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RESEARCH ARTICLE

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### TEMPERATURE EFFECT ON PH AND RESIDENCE TIME AS ESSENTIAL PARAMETERS FOR BIOGAS PRODUCTION. A REVIEW

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#### ABSTRACT

This study presents a systematic review of literature on the influence of temperature on methane production as biogas. A total of 337 scientific documents were collected from the Web of Science database and processed using tools such as Excel, Mendeley, and PRISMA approach literature review tool as PICOT method to identify the articles that best match the objectives of the study by eligibility criteria. The systematic review investigated 25 relevant studies, comparing mesophilic (30–40°C) and thermophilic (50–100°C) conditions. It was found that, although temperature is a determining factor in the efficiency of methanogenesis, its effect is conditioned by variables such as substrate type, residence time, pH, and the pretreatment methods applied. The results show that the mesophilic regime tends to offer greater operational stability, while the thermophilic regime can accelerate anaerobic digestion, but with a higher risk of inhibition by compounds such as free ammonia and volatile fatty acids. The synergy between adequate pretreatments and substrate selection is key to optimizing methane production. This work provides an updated overview of scientific knowledge in this area, identifying gaps and opportunities for future research aimed at the efficient design of thermally optimized anaerobic digestion systems.



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#### I. INTRODUCTION

In the context of growing concerns regarding sustainability and the transition toward renewable energy sources, biogas has emerged as a key alternative for sustainable energy generation. This gas, composed mainly of methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>), is produced through the anaerobic digestion of organic matter, making it a viable solution for waste management and the reduction of greenhouse gas emissions [1-4]. According to the International Energy Agency (IEA), biogas has the potential to meet up to 20% of global energy demand if its production and utilization processes are adequately optimized [5-7]. Temperature is one of the critical factors affecting the efficiency of methanogenesis, as it directly affects the activity of the microorganisms responsible for organic matter degradation [2],[8-10]. Therefore, understanding its impact is essential for improving biogas performance and maximizing its viability as a renewable energy source [11-16].

Numerous studies have addressed fundamental aspects of biogas production by exploring multiple optimization strategies. Research on the optimization of anaerobic digestion and biogas generation has examined variables such as the carbon-to-nitrogen (C/N) ratio, organic loading rate, hydraulic retention time, temperature control, and pH, as well as the application of kinetic models to enhance process efficiency [1-7], [17]. Anaerobic co-digestion, through the combination of different substrates such as manure, sewage sludge, and agricultural reisdues, has been shown to improve nutrient balance and process stability [7],[18-24]. In addition, various physical, chemical, and biological pretreatments have been investigated to improve biodegradability and increase biogas production, including thermal, alkaline, and enzymatic methods [11-16].

The influence of temperature on biogas production has been extensively studied, highlighting its effects on microbial community activity and overall process efficiency, particularly during transitions between mesophilic and thermotolerant conditions [2], [8-10]. Concurrently, studies have analyzed microbial community composition, evaluating the roles of fermentative and acetogenic bacteria, as well as methanogenic archaea, in methanogenesis under temperature regimes favorable to their development and energy potential. In this context, the present research conducts a comprehensive systematic review focused on reliable information on temperature-related trends in biogas production. To this end, it is essential to assess the impact of temperature on methane generation using data retrieved from the Web of Science database and analyzed with open-source tools such as Excel and SPSS, following the PRISMA bibliographic review framework. This approach enables the identification of key trends, the mapping of research related to temperature effects, and the detections emerging challenges that may guide future investigations. The fundamental objective guiding this research is as follows: A comprehensive review of extant scientific literature reveals a paucity of research regarding the influence of temperature on methane production. To provide a comprehensive approach to the main purpose, it is necessary first to address the following specific research questions (SRQ):

- SRQ 1: How does temperature have a correlational effect on the operating parameters of anaerobic digestion to enhance methanogenesis on biogas production?
- SRQ 2: How hydrogen potential (pH) and retention time of organic loading rate regarding anaerobic digestion (RT) are related to temperature?
- SRQ 3: What are the main areas of knowledge related to the influence of temperature on methane production?

A review of the literature reveals several seminal articles that have contributed to the consolidation of this field of study by settling down the approaches of temperature impact in biogas production. Responding to these questions aims to clarify the gaps in biogas production regarding considering operational parameters such as pH and RT, which may influence future trends in knowledge and establish the basis for new methodologies that yield better results in anaerobic digestion by considering temperature as a significant regulator of methanogenesis enhancement.

## II. MATERIALS AND METHODS

In this regard, a systematic review is a bibliographic synthesis that addresses a clearly formulated research question and applies systematic and explicit methods to identify, select, and critically evaluate relevant studies. It also involves the compilation and analysis of extracted data from the included studies in order to identify future research priorities, address questions that cannot be resolved by individual investigations, detect limitations in primary research that should be addressed in future studies, and generate or assess theories explaining how and why the phenomena of interest occur [25]. Within this scope, data extraction represents a key component of the PRISMA framework and is directly aligned with the research questions. In this study, data extraction was conducted through full-text analysis of the selected articles to identify PRISMA methodology ensures a transparent and robust selection process among the articles retrieved through the search strategy, thereby facilitating meaningful comparisons across studies and highlighting the significant contributions of meta-analytical evidence [26].

