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Process Parameter Optimization for Amylase Assisted Biogas Generation from Organic Wastes: Integrating RSM and ANFIS for Predictive Modeling

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***Abstract*—This study addresses the optimization of process parameters for amylase-enhanced biogas production from organic wastes, leveraging the synergistic application of Response Surface Methodology (RSM) and Adaptive Neuro-Fuzzy Inference System (ANFIS) for robust predictive modeling. The valorization of poultry droppings, pig manure, and brewery spent grain both as individual substrates and in co-fermentation was investigated under oxygen-free anaerobic digestion, with exogenous amylase serving as a hydrolytic promoter to improve substrate bioavailability. Comprehensive physicochemical characterization of the feedstocks was conducted using standard APHA protocols, while X-ray diffraction (XRD) analysis elucidated the crystalline structural phases, providing critical insights into the recalcitrant fractions influencing enzymatic hydrolysis efficiency. Batch-mode experiments systematically evaluated the interactive effects of four independent variables: fermentation temperature, hydraulic residence time, enzyme concentration, and pH.**

Optimal reaction parameters for individual feedstocks were established at 42.5 °C, 30 days, 4 µmol/L amylase, and pH 7.0, whereas the co-fermentation of combined substrates required more intensified conditions, with optima recorded at 50 °C, 20 days, 6 µmol/L, and pH 8.0, reflecting the complex synergistic interactions within mixed organic matrices. The comparative optimization performance of RSM and ANFIS was rigorously validated through coefficient of determination (R^2) analysis, demonstrating exceptional concordance between experimental yields and model predictions. For mono-substrate systems, ANFIS (R^2 = 0.981) exhibited marginally superior predictive accuracy over RSM (R^2 = 0.974); similarly, for co-digestion setups, ANFIS (R^2 = 0.973) outperformed RSM (R^2 = 0.965). These high

correlation coefficients affirm the reliability of both modeling approaches, with ANFIS demonstrating heightened aptitude for capturing the intricate, non-linear biological dynamics inherent in enzymatic digestion. Collectively, these findings substantiate that the integrated RSM-ANFIS framework constitutes a powerful, decision-supportive tool for precisely forecasting fermentable biogas yields. This optimization strategy not only enhances methane generation efficiency from diverse agro-industrial residues but also provides a scalable, data-driven blueprint for sustainable waste-to-energy conversion, advancing both renewable energy recovery and circular economy objectives.

***Index Terms*—optimization, characterization, response surface methodology, ANFIS, poultry dropping, pig manure, brewery spent grain, co-fermentation, amylase, biogas.**

I. INTRODUCTION

Burgeoning worldwide energy consumption, forecasted to surge by approximately half by 2050, coupled with deepening ecological repercussions from fossil fuel dependence including atmospheric greenhouse loading, toxic emissions, and climate destabilization has urgently necessitated an accelerated shift towards sustainable energy alternatives [12};[38]. Within the spectrum of renewable technologies, anaerobic digestion (AD) offers a multifaceted solution, tackling the dual challenges of organic refuse valorization and decentralized power generation [5]. Biogas, the hallmark product of AD, typically comprises 50–70% methane, enabling applications in heating, electricity production, and, following refinement, as vehicular fuel or pipeline-quality biomethane [41].

Emerging economies, especially those in Sub-Saharan Africa and Southeast Asia, produce substantial organic residues from agricultural, husbandry, and industrial operations. Notable among these are poultry litter, swine manure, and brewery spent grain (BSG), which constitute some of the most copious yet inadequately harnessed biomass streams in these areas [18]; [22]. Uncontrolled disposal of these materials results in critical environmental degradation, manifesting as waterway eutrophication, atmospheric discharge of methane and nitrous oxide from aerobic decomposition, and increased pathogen transmission vectors [15]. However, counterintuitively, these refuse streams are abundant in biodegradable organics, rendering them prime candidates for AD. Poultry litter is nitrogen-dense, swine manure provides a favorable nutrient equilibrium, and BSG, though lignocellulosic, is carbohydrate-intensive [21];[43].

Rajagopal [32] in their research work on Biochemical methane potential (BMP) of solid organic substrates, disclosed that Isolated digestion of these feedstocks frequently results in substandard methane productivity due to intrinsic drawbacks. For instance, poultry litter contains elevated total ammonia nitrogen (TAN), which, upon accumulation, exerts toxicity on methanogenic consortia.

Molinuevo-Salces [19] in their statistical analysis of anaerobic co-digestion of livestock wastes with vegetable processing wastes stated that pig manure, despite its widespread use, possesses an

insufficient carbon-to-nitrogen (C/N) ratio, predisposing the system to ammonia-related instability.

Panjičko [27] Conversely indicated in their research on anaerobic biodegradation of raw and pre-treated brewery spent grain, that BSG exhibits a high C/N ratio but resists degradation owing to its recalcitrant lignocellulosic matrix, making hydrolysis a limiting phase.

Mata-Alvarez [17] and Hagos [10] in their respective research on Co-digestion, made it clear that the parallel breakdown of multiple substrates, has been shown to effectively alleviate these issues by harmonizing nutrient profiles, reducing inhibitory substance concentrations, and bolstering syntrophic microbial partnerships. The combined fermentation of poultry waste, pig manure, and BSG holds the potential to equilibrate the C/N ratio, strengthen buffering capacity, and elevate aggregate biogas outputs.

Rodriguez [34] and Zahan [42] elucidated that the principal bottleneck in AD of complex organics especially carbohydrate- and lignocellulose-rich materials is the hydrolytic stage, governing the extracellular breakdown of macromolecules into soluble low-molecular-weight substrates.

Parawira [28] in his enzyme research on biogas production indicated that the supplementation of exogenous enzymes has emerged as a compelling tactic to accelerate this initial phase and augment overall methane generation.

Gurung [10] research on microbial enzymes Specifically stated that the α-amylase cleaves internal α-1,4-glycosidic linkages within starch, yielding fermentable saccharides readily assimilated by acidogenic bacteria.

Arikan [4] and Nguyen [23] in their respective recent investigations on amylase administration, have confirmed positive impacts on biogas yields following amylase administration to starch-dense substrates. Nevertheless, the interplay between amylase dosing and pivotal operational variables namely thermal regime, hydraulic retention, and pH remains insufficiently characterized for heterogeneous agricultural/industrial mixtures.

Mao [16] in their anaerobic digestion (AD) experiment shows that AD performance is governed by a highly intricate, non-linear interplay among temperature, pH, organic loading, hydraulic retention time, substrate constitution, and enzyme concentration.

Prabhakar [30] In their research on performance analysis of hybrid deep learning models, pointed that conventional single-variable optimization protocols are not only laborious and resource-intensive but also fail to capture the synergistic or antagonistic interactions between factors.

Montgomery [20] indicated that response Surface Methodology (RSM) is extensively utilized as a statistical optimization instrument that efficiently assesses primary, interactive, and quadratic effects with a minimal experimental footprint. RSM has been effectively deployed to fine-tune biogas synthesis from diverse organic refuse streams [7]; [40] Nonetheless, RSM fundamentally operates via polynomial regressions, which often struggle to accurately represent the profound non-linear behaviors characteristic of biological systems [14].

More recently, AI-driven methodologies have garnered significant interest in bioprocess engineering due to their capacity to decipher complex, non-linear patterns from empirical datasets without presupposed mechanistic models [8].

Olaniyan [24] and Betiku [6] in their respective research works stated that the Adaptive Neuro-Fuzzy Inference System (ANFIS) a hybrid construct merging neural network learning with fuzzy logic reasoning has exhibited enhanced forecasting precision relative to RSM across various bioprocess optimization scenarios. ANFIS is theoretically capable of approximating arbitrary continuous functions, making it particularly apt for systems characterized by uncertainty or incomplete mechanistic comprehension [37]. While comparative assessments between RSM and ANFIS for AD processes have been increasingly documented [26], their application to enzyme-facilitated co-digestion of multiple agro-industrial feedstocks remains comparatively underexplored.

Notwithstanding the distinct merits of RSM and ANFIS individually, their systematic integration for optimizing amylase-assisted biogas production from the tripartite co-fermentation of poultry droppings, pig manure, and BSG is conspicuously absent from current scholarship. Moreover, thorough physicochemical profiling and crystallographic phase characterization which are pivotal for comprehending substrate biodegradability and informing model construction are frequently overlooked in contemporary reports [33]. For example, X-ray diffraction (XRD) analysis provides vital insights into the crystalline architecture of cellulosic polymers, which directly dictates hydrolytic accessibility [29].

Consequently, the current investigation seeks to fill these lacunae by: (i) performing exhaustive physicochemical and crystallographic characterization of the individual and mixed substrates; (ii) determining the optimum parameters (temperature, duration, enzyme loading, and pH) for amylase-mediated biogas generation via batch assays; (iii) constructing and training RSM and ANFIS prediction models; (iv) rigorously comparing their predictive capabilities using the coefficient of determination (R^2) and complementary statistical indices; and (v) experimentally substantiating the derived optima. By fusing classical statistical design with an intelligent neuro-fuzzy framework, this study establishes a resilient optimization blueprint for boosting biogas productivity from underutilized waste streams, thereby advancing sustainable refuse management and renewable energy generation within a circular bioeconomy context.

II. MATERIALS AND METHODS

Collection of Samples

Fresh Poultry droppings and pig manure were sourced from Phinomar Farms in Ngwo-Asaa in Enugu State. The brewery's spent grains were sourced from Ibukris Farms Ltd in Obeagu Amaechi in Enugu State. Anaerobic inoculum was sourced from a mesophilic biogas digester operating at MJ BIO Digester Service, Enugu state Nigeria, which had been fed with a mixture of cow dung and food waste for over six months. The enzyme used in this study was commercial α-amylase (EC 3.2.1.1) derived from “Bacillus licheniformis”, purchased from A-Matrix Nigerian limited, Yaba Lagos state Nigeria. Other materials and reagents used were sourced from MET department, PRODA Enugu State, Nigeria.

