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Comparative Analysis of Energy Recovery and Digestate Quality from Anaerobic Co-Digestion of Fruit and Vegetable Wastes under Ambient and Mesophilic Conditions

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Abstract

Large quantities of fruits and vegetables are lost along the supply chain due to their perishable nature, with factors such as inadequate refrigerated transport, poor road conditions, and limited cold storage facilities contributing to this loss. Anaerobic digestion offers a promising solution for mitigating the negative impacts of fruits and vegetable wastes (FVW). This study investigated the anaerobic co-digestion of FVW under ambient and mesophilic conditions, focusing on biogas yield, digestate quality, and energy efficiency. A 50:50 mixture of FVW and cow dung at mesophilic temperatures resulted in the highest biogas yield of 1,328.4 mLg⁻¹VS, with a specific methane yield of 258.4 mL CH₄ g⁻¹ VS. The digestate from this mixture contained 64.56, 15.34, and 20.32 gkg⁻¹ of nitrogen (N), phosphorus (P), and potassium (K), respectively. The heavy metal concentrations of copper (Cu), zinc (Zn), lead (Pb), iron (Fe), and manganese (Mn) were 0.02, 0.51, non-detectable, 88.60, and 3.67 mgkg⁻¹, respectively, all falling within the acceptable limits for digestate/compost quality according to national and international standards. However, additional regulated metals such as As, Cr, Hg, Se, and Cd were not analyzed in this study. Therefore, compliance with international biofertilizer standards should be limited to the analyzed heavy metals. Although the average H₂S levels remained below 22 ppm, routine monitoring is advisable, as elevated levels can compromise biogas quality, necessitating additional costs for biogas purification. From an energy recovery perspective, integrating insulation and correctly sizing the tank was found to improve both energy yield and operational efficiency, thereby enhancing the overall sustainability of the anaerobic digestion system. Further research should focus on temperature adaptability experiments in different climate zones, co-digestion with other organic wastes, long-term operational stability testing at the pilot scale, heavy metals and microbial analysis of the solid digestate.

Keywords: Anaerobic digestion, Fruit Vegetable Waste, Co-digestion, System Scaling, Sustainability Index

1. Introduction

The fruit and vegetable (FV) production sector is a vital component of East Africa's agricultural economy, making significant contributions to food security, employment, and regional trade. For instance, in 2023, about 13.16 million tonnes of bananas, 10.91 million tonnes of pineapples, 9.19 million tonnes of potatoes, 4.12 million tonnes of mangoes, and 6.79 million tonnes of vegetables were produced in Eastern Africa [1]. Despite this high production, substantial postharvest losses ranging from 30 to 80wt% have been reported across the region [2], [3], [4], [5]. These losses not only result in the wastage of energy and valuable nutrients but also contribute to environmental degradation through the generation of greenhouse gases from open material decay [6].

The FV losses occur mainly during transportation and at markets because it is often expensive and impractical to provide adequate preservative conditions [7]. Although a small quantity of this waste is used as animal feed, most of it remains uncollected and is merely openly disposed of [8], [9]. For the collected portion (20-40wt%), most of it is mixed up with other wastes and deposited at landfills [6]. Fruit and vegetable wastes (FVW) are also illegally disposed of in open spaces such as wetlands and water channels, and such practices are associated with discharges of leachates polluting surface and underground water and exacerbating greenhouse gas emissions (GHG) [10], [11].

Anaerobic Digestion (AD) is one of the technologies that can mitigate the negative impacts of FV wastes [12], [13], [14], [15]. Specifically, AD adds value to these wastes through the production of energy and bio-fertilizer/soil conditioners, thus reducing the associated GHG emissions and making agricultural production more sustainable [16], [17], [18], [19]. Currently, the most common substrate used for AD in Eastern Africa is cow dung [20], [21]. However, the quantity of cow dung available and its competitive uses in construction and agriculture limit the broad adoption of AD technology. Moreover, existing AD technology is mainly localized at the household level among families with zero grazing animal systems [22]. As an alternative to the sole use of cow dung, other organic wastes, including agricultural residues, fresh food market waste, and the organic fraction of municipal solid waste (OFMSW) have been successfully tested [23], [24], [25]. Usually, the AD of organic materials is carried out in mesophilic conditions (35 ± 5 °C) or thermophilic conditions (55 ± 5 °C) [26], [27],[28]. Whereas some authors [29], [30], [31], [32] claim that it is far better to work in thermophilic rather than mesophilic conditions, the energy demand using the thermophilic approach is still a challenge, especially in energy-poor countries in sub-Saharan Africa (SSA). Published results are still divided regarding the AD of FV waste, with some researchers [24], [33] highlighting that there are no significant differences in process performance between

thermophilic and mesophilic reactors. In contrast, others affirm that the mesophilic reactor has the highest performance, with yields of up to 477 mL h⁻¹ compared to 79.5 mL h⁻¹ under thermophilic operation, representing nearly a six-fold increase in yield [34]. Other studies have highlighted the risks of over-acidification and trace element deficiencies when treating FV waste alone, recommending co-digestion with nutrient-rich substrates such as livestock and poultry manure [35], [36], [37].