### II.1 ELIGIBILITY AND ANALYSIS CRITERIA

The PICOT method was adopted for answering the research questions posed in the literature review with an adaptation of its scope using its components of "Population (P)," which refers to the operating temperature regimes that allow for biogas production. "Intervention (I)" is associated with the operating parameters of temperature impact that are most closely related to "Outcome (O)" with the improvement of methanogenesis, resulted in PIO structure (see Table 1).

Table 1: PICOT (PIO) structure.

COMPONENT	QUESTION	KEY WORDS
POPULATION (P)	What are the temperature regimes that have a direct impact on biogas production?	Biogas production, biogas generation
INTERVENTION (I)	In what way does process temperature influence the methanogenic efficiency on biogas production?	Temperature, t
OUTCOME (O)	Which are the crucial operational parameters on methanogenesis enhancement?	Methanogenesis, Methane production

Source: Authors, (2025).

Based on the modified PICOT structure, a search strategy was designed that included specific keywords to ensure comprehensive coverage of the topic. The search equation used was (TS=("Biogas production" OR "biogas generation") AND TS=(temperature OR t) AND TS=("methanogenesis" OR "methane production")) AND ((DT=("ARTICLE") AND LA=("ENGLISH") AND OAJ=("ALL OPEN ACCESS")) NOT (PY=("2025"))). This strategy was applied to the Web of Science (WoS) database, which was selected for its broad multidisciplinary scope and rigorous indexing process. The initial search yielded a total of 339 articles. However, following a manual revision, two articles corresponding to the year 2025 were identified; despite the applied exclusion criteria, these records were retrieved in the search results. After their removal, the final corpus consisted of 337 articles, providing a solid and refined basis for initial systematic analysis. Subsequently, the application of PRISMA systematic review methodology enabled the identification of the most relevant scientific literature addressing temperature and its influence on biogas production. During this process, inclusion and exclusion criteria were applied to further refine the final corpus (see Table 2). A total of 130 articles were excluded by title and abstract screening due to misalignment with the primary objective, resulting in 207 selected records. Of these, 92 articles could not be retrieved, leaving 115 studies for eligibility assessment.

Among them, 35 articles lacked relevance to the main objective of the study (EC 1), 10 were written in languages other than English (EC 2), and 45 corresponded to theoretical or non-experimental research, such as review papers, which could lead to limited empirical contribution to the present analysis (EC 3). Consequently, only 25 articles met all eligibility criteria and were included in the final review, as illustrated in the PRISMA flow diagram (see Figure 1). The original data were managed using specialized software Excel and Mendeley tool, improving the storage of the final corpus of studies for this review, and providing an enhancement in the analysis of the data consulted. All data processing, table construction, and figure generation were performed using the latest stable version of Python v2025.10.4. The analyses and graphical outputs were developed within the Visual Studio Code (VS Code) environment, ensuring reproducibility, efficient code management, and consistency in data visualization.

Table 2: Eligibility criteria.

EXCLUSION CRITERIA	INCLUSION CRITERIA
EC 1: Articles that do not fulfil research objective	IC 1: Assess temperature relevance on biogas production
EC 2: Non-English reports	IC 2: Determine numerical results to compare
EC 3: Non-empirical research (review)	IC 3: Empirical methods

Source: Authors, (2026).

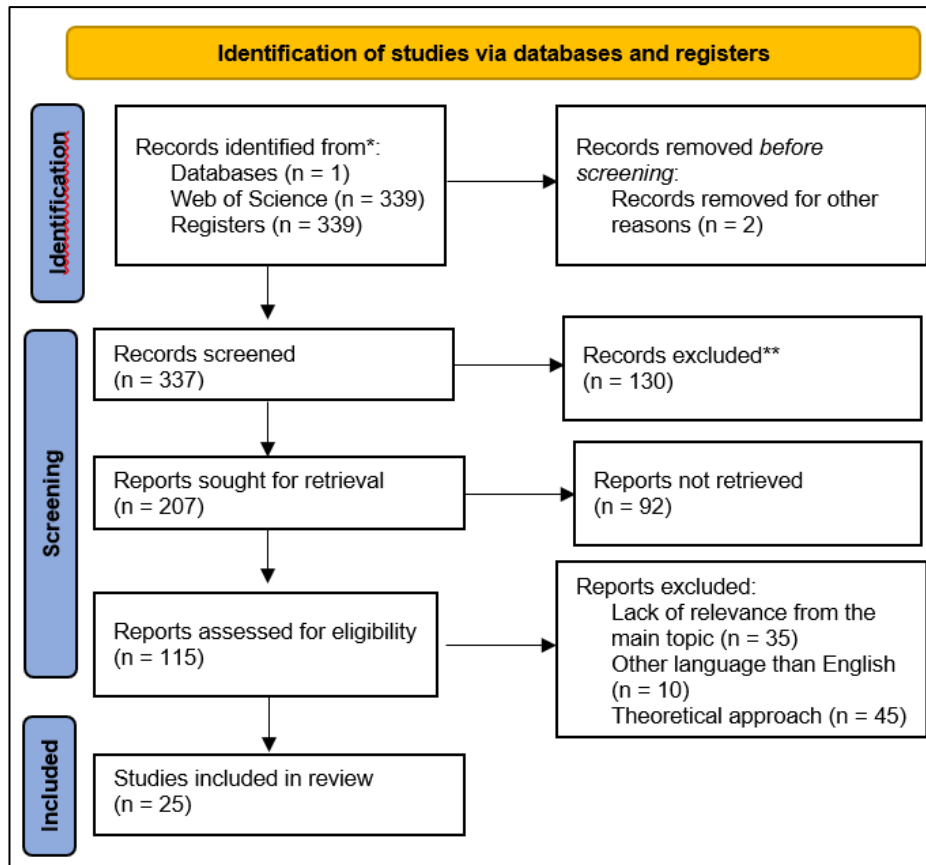


Figure 1: PRISMA flow diagram.