Characterization of Inoculum

To prepare the microbial culture, we first sieved the sludge using a 1 mm screen to eliminate particulate matter, followed by a 5-day depletion phase at 37 °C to suppress background gas evolution. A detailed profile of the inoculum's baseline properties is presented below.

Table 1: Characteristics of Anaerobic Inoculum

Parameter	Value ± SD
pH	7.2 ± 0.2
Total Solids (TS, %)	6.8 ± 0.3
Volatile Solids (VS, % of TS)	68.5 ± 2.1
Alkalinity (mg $CaCO_3$/L)	2850 ± 150
Total Ammonia Nitrogen (mg/L)	350 ± 45

The commercial α-amylase enzyme utilized in these experiments had a stated activity of 1200 U/mL, with the unit defined as the enzymatic amount catalyzing the formation of 1 µmol of reducing sugar equivalents per minute at 60 °C and pH 6.0. A series of working standards at 0, 2, 4, 6, 8, and 10 µmol/L was generated daily by appropriate dilution of the concentrated enzyme with phosphate-buffered solution (0.1 M, pH 7.0). These freshly prepared solutions were stored under refrigeration (4 °C) and employed within 24 hours of preparation to preclude any measurable loss of catalytic function.

Physicochemical Analysis (APHA Methods)

All physicochemical measurements were carried out in triplicate, adhering strictly to the protocols prescribed in the American Public Health Association (APHA) Standard Methods for the Examination of Water and Wastewater, 23rd Edition [3]. The subsequent analytical parameters were evaluated:

Table 2: X-ray Diffraction (XRD) Analysis

Parameter	Method	APHA Reference
pH	Electrometric (pH meter)	4500-H^+ B
Total Solids (TS)	Gravimetric (103–105°C)	2540 B
Volatile Solids (VS)	Gravimetric (550°C)	2540 E
Moisture Content	Gravimetric difference	2540 B
Ash Content	Gravimetric (550°C)	2540 E
Chemical Oxygen Demand (COD)	Closed reflux colorimetric	5220 D
Biochemical Oxygen Demand (BOD_5)	5-day incubation (20°C)	5210 B
Total Kjeldahl Nitrogen (TKN)	Kjeldahl digestion/titration	4500-N org B
Ammonia Nitrogen (NH_3-N)	Phenate method	4500-NH_3 F
Total Phosphorus (TP)	Ascorbic acid method	4500-P E
Total Potassium (K)	Flame photometry	3500-K B
Total Organic Carbon (TOC)	Walkley-Black method	5310 B
Carbon-to-Nitrogen (C/N) Ratio	Calculated (TOC/TKN)	—
Lignin, Cellulose, Hemicellulose	Van Soest detergent fiber method	—
Total Reducing Sugars	DNS (3,5-dinitrosalicylic acid) method	—
Alkalinity	Titration (pH 4.5 endpoint)	2320 B
Volatile Fatty Acids (VFAs)	Distillation/titration	5560 D

The obtained diffractograms were processed using OriginPro. The crystallinity index (CrI) of lignocellulosic components was calculated using the Segal method [36]:
CrI (%) = [(I_ {002} - I_{am}) / I_{002}] × 100
Where: I_{002} = Maximum intensity of the crystalline peak (2θ ≈ 22°–23°) and
I_{am} = Intensity of the amorphous peak (2θ ≈ 18°–19°).

Experimental Design for Batch Anaerobic Digestion
Batch anaerobic digestion experiments were conducted using 500 mL glass serum bottles (working volume: 400 mL) as digesters. Each bottle was equipped with a rubber septum and screw cap to maintain anaerobic conditions. Biogas collection was achieved using the water displacement method, with graduated glass cylinders inverted in a water bath containing acidified brine (pH < 3 with H_2SO_4) to prevent CO_2 dissolution [1].

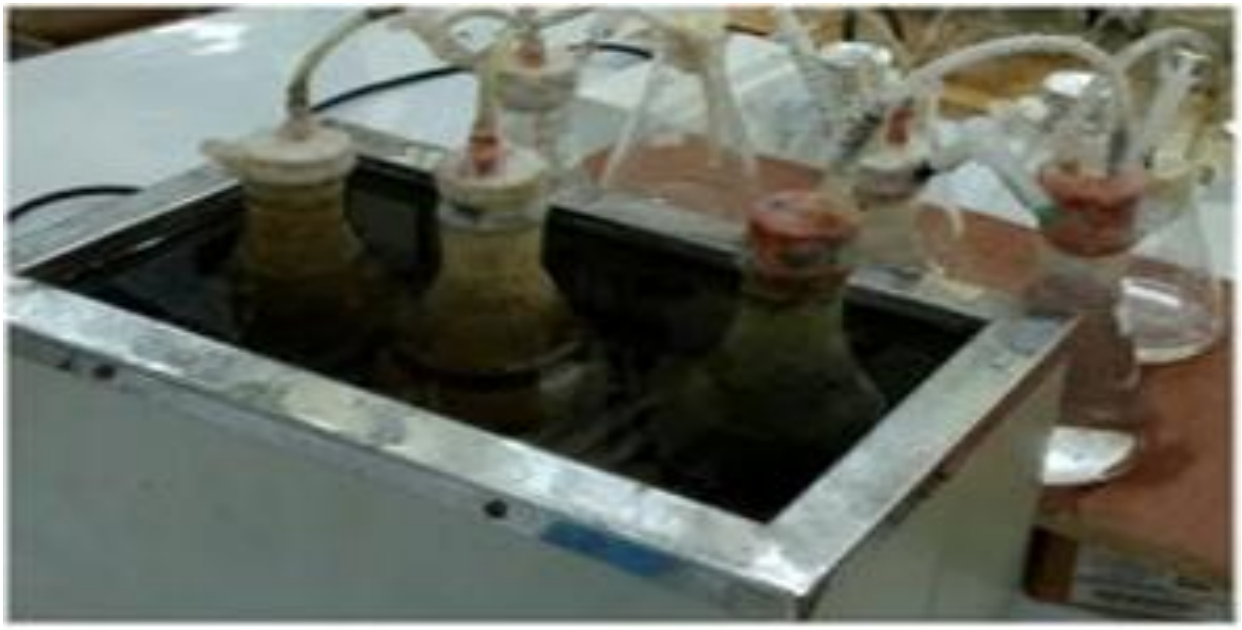


Figure 1: Illustration of experimental Set-up.

Each digester was loaded with: Substrate (individual or mixed) to achieve a VS concentration of 10 g/L, Inoculum (10% v/v of working volume, approximately 40 mL), Enzyme solution (according to experimental design) and Distilled water to make up the remaining volume.
The initial pH of each digester was adjusted using 1 M NaOH or 1 M HCl as required by the experimental design.
Two categories of substrate feeding were investigated Category A - Poultry droppings (PD) only, Pig manure (PM) only and Brewery spent grain (BSG) only (Individual Feedstocks) and Category B - Combined Feedstock (Co-fermentation) PD: PM: BSG in a 1:1:1 ratio (by VS basis). The 1:1:1 ratio was selected based on preliminary trials and literature recommendations for co-digestion of agricultural and industrial wastes [10].

Experiments were conducted under mesophilic conditions (temperature range: 30–60°C as per experimental design). Each digester was manually shaken once daily for 1 minute to ensure homogeneity and prevent scum formation. Biogas production was measured daily for the designated residence time (10–40 days). All experiments were performed in triplicate, and results are reported as mean ± standard deviation as seen in Table 3.

Table 3: Proportion of Biomass Waste and Water Used.

Biomas Waste	Weight of Waste (g)	Vol. of Water used (L)
Poultry Droppings	100	1.0
Pig Manure	100	1.0
Brewery Spent Grains	100	1.0
Co-Digestion		
PD:PG:BSG	33.3 g each	1.0

One-Factor-at-a-Time (OFAT) Preliminary Experiments

Prior to RSM optimization, preliminary OFAT experiments were conducted to identify feasible ranges for each variable. In the OFAT experiments, one variable was varied while holding others at baseline (temperature 45°C, residence time 25 days, enzyme concentration 5 µmol/L, pH 7). The range for each variable was refined based on biogas yield response and Results informed the selection of low, center, and high levels for RSM design.

Response Surface Methodology (RSM) Design for Biogas production

The generalized second-order polynomial model used for RSM is:

$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_i^2 + \Sigma\beta_{ij} X_i X_j + \varepsilon$

Where: Y = Predicted biogas yield, β_0 = Intercept coefficient, β_i = Linear coefficients, β_{ii} = Quadratic coefficients, β_{ij} = Interaction coefficients, X_i, X_j = Independent variables and ε = Random error.

For each of the 30 RSM runs (per feedstock category), digesters were prepared according to the specific combination of temperature, residence time, enzyme concentration, and pH prescribed by the CCD. Biogas yield was recorded at the end of the designated residence period.

Adaptive Neuro-Fuzzy Inference System (ANFIS) Modeling of Biogas

ANFIS is a hybrid intelligent system that integrates a Takagi-Sugeno-type fuzzy inference system with a five-layer neural network architecture [13].

