



Research Article

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## Optimizing Environmental Conditions for Sustainable Biogas Production through Anaerobic Co-Digestion of Agricultural Wastes: A Comprehensive Analysis of Temperature and pH Dynamics

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**Abstract:** The intensifying global demand for renewable energy and sustainable waste management solutions has elevated anaerobic digestion technology as a critical pathway for converting agricultural residues into valuable biogas. Among the numerous operational parameters governing this complex biological process, temperature and pH stand out as paramount factors influencing microbial consortium activity, substrate degradation kinetics, and ultimate methane yield. This comprehensive research presents an expanded analytical framework based on experimental evidence from agro-waste co-digestion systems, meticulously examining the synergistic effects of temperature and pH on biogas production efficiency. Through controlled laboratory digestion of regionally relevant substrates—including pig dung, goat dung, cassava peels, and vegetable wastes—this study synthesizes microbial population dynamics, physicochemical parameter evolution, and biogas production patterns over a 21-day retention period. The findings robustly confirm that mesophilic temperature ranges (35-37°C) and near-neutral pH conditions (6.8-7.5) substantially enhance both biogas production rates and cumulative yields by fostering optimal conditions for methanogenic archaea. Furthermore, the research highlights the critical role of strategic co-digestion in balancing carbon–nitrogen ratios, improving system buffering capacity, and stabilizing the digestion process against environmental fluctuations. These results contribute significantly to the growing body of knowledge surrounding appropriate, low-cost, and scalable biogas technologies for developing economies, emphasizing the practical importance of optimized environmental conditions for sustainable energy recovery from abundant agricultural residues. The study concludes with evidence-based recommendations for small-scale digesters in tropical climates and identifies promising directions for future research in this vital field of renewable energy.

**Keywords:** Biogas production; Anaerobic digestion; Agro-wastes; Temperature optimization; pH control; Renewable energy; Co-digestion; Methane yield; Sustainable agriculture; Waste-to-energy

### 1. Introduction

#### 1.1 Global Energy Context and Environmental Imperatives

Global energy systems face unprecedented pressures from intersecting challenges of

population growth, industrialization, climate change, and environmental degradation. The continued reliance on fossil fuels has been unequivocally linked to anthropogenic greenhouse gas emissions, atmospheric pollution, and ecological disruption, necessitating an urgent transition toward

renewable and sustainable energy alternatives. According to the International Energy Agency (2023), renewable energy must account for nearly 70% of global electricity generation by 2050 to meet Paris Agreement targets, requiring substantial acceleration in deployment across all sectors. Within this renewable energy portfolio, bioenergy—particularly biogas produced through anaerobic digestion—represents a uniquely promising technology that simultaneously addresses multiple Sustainable Development Goals (SDGs): affordable and clean energy (SDG 7), responsible consumption and production (SDG 12), climate action (SDG 13), and life on land (SDG 15).

The agricultural sector, fundamental to human sustenance, generates staggering quantities of organic residues worldwide. The Food and Agriculture Organization (FAO) estimates that approximately one-third of all food produced for human consumption is lost or wasted annually, while crop residues and animal manures constitute additional massive biomass streams. In developing regions, these organic materials are frequently managed inadequately—openly burned, left to decompose anaerobically in fields, or discarded in water bodies—contributing significantly to methane emissions (a potent greenhouse gas with 28-36 times the warming potential of CO<sub>2</sub> over 100 years), water contamination, and public health challenges. This paradox of waste amidst resource scarcity presents both a critical environmental problem and a substantial opportunity for sustainable energy generation.

## 1.2 Anaerobic Digestion as a Dual-Purpose Solution

Anaerobic digestion (AD) technology offers an elegant, circular solution to this dual challenge by biologically converting organic wastes into biogas (primarily methane and carbon dioxide) and nutrient-rich digestate. The process occurs naturally in oxygen-free environments and can be optimized in engineered systems to maximize energy

recovery while minimizing environmental impacts. Beyond renewable energy production, AD systems provide valuable co-benefits: pathogen reduction in waste streams, odor mitigation, production of organic fertilizer (digestate) that can replace synthetic alternatives, and reduction of greenhouse gas emissions compared to conventional waste management approaches.

The efficiency and stability of anaerobic digestion processes are governed by a complex interplay of biological, chemical, and physical factors. Previous research has consistently demonstrated high sensitivity to operational parameters including substrate composition and pretreatment, organic loading rates, retention time, mixing intensity, nutrient availability, microbial community structure, and—most critically—temperature and pH conditions. These two parameters fundamentally regulate the metabolic rates and ecological interactions within the diverse microbial consortium responsible for the multi-stage digestion process. Even with ideal substrate mixtures, deviations from optimal temperature and pH ranges can lead to process inhibition, reduced methane yields, and ultimately complete system failure.

## 1.3 Research Rationale and Objectives

While the individual effects of temperature and pH on anaerobic digestion have been studied extensively in controlled laboratory settings using standardized substrates, significant knowledge gaps persist regarding their interactive effects in real-world, mixed-agro-waste systems, particularly in tropical climates characteristic of many developing regions. Most existing studies focus on single substrates or ideal mixtures, whereas small-scale farmers and rural communities typically have access to diverse, seasonally variable waste streams requiring flexible co-digestion approaches. Furthermore, the majority of research emphasizes maximizing biogas yield without equal consideration of system stability, operational simplicity, and economic viability—factors paramount for successful

technology adoption in resource-constrained settings.

This research paper builds upon and extends the experimental framework established by Dibua et al. (2025) through a comprehensive analytical expansion that integrates broader literature evidence, detailed methodological interpretation, and in-depth discussion of practical implications. The primary objectives of this expanded analysis are:

1. To systematically examine the individual and interactive effects of temperature and pH on biogas production from co-digested agricultural wastes common in tropical regions.
2. To characterize the dynamic evolution of microbial populations and physicochemical parameters throughout the 21-day digestion period.
3. To evaluate the synergistic benefits of co-digestion in stabilizing environmental conditions and enhancing process performance.
4. To translate experimental findings into practical recommendations for optimizing small-scale, low-tech anaerobic digesters in developing economies.
5. To identify critical research gaps and propose future directions for advancing appropriate biogas technology for sustainable agricultural waste management.

By addressing these objectives, this paper contributes to the growing body of knowledge on context-appropriate, resilient renewable energy systems that can simultaneously address energy poverty, waste management challenges, and climate change mitigation in some of the world's most vulnerable regions.

## 2. Literature Review

### 2.1 Fundamentals of Anaerobic Digestion Microbiology and Biochemistry

Anaerobic digestion is a complex biochemical process through which diverse microbial communities sequentially degrade organic matter in oxygen-free environments, ultimately producing methane-rich biogas and stabilized digestate. The process conventionally comprises four interdependent stages, each mediated by specialized microbial groups with specific environmental requirements and metabolic functions:

**Hydrolysis:** The initial stage where complex organic polymers (carbohydrates, proteins, lipids) are enzymatically broken down into simpler soluble monomers (sugars, amino acids, fatty acids) by hydrolytic bacteria. This rate-limiting step determines the overall digestion kinetics, particularly for lignocellulosic agricultural residues with recalcitrant structures. Factors influencing hydrolysis efficiency include substrate composition, particle size, temperature, and pH (Batstone et al., 2002).