Source: Authors, (2026).

### II.1.1 Statistical Analysis

Due to the limited sample size, and the heterogeneity of experimental conditions across the studies, inferential statistics comparing thermal regimes were not considered informative. Therefore, results are primarily reported using descriptive statistics, while exploratory intra-regime analyses were conducted to assess the trends associated with temperature, pH, RT, by central tendency measures. In addition to central tendency reports, coefficient of variability (CV) was calculated to assess relative dispersion within each thermal regime. Relative difference ( $\nabla CH_4$ ) between mesophilic and thermophilic mean methane yields was also computed to evaluate the magnitude of change, given the limited inferential power of formal statistical tests.

Coefficient of variability

$$CV = \frac{SD}{\bar{x}} * 100 \tag{1}$$

Relative difference

$$\nabla CH_4 = \frac{CH_4 \text{ max} - CH_4 \text{ min}}{CH_4 \text{ min}} * 100 \tag{2}$$

Where:

SD: Standard deviation of the data collected by thermal range.

$\bar{x}$ : Mean of the methane production.

$CH_4 \text{ max}$ : Maximum value of methane production among studies collected by thermal range.

$CH_4 \text{ min}$ : Minimum value of methane production among studies collected by thermal range.

## II.2 AI – ASSISTED LANGUAGE EDITING

The manuscript was translated from Spanish into English and subsequently linguistically refined using an artificial intelligence-based language model (ChatGPT, OpenAI), exclusively for language-related purposes. The use of this tool was strictly limited to supporting translation accuracy, grammatical correction, stylistic consistency, and overall clarity of the English text. The AI system did not contribute to the formulation of research questions, study design, data collection, data processing, statistical analysis, interpretation of results, or the development of scientific conclusions. All scientific content, methodological decisions, analyses, and interpretations presented in this manuscript were conceived, executed, validated solely by the authors. The authors assume full responsibility for the accuracy, originality, and integrity of the work and confirm that the use of artificial intelligence did not influence the scientific validity, originality, or novelty of the study. This disclosure is provided in accordance with current transparency and ethical guidelines regarding the responsible use of artificial intelligence tools in scholarly publishing.

## III. RESULTS AND DISCUSSIONS

Temperature is a critical factor in anaerobic digestion due to its influence on process dynamics, inoculum acclimatization, microbial metabolic activity, methane yield, and overall process stability [27]. Literature generally distinguishes two thermal regimes. The mesophilic range (30-49 °C) is characterized by relatively slower but more stable digestion process with lower energy requirements, whereas the thermophilic range ( $\geq 50$  °C) promotes enhanced biokinetic activity and higher reaction rates, albeit at the expense of increased energy demand and reduced process stability. Thermophilic systems are more susceptible to fluctuations in temperature and pH, as well as to inhibition by elevated concentrations of free ammonium concentration [28-30]. Several studies report that temperature directly affects the optimization of biogas production by accelerating the metabolic rates of microbial communities involved in organic matter degradation and methanogenesis, thereby increasing methane yields [31-34]. Consequently, temperature represents a key parameter governing methane accumulation, as its interaction with other operational variables, such as residence time (RT) and pH, ultimately determines the magnitude of cumulative methane production (see Table 3).

Table 3: Methane peak results associated with temperature, pH and residence time (RT) from the articles included in PRISMA.

THERMAL RANGE	TEMPERATURE (°C)	RT (days)	pH	METHANE PEAK (mL CH <sub>4</sub> /g VS)	REFERENCE
Mesophilic	35	-	7.57	333	[35]
	35 ± 1	40	-	450 ± 22	[36]
	35	37	7.6	132.3	[37]
	35	30	7,8	218.88 ± 6.77	[38]
	37	55	8	273.2	[39]
	37 ± 0.5	30	8	389.1 ± 8.5	[32]
	37	20	7 ± 0.1	260.83 ± 15.02	[40]
	39	36	8	189 ± 6	[41]
Thermophilic	40	-	-	416.8 ± 6.77	[42]
	51.5	20	7 ± 0.1	275.66	[40]
	55	30	7.3 A	212.4	[43]
	55	26	6	253.8	[44]
	55	46	8	279.9	[39]
	55 ± 0.5	30	8	110.9 ± 22.1	[32]
	100	20	7.43	264.64	[33]

A: Value estimated by visual extraction of the figure indicated in the article, as numerical data are not available in tabular format.

Source: Authors, (2026).

Table 4: Descriptive statistical reports of methane production within the thermal regimes.

REGIME	MEAN ( $\bar{x}$ )CH <sub>4</sub> ((mL CH <sub>4</sub> /g VS)	SD	CH <sub>4</sub> min	CH <sub>4</sub> max	CV (%)	VCH <sub>4</sub> (%)
Mesophilic	295.901	108.568	132.3	450	37%	240%
Thermophilic	232.883	64.476	110.9	279.9	28%	152%

Source: Authors, (2026).