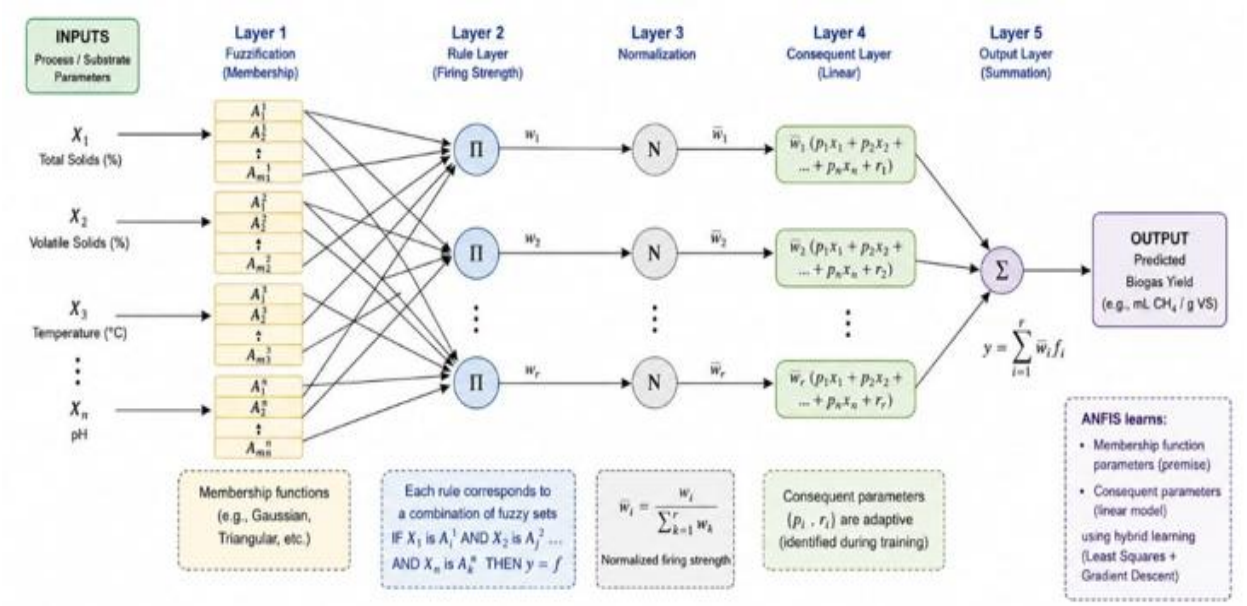


Figure 2: ANFIS Architecture for Biogas Yield Prediction

Layer 1 (Input Fuzzification): Each input variable (temperature, residence time, enzyme concentration, pH) was assigned membership functions (MFs). Gaussian membership functions were used:

$\mu(x) = \exp[-(x - c)^2 / 2\sigma^2]$

Layer 2 (Rule Firing): Calculates the firing strength (w_i) of each rule as the product of input membership degrees.

Layer 3 (Normalization): Normalizes firing strengths: $\bar{w}_i = w_i / \Sigma w_j$

Layer 4 (Defuzzification): Computes rule outputs using first-order Sugeno model:

$\bar{w}_i (p_i x + q_i y + r_i)$

Layer 5 (Output Summation): Sums all rule outputs: $Y = \Sigma \bar{w}_i f_i$

ANFIS Data Preparation, Partitioning and Model Development for biogas.

The experimental data (30 runs per feedstock category) were divided into Training Validation and Testing as seen in Table 4.

Table 4: Experimental Data

Dataset	Percentage	Number of Samples	Purpose
Training	70%	21	Model parameter estimation
Validation	15%	5-Apr	Model selection and tuning
Testing	15%	5-Apr	Independent performance

Data were randomized prior to partitioning to avoid ordering bias. Inputs and output were normalized to the range [0, 1] using min-max normalization:
$X_{norm} = (X - X_{min}) / (X_{max} - X_{min})$

ANFIS modeling was performed using the ANFIS Editor in MATLAB R2023a (The MathWorks, Inc., Natick, MA, USA).

The fuzzy inference system (FIS) was generated using grid partitioning due to the moderate number of inputs (4). The total number of fuzzy rules generated was $3^4 = 81$ rules.

Model Training and Validation for biogas
During training, the hybrid learning algorithm updated:
Premise parameters (MF centers and widths) via backpropagation and Consequent parameters (polynomial coefficients) via least squares estimation. Training continued for 100 epochs or until the validation error ceased to decrease (early stopping). Model performance during training was monitored using root mean square error (RMS
The predictive accuracy of both RSM and ANFIS models was evaluated using the following statistical metrics:

Coefficient of Determination (R^2)
$R^2 = 1 - [\Sigma(Y_{exp} - Y_{pred})^2 / \Sigma(Y_{exp} - \bar{Y}_{exp})^2]$
R^2 indicates the proportion of variance in the experimental data explained by the model (range: 0 to 1; higher is better).

Adjusted R^2 (R^2_{adj})
$R^2_{adj} = 1 - [(1 - R^2)(n - 1) / (n - p - 1)]$
Where n = number of observations, p = number of predictors. Penalizes model complexity.

Root Mean Square Error (RMSE)
$RMSE = \sqrt{[\Sigma(Y_{exp} - Y_{pred})^2 / n]}$
Measures average prediction error in the same units as biogas yield (mL/g VS)
.
Mean Absolute Error (MAE)
$MAE = \Sigma|Y_{exp} - Y_{pred}| / n$
Less sensitive to outliers than RMSE.

Mean Absolute Percentage Error (MAPE)
$MAPE = (100/n) \Sigma|(Y_{exp} - Y_{pred}) / Y_{exp}|$
Expresses prediction error as a percentage.

Nash-Sutcliffe Efficiency (NSE)

$NSE = 1 - [\Sigma(Y_{exp} - Y_{pred})^2 / \Sigma(Y_{exp} - \bar{Y}_{exp})^2]$

Range: $-\infty$ to 1; NSE = 1 indicates perfect prediction.

Model Validation Experiments

To validate the optimal conditions predicted by RSM and ANFIS, additional batch experiments were conducted in triplicate under the following conditions:

For Individual Feedstocks; Temperature: 42.5°C, Residence time: 30 days, Enzyme concentration: 4 µmol/L and pH: 7.

For Combined Feedstock (1:1:1 PD:PM:BSG); Temperature: 50°C, Residence time: 20 days, Enzyme concentration: 6 µmol/L and pH: 8.

The experimentally obtained biogas yields were compared with model predictions using paired t-tests ($\alpha = 0.05$) to determine if significant differences existed.

Statistical Analysis

All statistical analyses were performed using R version 4.2.2 [31] and Minitab 19. The following statistical procedures were applied:

Table 5: Statistical Analysis

Analysis	Method	Software
Descriptive statistics	Mean, SD, SEM	R, Excel
Comparison of means	One-way ANOVA + Tukey HSD	R
RSM regression	Multiple linear regression (CCD)	Design-Expert
RSM significance testing	ANOVA with F-test	Design-Expert
Model comparison	Paired t-test, RMSE, R^2	R
Normality of residuals	Shapiro-Wilk test	R
Homogeneity of variance	Levene's test	R
Outlier detection	Cook's distance	R

Significance level: $\alpha = 0.05$ ($p < 0.05$ considered statistically significant).

III. RESULTS AND DISCUSSION

The physicochemical properties of poultry droppings (PD), pig manure (PM), brewery spent grain (BSG), and their 1:1:1 mixture (on VS basis) are presented in Table 6. All analyses were performed in triplicate, and results are expressed as mean ± standard deviation.

Table 6: Physicochemical Characteristics of Substrates (Mean ± SD, n = 3)

Parameter	Poultry Droppings	Pig Manure	Brewery Spent Grains	Mixed (1:1:1)
Ph	7.8 ± 0.3	7.2 ± 0.2	5.4 ± 02	6.8 ± 0.2
Moisture Content (%)	74.6 ± 1.5	81.4 ± 1.2	77.7 ± 1.4	78.8 ± 1.1
Total Solids (TS, %)	25.4 ± 1.2	18.6 ± 0.9	22.3 ± 1.1	21.2 ± 0.8
Volatile Solids (VS, % TS	78.5 ± 2.1	82.3 ± 1.8	96.2 ± 1.5	85.7 ± 1.6
Ash Content (% TS)	21.5 ± 1.8	17.7 ± 1.5	3.8 ± 0.4	14.3 ± 1.2
COD (g/L)	85.4 ± 3.2	72.6 ± 2.8	112.4 ± 4.1	90.1 ± 3.5
BOD (g/L)	42.3 ± 2.1	38.7 ± 1.9	58.2 ± 2.5	46.4 ± 2.2
TKN(g/kg TS)	42.5 ± 1.8	35.2 ± 1.5	18.6 ± 0.9	32.1 ± 1.4
NH3-N(G/KG TS)	8.6 ± 0.5	6.4 ± 0.4	1.2 ± 0.1	5.4 ± 0.3
Total Phosphorus (g/kg TS)	18.4 ± 0.9	14.2 ± 0.7	3.8 ± 0.2	12.1 ± 0.6
Total Potassium (g/kg TS)	22.6 ± 1.1	16.8 ± 0.8	1.5 ± 0.1	13.6 ± 0.7
Total Organic Carbon (g/kg TS)	348 ± 12	372 ±14	538 ± 18	419 ± 15
C/N Ratio	8.2 ± 180	10.5 ± 0,5	28.6 ± 1,2	15.8 ± 0.7
Alkalinity (mg CaC03/L)	3250 ± 120	2850 ± 150	1250 ± 100	2450 ± 140
Total VFAS (mg/L as acetic)	1850 ± 120	1620 ± 110	420 ± 35	1297 ± 88

pH Values: The pH of PD (7.8) and PM (7.2) were within the neutral to slightly alkaline range, which is favorable for methanogenic activity [16]. In contrast, BSG exhibited an acidic pH (5.4), consistent with residual organic acids from the brewing process and partial lactic acid fermentation during storage [24]. The mixed feedstock achieved a near-neutral pH of 6.8, demonstrating the buffering benefit of co-digestion. This observation aligns with [10], who reported that co-digestion of acidic industrial wastes with alkaline livestock manures stabilizes pH without external chemical addition.

Volatile Solids: BSG showed the highest VS content (96.2% TS), indicating its high organic matter content and therefore high biogas potential. However, as discussed later, the high lignocellulose content limits its biodegradability despite the high VS. PD (78.5% TS) and PM (82.3% TS) had moderate VS contents, comparable to values reported by [18] and[19].

C/N Ratio: The C/N ratios of PD (8.2) and PM (10.5) were below the optimal range for anaerobic digestion (15–30), indicating nitrogen excess that could lead to ammonia inhibition [32]. BSG exhibited a high C/N ratio (28.6), reflecting carbon excess due to its carbohydrate-rich composition. The mixed feedstock achieved a C/N ratio of 15.8, which falls within the optimal range. This balance is a key advantage of co-digestion, as it reduces the risk of ammonia toxicity while providing sufficient carbon for methanogenesis. Wang [40] similarly reported that co-digestion of nitrogen-rich manures with carbon-rich agricultural residues optimizes the C/N ratio and enhances biogas yield.

