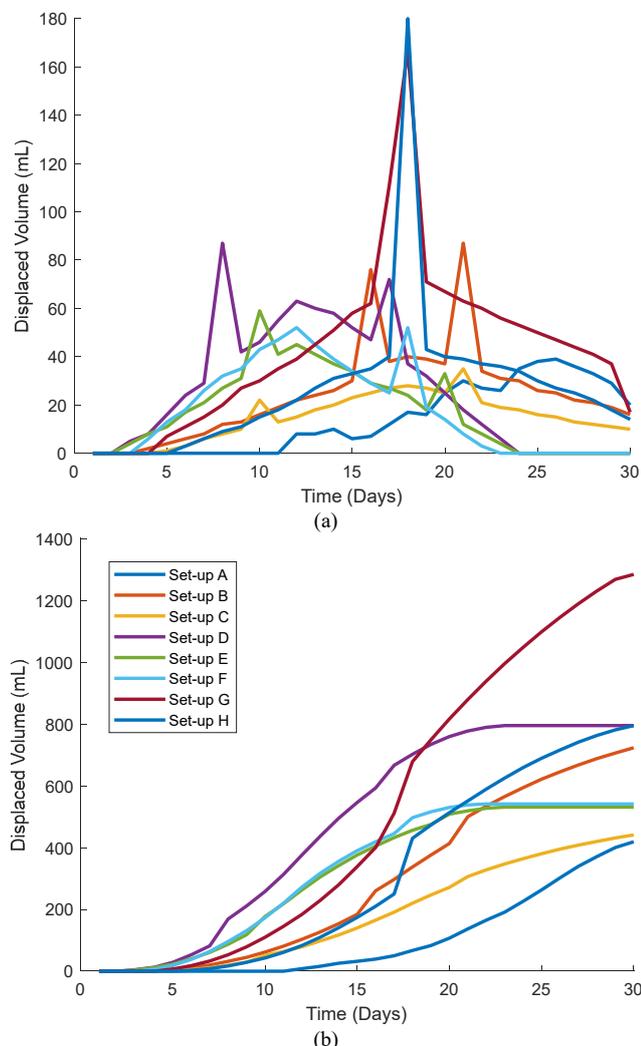


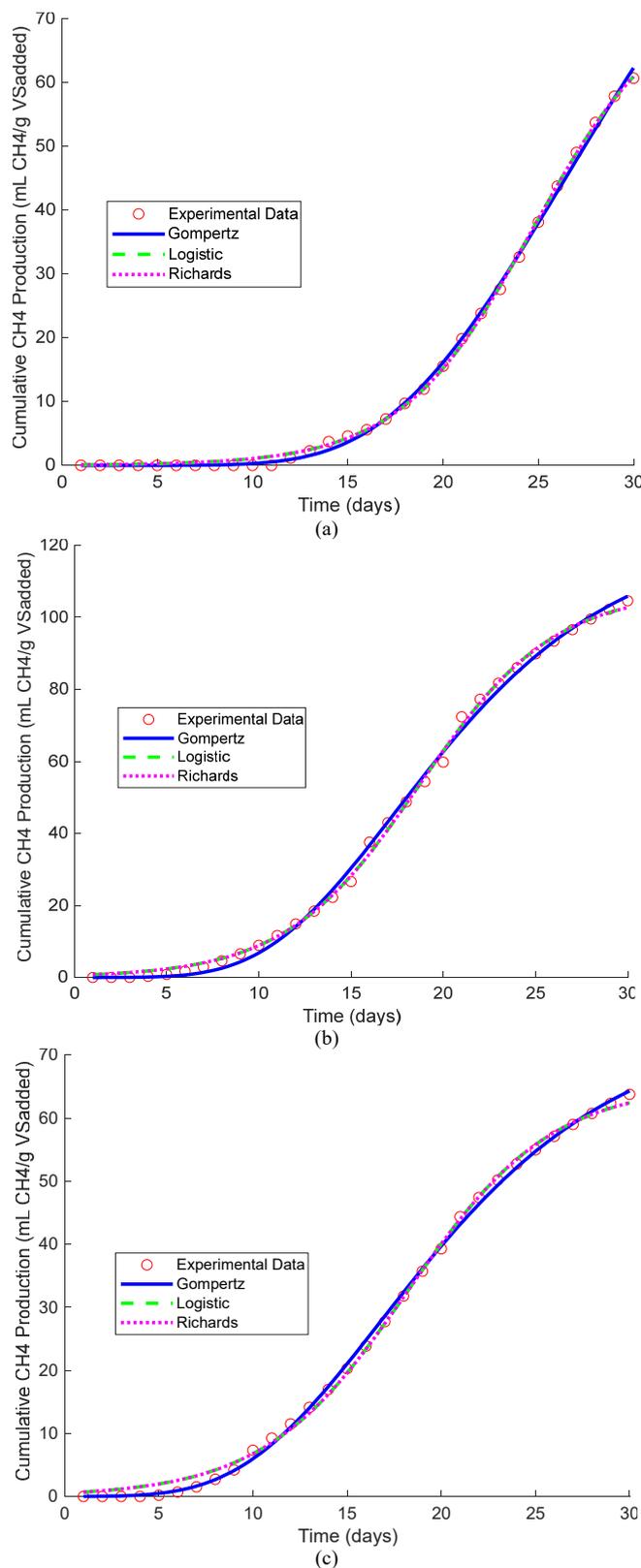
Fig. 5 Methane production profile: (a) Daily profiling for set-ups A to H; (b) Cumulative profiling for set-ups A to H