Table 3 presents a ranking of data extracted from 13 studies included in this review (52%), with three of these studies reporting results under both mesophilic and thermophilic temperature regimes [32], [39],[40]. To enable accurate comparisons between temperature and methane production, standardized units widely accepted in the literature were used, specifically milliliters of methane per gram of volatile solids (mL CH<sub>4</sub>/ g VS). This metric allows for a more direct comparison between mesophilic and thermophilic operating conditions and facilitates the identification of temperature-related trends in methane generation.

Marked variability is observed between the mesophilic range (35-37 °C) and the thermophilic range (50-100 °C), with the highest methane yields occurring at the lowest temperatures within the dataset. This finding indicates that temperature alone does not govern

methane production. Instead, the reported outcomes reflect a multifactorial interaction in which temperature acts in combination with other operational parameters, particularly pH and residence time (RT), which are essential for a more comprehensive interpretation of methane yields under different thermal conditions.

### III.1 MESOPHILIC TEMPERATURE RANGE

Among the 13 studies considered in Table 3, 69.23% (nine articles) examine the relationship between mesophilic temperature conditions and methane production. As shown in Table 4, the coefficient of variation (CV) of the methane production data indicates high heterogeneity ( $CV > 33\%$ , specifically 37%), suggesting that the mean value is not representative and that methane yields are highly dispersed. Furthermore, an analysis of the maximum and minimum recorded methane values reveals that, within the mesophilic range of 35–40 °C, the relative difference in methane production reaches approximately 240%, representing the highest variability observed across the evaluated thermal ranges. This pronounced variability indicates gaps in the control of study variables, highlighting the need to account for factors such as digester configuration, substrate characteristics, and applied pretreatment methods to improve biogas quality and process consistency [36],[45],[46]. In contrast, studies operating at a constant temperature generally report more comparable methane production levels, with reduced dispersion when pretreatment conditions and substrate types are similar, as illustrated in Figure 2.

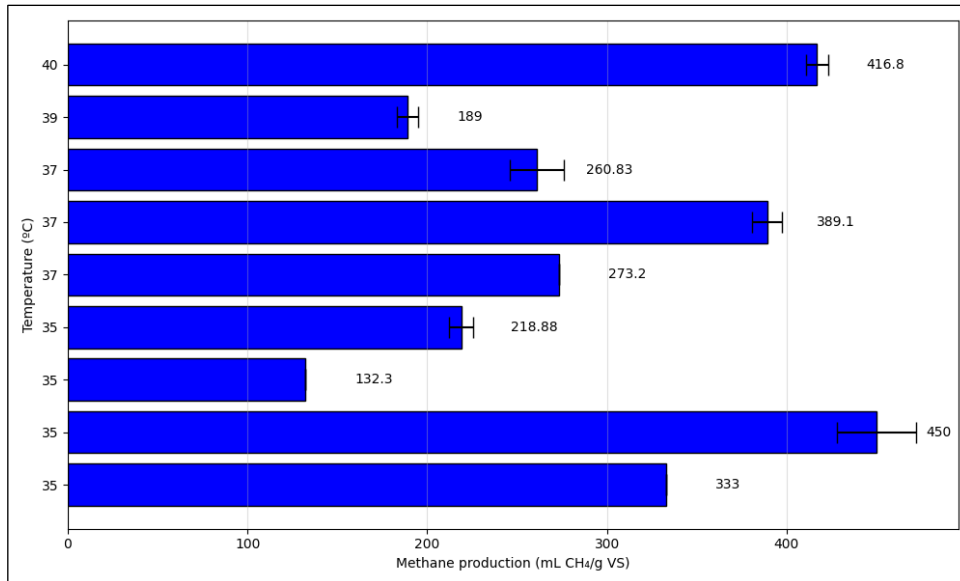


Figure 2: Mesophilic thermal range.

Source: Authors, (2026).

On the one hand, [36] evaluated food waste digestion at 35 °C by comparing conventional thermal pretreatment with electromagnetic microwave radiation (EMR) pretreatment. Although a methane peak of 450 mL CH<sub>4</sub>/g VS was achieved using EMR, the difference relative to the conventional method was modest ( $\approx 424$  mL CH<sub>4</sub>/g VS). This limited improvement was attributed to the possible accumulation of volatile fatty acids (VFAs), which can acidify the digestion medium. Moreover, microwave radiation may influence the composition and activity of methanogenic microorganisms by affecting enzymatic activity, inducing changes in DNA and RNA, and altering protein synthesis, depending on power, frequency, and exposure time [47]. Nevertheless, several studies have reported that the application of this type of pretreatment enhances the solubilization and degradation of organic solids, thereby promoting anaerobic digestion and increasing methanogenic potential [38], [48-50]. In this study, the estimated variation between heating methods was approximately 6%, which is consistent with previously reported findings [51], [52], indicating a positive effect of this heating approach on the biodegradability of the feedstock used in anaerobic digestion. On the other hand, both [42] and [37] investigated the anaerobic digestion of water hyacinth (*Eichhornia crassipes*) at 40 and 35 °C, respectively.