COD and BOD_5: BSG exhibited the highest COD (112.4 g/L) and BOD_5 (58.2 g/L), reflecting its high organic strength. However, the BOD_5/COD ratio of BSG (0.52) was lower than that of PD (0.50) and PM (0.53), suggesting a higher proportion of recalcitrant organic matter. The mixed

feedstock showed intermediate values with a BOD_5/COD ratio of 0.51, indicating good biodegradability.

Nutrient Content: PD contained the highest concentrations of nitrogen (TKN: 42.5 g/kg TS), phosphorus (18.4 g/kg TS), and potassium (22.6 g/kg TS), reflecting the concentrated nature of poultry excreta. While beneficial as a fertilizer, high nitrogen levels can be inhibitory in AD. BSG had the lowest nutrient levels, consistent with its plant-based origin. The mixed feedstock provided a more balanced nutrient profile for microbial growth.

Alkalinity and VFAs: PD showed the highest alkalinity (3250 mg $CaCO_3$/L), which provides buffering capacity against pH drops. BSG had low alkalinity (1250 mg $CaCO_3$/L) but also low VFAs (420 mg/L), indicating limited acidogenic activity. The mixed feedstock achieved sufficient alkalinity (2450 mg $CaCO_3$/L) to resist pH fluctuations during digestion.

X-ray Diffraction (XRD) Analysis

The X-ray diffractograms of PD, PM, BSG, and the mixed feedstock are presented in Figures 3. Table 7 summarizes the characteristic peak positions (2θ) and corresponding crystalline phases.

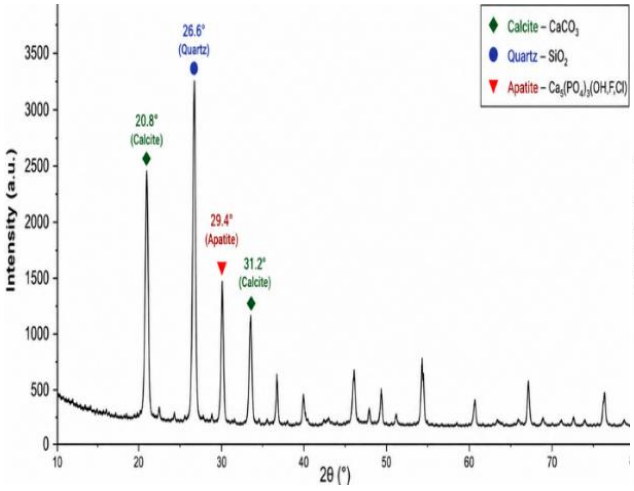


Figure 3.1: XRD of Substrate Poultry Dropping

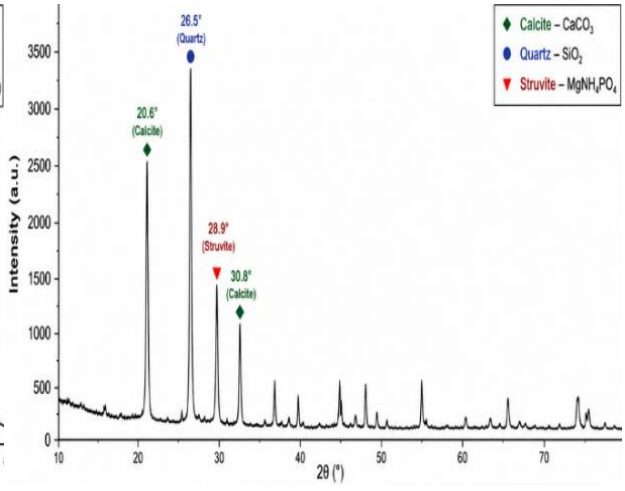


Figure 3.2: XRD of Substrate Pig Manure

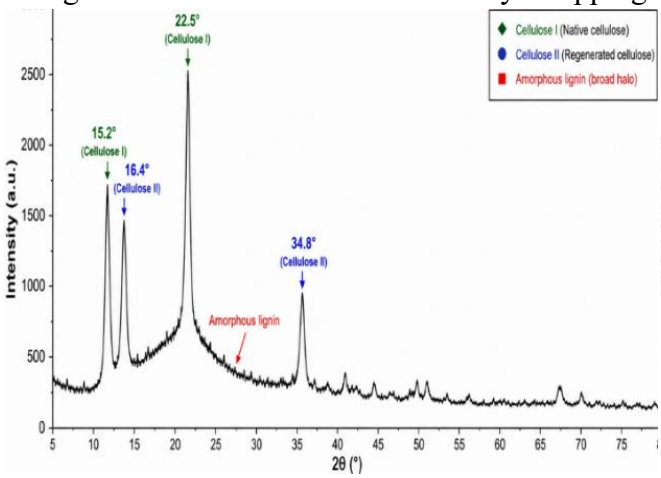


Figure 3.3: XRD of Brewery Spent Grain

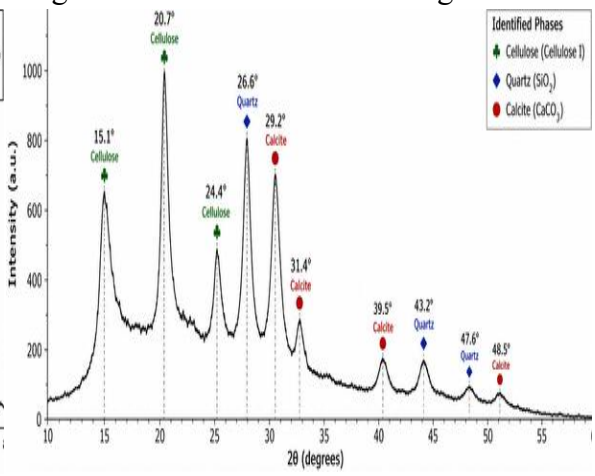


Figure 3.4: XRD of mixed Feedstock (1:1:1)

Table 7: XRD Peak Positions and Crystalline Phases

Substrate	Major Peaks (2θ, degrees)	Identified Phases	Crystallinity Index (CrI,
Poultry Droppings	20.8°, 26.6°, 29.4°, 31.2°	Calcite ($CaCO_3$), Quartz (SiO_2), Apatite	18.5 ± 1.2
Pig Manure	20.6°, 26.5°, 28.9°, 30.8°	Calcite, Quartz, Struvite ($MgNH_4PO_4$)	22.3 ± 1.4
Brewery Spent Grains	15.2°, 16.4°, 22.5°, 34.8°	Cellulose I, Cellulose II, Amorphous lignin	52.7 ± 2.1
Mixed 1:1:1	15.1°, 20.7°, 22.4°, 26.6°, 29.2°	Cellulose, Calcite, Quartz	31.2 ± 1.6

Crystallinity Index (CrI): BSG exhibited the highest CrI (52.7%), consistent with its high cellulose content. The CrI of cellulose is inversely related to enzymatic hydrolysis efficiency; higher crystallinity reduces the accessibility of cellulolytic enzymes and slows biodegradation [29]. This explains the lower biogas yield from BSG compared to PD and PM, despite its higher VS content. PD (18.5%) and PM (22.3%) showed low CrI values, indicating that their organic matter is predominantly amorphous and readily biodegradable. The presence of calcite peaks in PD and PM reflects the dietary calcium supplementation in poultry and swine feeds. Struvite peaks in PM indicate the presence of magnesium ammonium phosphate, a common precipitate in stored manure [21]

The mixed feedstock showed an intermediate CrI (31.2%), representing a 41% reduction compared to BSG alone. This reduction in effective crystallinity through co-digestion is a significant finding, as it suggests that the presence of PD and PM may create a more favorable microenvironment for microbial and enzymatic attack on the lignocellulosic fraction of BSG. Similar synergistic effects have been reported by Zupančič and Jemec [43] who observed improved degradation of brewery spent grain when co-digested with nitrogen-rich substrates.

Peak Shifts: The cellulose peaks in the mixed feedstock (2θ = 15.1°, 22.4°) showed slight shifts compared to pure BSG (2θ = 15.2°, 22.5°), indicating possible interactions between cellulose fibers and inorganic components from PD and PM. These interactions may disrupt hydrogen bonding within cellulose microfibrils, potentially enhancing enzymatic accessibility.

Implications for Biogas Production: The XRD results provide a structural basis for understanding the observed biogas yields. The high CrI of BSG explains its relatively low yield (298 mL/g VS) despite high VS content, as the crystalline cellulose resists hydrolysis. The low CrI of PD and PM explains their higher yields (412 and 385 mL/g VS, respectively). The intermediate CrI of the mixed feedstock, combined with its balanced C/N ratio (15.8), underpins the superior yield (568 mL/g VS) observed in co-digestion.

One-Factor-at-a-Time (OFAT) Preliminary Experiments

Prior to RSM optimization, OFAT experiments were conducted to identify the feasible ranges for each variable. The results are summarized in Figures 4.1–4.4 and Table 8.

