
ENHANCED ANAEROBIC DIGESTION OF COW DUNG AND POULTRY DUNG VIA A COUPLED MICROBIAL ELECTROLYTIC CELL: OPTIMIZATION, KINETICS, AND THERMODYNAMIC STUDIES.

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ABSTRACT

Coupling a microbial electrolysis cell (MEC) with anaerobic digestion (AD) is an effective approach to improving methane yield from agricultural waste. The current research focuses on the optimization, kinetics, and thermodynamic analysis of the MEC–AD hybrid system using cow dung and poultry dung as substrates. The physicochemical characterization results show that cow dung has higher volatile solids (78.20%) and a C:N ratio of 26.0, while poultry dung contains 62.35% volatile solids and a C:N ratio of 6.26. Based on mass balance calculations, the optimum substrate mixture containing 98.3% cow dung and 1.7% poultry dung is obtained to reach the optimum C:N ratio of 25:1. The optimization process was carried out by applying Response Surface Methodology using Central Composite Design involving applied voltage (0.3–1.5 V), pH (6–8), and C:N ratio (15–35). The proposed quadratic equation showed a high prediction accuracy ($R^2 = 0.9997$) and obtained the optimum values of 0.97 V, pH 7.0, and C:N ratio of 25 with the optimum methane yield of 350.73 mL $\text{CH}_4 \text{ g}^{-1}$ VS. Based on kinetic analysis using the Modified Gompertz model, the ultimate methane potential, maximum methane production rate, and lag phase were found to be 378.33 mL $\text{CH}_4 \text{ g}^{-1}$ VS, 41.25 mL $\text{CH}_4 \text{ g}^{-1}$ VS day^{-1} , and 1.36 days, respectively. Thermodynamic analysis showed that the process achieved 91.02% energy efficiency, 73.8% exergy efficiency, and an EROI of 29.55, confirming its favorable energetic and thermodynamic performance. Overall, the hybrid MEC–AD process significantly improved the methane yield.

Keywords: Anaerobic digestion; Microbial electrolysis cell; Cow dung; Poultry waste; Methane production

1. INTRODUCTION

The growing demand for energy and the environmental effects of fossil fuels have attracted the attention of researchers toward renewable energy generation systems. Anaerobic digestion (AD) is one of the promising approaches to produce methane, the main component of biogas, from organic wastes and address waste management issues (Angelidaki et al., 2009; Mao et al., 2015a). However, the conventional AD process can be affected by low methane production, substrate inhibition, and slow degradation (Appels et al., 2008).

Coupling AD with a microbial electrolysis cell (MEC) has been proposed to overcome the above problems. Low current input can increase the activity of electroactive microorganisms, facilitate direct interspecies electron transfer (DIET), and increase methane formation from organic waste (Ao et al., 2024; Wang et al., 2024). The applied voltage can accelerate the metabolism of microorganisms, increase electron transfer between them, and reduce the lag time before methane generation (Kaneko et al., 2025; Worajo et al., 2025).

Cow dung and poultry dung can be considered as suitable substrates for MEC-AD technology, since these are abundant agricultural wastes which contain biodegradable organic compounds (Kafle & Chen, 2016; Telo & Yigezu, 2025). Nevertheless, each of these substrates has certain drawbacks, which can affect the digestion performance. High nitrogen concentration in poultry dung can cause the accumulation of ammonia and inhibit methanogenic bacteria (Zhao et al., 2021). Moreover, the presence of lignocellulose in cow dung slows down its decomposition and limits the digestion rate (Zhang et al., 2025). The co-digestion process can increase the balance of nutrients and the stability of the microenvironment for methane-producing microorganisms (Mao et al., 2015b).

In this study, the possibility of improvement in methane production in the integrated MEC-AD system was investigated. First of all, the substrates were characterized from physicochemical and elemental perspectives. Additionally, Response Surface Methodology (RSM) was used for optimization of the operational parameters of the system. Kinetics of methane production was modeled using the Modified Gompertz model and validated with independent experiments (Sheron et al., 2026). Finally, energy, thermodynamic, and exergy analysis of the MEC-AD system was conducted.

