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ORIGINAL RESEARCH ARTICLE

Anaerobic Co-Digestion of Cattle Dung and *Typha latifolia*: Influence of Biomass Form on Biogas Yield, Gas Quality and Digestion Kinetics

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ABSTRACT

Anaerobic digestion provides an effective pathway for converting organic waste into renewable energy while simultaneously addressing environmental management challenges. This study evaluates the mesophilic anaerobic co-digestion of cattle dung with *Typha latifolia* biomass in two physical forms: fresh and powdered. Batch digesters were operated for 38 days, and reactor performance was assessed through daily and cumulative biogas production, methane concentration, hydrogen sulfide levels, and digestion kinetics. The results showed that co-digestion with fresh *Typha latifolia* (FTCD) produced the highest cumulative biogas yield (351.74 mL per reactor), representing a substantial improvement over cattle dung mono-digestion (234.05 mL). In contrast, reactors containing powdered biomass (PTCD) exhibited extremely low gas production (3.50 mL), indicating strong process inhibition. Statistical analysis using one-way ANOVA confirmed a significant effect of substrate composition on daily biogas production ($p < 0.001$). Methane concentration was consistently higher in FTCD reactors, suggesting improved metabolic stability during co-digestion. Kinetic modelling using the Modified Gompertz equation indicated higher biogas potential and production rate for the FTCD system, while the PTCD system exhibited a prolonged lag phase and minimal biodegradation. The findings demonstrate that fresh *Typha latifolia* can serve as an effective co-substrate with cattle dung under mesophilic conditions, whereas drying and powdering the biomass negatively affect digestion performance. These results highlight the importance of substrate form in macrophyte-based biogas systems and support the use of minimally processed wetland biomass for renewable energy production.

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INTRODUCTION

The global transition toward low-carbon energy systems has intensified interest in renewable bioenergy technologies that can simultaneously address waste management, energy security, and greenhouse gas mitigation. Anaerobic digestion (AD) is widely recognized as one of the most mature and scalable bioenergy pathways due to its ability to stabilize organic residues while producing biogas, a renewable fuel primarily composed of methane and carbon dioxide (Meegoda et al., 2018; Li et al., 2021; Salihawa et al., 2024). In agricultural and rural contexts, AD offers additional benefits by converting locally available biomass into energy while generating nutrient-rich digestate suitable for soil amendment.

Cattle dung remains one of the most commonly used substrates in small- and medium-scale biogas systems because of its favorable buffering capacity, high microbial inoculum density, and widespread availability in livestock-dominated regions (Gupta et al., 2016; Font-Palma, 2019). However, mono-digestion of cattle dung often results in moderate methane yields due to its relatively low carbon content and limited biodegradable fraction, particularly when animals are fed lignified or low-quality feedstocks (Li et al., 2021). To overcome these limitations, anaerobic co-digestion has emerged as an effective strategy to improve biogas yield, enhance process stability, and optimize the carbon-to-nitrogen (C/N) ratio by combining complementary substrates (Wei et al., 2019; Panigrahi et al., 2020).

In recent years, attention has increased toward freshwater macrophytes as potential co-digestion substrates. These plants grow rapidly, accumulate substantial organic matter, and are often harvested as waste biomass from wetlands, drainage channels, and irrigation systems. Comprehensive reviews have highlighted the energetic potential of macrophytes such as cattails (*Typha* spp.), reeds, and

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sedges, while also noting their structural recalcitrance and nutrient imbalance when digested alone (Hartung & Huber, 2020; Nagy, 2024). In many regions, particularly in Africa, uncontrolled macrophyte proliferation disrupts hydrological systems, reduces agricultural productivity, and degrades wetland ecosystems, necessitating regular removal and disposal.

Among emergent macrophytes, *Typha latifolia* (broadleaf cattail) is of particular concern due to its aggressive colonization of wetlands and irrigation canals. The plant exhibits high cellulose and hemicellulose content, which contribute to elevated organic carbon levels, but is typically deficient in Nitrogen, resulting in high C/N ratios that limit methanogenic activity during monodigestion (Mustafa & Scholz, 2011; Malovanyy et al., 2023). Several studies have demonstrated that cattail and other wetland biomass can be anaerobically digested, but methane yields are often constrained by lignocellulosic rigidity and slow hydrolysis rates unless co-digested with nitrogen-rich substrates or subjected to advanced pretreatment (Czubaszek et al., 2021; Dębowski et al., 2023).