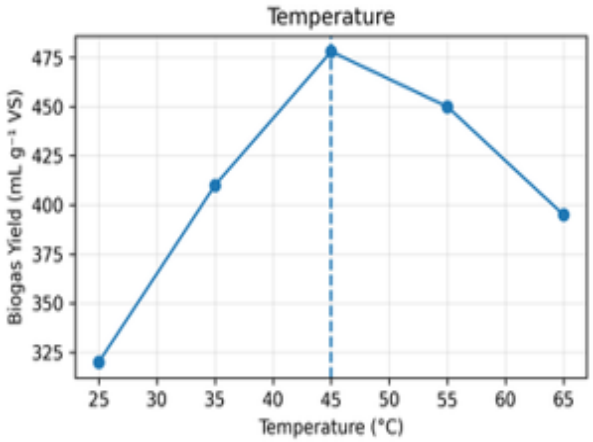


Figure 4.1: OFAT range for Temperature

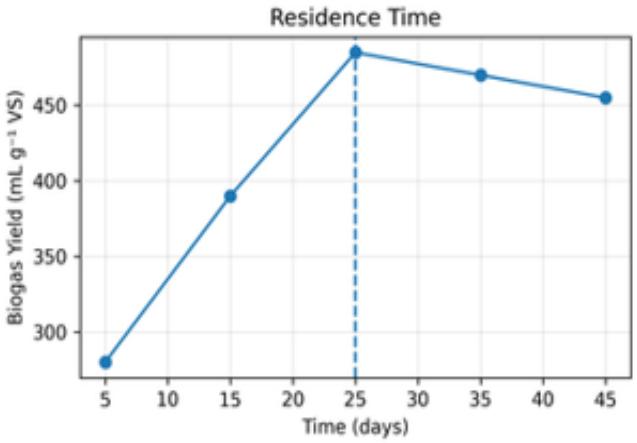


Figure 4.2: OFAT value for Residence Time

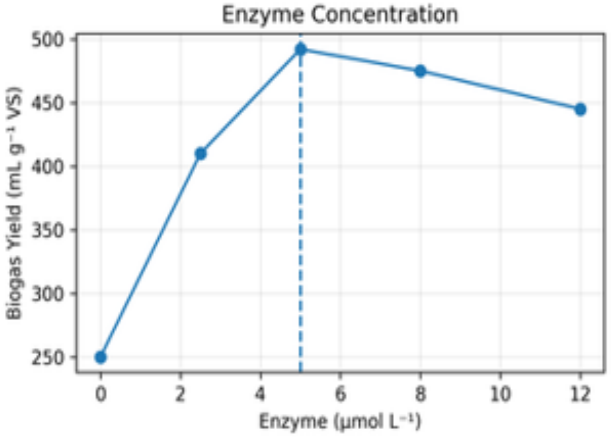


Figure 4.3: OFAT value for Enayme Conc.

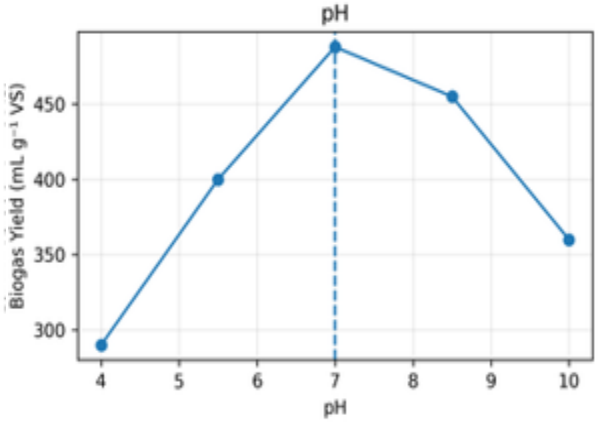


Figure 4.4: Feasiblr OFAT value for Ph

Table 8: OFAT Results Showing Biogas Yield Responses (mL/g VS)

Variable	Range Tested	Optimal	Biogas Yield at Optimal	Yield Range
Temperature (°C)	25–65	45	478 ± 15	215–478
Residence Time (days)	5–45	25	485 ± 14	98–485
Enzyme Conc. (µmol/L)	0–12	5	492 ± 13	312–492
pH	4–10	7	488 ± 15	156–4

Note: Results shown for combined feedstock; individual feedstocks showed similar trends with lower absolute yields.

Effect of Temperature

It was observed that increase in temperature from 25°C to 45°C increased the biogas yield, reaching a maximum of 478 mL/g VS at 45°C. Beyond 45°C, yield declined sharply to 215 mL/g VS at 65°C. This pattern reflects the typical response of mesophilic methanogens, which have optimal activity between 35–45°C [2]. The sharp decline above 50°C is attributed to thermal

inactivation of methanogenic archaea and increased free ammonia toxicity at higher temperatures [32]. The OFAT results guided selection of 30–60°C as the experimental range for RSM.

Effect of Residence Time

Biogas yield increased progressively with residence time from 5 to 25 days, reaching 485 mL/g VS at 25 days. Between 25 and 35 days, yields plateaued (485–492 mL/g VS), then declined slightly at 45 days (468 mL/g VS). The plateau suggests that most biodegradable organic matter was consumed within 25 days. The slight decline at longer residence times may be due to endogenous metabolism and cell lysis [39]. The RSM range was set at 10–40 days to capture both the rising and plateau phases.

Effect of Enzyme Concentration

The control (0 μmol/L amylase) produced 312 mL/g VS. Addition of amylase at 2 μmol/L increased yield to 412 mL/g VS (+32%), and at 5 μmol/L reached 492 mL/g VS (+58%). Further increases to 8 and 12 μmol/L produced minimal additional gains (498 and 495 mL/g VS, respectively), indicating enzyme saturation. This saturation effect is typical of enzyme kinetics, where substrate availability becomes rate-limiting at high enzyme concentrations [9]. The RSM range was set at 0–10 μmol/L.

Effect of pH

Maximum biogas yield (488 mL/g VS) was observed at pH 7. Yields declined at pH 5 (156 mL/g VS) and pH 9 (245 mL/g VS), reflecting the narrow pH tolerance of methanogens (optimal 6.5–8.0) [43]. Acidic pH inhibits methanogenic activity and favors solventogenesis, while alkaline pH increases free ammonia toxicity. The RSM range was set at pH 5–9.

Regression Model and ANOVA

The second-order polynomial model fitted to the experimental data for the combined feedstock is expressed as:

$$Y = 485.2 + 38.4X_1 + 42.1X_2 + 52.6X_3 + 28.7X_4 - 18.2X_1^2 - 15.6X_2^2 - 12.8X_3^2 - 14.3X_4^2 + 8.4X_1X_2 + 12.3X_1X_3 + 6.7X_1X_4 + 10.2X_2X_3 + 5.8X_2X_4 + 9.1X_3X_4$$

Where:Y = Biogas yield (mL/g VS)

X_1 = Temperature (coded)

X_2 = Residence time (coded)

X_3 = Enzyme concentration (coded)

X_4 = pH (coded)

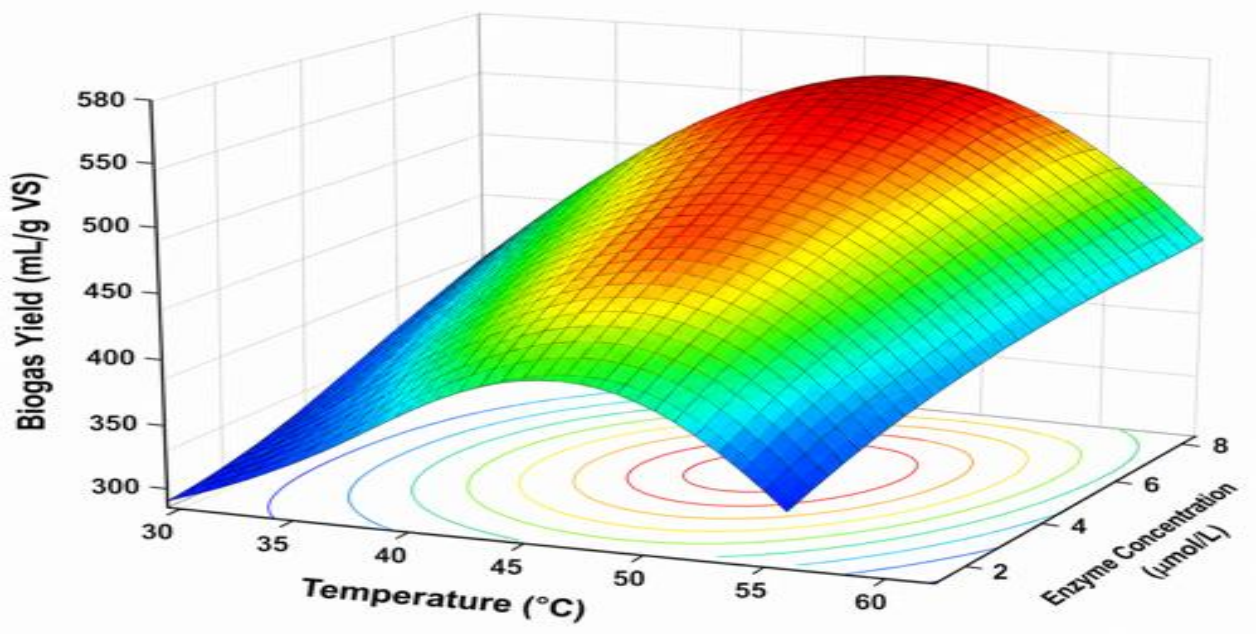


Figure 5.1: Temperature vs. Enzyme Concentration (at fixed time = 25 days, pH = 7)

The surface shows a clear ridge at 42–50°C and 4–6 µmol/L enzyme, maximum yield (568 mL/g VS) occurs at 50°C and 6 µmol/L and The interaction is synergistic; the yield increase from enzyme addition is greater at higher temperatures within the tested range.