Both studies agreed that the high lignin content of this substrate limits the degradation of cellulose and hemicellulose, thereby reducing the availability of fermentable sugars and microbial diversity, and adversely affecting the acidogenesis and methanogenesis stages [53], [54]. However, their findings differed with respect to operational conditions. Specifically, [42] reported that a low feed-to-inoculum ratio ( $F/I = 1.0$ ) favored methane production (416.8 mL CH<sub>4</sub>/g VS), whereas a high ratio ( $F/I=30.0$ ) inhibited the process due to the accumulation of VFA and ammonia. In contrast, [37] observed that solids concentrations exceeding 6.3% led to system saturation and reduced methane production to 132.3 mL CH<sub>4</sub>/g VS. Both studies concluded that, despite operating within optimal mesophilic temperature ranges (35–40 °C), lignin content and inhibitory compounds constrain digestion efficiency, making it essential to control organic loading or incorporate more readily degradable co-substrates. From this perspective, although methane yields ranging from 100 to 450 mL CH<sub>4</sub>/g VS have been reported under constant thermal conditions, the observed variability in methane production is primarily associated with the type of substrate, the pretreatment strategy, and the digestion approach, rather than with temperature as an isolated operational parameter.

### III.2 THERMOPHILIC TEMPERATURE RANGE

Conversely, among the 13 articles, 53.84% examined thermophilic operating conditions. Based on their distribution, a relatively homogeneous pattern is observed within this thermal regime, with a coefficient of variation below 33% (CV =28%), indicating reasonably stable performance across studies despite underlying methodological heterogeneity. In this context, the mean methane yield (232.88 mL CH<sub>4</sub>/g VS) can be considered representative of the overall data dispersion (Figure 3). Notably, most data points are clustered around 55 °C, where no clear or statistically significant differences in methane production can be attributed solely to temperature. Nevertheless, the wide range of reported yields (110.9 ± 22.1 – 279.9 mL CH<sub>4</sub>/g VS) indicates that variability is primarily governed by operational parameters such as pH regulation, residence time (RT), and substrate composition rather than by temperature alone. Overall, 55 °C emerges as a robust thermophilic operating condition, capable of achieving high methane productivity while remaining sensitive to process control and operational management.

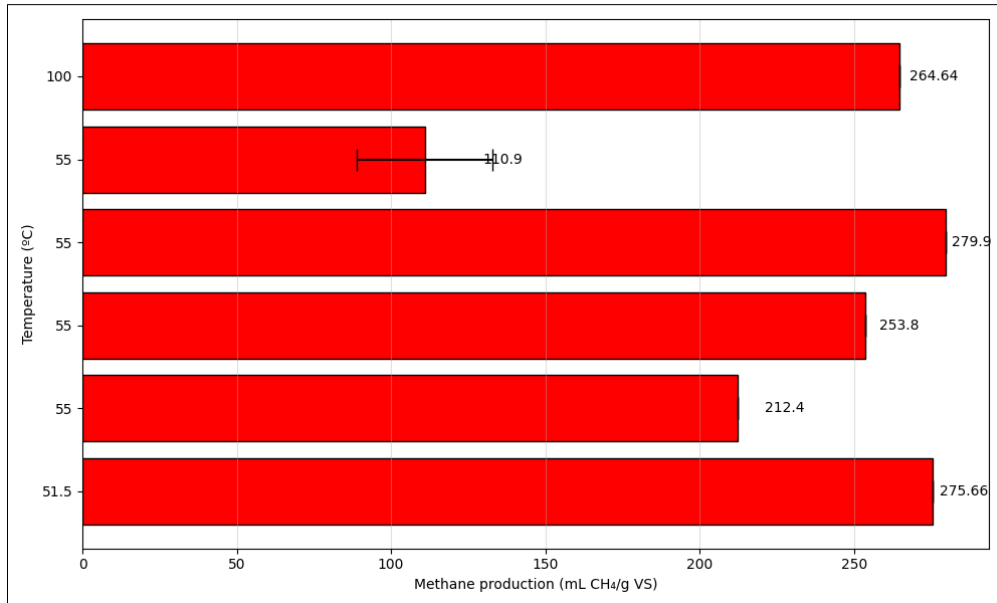


Figure 3: Thermophilic thermal range.

Source: Authors, (2026).

As an explanation for the observed variability, [32] reported a methane yield of 110.9 ± 22.1 mL CH<sub>4</sub>/g VS after acid-thermal pretreatment. The resulting acidic conditions strongly inhibited anaerobic digestion, likely due to insufficient acid removal and pH values below neutrality, which are toxic to anaerobic microorganisms [55]. Additionally, the use of whiskey by-products, also reported by [39], implied a low C:N ratio, favoring inhibition through elevated free ammonium (> 1000 mg/L) and volatile fatty acid (VFA) accumulation (> 4000 mg/L) [32], [56]. These results indicate that under thermophilic conditions, aggressive pretreatments combined with unstable C:N ratios can severely impair methanogenic activity [57-59]. Conversely, [39] showed that thermal pretreatment of microalgae at 90 °C coupled with digestion at 55 °C resulted in only a modest 6% increase in methane production compared to mesophilic conditions. This limited improvement was attributed to ammonium inhibition associated with the low C:N ratio of microalgae and the release of free ammonium following cell disruption during thermal pretreatment [60].