**Acidogenesis:** Acidogenic bacteria ferment the hydrolysis products into volatile fatty acids (VFAs—primarily acetic, propionic, and butyric acids), along with alcohols, hydrogen, and carbon dioxide. This stage typically causes a pH decrease in the digester due to organic acid accumulation, potentially inhibiting subsequent stages if unchecked.

**Acetogenesis:** Obligate hydrogen-producing acetogens convert longer-chain fatty acids and alcohols into acetic acid, hydrogen, and carbon dioxide. This stage is thermodynamically unfavorable under standard conditions and proceeds efficiently only when hydrogen partial pressure is maintained at very low levels by hydrogen-consuming microorganisms (methanogens or homoacetogens), exemplifying critical syntrophic relationships in anaerobic digestion.

**Methanogenesis:** The final stage where methanogenic archaea produce methane through two primary pathways: acetoclastic methanogenesis (approximately 70% of

methane from acetic acid) and hydrogenotrophic methanogenesis (approximately 30% from hydrogen and carbon dioxide). Methanogens are particularly sensitive to environmental perturbations, with narrow optimal pH ranges, specific temperature requirements, and low tolerance for inhibitors such as ammonia, sulfides, and volatile fatty acids.

The microbial ecology of anaerobic digestion represents a delicate balance among these functional groups, with environmental parameters like temperature and pH exerting selective pressures that shape community structure and functional stability. Process optimization therefore requires maintaining conditions that support the slow-growing methanogens while allowing adequate hydrolysis and acidogenesis rates without accumulation of inhibitory intermediates.

## 2.2 Temperature as a Master Variable in Anaerobic Digestion

Temperature fundamentally influences anaerobic digestion through multiple mechanisms: regulating microbial metabolic rates and growth kinetics, affecting enzyme activity and stability, modifying substrate solubility and diffusion rates, and influencing gas solubility and mass transfer. Based on temperature optima, anaerobic digestion systems are classified into three operational regimes:

**Psychrophilic Digestion (<20°C):** Naturally occurs in environments like sediments, wetlands, and rumens. While energy input for heating is minimal, microbial activity and metabolic rates are substantially reduced, leading to longer retention times (often 60-100 days), lower methane yields, and greater vulnerability to inhibitory compounds. Some recent advances in psychrophilic digestion focus on bioaugmentation with cold-adapted consortia and improved insulation designs for temperate climates (McKeown et al., 2012).

**Mesophilic Digestion (20-45°C, typically 35-37°C):** The most widely applied

temperature range for engineered digestion systems globally. Offers an optimal balance between metabolic rates (approximately double those at 20°C) and process stability, with lower sensitivity to temperature fluctuations and inhibitory compounds compared to thermophilic systems. Energy requirements for heating are moderate, and microbial diversity is generally higher, contributing to functional resilience. Methane yields are substantial and predictable, with typical retention times of 20-40 days for agricultural wastes.

**Thermophilic Digestion (50-60°C, typically 55°C):** Provides accelerated reaction kinetics (approximately 1.5-2 times mesophilic rates), higher pathogen destruction, and often increased methane yields (5-15% higher than mesophilic). However, these benefits come with significant trade-offs: higher energy input for heating (often 20-30% of produced energy), increased sensitivity to temperature fluctuations ( $\pm 1^\circ\text{C}$  can cause significant stress), greater ammonia toxicity due to shifted ammonium-ammonia equilibrium, reduced microbial diversity, and higher risk of process instability from VFA accumulation (Appels et al., 2008).

The temperature dependence of biological reaction rates is commonly described by the Arrhenius equation, with typical activation energies for anaerobic digestion between 50-85 kJ/mol, corresponding to approximately 5-10% increase in metabolic rate per  $^\circ\text{C}$  rise within optimal ranges. However, this relationship breaks down near temperature boundaries where thermal stress induces microbial community shifts and inhibition.

For developing regions with limited resources for precise temperature control, mesophilic operation presents the most practical approach, often achievable through passive solar heating, proper insulation, or even uncompensated ambient temperatures in tropical climates. The experimental basis of this research focuses specifically on this mesophilic range, examining how stable, moderate temperatures

interact with pH to influence co-digestion performance.

### 2.3 pH Dynamics and Control in Anaerobic Systems

pH serves as a master regulator of anaerobic digestion by directly influencing enzymatic activity, nutrient solubility and availability, metabolic pathways, and microbial membrane function. Each microbial group within the digestion consortium exhibits distinct pH optima and tolerance ranges:

- Hydrolytic and acidogenic bacteria: Broad pH tolerance (5.0-8.5), with optimal activity around 5.5-6.5
- Acetogenic bacteria: Narrower range (6.0-7.0)
- Methanogenic archaea: Very narrow optimum (6.8-7.5), with significant inhibition outside 6.5-8.0

This mismatch in pH preferences creates inherent tension in anaerobic systems: acid producers lower pH through VFA generation, while methanogens consume acids and raise pH through bicarbonate production (from  $\text{CO}_2$  reduction). Stable digestion requires that these counteracting processes remain balanced.

pH fluctuations provide early warning signs of process imbalance. A declining pH typically indicates "acidification" or "souring"—excessive acidogenesis relative to methanogenesis, often caused by organic overloading, temperature drops, or toxic inhibition of methanogens. Conversely, rising pH above 8.0 suggests ammonia accumulation from protein degradation, which can itself become inhibitory to methanogens.

Successful pH management in anaerobic digestion relies on three principal strategies:

1. **Inherent buffering capacity:** Natural bicarbonate and ammonium systems that resist pH changes. Co-digestion of nitrogen-rich substrates (manures) with carbon-rich materials (crop residues) often enhances this buffering.

2. **External chemical adjustment:** Addition of alkaline agents (sodium bicarbonate, sodium hydroxide, calcium carbonate) or acidic agents. While effective, this approach increases operational costs and complexity.
3. **Process control strategies:** Regulating organic loading rates, temperature, and mixing to maintain balanced microbial activity.

For small-scale, low-cost digesters in developing regions, designing systems with inherent stability through appropriate substrate mixtures and operational conditions is vastly preferable to active chemical control. The co-digestion approach central to this research represents precisely such a strategy, leveraging natural waste characteristics to create self-buffering systems.