The methane production profiles revealed clear distinctions in digestion performance across the different setups, driven by substrate composition, pretreatment, and FeCl<sub>3</sub> dosing. Setup G emerged as the optimum configuration, producing the highest daily peak (~165 mL) and cumulative methane volume (1284 mL), with sustained methane generation over the 30-day digestion period. Setup H followed a similar trajectory but consistently trailed behind, displacing 795 mL of Methane. This slight reduction in performance, despite being among the higher-yielding groups, is attributed to inhibitory effects from the elevated FeCl<sub>3</sub> dosage (0.30 g/L), which—while beneficial for sulfide suppression and phosphate precipitation—may impose oxidative stress on methanogens at excessive concentrations.

## 2) Kinetic Modeling

Three sigmoidal growth functions, the Modified Gompertz

Model (MGM), modified Richards model (MRM), and logistic function (LF), were used to provide critical insights about reaction rates, as well as model fitting. These models take into account various parameters, including R<sub>m</sub> (maximum reaction rate), λ (lag phase duration), R<sup>2</sup> (coefficient of determination), and RMSE (root mean square error), which collectively determine the models' accuracy and applicability. Calculated kinetic model parameter values are depicted in Table A1. The methane production model was fitted and graphed in MATLAB as shown in Fig. 6.



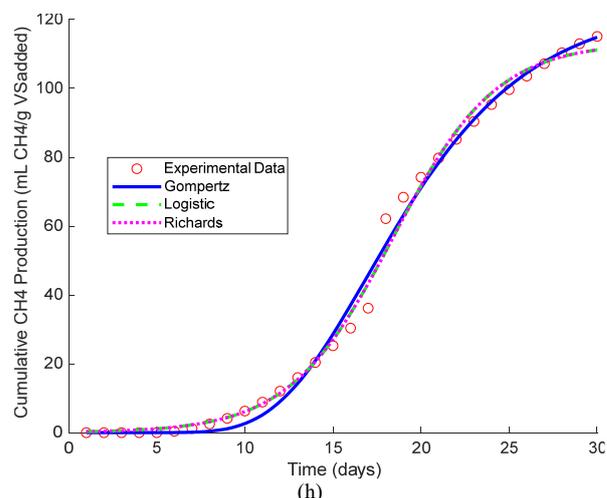
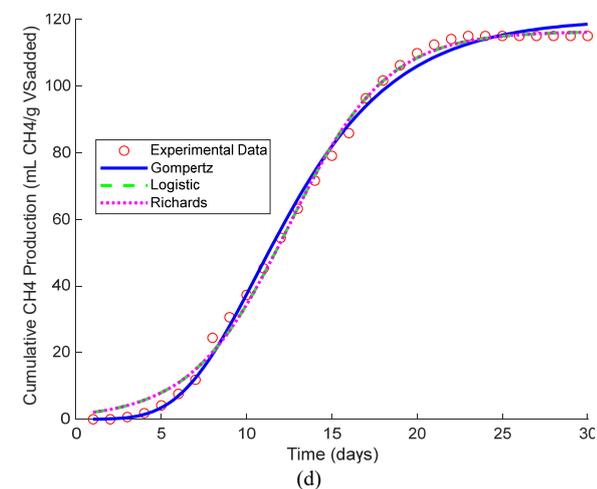
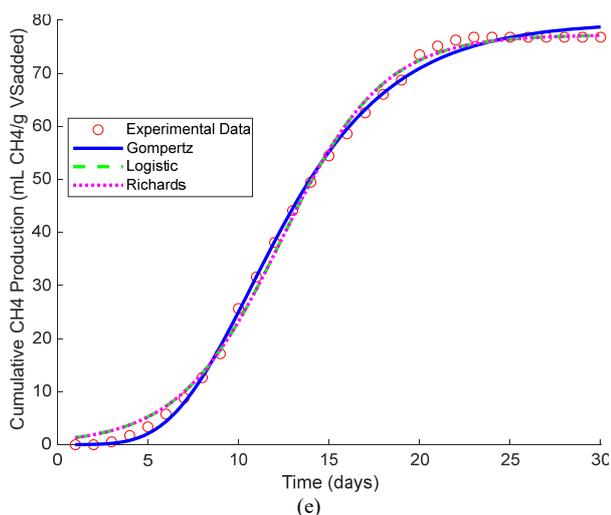
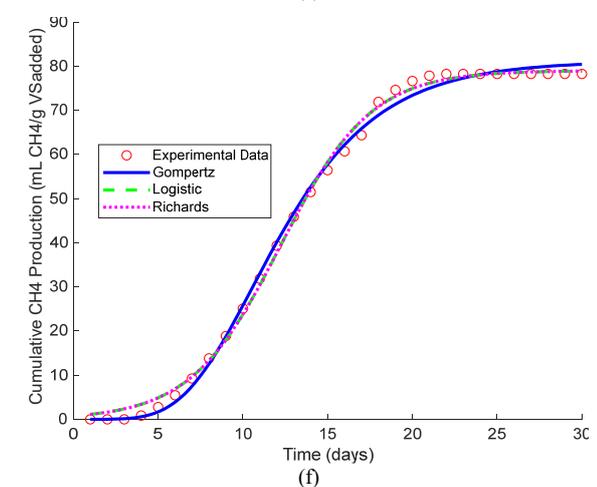


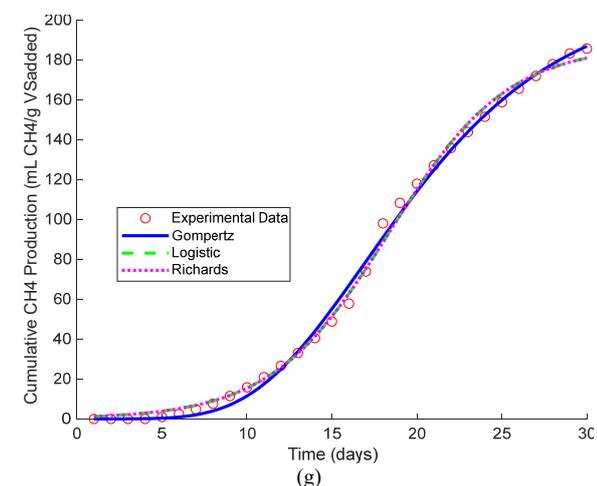
Fig. 6 Methane production fitting models for individual set-ups: (a) Setup A; (b) Setup B; (c) Setup C; (d) Setup D; (e) Setup E; (f) Setup F; (g) Setup G; (h). Setup H.