Co-digestion of macrophytes with animal manure has been shown to improve nutrient balance, accelerate microbial adaptation, and enhance methane production. Experimental work on paludiculture and wetland biomass indicates that blending emergent plants with manure can significantly improve digestion performance under mesophilic conditions, provided that substrate composition and processing are carefully managed (Hartung & Huber, 2020). However, the effectiveness of co-digestion is strongly influenced by biomass form and pre-processing method. Thermal drying and milling, often applied to reduce volume and improve handling, can alter lignin structure, increase crystallinity, and promote the formation of inhibitory phenolic compounds, potentially suppressing anaerobic biodegradability (Panigrahi et al., 2020; Malovanyy et al., 2023).

Despite growing interest in macrophyte-based biogas systems, several important knowledge gaps remain. First, there is limited experimental evidence directly comparing the anaerobic co-digestion performance of fresh versus dried or powdered *Typha latifolia* biomass when combined with cattle dung under mesophilic conditions. Second, while many studies report cumulative biogas yields, fewer assess gas quality parameters such as methane concentration and hydrogen sulfide content, which are critical for practical energy utilization. Third, although kinetic models such as the Modified Gompertz equation are widely used to interpret batch digestion behavior, their applicability to structurally complex or partially inhibited systems remains underexplored (Achinas & Euverink, 2019).

Addressing these gaps is particularly relevant for regions where *Typha latifolia* infestation and cattle farming coexist, offering opportunities for integrated waste-to-energy solutions. Valorizing invasive macrophyte biomass through anaerobic co-digestion not only contributes to renewable energy generation but also supports wetland restoration and sustainable biomass management, aligning with circular bioeconomy principles (Nagy, 2024).

Therefore, this study investigates the mesophilic anaerobic co-digestion performance of cattle dung with fresh and powdered *Typha latifolia* biomass in batch reactors. The objectives were to (i) characterise the physicochemical properties of cattle dung and *Typha* biomass, (ii) compare daily and cumulative biogas production from mono-digestion and co-digestion systems, (iii) evaluate methane concentration and hydrogen sulfide levels in the produced biogas, (iv) assess process stability through pH monitoring, and (v) apply the Modified Gompertz model to interpret kinetic behaviour and identify enhancement or inhibition effects. By providing a direct comparison of biomass forms, this work aims to clarify the role of substrate structure in macrophyte-based co-digestion and to inform practical strategies for sustainable bioenergy production from invasive wetland plants.

MATERIALS AND METHODS

3.1 Study Area and Biomass Collection

The study was conducted using biomass collected in Funtua, Katsina State, Nigeria (11°31'N, 7°18'E), a region characterized by widespread cattle rearing and seasonal proliferation of wetland macrophytes. Fresh cattle dung (CD) was obtained from open-lot herds near the Funtua municipal abattoir in Sabon Gari Ward, where traditional livestock husbandry practices provide nutrient-rich manure suitable for anaerobic digestion.

Typha latifolia (TL) biomass was harvested manually from wetland zones in Makera, an area experiencing increasing Typha encroachment. The entire above-ground plant—comprising stems and leaves—was collected to reflect realistic biomass removal practices used for wetland restoration. Fruiting bodies were excluded because of their low digestible matter content. Samples were transported within one hour in airtight, clean polyethylene containers to prevent premature degradation or moisture loss.

3.2 Substrate Preparation and Pretreatment

Upon arrival in the laboratory, TL biomass was rinsed with distilled water to remove debris, silt, and epiphytes that could affect digestion chemistry. The biomass was then chopped into 2–3 cm fragments to enhance microbial accessibility. Two forms of TL were prepared:

1. **Fresh *T. latifolia*:** sliced and used without drying.

2. **Powdered *T. latifolia*:** oven-dried at 60 °C to constant weight and milled to a particle size of <2 mm.

This dual preparation enabled comparison between unprocessed macrophyte biomass and a common pre-processing method. Drying and milling are known to alter lignocellulosic structure and potentially increase phenolic concentration or lignin crystallinity, which may influence anaerobic biodegradability.

Fresh cattle dung was homogenized manually to remove stones and fibrous debris, ensuring uniformity. The cattle dung served as both the substrate and the sole inoculum in all reactors; no external bacterial seed was added. The inoculum contribution was inherently embedded within the CD fraction in co-digestion systems.