The introduction of an additional biological pretreatment mitigated this effect, increasing methane yield by 12% (279.9 mL CH<sub>4</sub>/g VS). Overall, the comparison between mesophilic and thermophilic regimes indicates that temperature alone does not govern methane production. While mesophilic digestion (35-40 °C) exhibits high variability driven by substrate type, organic loading, and pretreatment strategy, thermophilic operation (50 -100 °C) demonstrates greater consistency and faster kinetics, particularly around 55 °C. However, thermophilic stability is highly sensitive to substrate composition, especially low C:N ratios and the use of aggressive pretreatments, which can promote ammonium and VFA accumulation. Therefore, optimal methane yields across both thermal regimes depend on a multifactorial balance between temperature, substrate characteristics, pretreatment selection, and strict control of key physicochemical parameters, rather than on temperature as an isolated variable.

### III.3 TEMPERATURE INFLUENCE ON pH

Across both mesophilic and thermophilic regimes, methane production was generally reported within a relatively narrow pH range of 6.0 to 8.0. Under mesophilic conditions, Figure 4 indicates that most studies operated at neutral to mild alkaline pH values (7.0-8.0), within which methane yields were commonly observed. However, [41] reported negligible methane production at 39 °C despite operating at pH 8.0. This outcome is primarily associated with the characteristics of the substrate, particularly its high lignin content, which limits biodegradability, as well as potential inhibition caused by VFA accumulation and the use of mono-digestion strategies. These findings challenge the assumption that maintaining a neutral to slightly alkaline pH (7.0-8.0) invariably enhances methane production. Although methanogenic archaea typically exhibit optimal activity within a pH range of 6.0-8.0 [44], [61-64], the influence of pH is strongly modulated by substrate quality, pretreatment approach, biodegradability, and the presence of inhibitory compounds [29], [45], [46], [65], [66].

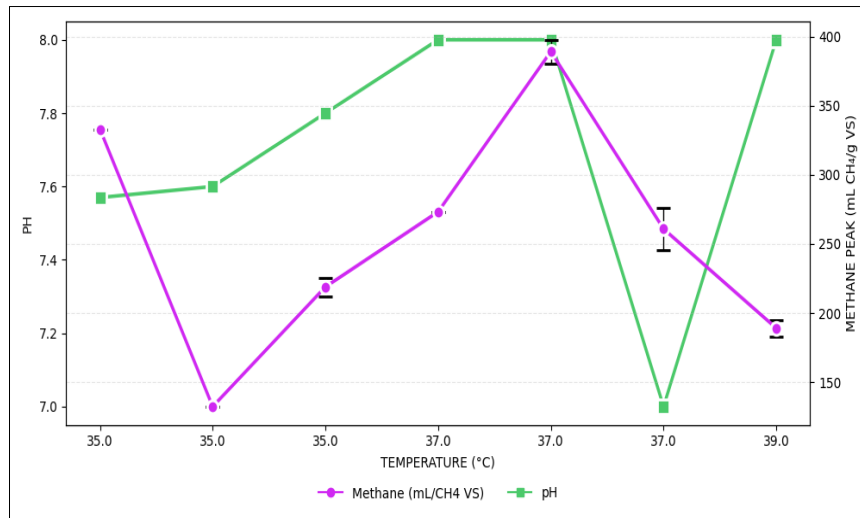


Figure 4: Mesophilic regime effect on pH.  
Source: Authors, (2026).

To support this notion, Figure 5 illustrates that under thermophilic conditions there is a clear tendency from acidic (pH 6.0) to alkaline conditions (pH 8.0) at 55 °C. In this context, [32] reported that, regardless of the pretreatment strategy applied, pH is strongly associated with the substrate composition under thermophilic operation, as temperature critically influences protein hydrolysis, leading to the formation of  $\text{NH}_4^+$  and  $\text{NH}_3$ . The latter acts as a weak base, stabilizing the digestion medium at alkaline pH values (around 8.0). Nonetheless, this alkalinity can inhibit methanogenesis due to increased concentrations of free ammonia nitrogen (FAN), as well as the accumulation of VFA and free ammonium, thereby generating a toxic and unstable environment that restricts methane production, irrespective of the pretreatment applied [67] ,[68]. Additionally, it is reported in [40] that with a digestion temperature of 51.5 °C, it associates the pH value (5.8) to the nutrient content of sewage sludge; nevertheless, its methane production is the highest among thermophilic studies, suggesting that the synergy in its substrates (SS/GTS), due to the easy degradation of VS and TS in GTS, results in better methanogenesis. Finally, there are authors who have not considered this operational parameter [33], [36] ,[37] ,[39] ,[42] ,[43], [69]. These findings may involve prioritizing the analysis of critical variables such as temperature, hydraulic retention of organic content, pretreatments, substrate type, overall energy efficiency, and technical feasibility, which is why this physicochemical parameter could be omitted from the results. Some researchers may also consider it a non-limiting parameter and choose not to report it if no significant variation is observed during the process or through automatic adjustment in controlled tests. Likewise, in some processes, such as the one reported by [36], the pretreatment methodologies and the layout of the digester make it difficult to measure physicochemical parameters such as pH and COD, so they are not taken into consideration.