Based on the visual analysis of the methane production model fitting for each setup (Fig. 6), distinct differences in the kinetic behavior of anaerobic digestion were observed. The variation in curved slopes reflects differences in methane production rates, while the differing plateau levels indicate variability in the total methane yield achieved across setups. Additionally, the presence and duration of initial lag phases prior to significant gas production further emphasize the influence of specific experimental conditions on the digestion process. These kinetic variations observed across Setups A to H suggest that the applied treatments significantly affected both the progression and efficiency of methane production. Notably, specific setups demonstrated higher production rates and overall methane yields, warranting further investigation into the factors underlying these enhanced performances.



The kinetic modeling of cumulative methane production demonstrates that all 3 models (Modified Gompertz, Richards, and Logistic) achieved excellent fits, with coefficients of determination ranging from 0.9947 to 0.9993. This high degree of correlation confirms that the experimental data follow well-defined sigmoidal kinetics. While residual plots and AIC/BIC model selection criteria were not generated due to analytical constraints, model evaluation was based on  $R^2$  and RMSE, which are commonly used and acceptable metrics in anaerobic digestion kinetic modeling. The graphical representation of methane production kinetic modelling for the individual setups is shown in Fig. 7, and the calculated kinetic modelling parameters are listed in the appendix.



The parameters A, maximum methane yield, and  $R_m$ , maximum methane production rate, provide the most direct insight into process performance. Notably, Setup G (0.20 g/L ferric chloride) exhibited the highest values for both A and  $R_m$ , indicating that this dosing level maximized substrate conversion and enhanced methanogenic activity. In contrast, intermediate dosing levels (0.05–0.10 g/L, Setups E and F) were associated with reduced methane potential and lower production rates, suggesting inhibitory effects at these concentrations.

The lag phase parameter  $\lambda$  further illustrates the influence of alkaline pretreatment and ferric chloride dosing on microbial adaptation. Setups D through F showed shorter lag phases (6–13 days) than Setups A, B, and H (10–17 days),

supporting the conclusion that alkaline pretreatment facilitated faster hydrolysis and microbial activation. However, the reduction in lag did not consistently correspond to higher methane yields. For example, Setups E and F achieved shorter lag phases yet produced comparatively low methane volumes, implying that while pretreatment improved early-stage kinetics, subsequent methanogenesis was constrained by inhibitory or imbalanced conditions introduced by suboptimal ferric chloride concentrations.