2. METHODOLOGY

2.1 Substrate Collection and Preparation

Fresh cow dung and poultry dung were obtained from the Teaching and Research Farm at Ahmadu Bello University in Zaria, Nigeria. The poultry dung included both droppings and bedding materials used in poultry houses. The samples were placed in sealed containers, taken to the lab, and kept at 4°C to prevent changes before testing. Before starting the experiments, each type of material was mixed well. The mixture was passed through a 2 mm sieve to remove large fragments. Distilled water was added to dilute the solids to between 8% and 10%. This range is known to be good for digestion (Chojnacka & Moustakas, 2024).

2.2 Physicochemical and Elemental Analysis

The physicochemical tests followed the methods from APHA (2017). Total solids were found by drying the samples in an oven at 105°C. Volatile solids were measured by burning the dried samples at 550°C. Chemical Oxygen Demand was tested using the reflux titrimetric method. Total Organic Carbon was found with the Walkley- wet oxidation method (Nelson & Sommers, 1996). Total Nitrogen (Kjeldahl) was measured using Kjeldahl digestion and distillation (Evers & Hughes, 2002). The carbon-to-nitrogen ratio was calculated from the values of total organic Carbon and total Nitrogen as:

$$C:N = \frac{\%TOC}{\%TKN} \quad 1$$

To get a carbon-to-nitrogen ratio of 25 to 1, the amounts of cow dung and poultry waste were estimated using this mass-balance equation:

$$\frac{M_{CD}}{M_{PW}} = \frac{N_{PW} \times 25 - C_{PW}}{C_{CD} - N_{CD} \times 25} \quad 2$$

2.3 MEC-AD Reactor Design and Setup

Experiments were carried out on a lab-scale apparatus that coupled microbial electrolysis with anaerobic digestion. The reactor consisted of a double-chamber configuration with a working volume of 1.5L. A graphite rod (10 × 100 mm) was used as the anode, while a 50 × 50 mm 304 stainless-steel mesh was used as the cathode. Both parts were connected to a power supply that could provide 0-2 V to maintain a steady voltage. The anode was inserted into the substrates to help transfer electrons through the microbes. The cathode was placed in the part of the reactor where hydrogen formed and then was used by other microbes to make methane (Shao et al., 2025). All experiments were done in batch mode at a temperature of 30 to 37 degrees Celsius.

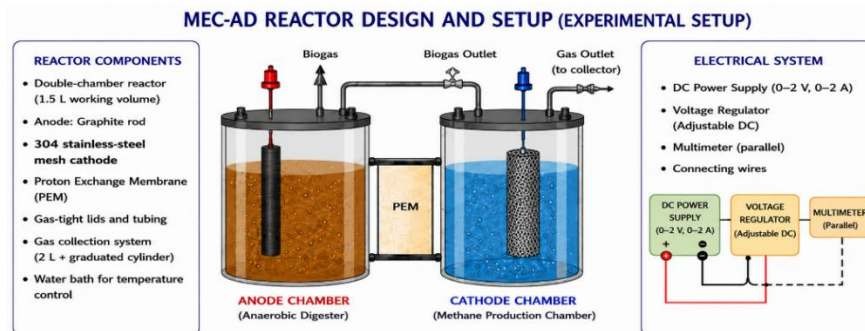


Figure 1: Schematic Diagram of the Coupled MEC-AD Reactor and Experimental Setup

2.4 Experimental Design and Optimization

The process was optimized using Response Surface Methodology based on a Central Composite Design. Three factors were considered: voltage (0.3 to 1.5), pH (6 to 8), and C:N (15 to 35). There were 20 runs in total. These included eight factorial points, six axial points, and six center points. The amount of methane produced was the response.

The data were fitted into a second-order model that had the equation:

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} X_i X_j + \epsilon \quad 3$$

Analysis of variance was done at a 95% confidence level to check how good the model was and which factors were important. This was done using Design-Expert® Version 13 (Stat-Ease Inc.).

2.5 Kinetic Modeling

The methane production was described using the Modified Gompertz model:

$$B(t) = B_0 \times \exp \left\{ - \exp \left[\frac{R_{\max} \times e}{B_0} (\lambda - t) + 1 \right] \right\} \quad 4$$

where $B(t)$ is the total methane produced at time t , B_0 is the maximum methane possible, $R_{\max}$ is the maximum rate of methane, λ is the lag phase, and e is Euler's constant.