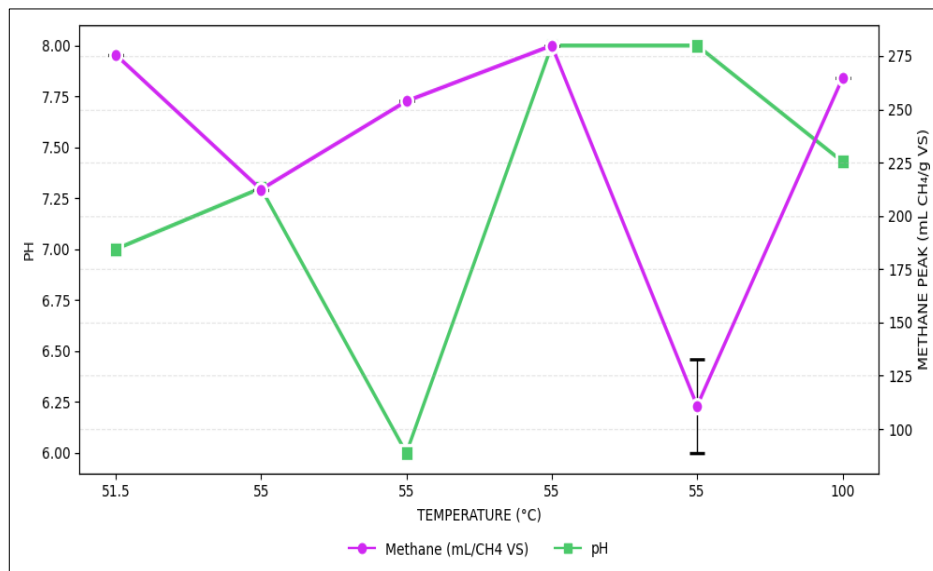


Figure 5: Thermophilic regime effect on pH.  
Source: Authors, (2026).

### III.4 TEMPERATURE INFLUENCE ON RT

Within both thermal regimes, methane production was reported under a broad range of residence time, reflecting heterogeneous operational strategies rather than standardized process conditions. On the one hand, Figure 6 shows the dispersion of temperature data based on their corresponding methane values and the influence of RT as a parameter established in each study.

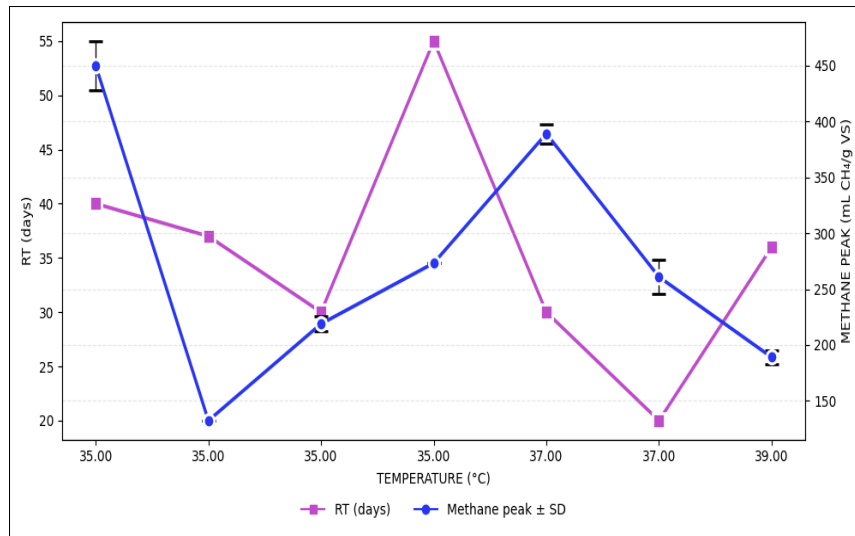


Figure 6: Mesophilic influence on RT (days).

Source: Authors, (2026).

At 39 °C, with a retention time (RT) of 36 days, [41] reported lower methane production (189 mL CH<sub>4</sub>/g VS) when using rumen as the base substrate. This indicates that RT does not act directly on temperature but rather interacts with substrate characteristics and organic loading, depending on the pretreatments applied to enhance anaerobic digestion (AD) performance. In this context, a longer RT does not necessarily guarantee higher methane yields across temperatures, as substrate composition and pretreatment efficiency play a decisive role. Furthermore, at a constant temperature of 35 °C, [39] reported lower methane production at an RT of 55 days compared to the 40-day process reported in [36], yielding 273.2 and 450-mL CH<sub>4</sub>/g VS, respectively. This relative difference (64.7%) contradicts the conventional assumption that shorter RTs necessarily result in reduced methane production [70]. Similarly, [40] reported a methane yield of 260.83 mL CH<sub>4</sub>/g VS, at an RT of 20 days, which is comparable to the values reported by [39].

These differences can be attributed to variations in operating conditions, substrate quality, and digester configuration, particularly when strategies such as co-digestion are applied [38]. Likewise, at a constant temperature of 37 °C, [32] observed higher methane production (389.1 mL CH<sub>4</sub>/g VS) after 30 days of digestion. Overall, these findings highlight the need to standardize experimental conditions across studies and to systematically compare pretreatment strategies and substrate types to establish robust correlations. Methane production trends tend to vary according to substrate complexity, pretreatment intensity, and RT, even when temperature is held constant. Conversely, Figure 7 shows that under thermophilic temperatures, RTs will be lower due to the rapid hydrolytic reaction and breakdown of the cell walls of organic matter, providing higher methane peaks in a very short time [30]. Notably, at 51.5 °C, the methane peak was one of the highest among the studies in this temperature range (275.66 mL CH<sub>4</sub>/g VS), considering a RT of 20 days. This may confirm that, under high temperature conditions, methane production will be enhanced, with a lower RT or contact time with the organic load.