It is also important to note that maintaining mesophilic conditions may not be feasible in the low-resource settings and energy-poor countries of SSA [38], [39]. Ambient conditions (25±2 °C), as investigated by Deago et al [40], have demonstrated a practical alternative to conventional, energy-intensive practices. In their study, biogas production at 25 °C was shown to meet considerable energy demands of up to 9,528 kWhmonth⁻¹, although this is lower than the 21,912 kWhmonth⁻¹ achieved under mesophilic conditions. The authors recommended co-digestion as a strategy worth evaluating at varying temperatures, based on the energy output at 25 °C. This supports the rationale for comparing co-digestion performance under both ambient and mesophilic conditions, particularly to assess trade-offs in biogas yield, energy recovery, and system feasibility for decentralized waste-to-energy solutions in the context of Eastern Africa.

In addition to technical considerations, an integral component of sustainable and integrated FVW, critical for the broader scaling up of AD systems, is public participation in source separation. One critical challenge in most developing countries remains the low perception and motivation among citizens to practice source waste separation [41]-[42]. Several factors contribute to the low source separation of municipal solid waste (MSW). These include low awareness and motivation among citizens, the absence of markets for recyclables and biodegradable materials, outdated laws, policies, and regulations, as well as the unavailability of suitable facilities and infrastructure, among others [43], [44], [45]. A potential solution lies in actively engaging youth as agents of change through primary, secondary, and university curricula, as well as launching intensive public education campaigns to demonstrate, in simple terms, the bioenergy potential of FVW [42], [46]. These measures are likely to reduce operational costs and improve FVW recovery at source in the long term.

Significantly, decentralized biogas systems could contribute towards achieving Sustainable Development Goal (SDG) 7: “Ensure access to affordable, reliable, sustainable and modern energy for all.” Despite global energy access rising to 92% in 2023, 666 million people still lack electricity, the majority of whom live in SSA, which continues to lag significantly behind [47]. Notably, approximately 85% of those without electricity live in rural areas of SSA [47], highlighting the urgent need for localized, renewable energy solutions such as small-scale biogas systems.

Digestate also referred to as effluent in wet systems is another valuable product from the anaerobic digestion that can be used as a soil conditioner [48]. However, it must meet the bio-fertilizer certification scheme for the World Health Organization (WHO), national and international standards, especially for ammonium, heavy metals, and sulfides [49], [50], [51], [52], [53]. Specifically, the heavy metal content of Cu, As, Cr, Cd, Zn, Fe, Mn, Ni, and Pb should be within the acceptable limits, as shown in **Table 1**. Various researchers have studied the use of AD digestate, including Liedl et al [54], Liedl et al [55], Tokuda et al [56], Liu et al [57], CCME [58], UNBS [59], and indicated that concentrations exceeding these limits may be detrimental or injurious to vegetation, animals, public health, or the environment due to long-term soil accumulation. Nevertheless, certain elements such as copper, cobalt, molybdenum, and zinc are essential plant micronutrients and are beneficial in digestate when present in appropriate amounts [58].

Despite extensive literature on the anaerobic co-digestion of fruit and vegetable waste with animal manure, most studies prioritize biogas production under controlled mesophilic conditions, with limited focus on the comparative energy sustainability assessment with scaling analysis and digestate quality assessment under ambient and mesophilic conditions within the framework of circular economy. Thus, the novelty of this study lies in the comparative assessment of anaerobic co-digestion of locally available substrates (FVW with cow dung) under ambient and mesophilic conditions using an Energy Sustainability Index (ESI)-driven approach, with a particular focus on how scaling analysis across varying digester sizes in an East African context influences biogas yield and quality, kinetics of biogas production, energy consumption, energy efficiency, and digester quality of AD systems in Sub-Saharan Africa. The ESI parameter is a comparative thermodynamic indicator defined as a ratio between the energy obtained in the form of H_2 and/or CH_4 and that spent as direct energy to heat and stir the fermenting broth at the working temperature and, when present, the energy spent as heat in the broth pretreatment [27]. Overall, this study answers a question that most AD studies (at least the ones reviewed in this manuscript) miss, i.e., “How can anaerobic Co-digestion systems be thermally and energetically configured to maximize net energy recovery using an Energy Sustainability Index (ESI)-driven approach?” The study aimed to offer insights into optimizing AD systems for large-scale use in Sub-Saharan Africa. It also examined their potential to solve FV waste management issues and support sustainable energy production.