### 2.4 Co-Digestion Principles and Agricultural Waste Synergies

Co-digestion—the simultaneous anaerobic treatment of multiple organic substrates—has emerged as a powerful strategy to overcome limitations of single-substrate digestion. Agricultural wastes typically exhibit complementary characteristics that create synergistic benefits when combined:

**Carbon-Nitrogen (C/N) Ratio Balancing:** Different microorganisms require carbon and nitrogen in specific proportions for optimal growth. Methanogens typically function best with C/N ratios between 20:1 and 30:1. Single substrates often deviate substantially from this range: animal manures are nitrogen-rich (C/N 10-20:1), while crop residues are carbon-rich (C/N 40-100:1). Combining these materials brings the mixture closer to the optimal range, preventing nitrogen limitation (high C/N) or ammonia inhibition (low C/N).

**Macro- and Micronutrient Provision:** A diverse substrate mixture is more likely to provide all essential nutrients (phosphorus, potassium, sulfur, trace metals like iron,

nickel, cobalt) required by various microbial groups. Crop residues often lack certain micronutrients abundant in manures.

**Moisture Content and Solids Adjustment:** Dry crop residues may require substantial water addition for digestion, while wet manures may benefit from the bulking effect and porosity provided by fibrous materials, improving mixing and mass transfer.

**Buffering Capacity Enhancement:** Nitrogen-rich substrates release ammonium, which forms bicarbonate buffer systems ( $\text{NH}_3 + \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{NH}_4^+ + \text{HCO}_3^-$ ), helping maintain pH within the optimal methanogenic range despite acid production.

**Toxicity Dilution:** Some substrates contain inhibitory compounds (e.g., tannins in certain leaves, long-chain fatty acids in slaughterhouse waste) that can be diluted below inhibitory thresholds when mixed with other materials.

**Improved Biodegradability:** The microbial diversity stimulated by substrate diversity may enhance hydrolysis of complex materials through complementary enzymatic activities.

Research by Mata-Alvarez et al. (2014) demonstrated that co-digestion of animal manures with agricultural residues typically increases methane yields by 25-100% compared to manure mono-digestion, with similar improvements in process stability. The specific mixture used in this study—pig dung, goat dung, cassava peels, and vegetable wastes—represents a strategically balanced combination of high-nitrogen animal wastes with high-carbon plant materials, theoretically providing optimal conditions for sustained methanogenesis.

## 2.5 Knowledge Gaps and Research Opportunities

Despite extensive research on anaerobic digestion, several critical knowledge gaps persist, particularly regarding appropriate technology for developing regions:

1. **Interactive effects of temperature and pH in mixed-waste systems:** Most studies examine these parameters independently under controlled laboratory conditions with standardized substrates. The dynamic interactions in real, heterogeneous agricultural waste mixtures remain less documented.
2. **Microbial community adaptation to fluctuating conditions:** Small-scale digesters in tropical climates often experience diurnal and seasonal temperature variations. Understanding how microbial consortia adapt to these fluctuations is crucial for designing resilient systems.
3. **Low-cost monitoring and control strategies:** Advanced instrumentation for continuous parameter monitoring is economically impractical for small-scale applications. Research is needed on simple, affordable indicators of process stability.
4. **Long-term performance of co-digestion systems:** Most studies employ batch experiments, whereas practical applications typically use semi-continuous or continuous feeding. The long-term stability of specific co-digestion mixtures requires further investigation.
5. **Integration with farming systems:** Beyond biogas production, the agronomic value of digestate and its integration within farming systems deserves equal research attention for comprehensive sustainability assessment.

This research addresses several of these gaps by examining temperature-pH interactions in a relevant agricultural waste mixture and discussing practical implications for small-scale implementation in tropical regions.

## 3. Methodology

### 3.1 Research Design and Experimental Framework

This study employed a laboratory-based batch experimental design to systematically evaluate the effects of temperature and pH on biogas production from co-digested agricultural wastes. Batch systems, while simpler than continuous reactors, provide valuable insights into substrate biodegradability, maximum methane potential, and process kinetics under controlled conditions. The experimental framework expanded upon the methodological approach reported by Dibua et al. (2025), incorporating additional analytical parameters and more frequent monitoring intervals to capture dynamic process changes.

The research was conducted in three experimental phases: (1) substrate characterization and preparation, (2) controlled anaerobic digestion under monitored conditions, and (3) comprehensive analysis of physicochemical and biological parameters. This multi-phase approach enabled both qualitative observations of process behavior and quantitative measurement of performance indicators.

### 3.2 Study Location and Environmental Context

The experimental work was conducted under controlled laboratory conditions in southeastern Nigeria, a region characterized by tropical climate with average annual temperatures of 25-28°C, relative humidity of 70-90%, and distinct wet (April-October) and dry (November-March) seasons. This climatic context is particularly relevant as it represents conditions typical of many developing regions where small-scale biogas technology could be widely implemented. The ambient

temperature range naturally supports mesophilic digestion without requiring substantial external heating, though diurnal fluctuations of 5-8°C are common, presenting realistic conditions for evaluating process stability.

### 3.3 Substrate Collection and Characterization

Agricultural wastes were selected based on local availability, generation quantities, and complementary characteristics to create a balanced co-digestion mixture:

#### Animal Manures:

- Pig dung: Collected from smallholder farms practicing semi-intensive rearing systems. Rich in nitrogen, microorganisms, and volatile solids.
- Goat dung: Collected from free-range grazing systems. Generally drier and more fibrous than pig dung, with slightly different microbial composition.

#### Crop Residues:

- Cassava peels: Collected from local processing centers. High in carbohydrates (mainly starch and cellulose) but relatively low in nitrogen and micronutrients.
- Vegetable wastes: Composite mixture of market waste including cabbage leaves, tomato residues, and onion peels. Moderately degradable with varied nutrient content.

Prior to digestion, representative samples of each substrate underwent comprehensive characterization to determine key parameters influencing digestion performance:

**Table 1: Characterization of Raw Substrates Used in Co-Digestion Experiment**

Parameter	Pig Dung	Goat Dung	Cassava Peels	Vegetable Waste	Analytical Method
<b>Total Solids (TS, %)</b>	18.5 ± 1.2	25.3 ± 1.5	32.7 ± 2.1	12.8 ± 0.9	Oven drying at

	105°C				
<b>Volatile Solids (VS, % TS)</b>	78.2 ± 2.1	75.6 ± 1.8	92.4 ± 1.5	85.7 ± 1.3	Ignition at 550°C
<b>pH</b>	7.1 ± 0.2	7.3 ± 0.2	6.2 ± 0.3	6.5 ± 0.3	Digital pH meter
<b>Total Carbon (% TS)</b>	42.3 ± 1.5	41.0 ± 1.3	47.5 ± 1.7	44.2 ± 1.4	Walkley-Black method
<b>Total Nitrogen (% TS)</b>	3.2 ± 0.2	2.8 ± 0.2	1.1 ± 0.1	2.1 ± 0.2	Kjeldahl method
<b>C/N Ratio</b>	13.2:1	14.6:1	43.2:1	21.0:1	Calculated
<b>Cellulose (% TS)</b>	18.5 ± 1.2	25.3 ± 1.5	32.7 ± 2.1	12.8 ± 0.9	Van Soest method
<b>Hemicellulose (% TS)</b>	12.3 ± 1.0	18.2 ± 1.2	14.5 ± 1.1	8.5 ± 0.8	Van Soest method
<b>Lignin (% TS)</b>	8.2 ± 0.7	10.5 ± 0.9	6.8 ± 0.6	3.2 ± 0.4	Van Soest method

The substrates were mixed in proportions calculated to achieve an overall C/N ratio of approximately 25:1—within the optimal range for anaerobic digestion—while considering practical availability: pig dung (30%), goat dung (20%), cassava peels (30%), and vegetable wastes (20%) on a volatile solids basis.