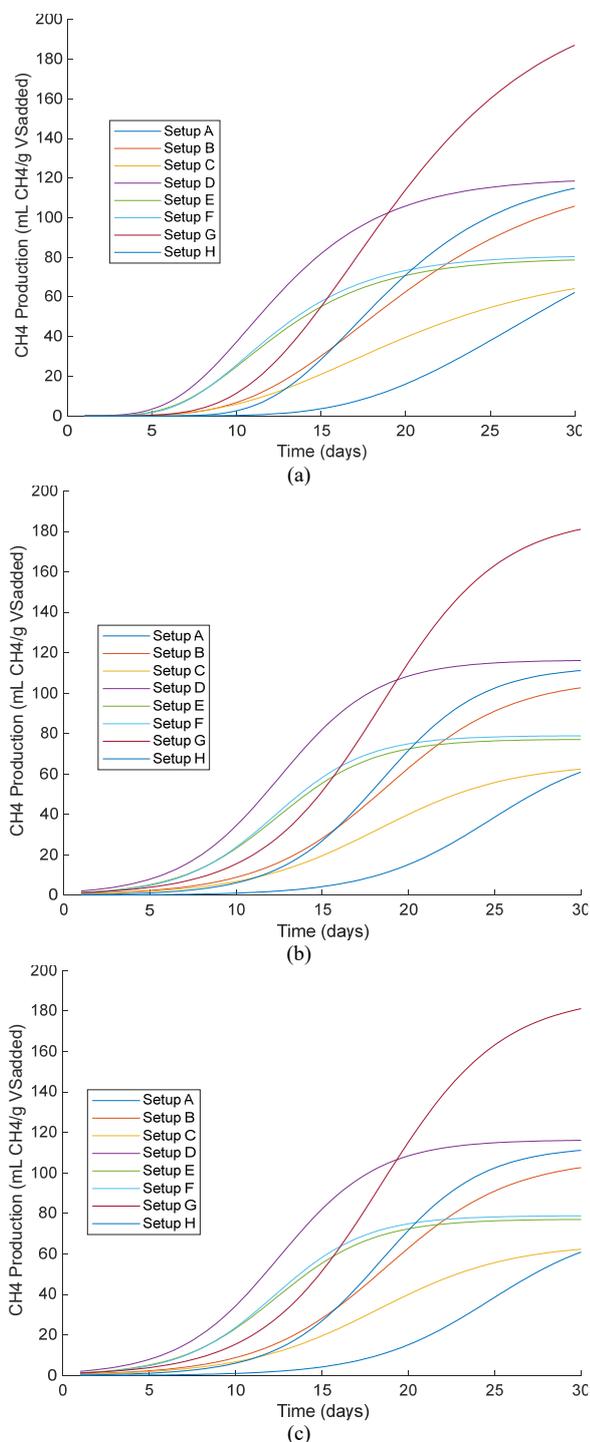


Fig. 7. Methane production kinetic modelling: (a). Gompertz kinetic model for setups A to H; (b). Logistic kinetic model for setups A to H; (c). Richards kinetic model for setups A to H.

### 3) Correlation on the use of Ferric Chloride and specific methane production

The total methane production across the different setups

shows variability with respect to the presence and concentration of ferric chloride ( $\text{FeCl}_3$ ) dosing, as well as to other treatments. Table 7 presents the total Methane produced in the control setups (A, B, and C) for comparison.

Table 7. Control set-ups and total methane produced

Set-up	Alkaline-Pre-treatment	Addition of Ferric Chloride	Total Methane Produced (mL)
A	✗	✗	419.47
B	✓	✗	722.59
C	✗	0.20 g/L	440.51

As shown in Table 8, methane production varied across setups with different  $\text{FeCl}_3$  dosages, but the changes in total methane yield did not follow a clear, statistically significant trend. This suggests that the impact of  $\text{FeCl}_3$  dosing on methane production may not be linear or consistent across all dosages.

Table 8. Ferric chloride dosing and total methane produced

Set-up	Ferric Chloride Dosing (g/L)	Total Methane Production (mL)
B	0.00	722.59
D	0.01	794.97
E	0.05	530.60
F	0.10	540.65
G	0.20	1284.44
H	0.30	795.33

To evaluate the relationship between  $\text{FeCl}_3$  dosing and methane production, Pearson correlation analysis was applied and listed in Table 9. The resulting correlation coefficient,  $r = 0.434$ , indicates a weak positive association between  $\text{FeCl}_3$  dosage and total methane production. However, the correlation was not statistically significant ( $p = 0.390$ ,  $n = 6$ , two-tailed), indicating that variations in  $\text{FeCl}_3$  concentration within the tested range did not significantly affect methane yield. The low t-statistic ( $t = 0.962$ ,  $df = 4$ ) further supports the conclusion that the observed differences in methane production are more likely due to random variation rather than a meaningful dose-response effect.

Table 9. Pearson correlation statistics on ferric chloride dosing and total methane produced

Pearson product moment correlation coefficient, $r$	0.433516
Number of data sets	6
T-stat	0.962144
Degrees of freedom (df)	4
p-value	0.390463

These findings indicate that 0.20 g/L  $\text{FeCl}_3$  yielded the highest methane yield in this study, but the effect was not statistically significant ( $p > 0.05$ ). Therefore, this Dosage should be interpreted as the highest observed performance, not a definitive optimum. However, a slight increase in methane production was observed in Setup C compared to Setup A, though this effect was not as pronounced as in Setup B. This suggests that alkaline treatment may have a greater effect on methane production than  $\text{FeCl}_3$  dosing alone. Despite a positive correlation between  $\text{FeCl}_3$  dosing and total methane production, the p-value was greater than 0.05, indicating that the effect was not statistically significant.

### 4) Statistical analysis of pH, BOD, and COD changes before and after digestion

The results of the statistical analysis using a t-test on pH, BOD, and COD before and after digestion are summarized in

Table 10. The t-test was conducted to evaluate whether the observed changes in these parameters were statistically significant, with a significance level set at  $p < 0.05$ .