The model parameters were estimated using nonlinear regression in Python Version 3.9. with optimization carried out using the Levenberg-Marquardt algorithm

2.6 Development of Enhancement Factors and Global Model Integration

Voltage, pH, and carbon-to-nitrogen ratio all influence methane production. To account for changes in reactor performance across operating conditions, explicit enhancement factors were fitted and integrated into the Modified Gompertz parameters (B_0 , $R_{\max}$, and λ).

2.6.1 Voltage Enhancement Factors

Raw experimental data for ultimate methane potential (B_0) and production rate ($R_{\max}$) at different applied voltages (0.3 – 1.5 V) obtained from the literature were fitted using polynomial regression in Python (Version 3.9)(Zou et al., 2021). The resulting unnormalized polynomial models showed strong coefficients of determination ($R^2 = 0.9897$ for B_0 and $R^2 = 0.9884$ for $R_{\max}$). To derive dimensionless enhancement multipliers ($f(V)$), the raw curves were normalized to their baseline values at the optimal reference voltage ($V_{\text{ref}} = 1.0$ V, where $B_{0,\text{raw}}(1.0) = 328.9$ mLCH₄ g⁻¹VS and $R_{\max,\text{raw}}(1.0) = 24.312$ mLCH₄ g⁻¹VS day⁻¹)

$$f_{B_0}(V) = -1.207V^4 + 4.504V^3 - 6.815V^2 + 4.520V \quad 5$$

$$f_{R_{\max}}(V) = 3.933V^5 - 17.315V^4 + 27.680V^3 - 19.827V^2 + 6.525V \quad 6$$

For the lag phase (λ), applied voltage reduces adaptation time at lower voltages ($V \leq 1.0$ V) but induces electrochemical stress at higher voltages ($V > 1.0$ V). A hybrid piecewise function was

$$\text{developed: } \lambda(V) = \begin{cases} \frac{1}{k(1+\alpha V+\beta V^2)}, & V \leq V_{\text{opt}} \\ \frac{1+\left(\frac{V-V_{\text{opt}}}{K_I}\right)^n}{k(1+\alpha V+\beta V^2)}, & V > V_{\text{opt}} \end{cases} \quad 7$$

A two-step regression approach was applied: linear transformation ($y = \frac{1}{\lambda}$) determined quadratic growth constants (k, α, β), while non-linear regression fitted the inhibition threshold (K_I) for $V > 1.0$ V, before normalizing relative to $\lambda(1.0$ V).

2.6.2 pH and C:N Ratio Enhancement Factors

The effects of pH and C:N ratio were modeled using Gaussian functions centered around their optimal values ($\mu = 7.0$ for pH; $\mu = 25$ for C:N) to reflect symmetric performance decay away from ideal conditions:

$$f_{B0}(\text{pH}) = \exp\left(-0.5 \times \left[\frac{\text{pH}-7.0}{0.8}\right]^2\right) \quad 8$$

$$f_{B0}(\text{C:N}) = \exp\left(-0.5 \times \left[\frac{\text{C:N}-25}{7.0}\right]^2\right) \quad 9$$

Standard deviations (σ) for $R_{\max}$ were set to 0.7 (pH) and 9.0 (C:N), while σ values for λ were 1.0 (pH) and 11.0 (C: N).

2.6.3 Global Kinetic Integration

Following autonomous multiplicative bioprocess modeling frameworks (such as IWA ADM1), the individual enhancement factors modify baseline kinetic parameters ($B_{0,\text{base}} = 498 \text{ mL g}^{-1} \text{ VS}$, $R_{\max,\text{base}} = 23 \text{ mL g}^{-1} \text{ VS day}^{-1}$, $\lambda_{\text{base}} = 3.2 \text{ days}$):

$$B_{\text{total}} = B_{0,\text{base}} \times f_{B0}(V) \times f_{B0}(\text{pH}) \times f_{B0}(\text{C:N}) \quad 10$$

$$R_{\text{total}} = R_{\max,\text{base}} \times f_{R\max}(V) \times f_{R\max}(\text{pH}) \times f_{R\max}(\text{C:N}) \quad 11$$

$$\lambda_{\text{total}} = \lambda_{\text{base}} \times f_{\lambda}(V) \times f_{\lambda}(\text{pH}) \times f_{\lambda}(\text{C:N}) \quad 12$$