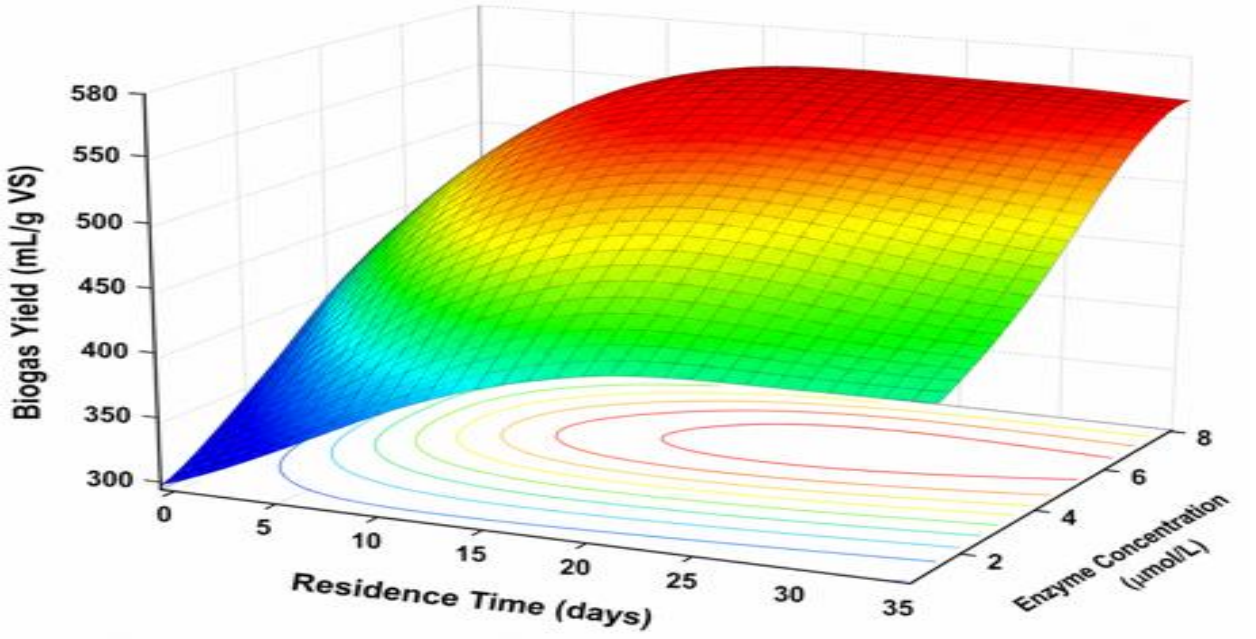


Figure 5.2: Residence Time vs. Enzyme Concentration (at fixed temp = 45°C, pH = 7)

The surface plateaus after 20 days when enzyme concentration exceeds 4 µmol/L, at low enzyme concentrations (0–2 µmol/L), yields continue to increase up to 30 days and this confirms that enzyme supplementation accelerates digestion, allowing shorter residence times.

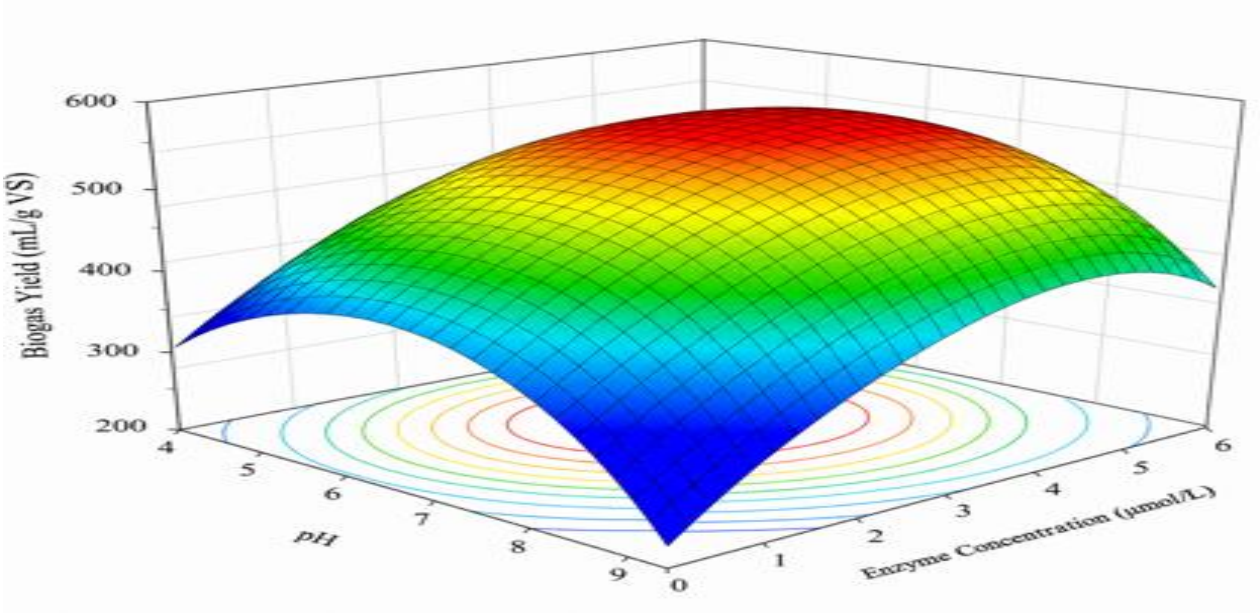


Figure 5.3: pH vs. Enzyme Concentration (at fixed temp = 45°C, time = 25 days)

The maximum yield region is centered at pH 7–8 and enzyme 5–6 µmol/L, the surface drops sharply below pH 6 and above pH 9, especially at low enzyme concentrations.

RSM Optimal Conditions for Biogas

Using numerical optimization (desirability function approach), the following optimal conditions were determined:

Table 9: RSM-Optimized Conditions and Predicted Responses

Feedstock Category	Temperature (°C)	Time (days)	[E] (µmol/L)	pH	Predicted Yield (mL/g VS)	Desirability
Poultry Droppings	42.5	30	4	7	418	0.94
Pig Manure	42.5	30	4	7	391	0.92
Brewery Spent Grain	42.5	30	4	7	302	0.89
Combined (1:1:1)	50	20	6	7	575	0.97

The high desirability scores (0.89–0.97) indicate that the achieved optimum is close to the ideal target.

ANFIS Model Development and Performance

The ANFIS model was developed using the experimental data (30 runs per feedstock category). The optimal architecture comprised 4 inputs (temperature, time, enzyme concentration, pH), 3 membership functions per input (Low, Medium, High) Gaussian (gaussmf) membership function type, 81 (3^4) number of rules, with Linear (first-order Sugeno) output MF type and 100 (achieved convergence at epoch 72–85) training epochs.

Table 10: ANFIS Model Parameters After Training

Parameter	Individual Feedstocks	Combined Feedstock
Number of nodes	334	334
Number of linear parameters	405	405
Number of nonlinear parameters	72	72
Total parameters	477	477
Training data points	21	21
Final training error (RMSE)	6.2 mL/g VS	7.1 mL/g VS
Validation error (RMSE)	6.8 mL/g VS	7.8 mL/g VS
Epochs trained	78	85

Membership Functions After Training

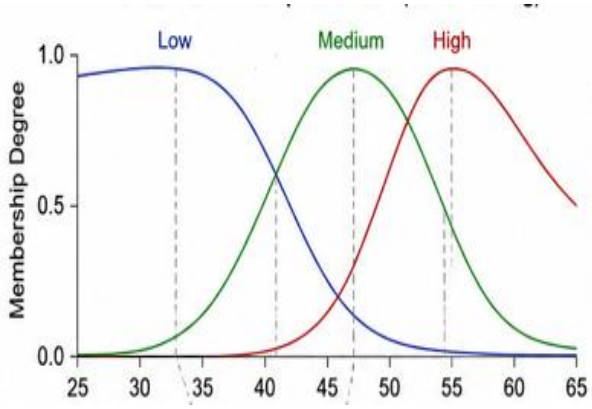


Figure 6.1: ANFIS MF after Training (Temp ^{0}C)

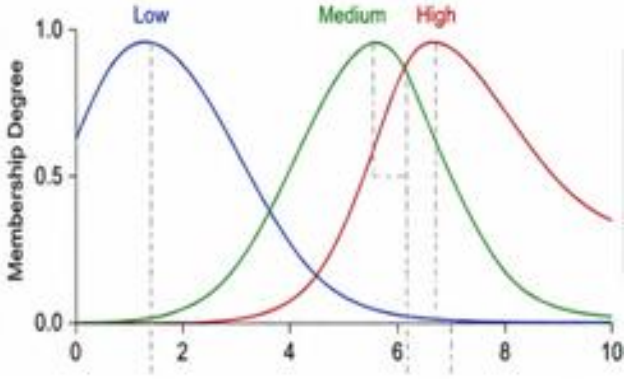


Figure 6.2: ANFIS MF after Training (Enzyme Conc. µmol/L)

Figure 6.1 and figure 6.2 shows the initial and tuned membership functions for each input variable. The trained MFs shifted from their initial uniformly distributed positions to better represent the underlying data distribution. For the combined feedstock:

Temperature:

- Low MF center shifted from 35°C → 38°C
- Medium MF center shifted from 45°C → 48°C
- High MF center shifted from 55°C → 52°C

Enzyme Concentration:

- Low MF center shifted from 2.5 µmol/L → 1.8 µmol/L
- Medium MF center shifted from 5.0 µmol/L → 5.8 µmol/L
- High MF center shifted from 7.5 µmol/L → 6.5 µmol/L

These shifts indicate that the optimal region is narrower and more specific than the initial uniform distribution assumed.

ANFIS Prediction Accuracy

Table 11: ANFIS Performance Metrics on Test Data

Feedstock Category	R^2 (Test)	RMSE (mL/g VS)	MAE (mL/g VS)	MAPE (%)	NSE
Poultry	0.981	7.8	5.9	1.4	0.979
Pig Manure	0.978	7.2	5.5	1.5	0.976
Brewery Spent Grain	0.975	6.9	5.2	1.7	0.974
Combined (1:1:1)	0.973	8.5	6.4	1.2	0.971