3.3 Experimental Setup and Reactor Configuration

Three batch digestion systems were formulated on a volatile solids (VS) basis, each representing a different substrate composition:

1. **CD (Control):** 100% cattle dung
2. **FTCD:** 50% cattle dung + 50% fresh *T. latifolia*
3. **PTCD:** 50% cattle dung + 50% powdered *T. latifolia*

The selected 50:50 VS-based substrate ratio was chosen to balance nitrogen availability from cattle dung with the high organic carbon content of Typha biomass. Previous studies on macrophyte–manure co-digestion indicate that macrophyte fractions between 25–50% typically optimize nutrient balance and process stability without inducing inhibition (Hartung & Huber, 2020; Nagy, 2024)

The mixtures were adjusted to approximately 85% moisture content and loaded into 25-L laboratory-scale batch digesters, each equipped with (Figure 1):

- an airtight feeding inlet,
- a gas outlet linked to a water-displacement apparatus, and
- a sampling port for pH measurement and slurry collection.

Digesters were operated under mesophilic conditions (35 ± 2 °C) and manually mixed once daily to prevent sedimentation and improve substrate–microbe contact. Retention time was 38 days, defined as the point at which daily biogas production fell below 1% of cumulative volume for three consecutive days.

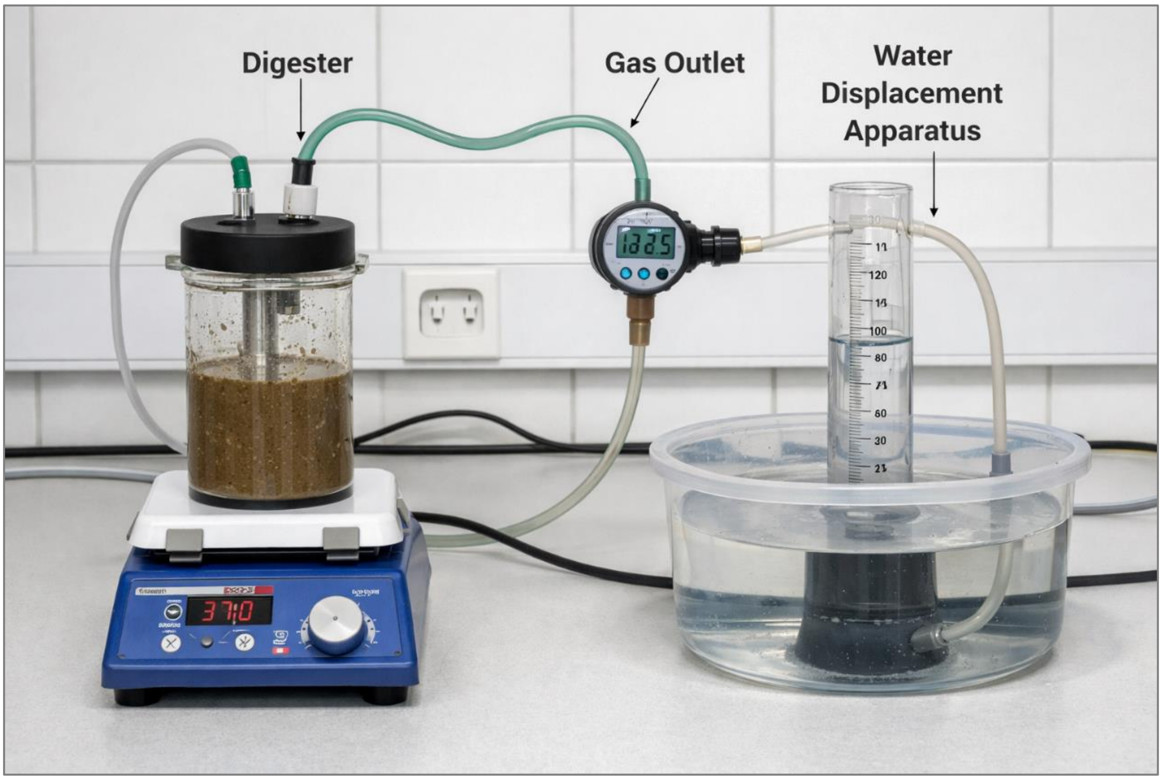

Figure 1: Schematic representation of the laboratory-scale batch anaerobic digestion system used in this study, illustrating the digester vessel, gas outlet line, and water displacement apparatus used for daily biogas measurement.

No pH adjustments or chemical additives were introduced, ensuring that digestion performance reflected inherent feedstock characteristics rather than engineered modification.

3.4 Biogas Volume Measurement

Biogas production was monitored daily using the acidified brine water-displacement method, which minimizes CO_2 dissolution and ensures accurate volumetric readings. Acidified brine (pH ≈ 2) was prepared by dissolving NaCl in distilled water and adjusting acidity with HCl.

Gas volumes were corrected to standard temperature and pressure (STP; 0 °C, 1 atm) using the ideal gas equation. Prior to experimentation, digesters underwent leak testing through submerged bubble inspection to guarantee airtight operation. Daily gas production from each reactor was recorded throughout the 38-day digestion period. The complete dataset of daily measurements used to generate cumulative production curves and statistical analysis is provided as Supplementary Table S1.