Substituting these into Equation (4) yields the global predictive kinetic equation:

$$B(t, V, \text{pH}, \text{C:N}) = B_{\text{total}} \cdot \exp\left(-\exp\left[\frac{R_{\text{total}} \cdot e}{B_{\text{total}}}(\lambda_{\text{total}} - t) + 1\right]\right) \quad 13$$

2.7 Thermodynamic and Exergy Analysis

Methane energy was calculated using:

$$E_{\text{CH}_4} = V_{\text{CH}_4} \times \rho_{\text{CH}_4} \times \text{LHV}_{\text{CH}_4} \quad 14$$

where the lower heating value (LHV) of methane was taken as **50 MJ/kg**, while methane density was taken as **0.716 kg/m³** (Nindhia et al., 2021).

Overall energy efficiency was determined from:

$$\eta_{\text{energy}}(\%) = \left(\frac{E_{\text{CH}_4}}{E_{\text{in}}}\right) \times 100 \quad 15$$

Exergy analysis was also performed to determine how effectively the available energy was converted into useful work. Electrical energy was assigned an exergy factor of **1.0**. Methane was assigned an exergy factor of **0.94**, making it possible to evaluate energy losses occurring within the coupled MEC-AD system (Dinçer & Rosen, 2021).

3. RESULTS AND DISCUSSION

3.1 Substrate Characterization

Table 1: Substrate Characterization

Parameter	Cow Dung	Poultry Dung
Total Solids (TS, %)	9.85	8.40
Volatile Solids (VS, % TS)	78.20	62.35
COD (g L ⁻¹)	95.40	68.20
Ash Content (% TS)	21.80	37.65
Carbon (%)	38.5	31.3
Nitrogen (%)	1.48	5.00
C:N ratio	26.0	6.26

Cow dung demonstrated higher volatile solids (VS) content (78.20%) and chemical oxygen demand (COD) (95.40 g/L) compared to poultry dung (62.35% and 68.20 g/L), indicating the presence of a greater amount of biodegradable organic matter that could be used to produce methane. The VS content of cow dung fits the typical 70-85% range of ruminant manure, reflecting the partial

lignocellulosic breakdown before being excreted. The C:N ratio of cow dung is 26.0, falling within the recommended 20-30 range of anaerobic digestion. On the contrary, poultry dung possessed a C:N ratio of 6.26 because of its increased nitrogen content (5.00%), which could promote the formation of ammonia and inhibit methanogenesis. Mass balance shows that 98.3% of cow dung and 1.7% of poultry dung satisfied the requirement of the target C ratio of 25:1.

3.2 Optimization of MEC-AD Operating Parameters

In the 20 central composite design (CCD) experiments, methane yield ranged from 50.69 to 350.73 mL CH₄/g VS, showing a strong dependence on process conditions. The six center-point runs at 0.90 V, pH 7.0, and C:N ratio of 25 produced the maximum methane yield of 350.73 mL CH₄ g⁻¹ VS, with a standard deviation below 0.01, demonstrating excellent reproducibility. The quadratic model proved to be highly significant ($F = 3201.86$; $p < 0.0001$), with $R^2 = 0.9997$, Adjusted $R^2 = 0.9993$, Predicted $R^2 = 0.9982$, and Adequate Precision = 128.074, which demonstrates a good correlation and ratio of signal to noise. The reduced quadratic model in coded variables was expressed as:

$$\ln(Y) = 5.85 + 0.0669A - 0.4013A^2 - 0.9378B^2 - 0.5261C^2$$

Denoting Y as the methane yield (mL CH₄ g⁻¹ VS), A, B, and C stand for the coded values of applied voltage, pH, and C:N ratio, respectively. Since there is a positive linear coefficient related to voltage, the initial increase in methane yield with the increase in applied voltage can be expected. However, due to the presence of the negative quadratic coefficients, it can be deduced that there are optimal operation ranges after which the methane production decreases.

3.3 Interactive Effects of Process Variables

3.3.1 pH and Carbon-to-Nitrogen Ratio

The pH–C response surface revealed a dome-shaped graph, characterized by a sharp peak. Maximum methane production happened at pH 6.8-7.2; pH lower than 6.5 caused the accumulation of volatile fatty acids and inhibited methanogenesis, whereas pH higher than 7.5 shifted the ammonium-ammonia equilibrium towards the inhibitory free ammonia (NH₃).