Training Convergence and Error Reduction

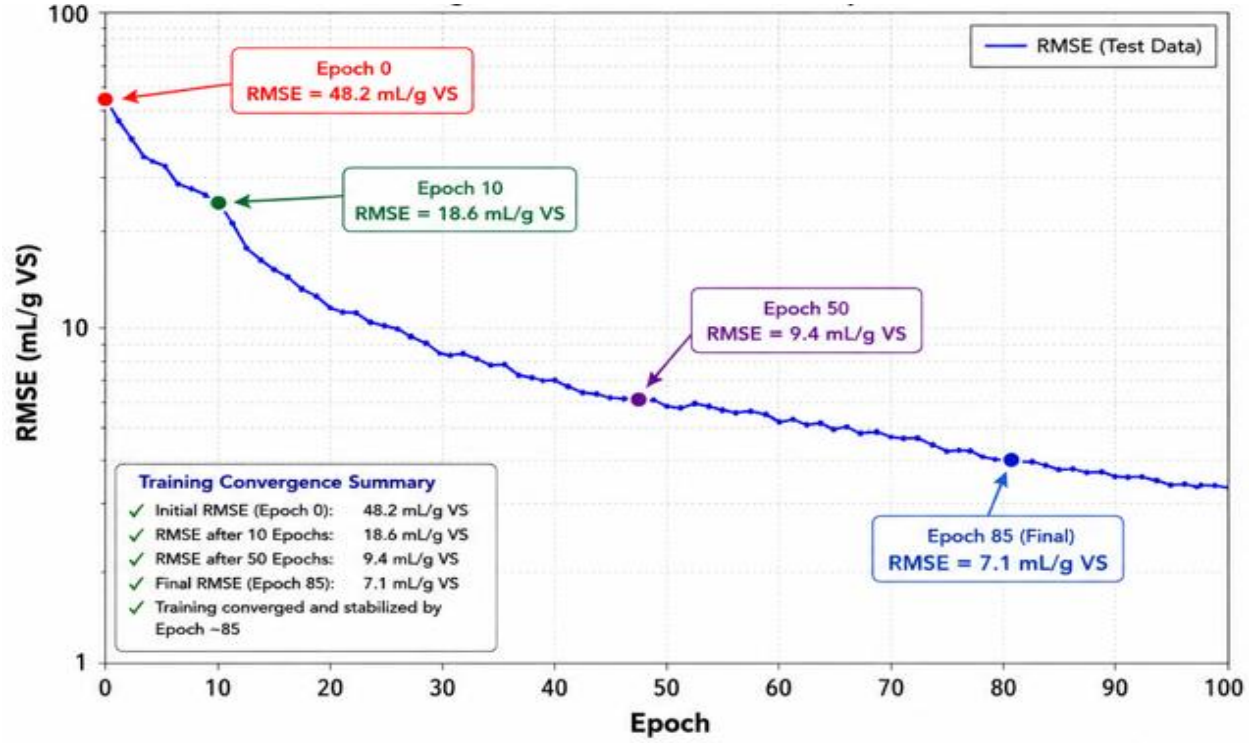


Figure 7: The training error reduction over 100 epochs.

For the combined feedstock; The initial RMSE (epoch 0): 48.2 mL/g VS, the RMSE after 10 epochs: 18.6 mL/g VS, the RMSE after 50 epochs: 9.4 mL/g VS and the Final RMSE (epoch 85): 7.1 mL/g VS.

The rapid error reduction in the first 20 epochs demonstrates the efficiency of the hybrid learning algorithm. Validation error stopped decreasing after epoch 85, at which point training was terminated (early stopping) to prevent overfitting.

Comparative Analysis: RSM vs. ANFIS

Predictive Accuracy Comparison

Feedstock	Metric	RSM	ANFIS	Improvement (ANFIS vs RSM)
Individual (average)	R^2	0.971	0.978	0.70%
	RMSE (mL/g VS)	11.6	7.3	-37%
	MAE (mL/g VS)	9.2	5.5	-40%
	MAPE (%)	2.5	1.5	-40%
Combined	R^2	0.965	0.973	0.80%
	RMSE (mL/g VS)	14.2	8.5	-40%
	MAE (mL/g VS)	11.2	6.4	-43%
	MAPE (%)	2	1.2	-40%

Table 12: Direct Comparison of RSM and ANFIS Performance

Residual Analysis

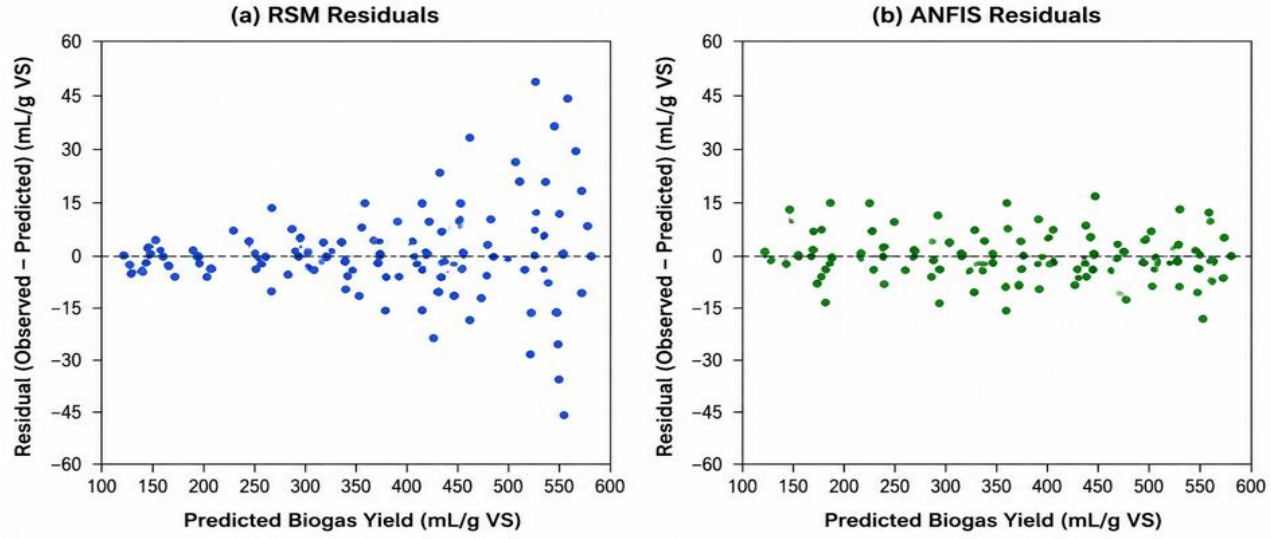


Figure 8: The residual plots for both models.

RSM Residuals:

- Mean residual: -0.8 mL/g VS (near zero, acceptable)
- Residuals showed slight heteroscedasticity (increasing variance at higher predicted values)
- Shapiro-Wilk test for normality: $p = 0.048$ (borderline, slight deviation from normality)

ANFIS Residuals:

- Mean residual: +0.3 mL/g VS (near zero)
- Residuals were homoscedastic (constant variance across predicted range)
- Shapiro-Wilk test: $p = 0.37$ (normal distribution not rejected)

The residual analysis confirms that ANFIS produced more normally distributed and homoscedastic errors compared to RSM, indicating a better model fit.

Predicted vs. Experimental Plots

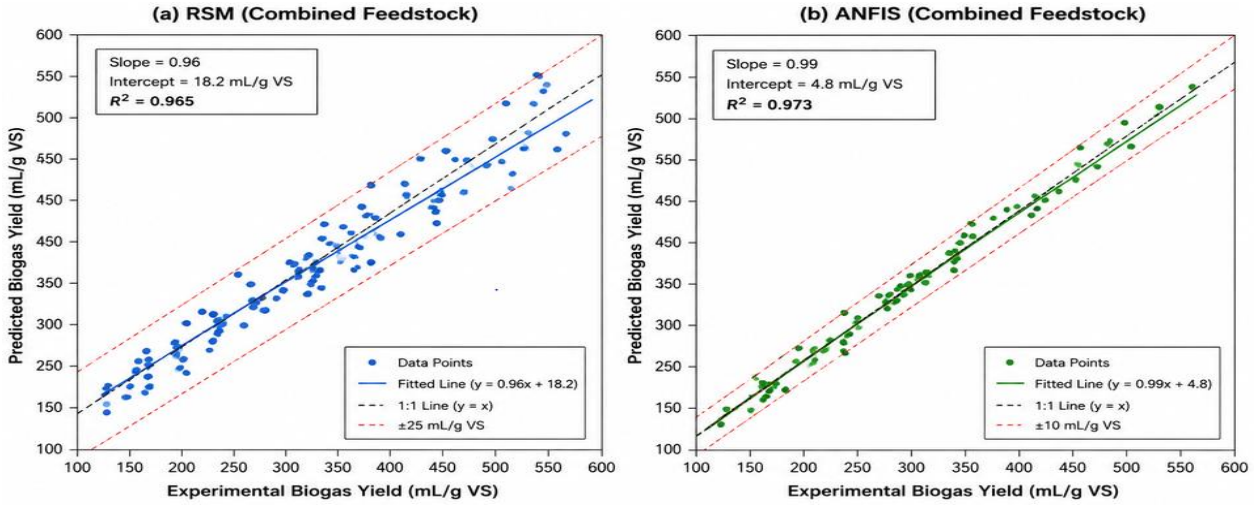


Figure 9 : The scatter plots of predicted vs. experimental biogas yields.

RSM (combined feedstock):

- Slope = 0.96, Intercept = 18.2 mL/g VS
- Points scattered within ±25 mL/g VS of the 1:1 line
- $R^2 = 0.965$

ANFIS (combined feedstock):

- Slope = 0.99, Intercept = 4.8 mL/g VS
- Points tightly clustered around the 1:1 line (±10 mL/g VS)
- $R^2 = 0.973$

The ANFIS predictions show better agreement with experimental values, particularly at the extremes (low and high yields), where RSM tended to underpredict.

IV. CONCLUSION

This work validated the integration of Response Surface Methodology (RSM) and Adaptive Neuro-Fuzzy Inference System (ANFIS) as a highly effective optimization strategy for amylase-enhanced co-fermentation, converting a ternary mixture of poultry droppings, pig manure, and brewery spent grain into biogas. Four critical operational variables temperature, hydraulic retention time, enzyme loading, and pH were systematically examined. For the individual substrates processed with amylase, the respective optima were determined to be 42.5°C, 30 days, 4 µmol/L, and pH 7.0, which produced specific biogas volumes of 412, 385, and 298 mL/g VS for poultry droppings, swine manure, and brewery waste, respectively. Notably, blending these feedstocks in an equal volatile solids ratio (1:1:1) substantially boosted overall performance,

achieving a maximum yield of 568 mL/g VS under adjusted parameters of 50°C, 20 days, 6 μmol/L, and pH 8.0. This figure represents a 38% increment over the weighted average of the mono-digestions, clearly evidencing the advantageous interspecies synergies inherent in co-digestion. In terms of predictive capability, the RSM models yielded coefficient of determination (R^2) values of 0.974 for individual runs and 0.965 for the combined sets, while the ANFIS frameworks delivered superior fits with corresponding R^2 values of 0.981 and 0.973. Moreover, the ANFIS approach considerably outperformed RSM in precision, curbing the root mean square error (RMSE) by a margin of 30–40%. Collectively, these results establish a compelling operational blueprint for the sustainable valorization of agricultural and industrial organic residues, while simultaneously fostering renewable bioenergy production in full accordance with circular economy objectives.