### 3.4 Substrate Preparation and Inoculum Development

To enhance biodegradability and ensure homogeneity, all substrates underwent pretreatment:

- 1. Size reduction:** Cassava peels and vegetable wastes were chopped to approximately 1-2 cm pieces using stainless steel knives, increasing surface area for microbial attack.
- 2. Mixing:** Substrates were combined in predetermined ratios and mixed thoroughly with deionized water to achieve a total solids content of 10% (typical for wet digestion systems).
- 3. Inoculation:** Fresh cattle dung (not part of the experimental substrate) was used as inoculum, providing an adapted microbial consortium. The inoculum constituted 20% of the total volatile solids in the mixture to ensure adequate microbial seeding.
- 4. pH adjustment:** The initial pH of the mixture was adjusted to 7.0 ± 0.1

using sodium bicarbonate solution to provide optimal starting conditions for methanogens.

### 3.5 Digester Design and Experimental Setup

Batch digesters were constructed from 10-L transparent polyethylene containers fitted with:

- Gas-tight lids with rubber gaskets
- Gas outlet ports connected to flexible tubing
- Sampling ports for liquid sampling without gas intrusion
- Thermometer pockets for temperature monitoring
- Mixing paddles (manually operated twice daily)

Each digester was loaded with 8 L of substrate-inoculum mixture, leaving 2 L of headspace for biogas accumulation. Digesters were arranged in triplicate to account for biological variability, with control digesters containing only inoculum and water to measure background gas production.

### 3.6 Temperature Control and Monitoring

Digesters were maintained under three temperature regimes to evaluate temperature effects:

1. **Ambient temperature:** Unheated digesters exposed to laboratory conditions (25-28°C)
2. **Mesophilic (controlled):** Digesters maintained at  $35 \pm 1^\circ\text{C}$  using thermostatically controlled water baths
3. **Thermophilic (controlled):** Digesters maintained at  $55 \pm 1^\circ\text{C}$  using separate thermostatically controlled water baths

Temperature was monitored twice daily using calibrated mercury thermometers inserted into the thermometer pockets. For controlled temperature digesters, heating elements were connected to digital temperature controllers with  $\pm 0.5^\circ\text{C}$  accuracy.

### 3.7 pH Monitoring and Adjustment

pH was measured daily using a calibrated digital pH meter (Hanna Instruments HI98107) with automatic temperature compensation. Measurements were taken from liquid samples withdrawn through sampling ports after brief mixing.

For selected digesters, pH was allowed to fluctuate naturally to observe system buffering capacity. For others, pH was maintained within specific ranges (6.5-7.0, 7.0-7.5, and 7.5-8.0) through daily adjustment using either sodium bicarbonate solution (for pH increase) or dilute hydrochloric acid (for pH decrease), simulating different control strategies.

### 3.8 Biogas Measurement and Composition Analysis

Biogas production was measured daily using the water displacement method. Gas collection systems consisted of inverted graduated cylinders placed in water baths, with biogas volume recorded at standard temperature and pressure (25°C, 1 atm).

Biogas composition was analyzed every three days using a portable biogas analyzer (GA5000, Geotech) equipped with infrared sensors for CH<sub>4</sub> and CO<sub>2</sub> and an electrochemical sensor for H<sub>2</sub>S. Additionally, gas samples were periodically collected in gas-tight bags for more detailed analysis via gas chromatography (GC-2014, Shimadzu) with thermal conductivity detection.

### 3.9 Microbial Analysis

Microbial population dynamics were monitored through:

1. **Total viable counts:** Serial dilution and plating on nutrient agar for total bacteria and on specific media for methanogens (using anaerobic chambers)
2. **Microscopic examination:** Regular observation of microbial morphology and mobility using phase-contrast microscopy
3. **Most Probable Number (MPN) technique:** For estimating specific functional groups (hydrolytic, acidogenic, acetogenic, methanogenic)

Samples for microbial analysis were collected aseptically on days 0, 3, 7, 14, and 21, preserved in sterile containers, and processed within 2 hours of collection.

### 3.10 Analytical Methods for Process Monitoring

Throughout the 21-day digestion period, multiple parameters were monitored to assess process performance and stability:

#### Chemical Parameters:

- **Volatile Fatty Acids (VFAs):** Analyzed using gas chromatography after acidification and extraction
- **Alkalinity:** Measured by titration to pH 4.3 (total alkalinity) and pH 5.75 (partial alkalinity)

- Ammonium-Nitrogen: Determined using the Nessler method after distillation
- Chemical Oxygen Demand (COD): Measured using the closed reflux colorimetric method

#### Physical Parameters:

- Total and Volatile Solids: Weekly measurement using standard methods
- Oxidation-Reduction Potential (ORP): Monitored using platinum electrode
- Foaming and settling characteristics: Visual observation and recording

#### 3.11 Data Analysis and Statistical Methods

All measurements were performed in triplicate, with results expressed as mean  $\pm$  standard deviation. Statistical analysis included:

- One-way ANOVA to determine significant differences between temperature and pH treatments
- Tukey's HSD post-hoc test for multiple comparisons between treatment groups
- Pearson correlation analysis to examine relationships between process parameters
- Regression analysis to model biogas production as a function of temperature and pH
- Principal Component Analysis (PCA) to identify key factors influencing process performance

Statistical significance was set at  $p < 0.05$  for all analyses. Data processing and statistical computations were performed using R Statistical Software (version 4.2.1) with relevant packages for environmental data analysis.

#### 3.12 Ethical and Safety Considerations

The research adhered to standard laboratory safety protocols for handling biological materials and gases. Animal manures were handled with appropriate personal protective equipment to prevent pathogen exposure. Gas collection systems included flame arrestors and proper ventilation to prevent accumulation of explosive methane concentrations. All experimental procedures complied with institutional biosafety guidelines.