3.5 Methane and Gas Composition Analysis

Biogas composition was analyzed using a Gas Chromatograph (Agilent 7890B) fitted with a Thermal Conductivity Detector (TCD). The GC employed a Porapak Q column (80/100 mesh, 2 m × 3 mm) operated isothermally at 50 °C. Helium served as the carrier gas at a flow rate of 30 mL·min^{-1}.

Gas samples (50 mL) were collected with gas-tight syringes and analyzed for:

- methane (CH_4),
- carbon dioxide (CO_2),
- hydrogen sulfide (H_2S),
- and trace nitrogen (N_2).

Prior to sample analysis, the gas chromatograph was calibrated using certified methane and carbon dioxide standard gas mixtures. Calibration curves were generated to verify detector response and ensure analytical accuracy. Gas samples collected from each digester were analyzed in duplicate, and the average values were used to represent methane and gas composition for each sampling event.

Calibration was performed using certified standard mixtures to ensure analytical accuracy. Methane and carbon dioxide results were expressed as % v/v, while H_2S was recorded in parts per million (ppm).

3.6 Physicochemical Characterization of Substrates

Feedstock characterization followed APHA Standard Methods (2012). The analyses conducted include:

- **Total solids (TS)** — dried at 105 °C
- **Volatile solids (VS)** — combusted at 550 °C
- **Ash content** — residual after combustion
- **Organic carbon** — Walkley–Black dichromate oxidation
- **Total Nitrogen (TN)** — Kjeldahl digestion
- **C/N ratio** — computed from measured OC and TN
- **pH** — determined on a 1:10 (w/v) slurry

All analyses were executed in **triplicate**, and results are presented as mean ± standard deviation.

3.7 Kinetic Modeling

The Modified Gompertz model was applied to cumulative biogas production data to evaluate digestion kinetics and derive the parameters: P, the maximum cumulative biogas potential (mL); Rm, the maximum biogas production rate (mL day^{-1}); and λ, the lag phase duration (days). Model fitting was performed using nonlinear regression, and goodness-of-fit was evaluated using the coefficient of determination (R^2).

3.8 Statistical Analysis

All digestion experiments were conducted in triplicate reactors for each treatment (n = 3) to ensure experimental reliability. Daily gas production data from the replicate reactors were averaged, and the results are presented as mean ± standard deviation. Differences in daily biogas production among treatments were evaluated using one-way analysis of variance (ANOVA). Where significant differences were detected ($p < 0.05$), Tukey's honestly significant difference (HSD) test was applied for post-hoc pairwise comparison. Statistical analyses were performed using standard statistical software. Statistical analyses were conducted using the daily reactor measurements. The raw dataset used for these analyses has been provided in the supplementary materials to ensure transparency and reproducibility of the reported results.

RESULTS AND DISCUSSION

4.1. Physicochemical Characteristics of Substrates

The physicochemical properties of cattle dung and *Typha latifolia* biomass are presented in Table 1 and provide a mechanistic basis for the observed anaerobic digestion performance. Cattle dung exhibited a total solids (TS) content of 18.3 ± 0.4% and a volatile solids (VS) fraction of 73.8 ± 0.9% TS, indicating a substantial biodegradable organic component (Gupta et al., 2016; Li et al., 2021). Its near-neutral pH (7.4 ± 0.1) and moderate C/N ratio (20.7 ± 0.7) are within the optimal range for methanogenic activity, supporting its suitability as both substrate and inoculum.

Table 1 Physicochemical characteristics of cattle dung and *Typha latifolia* biomass used in anaerobic digestion

Parameter	Fresh *Typha latifolia*	Cattle dung	Powdered *Typha latifolia*
Total solids (TS, %)	19.8 ± 0.7	18.3 ± 0.4	84.5 ± 1.2
Volatile solids (VS, % TS)	78.6 ± 1.1	73.8 ± 0.9	72.2 ± 1.4
Ash content (%)	21.4 ± 0.8	26.2 ± 1.1	27.8 ± 0.9
Organic carbon (%)	41.8 ± 0.6	38.1 ± 0.8	39.6 ± 0.7
Total Nitrogen (%)	0.81 ± 0.03	1.84 ± 0.04	0.68 ± 0.02
C/N ratio	51.6 ± 1.3	20.7 ± 0.7	≈58 ± 2.1
pH	6.3 ± 0.1	7.4 ± 0.1	4.9 ± 0.2