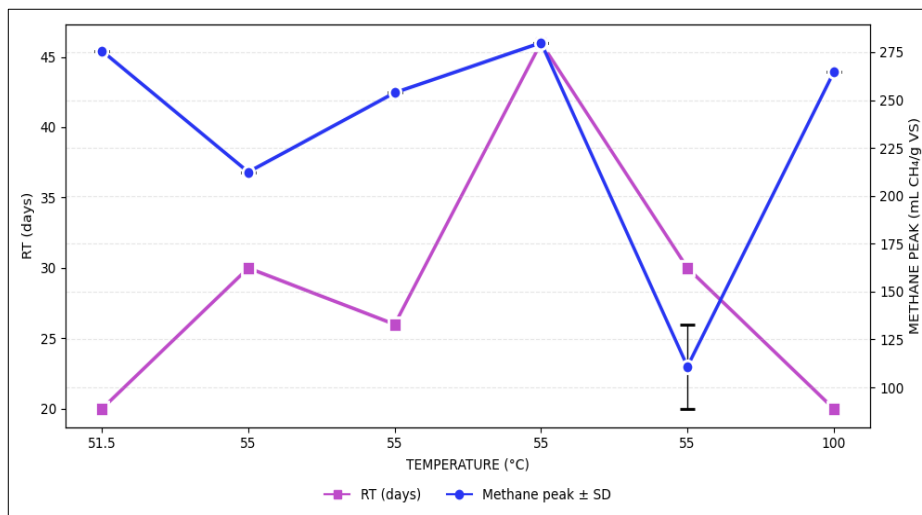


Figure 6: Thermophilic influence on RT (days).

Source: Authors, (2026).

For instance, temperatures of 55 °C that reported longer RT's, such as 26 and 30 days, estimate a projection on methane production, theoretically. The relative difference between studies, considering methane production results, with 30 days of RT, is 171.9%. This disproportionate variation suggests that the type of pretreatment and substrate have a greater effect on AD than RT alone as a function of temperature, as independent variables [32],[43].

In contrast, other studies at 55°C show that higher RT (46 days) can also yield good results (279.9 mL CH<sub>4</sub>/g VS); however, the stability of the process suggests higher energy demand and difficulties in operational control [39], [71]. Similarly, at a higher temperature of 100 °C and only 20 days of RT, one study shows that yields are optimal (264.64 mL CH<sub>4</sub>/g VS, respectively), reinforcing the idea of greater efficiency in biodegradability in terms of methane production levels and speed in the process [33]. Finally, the results of both figures show a contrast between the mesophilic and thermophilic ranges with respect to their impact on RT. The results in Figure 7 show that, in a thermophilic range, the RT are relatively lower compared to the results of the mesophilic range in Figure 6. This suggests that the thermophilic regime positively influences the extracellular enzymatic activity of the organic load, promoting higher hydrolysis rates than at mesophilic temperatures [72]. Nevertheless, yields at methane peaks in the thermophilic regime are lower due to susceptibility to temperature variations, compared to the mesophilic regime [27]. This reflects what was reported by [62], although a higher operating temperature led to greater microbial activity initially, when maximum methane production was reached, prolonging the RT under these conditions can cause a decrease in production, since most methanogenic microbial consortia do not have thermotolerant characteristics [34], [73].

### III.5 FUTURE PERSPECTIVES ON BIOGAS PRODUCTION RESEARCH

Future research on anaerobic digestion should prioritize the standardization of experimental conditions to enable robust comparisons across mesophilic and thermophilic regimes. Given that methane production variability is more strongly influenced by substrate characteristics and pretreatment strategies than by temperature or retention time alone, future studies should adopt harmonized reporting of key operational parameters, including C:N ratio, organic loading rate, pH control strategies and inhibitor thresholds. Further investigation is required on the development of adaptive process control strategies capable of mitigating thermophilic instability, especially under fluctuating temperature and pH conditions. Integrating real-time monitoring of physicochemical indicators with predictive modeling may enhance process resilience and methane productivity, as well as a mathematical model that integrates deeper approaches into the correlation among temperature and other operational parameters, such as pH and retention time that reflects a multifactorial influence in the heating field.

## IV. CONCLUSIONS

The results of the literature review allow us to affirm that the efficiency of AD does not depend solely on temperature, but on a multivariable interaction between pH and RT, which is evident in the natural behavior between the temperature ranges specified in this analysis. To this end, its relevance lies in understanding pH and RT not as fixed values, solely related to temperature, but as variables that respond to the type of substrate, the pretreatment applied, and the presence of inhibitory compounds such as VFA or FAN. Evidence shows that high yields can be achieved even in suboptimal ranges of these parameters, if the stability of the medium is ensured, the organic load is optimized, and/or strategies such as co-digestion, and physical, chemical, and biological pretreatments are employed. For future research it should be considered greater rigor in reporting other highly influential operational variables, such as organic load content based on substrate characteristics and the relationships between biological and/or microbiological inoculants, to build more robust databases for systematic reviews of literature concerning temperature as the main factor in the AD process. Likewise, it is necessary to compare the methodologies for analyzing accumulated methane production provided by biogas kinetics reports and to provide a mathematical model that allows methane production to be estimated, considering temperature as the main variable, correlated to other operational parameters.

## V. AUTHOR'S CONTRIBUTION

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