Methane yield was higher than 300 mL CH₄/g VS in the range of C 25. The C ratios lower than 20 contributed to ammonia formation due to the excess of nitrogen, whereas those higher than 30 inhibited microbial growth because of the deficiency of nitrogen.

3.3.2 Applied Voltage and Carbon-to-Nitrogen Ratio

The voltage range optimal for methane production was about 0.6-1.2 V, where the electrical stimulation promoted electron transfer and organic matter degradation.

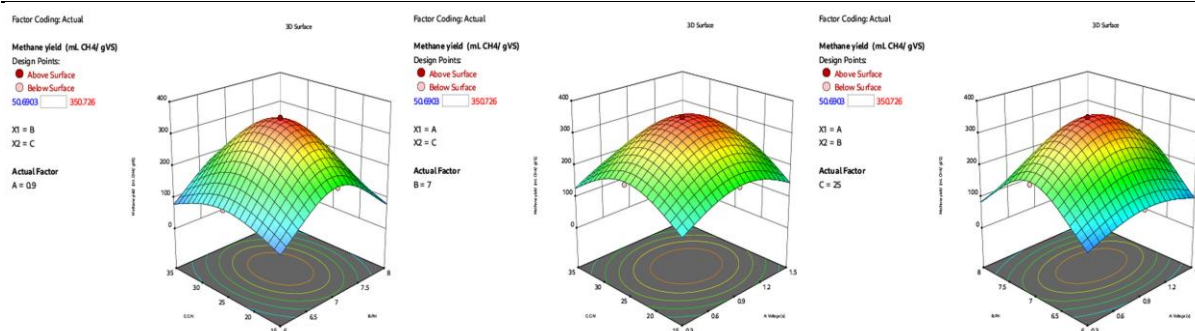
Below 0.6V, the thermodynamic driving force was not sufficient to stimulate the electroactive microorganisms, and above 1.2 V, the performance decreased probably due to electrochemical stress and water electrolysis.

3.3.3 Applied Voltage and pH

The voltage–pH response showed that electrical stimulation was most effective under favorable microbial conditions.

The combination of 0.9 V and pH 7.0 facilitated the methanogenic activity. Increasing the voltage without appropriate pH weakened the electrochemical effects; thus, it was necessary to optimize both voltage, pH, and C:N ratio.

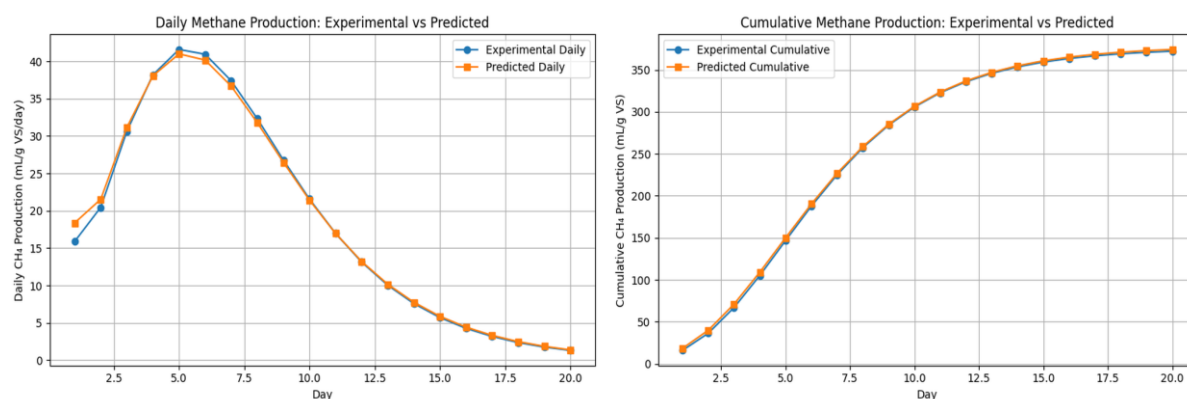
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3.4 Kinetic Modeling

The Modified Gompertz model was built under optimized conditions (0.97 V, pH 7.0, C 25) based on the cumulative, daily, and joint methane data. The joint fit revealed the true behavior of the reactor better than the cumulative and daily ones and provided the ultimate methane potential (P) of 378.33 mL CH₄/g VS, maximum production rate (R_{max}) of 41.25 mL CH₄/g VS/day, and lag phase (λ) of 1.36 days. The R^2 values of the cumulative and daily fits were 0.99965 and 0.99497, respectively. Methane production attained 10%, 50%, and 90% of the calculated potential after 1.92, 5.97, and 12.33 days, respectively.