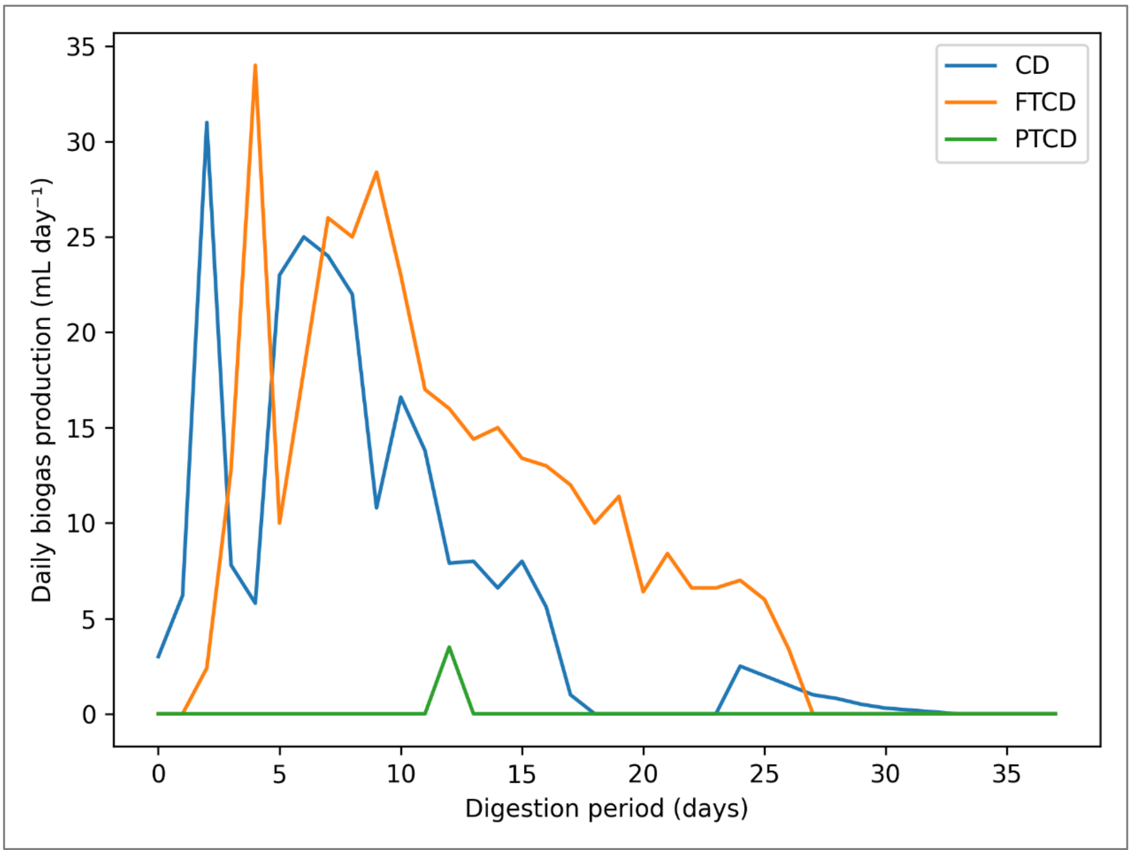

Figure 2: Daily Biogas Production Profiles for CD, FTCD, and PTCD Digesters during the 38-day digestion period.