Table 10. Statistical analysis using T-Test

Parameter	pH	BOD	COD
Mean (Before)	6.2363	89.4000	143.7750
Mean (After)	7.0138	54.0000	87.7875
t-value	-5.9689	2.5528	2.5659
p-value	0.000352	0.032675	0.031483
Significance ( $p < 0.05$ )	Confirmed	Confirmed	Confirmed
Change	Increase	Decrease	Decrease

V. CONCLUSION

This study investigated the effects of FeCl<sub>3</sub> dosing and alkaline pretreatment on methane production during mesophilic anaerobic digestion of OFMSW and WAS. Emphasis was placed on key indicators such as BOD and COD reductions, as well as the underlying mechanisms by which FeCl<sub>3</sub> enhances methane generation. Results showed that the optimal FeCl<sub>3</sub> concentration (0.20 g/L, Setup G) significantly increased cumulative methane yield (175.31 mL/gVS) and led to substantial reductions in both BOD and COD, indicating more effective substrate breakdown and bioconversion. These improvements suggest that FeCl<sub>3</sub> not only acts as a catalytic enhancer but also accelerates the degradation of organic matter within the reactor. However, findings also revealed that excessive FeCl<sub>3</sub> dosing can inhibit microbial activity, highlighting the importance of precise concentration control.

Furthermore, the combined use of alkaline pretreatment and FeCl<sub>3</sub> demonstrated synergistic effects, though the pH-buffering capacity of calcium hydroxide introduced masking effects that require careful management. Overall, this study underscores the critical balance between FeCl<sub>3</sub> dosing, pH stability, and microbial performance in optimizing anaerobic digestion. The insights gained provided a practical foundation for enhancing biogas production and improving waste treatment efficiency in full-scale applications by strategically integrating chemical additives and operational controls.

APPENDIX

Table A1. Calculated kinetic model parameters

Set-up	Model	Kinetic Parameters				
		A	R <sub>m</sub>	λ	R <sup>2</sup>	RMSE
A	MGM	122.05	4.9493	17.3245	0.9990	0.6140
	RM	74.33	5.0163	16.3526	0.9993	0.5186
	LF*	74.33	5.3432	17.7696	0.9993	0.5186
B	MGM	124.49	6.6034	10.4501	0.9981	1.6480
	RM*	107.29	8.6085	15.3192	0.9987	1.3674
	LF	107.29	8.6085	15.3192	0.9987	1.3674
C	MGM*	75.79	3.8147	9.5062	0.9993	0.6013
	RM	65.27	5.0154	14.8915	0.9983	0.9500
	LF	65.27	4.2687	10.5914	0.9983	0.9500
D	MGM	120.19	9.8356	6.1951	0.9970	2.4602
	RM*	116.41	13.314	12.9488	0.9973	2.3606
	LF	116.41	10.1385	6.7525	0.9973	2.3606
E	MGM	79.62	6.7356	6.2922	0.9985	1.1751
	RM*	77.24	9.0398	12.8777	0.9977	1.4562
	LF	77.24	6.8720	6.7556	0.9977	1.4562
F	MGM	81.13	7.2694	6.4530	0.9973	1.6038
	RM*	79.00	9.7575	12.7234	0.9977	1.4797

	LF	79.00	7.4170	6.9190	0.9977	1.4797
	MGM	214.20	12.1065	10.4729	0.9975	3.3894
G	RM*	187.54	15.8988	15.4488	0.9976	3.3375
	LF	187.54	13.4753	11.4214	0.9976	3.3375
	MGM	124.62	8.8411	11.8175	0.9947	3.1738
H	RM*	113.35	11.5554	16.2735	0.9952	3.0242
	LF	113.35	9.6545	12.5400	0.9952	3.0242

Note: Models marked with an asterisk (\*) indicate the best fit for each set up based on R<sup>2</sup> and RMSE.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Gabrielle Nicole Corteza: Conceptualization, Methodology, Investigation, Writing – Original Draft, Resources, Visualization. Kyara Christelle Riguerra: Validation, Formal Analysis, Investigation, Data Curation, Writing – Review & Editing. Michelle Almendrala: Conceptualization, Methodology, Supervision, Project administration; All authors had approved the final version.

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