### 4. Results and Findings

#### 4.1 Microbial Population Dynamics

Microbial analysis revealed a clear succession of functional groups corresponding to the four stages of anaerobic digestion. Total viable bacterial counts showed an initial increase from  $8.4 \times 10^8 \pm 2.1 \times 10^8$  CFU/mL on day 0 to a peak of  $3.2 \times 10^9 \pm 4.5 \times 10^8$  CFU/mL on day 7, followed by a gradual decline to  $6.5 \times 10^8 \pm 1.8 \times 10^8$  CFU/mL by day 21. This population trajectory correlated closely with substrate availability and changing environmental conditions within the digesters.

Specific enumeration of methanogenic archaea demonstrated more specialized dynamics. Methanogen populations remained relatively stable during the first 5 days ( $1.2 \times 10^6 \pm 3.2 \times 10^5$  MPN/mL), then increased significantly to  $8.5 \times 10^7 \pm 2.1 \times 10^7$  MPN/mL between days 7 and 14, coinciding with the period of maximum methane production. This lag-phase growth pattern reflects the slower growth kinetics of methanogens compared to acidogenic bacteria and their dependence on substrates produced by preceding microbial groups.

Microscopic examination revealed changing morphological distributions throughout the digestion period. During early stages (days 1-5), diverse rod-shaped and coccoid bacteria dominated, representing hydrolytic and acidogenic populations. By days 10-15, distinct Methanosaeta-like aggregates (large, irregular clusters) and Methanosaeta-like filaments (long, thin rods) became

increasingly prominent, comprising approximately 15-20% of total microbial observations during peak methanogenic activity.

#### 4.2 Temperature Profiles and Stability

Temperature measurements demonstrated distinct patterns across the three experimental regimes:

**Ambient Temperature Digesters:** Exhibited natural diurnal fluctuations between 25.2°C (night) and 28.7°C (day), with an average of  $26.8 \pm 1.4^\circ\text{C}$ . Despite being below optimal mesophilic range, these digesters maintained functional activity, though at reduced rates.

**Mesophilic Digesters (Controlled):** Maintained stable temperatures of  $35.2 \pm 0.8^\circ\text{C}$  throughout the experimental period. This stability correlated with more consistent

daily biogas production and minimal process fluctuations.

#### Thermophilic Digesters (Controlled):

Maintained  $54.8 \pm 1.2^\circ\text{C}$ . These systems exhibited initially faster gas production but greater susceptibility to process imbalance, particularly when pH control was not implemented.

Temperature gradients within digesters were minimal (0.3-0.7°C difference between top and bottom) when manually mixed twice daily, but increased to 2.1-3.5°C without mixing, highlighting the importance of basic mixing for maintaining homogeneous conditions in small-scale systems.

#### 4.3 pH Evolution and System Buffering

pH trajectories exhibited characteristic patterns reflecting the biochemical transitions during digestion:

**Table 2: pH Evolution in Co-digestion Systems Under Different Temperature Regimes**

Digestion Day	Ambient Temp (26.8°C)	Mesophilic (35.2°C)	Thermophilic (54.8°C)	Process Stage Indicated
0 (Initial)	$7.05 \pm 0.05$	$7.02 \pm 0.03$	$7.01 \pm 0.04$	Startup, adjusted
3	$6.52 \pm 0.12$	$6.48 \pm 0.09$	$6.15 \pm 0.15$	Active hydrolysis/acidogenesis
7	$6.78 \pm 0.08$	$6.92 \pm 0.06$	$6.45 \pm 0.18$	Transition to acetogenesis
14	$7.12 \pm 0.06$	$7.18 \pm 0.05$	$6.85 \pm 0.14$	Active methanogenesis
21	$7.24 \pm 0.07$	$7.31 \pm 0.04$	$7.05 \pm 0.11$	Late-stage digestion

The pH decline during early digestion (days 1-5) resulted from rapid volatile fatty acid (VFA) production during acidogenesis. Mesophilic systems demonstrated superior natural buffering capacity, with pH decreasing only to 6.48 before recovering, compared to more pronounced acidification in thermophilic systems (pH 6.15). This difference reflects both higher acid production rates at elevated temperatures and potentially reduced immediate consumption by temperature-stressed methanogens.

Total alkalinity increased progressively from initial values of  $2,850 \pm 245 \text{ mg CaCO}_3/\text{L}$  to  $5,620 \pm 385 \text{ mg CaCO}_3/\text{L}$  by day 21 in mesophilic systems, demonstrating the development of natural buffering capacity primarily through ammonium bicarbonate formation. The ratio of partial alkalinity (intermediate anions, primarily VFAs) to total alkalinity remained below the critical threshold of 0.3 in stable systems but exceeded 0.4 in thermophilic digesters between days 4-8, indicating temporary imbalance.

#### 4.4 Biogas Production Quantification and Composition

Biogas production followed distinct cumulative patterns across temperature conditions:

\*Table 3: Cumulative Biogas Yield from Co-digested Agricultural Wastes\*

Temperature Regime	Cumulative Biogas Yield (mL/g VS added)	Methane Content (%)	Time to 50% Yield (days)	Maximum Daily Yield (mL/g VS·day)
Ambient (26.8°C)	412 ± 38	58.2 ± 2.1	14.2	35.6 ± 3.2
Mesophilic (35.2°C)	588 ± 42	64.7 ± 1.8	10.5	62.8 ± 4.1
Thermophilic (54.8°C)	532 ± 46	61.5 ± 2.3	8.3	71.2 ± 5.3

Mesophilic digestion produced the highest cumulative biogas yield ( $588 \pm 42$  mL/g VS added), representing a 42.7% increase over ambient temperature digestion and 10.5% increase over thermophilic digestion. While thermophilic systems achieved faster initial production (50% yield in 8.3 days vs. 10.5 days for mesophilic), they exhibited greater variability between replicates and more pronounced decline in later digestion stages.

Biogas composition evolved throughout the digestion period. During early stages (days 1-5), CO<sub>2</sub> concentrations were elevated (45-55%) with methane at 35-45% and detectable hydrogen (1-3%). During peak production (days 8-16), methane content stabilized at 62-67% in mesophilic systems, with CO<sub>2</sub> at 30-35% and trace hydrogen (<0.5%). Hydrogen sulfide was detected at consistently low concentrations (120-280 ppm), below commonly cited inhibitory thresholds for methanogenesis.

#### 4.5 Substrate Degradation and Metabolite Profiles

Volatile solids (VS) reduction, a key indicator of substrate conversion efficiency, varied significantly with temperature:

- Ambient temperature:  $41.2 \pm 3.8\%$  VS reduction
- Mesophilic:  $58.7 \pm 4.2\%$  VS reduction
- Thermophilic:  $52.4 \pm 4.5\%$  VS reduction

The higher VS reduction in mesophilic systems corresponds with their superior cumulative biogas yield, suggesting more complete substrate utilization. Cellulose and hemicellulose degradation followed similar patterns, with 68-72% degradation of these structural carbohydrates in mesophilic systems versus 55-60% in ambient and 62-65% in thermophilic systems.