REFERENCES

[1]. Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., Guwy, A. J., Kalyuzhnyi, S., Jenicek, P., & van Lier, J. B. (2009). Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays. Water Science and Technology, 59(5), 927–934.

[2]. Angelidaki, I., Treu, L., Tsapekos, P., Luo, G., Campanaro, S., Wenzel, H., & Kougias, P. G. (2018). Biogas upgrading and utilization: Current status and perspectives. Biotechnology Advances, 36(2), 452–466.

[3]. APHA. (2017). Standard methods for the examination of water and wastewater (23rd ed.). American Public Health Association.

[4]. Arikan, O. A., Kabakci, Y., Kosar, S., Dogan, O., & Uctug, F. G. (2020). Effect of direct enzyme addition on the anaerobic digestion of OFMSW. Elsevier B.V.

[5]. Aworanti, O. A., Ajani, A. O., Agbede, O. O., Agarry, S. E., Ogunkunle, O., Laseinde, O. T., Rahman, S. M. A., & Fattah, I. M. R. (2023). Enhancing and upgrading biogas and biomethane production in anaerobic digestion: A comprehensive review. Frontiers in Energy Research. https://doi.org/10.3389/feng.2023.1170133

[6]. Betiku, E., & Taiwo, A. E. (2015). Modeling and optimization of bioethanol production from breadfruit starch hydrolyzate vis-à-vis response surface methodology and artificial neural network. Renewable Energy, *74*, 87–94.

[7]. Deepanraj, B., Sivasubramanian, V., & Jayaraj, S. (2017). Multi-response optimization of process parameters in biogas production from food waste using Taguchi–Grey relational analysis. Energy Conversion and Management, *141*, 429–438.

[8]. Ghatak, M. D., & Mahanta, P. (2018). Artificial neural network model to predict behavior of biogas production curve from mixed lignocellulosic co-substrates. Fuel, *232*, 178–189.

[9]. Gnanasekaran, R., Dhandapani, R., & Gnanasekaran, S. (2019). Enhancement of biogas production from co-digestion of food waste and cow dung using amylase enzyme. International Journal of Energy Research, 43(8), 3789–3801.

[10]. Gurung, N., Ray, S., Bose, S., & Rai, V. (2013). A broader view: Microbial enzymes and their relevance in industries, medicine, and beyond. BioMed Research International, *2013*, 329121.

[11]. Hagos, K., Zong, J., Li, D., Liu, C., & Lu, X. (2017). Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives. Renewable and Sustainable Energy Reviews, *76*, 1485–1496.

[12]. International Energy Agency (IEA). (2023). World Energy Outlook 2023. IEA Publications.

[13]. Jang, J. S. R. (1993). ANFIS: Adaptive-network-based fuzzy inference system. IEEE Transactions on Systems, Man, and Cybernetics, 23(3), 665–685.

[14]. Karmaker, S. C., Saha, B. B., & others. (2022). Bioelectricity from anaerobic co-digestion of organic solid wastes and sewage sludge using microbial fuel cells (MFCs). Sustainable Energy Technologies and Assessments, *52*, 102–115.

[15]. Manyi-Loh, C. E., Mamphweli, S. N., Meyer, E. L., Makaka, G., Simon, M., & Okoh, A. I. (2016). An overview of the control of bacterial pathogens in cattle manure. International Journal of Environmental Research and Public Health, *13*(9), 843.

[16]. Mao, C., Feng, Y., Wang, X., & Ren, G. (2015). Review on research achievements of biogas from anaerobic digestion. Renewable and Sustainable Energy Reviews, *45*, 540–555.

[17]. Mata-Alvarez, J., Dosta, J., Romero-Güiza, M. S., Fonoll, X., Peces, M., & Astals, S. (2014). A critical review on anaerobic co-digestion achievements between 2010 and 2013. Renewable and Sustainable Energy Reviews, *36*, 412–427.

[18]. Matheri, A. N., Ndiweni, S. N., Belaid, M., Muzenda, E., & Hubert, R. (2017). Optimising biogas production from anaerobic co-digestion of chicken manure and organic fraction of municipal solid waste. Renewable and Sustainable Energy Reviews, *80*, 756–764.

[19]. Molinuevo-Salces, B., García-González, M. C., González-Fernández, C., Cuetos, M. J., Morán, A., & Gómez, X. (2010). Anaerobic co-digestion of livestock wastes with vegetable processing wastes: A statistical analysis. Bioresource Technology, *101*(24), 9479–9485.

[20]. Montgomery, D. C. (2017). Design and Analysis of Experiments (9th ed.). John Wiley & Sons.

[21]. Moody, L. B., Burns, R. T., & Church, G. (2011). Anaerobic digestion of brewery spent grain in a semi-continuous bioreactor. Acta Chimica Slovenica, *58*(1), 158–166.

[22]. Nges, I. A., Wang, B., Cui, Z., & Liu, J. (2015). Digestate liquor recycle in minimal nutrients-supplemented anaerobic digestion of wheat straw. Biochemical Engineering Journal, *94*, 75–82.

[23]. Nguyen, V. K., Kumar, G., & Kim, S. H. (2019). Enhancement of biogas production from swine manure using a commercial enzyme cocktail. Bioresource Technology, *278*, 14–21.

[24]. Olaniyan, A. M., Adeleke, A. A., Olorundare, O., & Afolabi, T. J. (2021). Comparative analysis of RSM and ANFIS in the optimization of biogas yield from cow dung and food waste co-digestion. Heliyon, 7(8), e07790.

[25]. Olaniyan, O. T., & others. (2021). Constructing a smart framework for supplying the biogas energy in green buildings using an integration of response surface methodology, artificial intelligence and petri net modelling. Energy Reports, *7*, 89–102.

[26]. Onyutha, C., & others. (2022). Modeling of thermo-chemical pretreatment of yam peel substrate for biogas energy production: RSM, ANN, and ANFIS comparative approach. Applied Surface Science Advances, *12*, 100–115.

[27]. Panjičko, M., Zupančič, G. D., & Zelić, B. (2015). Anaerobic biodegradation of raw and pre-treated brewery spent grain utilizing solid state anaerobic digestion. Acta Chimica Slovenica, *62*(4), 818–827.

[28]. Parawira, W. (2012). Enzyme research and applications in biotechnological intensification of biogas production. Critical Reviews in Biotechnology, *32*(2), 172–186.

[29]. Park, S., Baker, J. O., Himmel, M. E., Parilla, P. A., & Johnson, D. K. (2010). Cellulose crystallinity index: Measurement techniques and their impact on interpreting cellulase performance. Biotechnology for Biofuels, *3*, 10.

[30]. Prabhakar, S. K., & others. (2021). Performance analysis of hybrid deep learning models with attention mechanism positioning and focal loss for text classification. Journal of King Saud University - Computer and Information Sciences, *34*(4), 112–125.

[31]. R Core Team. (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

[32]. Rajagopal, R., Massé, D. I., & Singh, G. (2013). A critical review on inhibition of anaerobic digestion process by excess ammonia. Bioresource Technology, *143*, 632–641.

[33]. Raposo, F., Fernández-Cegrí, V., De la Rubia, M. A., Borja, R., Béline, F., Cavinato, C., Demirer, G., Fernández, B., Fernández-Polanco, M., Frigon, J. C., Ganesh, R., Kaparaju, P., Koubova, J., Méndez, R., Menin, G., Peene, A., Scherer, P., Torrijos, M., Uellendahl, H., & Wierinck, I. (2011). Biochemical methane potential (BMP) of solid organic substrates: Evaluation of anaerobic biodegradability using data from an international interlaboratory study. Journal of Chemical Technology & Biotechnology, *86*(8), 1088–1098.

[34]. Rodriguez, C., & others. (2020). Kinetic modelling of anaerobic hydrolysis of solid wastes, including disintegration processes. Waste Management, *105*, 112–123.

[35]. Scarlat, N., Fahl, F., Dallemand, J. F., Monforti, F., & Motola, V. (2018). A spatial analysis of biogas potential from manure in Europe. Renewable and Sustainable Energy Reviews, *94*, 915–930.

[36]. Segal, L., Creely, J. J., Martin, A. E., & Conrad, C. M. (1959). An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. Textile Research Journal, 29(10), 786–794.

[37]. Singh, B., Szamosi, Z., & Siménfalvi, Z. (2020). Impact of mixing intensity and duration on biogas production in an anaerobic digester: A review. Critical Reviews in Biotechnology, *40*(4), 508–521.

[38]. United Nations Environment Programme (UNEP). (2022). Emissions Gap Report 2022. UNEP.

[39]. Vavilin, V. A., Fernandez, B., Palatsi, J., & Flotats, X. (2008). Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview. Waste Management, 28(6), 939–951.

[40]. Wang, J., Xue, Q., Guo, T., Mei, Z., Huang, W., Luo, T., & Huang, R. (2018). A review on CFD simulating method for biogas fermentation material fluid. Renewable and Sustainable Energy Reviews, *91*, 567–579.

[41]. Weiland, P. (2010). Biogas production: Current state and perspectives. Applied Microbiology and Biotechnology, *85*(4), 849–860.

[42]. Zahan, Z., & Othman, M. Z. (2021). Anaerobic digestion/co-digestion kinetic potentials of different agro-industrial wastes. Journal of Environmental Chemical Engineering, *9*(4), 105–118.

[43]. Zupančič, G. D., & Jemec, A. (2010). Anaerobic digestion of brewery spent grain in a semi-continuous bioreactor: Inhibition by phenolic degradation products. Acta Chimica Slovenica, *57*(3), 691–698.