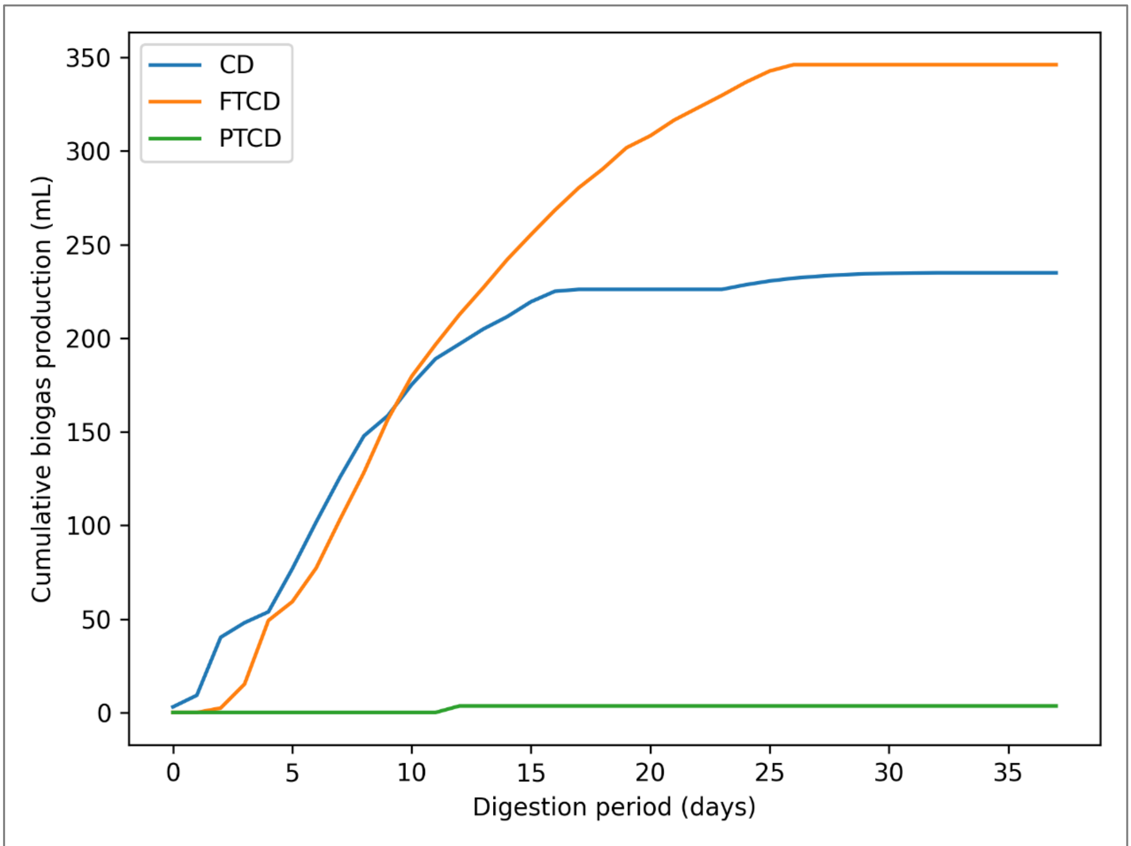

Figure 3 Cumulative Biogas Production from CD, FTCD, and PTCD Systems under Mesophilic Conditions.

Fresh *T. latifolia* showed comparable TS content (19.8 ± 0.7%) but a higher VS fraction (78.6 ± 1.1% TS), reflecting a large proportion of biodegradable organic matter. However, its elevated C/N ratio (51.6 ± 1.3) indicates nitrogen limitation when digested alone. The slightly acidic pH (6.3 ± 0.1) remained favorable for hydrolytic and acidogenic processes when co-digested with cattle dung, suggesting that nutrient complementarity and buffering effects would be critical for stable digestion (Hartung & Huber, 2020; Dębowski et al., 2023).