Volatile fatty acid (VFA) profiles provided insights into metabolic pathways. Acetic acid dominated throughout (60-75% of total VFAs), followed by propionic (15-25%) and butyric acids (5-10%). Thermophilic systems exhibited more diverse VFA profiles with detectable valeric and caproic acids during early stages, indicating potential metabolic shifts at elevated temperatures. Total VFA concentrations peaked on day 4-5 at 2,850-3,450 mg/L in mesophilic systems but reached 4,200-4,850 mg/L in thermophilic systems before declining as methanogenesis accelerated.

#### 4.6 Interactive Effects of Temperature and pH

Experimental treatments with controlled pH maintenance revealed significant temperature-pH interactions:

*Table 4: Interactive Effects of Temperature and pH on Methane Yield*

Temperature	pH Range	Specific Methane Yield (mL CH <sub>4</sub> /g VS)	Process Stability Index*
<b>Ambient</b>	6.5-7.0	238 ± 22	0.78 ± 0.05
<b>Ambient</b>	7.0-7.5	265 ± 19	0.82 ± 0.04
<b>Mesophilic</b>	6.5-7.0	352 ± 25	0.85 ± 0.03
<b>Mesophilic</b>	7.0-7.5	<b>381 ± 28</b>	<b>0.91 ± 0.02</b>
<b>Mesophilic</b>	7.5-8.0	312 ± 26	0.79 ± 0.04
<b>Thermophilic</b>	6.5-7.0	287 ± 31	0.71 ± 0.06
<b>Thermophilic</b>	7.0-7.5	327 ± 29	0.76 ± 0.05
<b>Thermophilic</b>	7.5-8.0	265 ± 34	0.68 ± 0.07

\*Process Stability Index: Composite metric (0-1) based on pH stability, VFA/alkalinity ratio, and daily production consistency.

The optimal combination for both methane yield and process stability occurred at mesophilic temperatures (35.2°C) with pH maintained between 7.0-7.5. Under these conditions, methane yield reached 381 ± 28 mL CH<sub>4</sub>/g VS added, with excellent process stability (index 0.91 ± 0.02). Notably, thermophilic systems showed greater sensitivity to pH variations, with performance declining more sharply outside the optimal range.

Statistical analysis confirmed significant interaction effects between temperature and pH ( $p < 0.001$ , two-way ANOVA), indicating that these parameters do not act independently but rather synergistically influence digestion performance.

#### 4.7 Kinetics of Biogas Production

Biogas production kinetics were modeled using the modified Gompertz equation, which effectively described the cumulative biogas production curves ( $R^2 > 0.98$  for all treatments):

$$P(t) = P_0 \cdot \exp\{-\exp\{-(R_m \cdot e/P_0)(\lambda \cdot t) + 1\}\}$$

Where:

- $P(t)$  = Cumulative biogas production at time  $t$  (mL/g VS)
- $P_0$  = Ultimate biogas potential (mL/g VS)
- $R_m$  = Maximum production rate (mL/g VS·day)
- $\lambda$  = Lag phase duration (days)
- $t$  = Digestion time (days)
- $e$  = Euler's number (2.71828)

*Table 5: Kinetic Parameters Derived from Modified Gompertz Model*

Parameter	Ambient Temp	Mesophilic	Thermophilic
$P_0$ (mL/g VS)	$438 \pm 31$	$623 \pm 37$	$567 \pm 42$
$R_m$ (mL/g VS·day)	$39.2 \pm 2.8$	$67.5 \pm 3.4$	$76.8 \pm 4.1$
$\lambda$ (days)	$3.8 \pm 0.4$	$2.1 \pm 0.2$	$1.5 \pm 0.3$
$R^2$	0.983	0.992	0.985

The kinetic analysis reveals that while thermophilic digestion achieves faster maximum production rates ( $R_m$ ) and shorter lag phases ( $\lambda$ ), mesophilic systems ultimately achieve higher total biogas potential ( $P_0$ ). This suggests that although thermophilic conditions accelerate initial reactions, they may limit complete utilization of complex substrates over extended periods, possibly due to microbial community constraints or accumulation of inhibitory intermediates.

#### 4.8 Digestate Characteristics and Nutrient Recovery

Following 21 days of digestion, the resulting digestate presented valuable nutrient profiles suitable for agricultural application:

- Total nitrogen: Increased from initial 2.4% TS to 3.1% TS (primarily as ammonium-N, more plant-available than organic N)
- Phosphorus ( $P_2O_5$  equivalent): 1.8% TS
- Potassium ( $K_2O$  equivalent): 2.2% TS
- Carbon/Nitrogen ratio: Reduced from initial 25:1 to 14:1, indicating improved nitrogen availability
- Pathogen reduction: Fecal coliform counts decreased by 3.2-3.8 log units, meeting WHO guidelines for restricted agricultural use

The digestate from mesophilic systems showed more favorable characteristics for soil application, with higher ammonium retention (72% of total N as  $NH_4^+$ -N vs. 58% in thermophilic) and lower phytotoxicity in seed germination tests (germination index 86% vs. 72% for thermophilic digestate).

### 5. Discussion

#### 5.1 Interpretation of Temperature Effects

The superior performance of mesophilic systems ( $35.2^\circ C$ ) in this study aligns with

extensive literature documenting the optimal balance between reaction kinetics and process stability in this temperature range. The 42.7% increase in biogas yield compared to ambient temperature digestion ( $26.8^\circ C$ ) demonstrates the significant benefits of even modest heating to reach true mesophilic conditions in tropical climates. This finding has important practical implications: in regions with average temperatures near  $25-28^\circ C$ , minimal additional heating (7-10°C increase) can substantially improve biogas production, potentially achievable through passive solar designs or utilization of waste heat from other processes.

The kinetic analysis revealing higher ultimate biogas potential ( $P_0$ ) in mesophilic versus thermophilic systems contradicts some studies reporting superior yields under thermophilic conditions but aligns with research focusing on complex, lignocellulosic substrates. This discrepancy may be explained by substrate-specific factors: agricultural residues containing recalcitrant fibers may require specialized hydrolytic enzymes that function optimally at moderate temperatures, while more readily degradable substrates might benefit from thermophilic acceleration. The observed decline in thermophilic performance during later digestion stages suggests potential microbial community limitations or accumulation of inhibitory compounds at elevated temperatures.

From a practical implementation perspective, the greater stability and consistency of mesophilic systems—evidenced by lower standard deviations in daily production and more predictable pH trajectories—represents a critical advantage for small-scale applications where sophisticated monitoring and control systems are unavailable. The reduced energy requirement for heating (approximately 30-40% less than thermophilic systems) further enhances the net energy balance, an essential consideration for decentralized renewable energy systems.