Compared with conventional anaerobic digestion reported in the literature, the MEC–AD system increased methane yield by approximately 17–40%, increased methane production rate by 64–118%, and reduced the lag phase by approximately 55–82%. These comparisons were based on literature-reported conventional AD performance rather than a parallel conventional-AD control experiment conducted in the present study



3.5 Model Validation

Three independent experiments out of the data set used for the model development provided the average prediction error of 6.26%. The R^2 value of the model validation of 0.9987 proves that the model explained 99.87% of the variance in the methane yield, which means a good predictive ability within the investigated operating range.

3.6 Thermodynamic Performance

For optimal working conditions, the consumption of electrical and thermal energies per batch in the MEC-AD process was 6.70×10^{-4} MJ and 0.0627 MJ, respectively. However, for substrate chemical and methane energy yields, these values were 1.99374 MJ and 1.87234 MJ, respectively. As a result, the net energy obtained is 0.18477 MJ, and an efficiency of 91.02% has been observed. The energy return on investment (EROI) is 29.55, and it is evident that there is significantly more energy yield from methane than the electrical energy input into the system. The total exergy of input was 2.381 MJ, where the contribution from the substrate was 2.380 MJ, 0.00067 MJ was attributed to the electrical input, and 0.000267 MJ was attributed to the thermal input. The exergy of methane energy output was 1.758 MJ, whereas that of irreversibility destruction was 0.623 MJ. Therefore, the exergy efficiency

was 73.8%, while the ratio of exergy destruction was 26.2%. This is because the exergy balance fulfilled the equation $1.758 + 0.6$.

4. CONCLUSIONS

This study demonstrated the potential of coupling microbial electrolysis with anaerobic digestion for enhanced methane recovery from cow dung and poultry dung. Cow dung contained 78.20% VS, 38.5% carbon, 1.48% nitrogen, and a C:N ratio of 26.0, while poultry dung contained 62.35% VS, 31.3% carbon, 5.00% nitrogen, and a C:N ratio of 6.26. Carbon–nitrogen balancing produced an optimum mixture of 98.3% cow dung and 1.7% poultry dung at a target C:N ratio of 25.

RSM optimization identified 0.97 V, pH 7.0, and C:N 25 as the optimum operating conditions, with a maximum observed methane yield of 350.73 mL CH₄ g⁻¹ VS. The reduced quadratic model provided an excellent fit with $R^2 = 0.9997$, adjusted $R^2 = 0.9993$, and predicted $R^2 = 0.9982$. The Modified Gompertz model gave an ultimate methane potential of 378.33 mL CH₄ g⁻¹ VS, maximum methane production rate of 41.25 mL CH₄ g⁻¹ VS Day⁻¹, and lag phase of 1.36 days. Independent validation produced an average prediction error of 6.26% and $R^2 = 0.9987$.

The thermodynamic analysis indicated 91.02% energy efficiency and an EROI of 29.55. The corrected exergy analysis gave 2.381 MJ total input exergy, 1.758 MJ methane exergy, 0.623 MJ exergy destruction, 73.8% exergy efficiency, and 26.2% exergy destruction ratio. These results demonstrate that the system was energy-positive and achieved substantial conversion of substrate exergy into methane.

For Nigeria, the approach has practical potential because livestock production generates large quantities of manure that can be difficult to manage safely and productively. A decentralized MEC–AD system could provide a pathway for converting manure into renewable methane while reducing uncontrolled decomposition and improving nutrient management. Nevertheless, the present work was conducted at laboratory scale and should not be interpreted as proof of immediate commercial viability. Scale-up challenges include electrode and membrane cost, electrode durability and fouling, electrical-control requirements, mixing, feedstock variability, continuous-operation stability, heat management, and the economics of integrating the MEC subsystem with existing digesters.

Future work should therefore include pilot- and semi-continuous-scale testing, long-term electrode performance assessment, optimization of electrode configuration, process automation, techno-economic analysis, and life-cycle assessment under Nigerian agricultural conditions. Such studies are needed to determine whether the observed laboratory-scale performance can be maintained at practical scale and under variable real-world feedstock conditions.

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