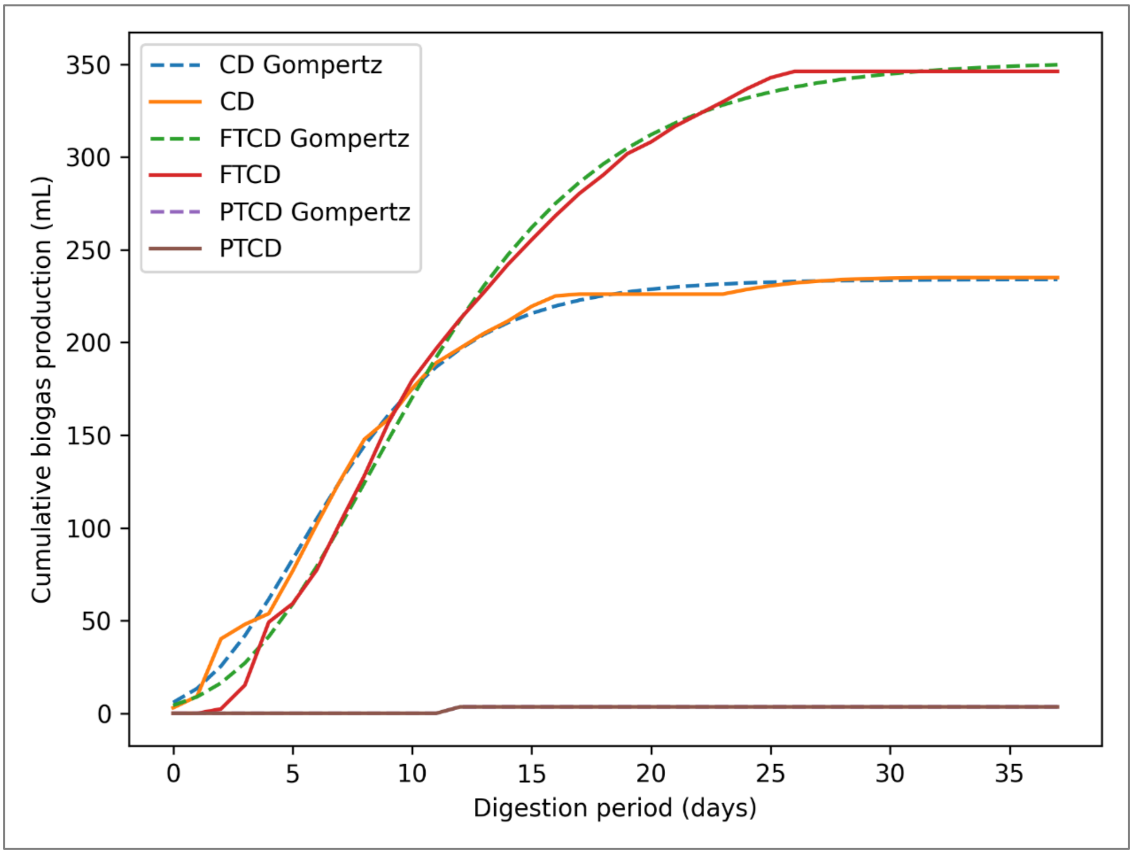

Figure 4: Modified Gompertz model fits of Cattle Dung (CD), Fresh *Typha latifolia* with Cattle Dung (FTCD), and Powdered *Typha latifolia* with Cattle Dung (PTCD).

Table 2 Modified Gompertz kinetic parameters for anaerobic digestion of cattle dung (CD), cattle dung with fresh *Typha latifolia* (FTCD), and cattle dung with powdered *Typha latifolia* (PTCD)

Digestion system	P – Maximum biogas potential (mL)	Rm – Maximum biogas production rate (mL day^{-1})	λ – Lag phase (days)
CD	234.05	21.87	1.20
FTCD	351.74	23.33	2.67
PTCD	3.50	42.02	11.07

In contrast, powdered *T. latifolia* exhibited extremely high TS content (84.5 ± 1.2%) and the lowest pH among the substrates (4.9 ± 0.2), alongside the highest C/N ratio (≈58 ± 2.1) (Czubaszek et al., 2021; Malovanyy et al., 2023). These conditions reflect high lignocellulosic concentration, limited moisture availability, and nitrogen deficiency, all of which are unfavorable for methanogenic activity and provide a strong indication of potential process inhibition.

4.2 Daily Biogas Production and Statistical Evaluation

Daily biogas production profiles (Figure 2) showed distinct temporal patterns among treatments. FTCD exhibited the highest and most sustained daily biogas production, while CD showed moderate production with a gradual decline. PTCD produced negligible daily biogas, limited to a brief early phase (Achinas & Euverink, 2019; Panigrahi et al., 2020). The raw daily biogas production dataset used to generate the production profiles presented in Figure 2 is provided in Supplementary Table S1.

One-way ANOVA confirmed that treatment type exerted a statistically significant effect on daily biogas production ($F = 16.58$, $p < 0.001$). Post-hoc Tukey HSD analysis revealed no statistically significant difference between FTCD and CD ($p = 0.686$), indicating overlapping daily production behavior during active digestion. In contrast, PTCD differed significantly from both FTCD and CD ($p < 0.001$), confirming that its poor performance was attributable to process inhibition rather than experimental variability.

These statistical outcomes corroborate the observed daily production trends and support the interpretation that fresh *T. latifolia* enhances digestion performance relative to powdered biomass (Hartung & Huber, 2020; Malovanyy et al., 2023).

4.3. Cumulative Biogas Production Performance

Cumulative biogas production trends (Figure 3) further highlight the influence of substrate properties and biomass form. FTCD achieved the highest cumulative biogas volume (351.74 mL per batch reactor), representing approximately a 50% increase relative to the CD system (234.05 mL). This enhancement demonstrates a clear synergistic effect arising from co-digestion, where the high organic carbon content of fresh *T. latifolia* (41.8 ± 0.6%) complements the nitrogen-rich cattle dung (1.84 ± 0.04% N), resulting in improved nutrient balance and microbial activity (Wei et al., 2019; Dębowski et al., 2023).

By contrast, the PTCD system produced only 3.50 mL of cumulative biogas, confirming severe inhibition. Despite having a comparable organic carbon content (39.6 ± 0.7%), the powdered biomass exhibited excessive TS concentration, low moisture, and acidic conditions, which together hindered hydrolysis and downstream methanogenesis (Czubaszek et al., 2021; Nagy, 2024). These findings demonstrate that particle size reduction through drying and powdering does not necessarily enhance anaerobic digestibility for lignocellulosic macrophytes.