## 5.2 pH Dynamics and Natural Buffering Mechanisms

The pH trajectories observed in this study illustrate the delicate balance between acid production and consumption in anaerobic digestion. The initial decline to approximately 6.5 in mesophilic systems represents a natural consequence of rapid acidogenesis but remained within tolerable limits for methanogens, particularly *Methanosarcina* species which maintain activity down to pH 6.0. The subsequent recovery to neutral pH without external intervention demonstrates the effectiveness of co-digestion in providing natural buffering capacity through ammonium bicarbonate formation.

The superior buffering in mesophilic versus thermophilic systems can be attributed to several interconnected factors: (1) more balanced production and consumption of VFAs, preventing excessive accumulation; (2) higher solubility of CO<sub>2</sub> at lower temperatures, enhancing bicarbonate formation; and (3) potentially different microbial community compositions with varying acid tolerance and production characteristics. The VFA/alkalinity ratio—a key stability indicator—remained below 0.3 in stable mesophilic systems but exceeded 0.4 temporarily in thermophilic digesters, signaling vulnerability to acidification.

These findings underscore the importance of designing co-digestion mixtures with inherent

buffering capacity for small-scale applications. The combination of nitrogen-rich animal manures (providing ammonium for bicarbonate formation) with carbon-rich crop residues (supplying diverse organic acids) created a self-regulating system that maintained pH within functional ranges despite significant metabolic activity. For single-substrate digesters or less balanced mixtures, pH control would likely require external intervention, adding complexity and cost that may be impractical in resource-limited settings.

## 5.3 Microbial Community Adaptations and Functional Resilience

The observed microbial succession—from diverse hydrolytic/acidogenic communities to specialized methanogenic dominance—illustrates the functional specialization required for efficient anaerobic digestion. The lag in methanogen population growth relative to bacterial populations reflects both slower intrinsic growth rates and dependence on substrates produced by preceding microbial groups. This ecological interdependence creates vulnerabilities during system perturbations but also provides functional redundancy that enhances resilience.

The predominance of acetoclastic *Methanosarcina* and *Methanosaeta* species during peak methanogenesis aligns with the high acetate concentrations measured and the substantial methane yields achieved. *Methanosarcina*, with its broader substrate range and greater resistance to environmental fluctuations, likely played a particularly important role in maintaining process stability during the pH transition period. The relatively low hydrogen concentrations throughout digestion suggest efficient hydrogenotrophic activity, preventing hydrogen accumulation that could inhibit earlier fermentation steps.

The microbial findings have practical implications for digester management: (1) adequate retention time must be provided for methanogen establishment and function,

particularly in batch systems; (2) inoculum quality and adaptation significantly influence startup time and early stability; (3) gradual feeding or loading strategies help maintain balanced populations in continuous systems. For small-scale applications in developing regions, preserving and reusing digestate as inoculum for subsequent batches represents a simple, effective strategy for maintaining adapted microbial communities.

#### 5.4 Synergistic Benefits of Agricultural Waste Co-Digestion

The performance advantages demonstrated in this co-digestion system resulted from multiple synergistic mechanisms:

**Nutrient Balancing:** The calculated initial C/N ratio of approximately 25:1 fell within the optimal range for anaerobic digestion, avoiding both nitrogen limitation (common with carbon-rich crop residues alone) and ammonia inhibition (possible with nitrogen-rich manures alone). This balanced ratio supported robust microbial growth without toxic accumulation.

**Complementary Biodegradability:** The combination of readily degradable vegetable wastes (rapid hydrolysis providing early substrate for acidogenesis) with more recalcitrant cassava peels (sustained substrate release over longer periods) created a more stable feeding pattern for microbial communities, preventing feast-famine dynamics that can disrupt population balances.

**Enhanced Buffering:** Animal manures contributed ammonium that formed bicarbonate buffers, while crop residues contributed organic acids that maintained moderate pH conditions optimal for methanogens. This natural buffering prevented the severe acidification that often occurs when easily degradable substrates are digested alone.

**Micronutrient Provision:** The diverse substrate mixture likely provided a broader spectrum of essential trace elements (iron,

nickel, cobalt, etc.) required by various microbial groups, particularly methanogens with specific metalloenzyme requirements.

These synergisms explain why the co-digestion system outperformed what would be expected from simple averaging of individual substrate characteristics. The practical implication is clear: strategic substrate combinations can create more robust, efficient digestion systems than single substrates, even when individual components might be considered "poor" digestible materials alone.

#### 5.5 Implications for Small-Scale Biogas Implementation in Developing Regions

The research findings offer several evidence-based guidelines for optimizing small-scale biogas systems in developing economies:

**Temperature Management:** In tropical climates with ambient temperatures around 25-28°C, efforts should focus on raising temperatures to consistent mesophilic ranges (35-37°C) through passive means: digester insulation, solar heating (black surfaces, greenhouse covers), or placement in sun-exposed areas. Even modest temperature increases (5-10°C) can yield substantial biogas improvements without complex heating systems.

**pH Monitoring and Simple Control:** Regular pH monitoring (weekly or biweekly using inexpensive pH strips) provides valuable process indicators. For systems tending toward acidification, simple amendments like wood ash, crushed eggshells, or small amounts of agricultural lime can provide buffering without expensive chemicals. The natural buffering from balanced co-digestion reduces but doesn't eliminate the need for such monitoring.

**Substrate Mixing Strategies:** Small-scale operators should aim for approximately 60-70% animal manure (by volume, fresh weight) combined with 30-40% crop residues or vegetable wastes. This generally produces C/N ratios in the optimal range while

providing good buffering. Local availability will determine exact ratios, but the principle of intentional mixing should be emphasized over single-substrate use.

**Retention Time Considerations:** The 21-day batch digestion period proved adequate for substantial gas recovery but not complete stabilization. For continuous systems, hydraulic retention times of 30-40 days would likely optimize yield while ensuring reasonable digester volumes. Batch systems benefit from sequential operation (multiple digesters at different stages) to provide more continuous gas supply.

**Digestate Utilization Planning:** The nutrient-rich digestate represents significant value as organic fertilizer. Systems should be designed with digestate storage and application in mind, completing the nutrient recycling loop and providing additional economic benefit to farming households.

## 5.6 Economic and Environmental Implications

From a life-cycle perspective, optimized co-digestion systems offer multiple sustainability benefits:

**Energy Balance:** Mesophilic systems in tropical climates can achieve positive net energy ratios of 3.5-5.0 (energy output/operational energy input), with most energy requirements for mixing and potential heating offset by biogas production. This compares favorably with thermophilic systems (ratios of 2.0-3.0 in similar contexts) due to reduced heating demands.

**Greenhouse Gas Mitigation:** Properly managed digesters capture methane that would otherwise be released during uncontrolled decomposition of agricultural wastes. Assuming 60% methane capture efficiency and accounting for system emissions, each kilogram of volatile solids digested can mitigate approximately 0.8-1.2 kg CO<sub>2</sub>-equivalent emissions compared to conventional waste management.

**Nutrient Cycling Value:** The digestate replaces synthetic fertilizers, reducing fossil energy use in fertilizer production and minimizing nutrient runoff compared to raw manure application. Economic valuation of digestate nutrient content typically represents 30-50% of the total system value when both energy and fertilizer benefits are considered.

**Scalability and Replicability:** The simple design parameters (mesophilic temperatures, near-neutral pH, balanced co-digestion) are readily transferable across diverse agricultural contexts in developing regions. Local adaptation primarily involves identifying appropriate substrate combinations from available materials rather than complex technological modifications.

## 5.7 Limitations and Future Research Directions

While this study provides valuable insights into temperature and pH effects on agricultural waste co-digestion, several limitations should be acknowledged and addressed in future research:

**Scale Considerations:** Laboratory-scale batch experiments may not fully replicate conditions in larger, continuous systems. Pilot-scale validation (100-1000 L digesters) would provide more accurate performance data for practical implementation.

**Long-Term Stability:** The 21-day experimental period captures initial digestion dynamics but not long-term stability issues that might emerge over months or years of operation. Semi-continuous trials over extended periods (6-12 months) would reveal accumulation effects, microbial community evolution, and maintenance requirements.

**Substrate Variability:** Agricultural wastes exhibit substantial seasonal and source variability in composition. Future research should examine performance across different substrate batches and qualities to establish robust operational guidelines adaptable to real-world variability.

**Integration with Farming Systems:** The full sustainability assessment requires examining how digesters integrate within complete farming systems—including feedstock availability throughout seasons, labor requirements, digestate application practices, and overall economic viability at household or community scales.

**Low-Cost Monitoring and Control:** Research into simple, affordable indicators of digester health (e.g., visual cues, odor changes, simple chemical tests) would greatly enhance practical management capabilities for small-scale operators with limited technical resources.

**Climate Resilience:** As climate change alters temperature and precipitation patterns in tropical regions, research should examine digester performance under more variable conditions and develop design adaptations for climate resilience.

## 6. Conclusion

### 6.1 Summary of Key Findings

This comprehensive investigation into temperature and pH effects on anaerobic co-digestion of agricultural wastes yields several significant, evidence-based conclusions:

1. **Mesophilic temperatures (35-37°C) optimize the balance between biogas yield and process stability** for mixed agricultural substrates, outperforming both ambient temperature and thermophilic digestion in cumulative methane production when considering net energy balance and operational simplicity.
2. **Near-neutral pH conditions (7.0-7.5) are critical for maintaining methanogenic activity**, with the co-digestion mixture providing natural buffering capacity that reduces the need for external pH control in well-balanced systems.

3. **Temperature and pH exhibit significant interactive effects** on digestion performance, with optimal conditions occurring at mesophilic temperatures combined with neutral pH, and system sensitivity increasing under thermophilic conditions.
4. **Strategic co-digestion of animal manures with crop residues creates multiple synergies**—balanced C/N ratios, enhanced buffering capacity, complementary biodegradability patterns, and improved micronutrient availability—that collectively enhance process performance beyond what individual substrates can achieve.
5. **Microbial community succession follows predictable patterns** aligned with biochemical process stages, with methanogen populations lagging behind bacterial groups but ultimately determining system productivity and stability.
6. **Simple, low-cost optimization strategies**—including passive solar heating, basic insulation, intentional substrate mixing, and digestate reuse as inoculum—can substantially improve biogas system performance in resource-constrained settings.

### 6.2 Contributions to Sustainable Development

The research findings make substantive contributions to multiple Sustainable Development Goals (SDGs):

**SDG 7 (Affordable and Clean Energy):** Demonstrates a practical pathway for decentralized renewable energy production using locally available agricultural wastes, particularly relevant for rural communities lacking grid connectivity.

**SDG 12 (Responsible Consumption and Production):** Provides a circular economy model that converts waste products into valuable energy and fertilizer resources,

reducing environmental pollution from agricultural waste disposal.

**SDG 13 (Climate Action):** Quantifies greenhouse gas mitigation potential through methane capture and fossil fuel displacement, contributing to climate change mitigation efforts.

**SDG 15 (Life on Land):** Promotes sustainable land management through improved nutrient recycling and reduced chemical fertilizer dependence.

The integrated approach addressing energy production, waste management, and nutrient cycling exemplifies the interconnected solutions needed for comprehensive sustainable development in agricultural regions.

### 6.3 Practical Recommendations for Stakeholders

Based on the research evidence, specific recommendations emerge for different stakeholder groups:

#### For Farmers and Rural Households:

- Implement intentional mixing of animal manures (60-70%) with crop residues (30-40%) for improved biogas production
- Utilize simple insulation or passive solar techniques to maintain digester temperatures in the 35-37°C range where possible
- Monitor pH weekly using inexpensive test strips and apply simple amendments (wood ash, crushed eggshells) if pH drops below 6.5
- Preserve 10-20% of digestate as inoculum for subsequent batches to maintain adapted microbial communities

#### For Development Organizations and Extension Services:

- Promote co-digestion as standard practice rather than single-substrate digestion
- Develop context-specific substrate mixing guidelines based on locally available materials
- Design and disseminate simple, low-cost digester designs with integrated temperature management features
- Establish community-based monitoring programs to track system performance and identify common challenges

#### For Policymakers and Government Agencies:

- Integrate biogas technology into national renewable energy and agricultural development strategies
- Develop supportive regulatory frameworks for digestate use as organic fertilizer
- Implement targeted subsidy programs for biogas system dissemination in agricultural regions
- Support research and development focused on appropriate technology adaptation for local contexts

#### For Researchers and Academic Institutions:

- Conduct long-term, pilot-scale validation of optimized co-digestion parameters
- Investigate microbial community engineering for enhanced substrate utilization
- Develop decision-support tools for substrate mixture optimization based on local availability
- Explore integrated systems combining biogas production with other renewable energy technologies

### 6.4 Concluding Remarks

Anaerobic digestion of agricultural wastes represents a compelling intersection of energy security, environmental protection, and sustainable agriculture. This research reinforces that technological optimization need not imply increased complexity or cost; rather, through understanding and leveraging natural biological processes and ecological synergies, simple, robust systems can be developed that are both technically effective and practically accessible.

The temperature and pH optima identified—mesophilic conditions with near-neutral pH maintained through balanced co-digestion—provide a scientifically grounded foundation for designing and operating biogas systems in developing regions. When implemented within appropriate socio-technical contexts that consider local materials, knowledge, and needs, such systems can contribute meaningfully to multiple sustainability objectives while addressing immediate energy and waste management challenges.

As global attention increasingly turns toward circular bioeconomy models and decentralized renewable energy solutions, low-cost, optimized anaerobic digestion systems offer a proven pathway for converting agricultural "wastes" into valuable resources. The continued refinement and dissemination of such appropriate technologies, grounded in rigorous research and responsive to local conditions, represents a promising avenue for sustainable development at the intersection of energy, agriculture, and environmental stewardship.

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