4.4. Biogas Quality and Hydrogen Sulfide Formation

Biogas quality analysis revealed consistently higher methane concentrations in FTCD compared to CD, indicating more efficient conversion of intermediate products into methane. Improved methane quality in co-digestion systems is commonly associated with balanced nutrient availability and stable syntrophic relationships between acidogenic and methanogenic microorganisms (Font-Palma, 2019; Li et al., 2021).

Hydrogen sulfide concentrations were lowest in FTCD, whereas higher and more variable levels were observed in CD and PTCD. Reduced H_2S formation in co-digestion systems has been attributed to moderated protein degradation and improved sulfur assimilation under stable digestion conditions (Font-Palma, 2019). In PTCD, unstable H_2S profiles further reflect microbial stress and inhibited methanogenesis, consistent with its poor biogas performance.

4.5. Process Stability and pH Evolution

The evolution of pH during digestion provides additional insight into process stability. All systems maintained pH values within the operational range for mesophilic anaerobic digestion, although notable differences were observed. FTCD exhibited minor pH fluctuations during the early digestion phase, followed by rapid stabilization near neutral values, reflecting effective buffering by cattle dung and efficient acid consumption by methanogens (Meegoda et al., 2018).

The PTCD system maintained comparatively lower pH values for an extended period, consistent with delayed methanogenic activity and accumulation of intermediate fermentation products. This behavior directly reflects the acidic nature (pH 4.9 ± 0.2) and high TS content of powdered *T. latifolia*, reinforcing the inhibitory effect of this biomass form (Czubaszek et al., 2021; Malovanyy et al., 2023).

4.6 Kinetic Modeling and Statistical Context

Modified Gompertz modeling, as shown in Figure 4, further supported the experimental and statistical findings. FTCD exhibited the highest biogas potential (P = 351.74 mL) and production rate (Rm = 23.33 mL day^{-1}), while CD showed moderate kinetic performance (P = 234.05 mL). PTCD exhibited an extremely low biogas potential (P = 3.50 mL) and a prolonged lag phase (11.07 days), consistent with statistically confirmed inhibition. The elevated Rm value estimated for PTCD reflects a modeling artifact commonly observed in inhibited systems (Achinas & Euverink, 2019; Wei et al., 2019).

The CD system (Table 2) showed moderate kinetic performance (P = 234.05 mL; Rm = 21.87 mL day^{-1}) and a short lag phase (1.20 days), consistent with the inherent microbial richness of cattle dung. In contrast, PTCD displayed an extremely low biogas potential (P = 3.50 mL) and a prolonged lag phase (11.07 days), providing strong kinetic evidence of inhibition. The elevated Rm value estimated for PTCD is a modeling artifact commonly observed when fitting classical kinetic models to severely inhibited systems (Panigrahi et al., 2020).

4.7. Implications for Macrophyte Valorization and Bioenergy Systems

The integrated results demonstrate that substrate form and physicochemical characteristics critically determine the anaerobic digestibility of *T. latifolia*. Fresh biomass enhances cumulative biogas production, methane quality, and kinetic performance when co-digested with cattle dung, whereas powdering and drying exacerbate lignocellulosic recalcitrance and inhibit microbial conversion. Similar conclusions have been drawn in recent reviews of macrophyte-based anaerobic digestion, which emphasize the advantages of minimal-processing strategies (Hartung & Huber, 2020; Nagy, 2024).

These findings emphasize that minimal processing strategies may be more effective and cost-effective than energy-intensive pretreatments for invasive macrophytes. Co-digestion of fresh wetland biomass with livestock manure offers a practical pathway for renewable energy generation and ecosystem management, particularly in regions where both resources are abundant.

Although the batch digestion experiments provide useful insights into substrate behavior, the results should be interpreted with consideration of laboratory-scale conditions. Reactor performance in continuous systems or full-scale digesters may vary due to operational factors, including hydraulic loading, mixing efficiency, and microbial adaptation. Future studies could therefore evaluate the long-term performance of fresh macrophyte co-digestion under continuous reactor operation.

CONCLUSIONS

This study demonstrates that substrate form significantly influences anaerobic digestion performance. One-way ANOVA confirmed significant differences in daily biogas production among treatments ($p < 0.001$), with post-hoc analysis showing that powdered *Typha latifolia* co-digestion is statistically inferior to both cattle dung mono-digestion and fresh biomass co-digestion.

Fresh *Typha latifolia* co-digestion produced the highest cumulative biogas volume and improved gas quality, while powdered biomass resulted in severe inhibition. These findings confirm that minimal processing strategies are more effective than drying and powdering for valorizing invasive macrophytes in anaerobic digestion systems.

SUPPLEMENTARY MATERIALS

Available in the online version

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