Table 1: Heavy metal limits of anaerobic digestate for agricultural use

Element	Ugandan Standards/ Limit (mgkg ⁻¹)	Canadian Standards/ Limit (mgkg ⁻¹)	German Standards/ Limit (mgkg ⁻¹)
Lead (Pb)	100	150	75
Copper (Cu)	300	400	150

Zinc (Zn)	-	700	500
Chromium (Cr)	50	210	150
Nickel (Ni)	-	62	50
Cadmium (Cd)	5	3	3
Mercury (Hg)	2	0.8	3
Iron (Fe)	-	-	-
Arsenic (As)	10	13	-
Cobalt (Co)	-	34	25
Selenium (Se)	-	2	-
Molybdenum (Mo)	-	5	-
Reference	[59]	[58]	[60]

2. Materials and Methods

2.1 Study Area

The study was conducted at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK), Uganda in the Department of Agricultural and Biosystems Laboratory. MUARIK is located at a latitude of 0.450 and a longitude of 32.620 in Wakiso district, approximately 21 km north of Kampala city. MUARIK is part of the Lake Victoria basin, characterized by a typical tropical climate with average maximum temperatures of 28.5 °C during the dry season and minimum temperatures of 21.5 °C and occasionally 14 °C during the wet seasons [61]-[62]. It has a mean annual rainfall of approximately 1,200 mm, distributed in a bimodal pattern, with the short rains occurring between March and June, and the long rains from September to December [63].

2.2 Substrate and inoculum preparation

Fruit and vegetable waste were collected from vendors at Kalerwe Market in Kampala City, Uganda. The selected waste, including bananas, potatoes, mangoes, and tomatoes, was purposively chosen based on a preliminary survey and their significant contribution to waste generation in a previous study on supply chain fruit and vegetable post-harvest loss [5]. Specifically, the FVWs included bananas (whole fingers, including peels), potato tubers, mango fruits, and whole tomatoes. No stems, leaves, or non-edible portions were included in the collection to maintain consistency and to reflect typical consumer-level waste. The FVW collection was thus designed to carefully select the discarded portions of each category at the point of sale. A total of 40 kg of fresh waste was collected over a two-day period for use in the experiments. The collected FVW were manually categorized and sorted on a tarpaulin and then weighed using a Torrey® L-PCR 40 electronic scale. The wastes were then washed with clean tap water, mixed with a spade, and shredded to a particle size of 10 mm [64], using a hammer mill (Brook Crompton Series 2000 Model).

Cow dung, used as a co-substrate, was sourced from the zero-grazed dairy cows unit at MUARIK. Meanwhile, digestate from a 6 m³ tubular biodigester, which was actively digesting cow manure and also located on the farm in MUARIK, served as the inoculum. The inoculum was prepared according to the procedures described by Filer et al.[37], which involved

sieving through a 5 mm sieve before storing it in 20 L containers at ambient conditions for 48 hours prior to use. During this period, the inoculum was gently mixed manually once daily to prevent sedimentation and promote uniform microbial distribution. No additional pre-degassing or re-activation steps were performed, as the inoculum was already actively digesting cow manure. This stabilization period aimed to ensure consistent microbial activity.

2.3 Substrate and inoculum characterization

The substrates and inoculum were characterized for physicochemical parameters, including nitrogen, phosphorus, potassium, total and volatile solids, carbon, and pH. The selection of N, P, and K was informed by their relevance as major limiting nutrients in many regions of SSA [65]. At the same time, VS, carbon, and pH were included as the widely recognized major indicators of the degree of conversion at different AD steps, microbial activity, and the quality of the residual digestate [66]. The methods used to determine each of these parameters followed established protocols, as shown in **Table 2**. Notably, three one-kilogram samples of the substrate, inoculum, and digestate were collected in triplicate, immediately packed, transported in ice boxes, and stored in a deep freezer for later laboratory analysis.

Table 2: Standardized procedures used for characterization

Parameters	Tools and equipment	Reference
Nitrogen (N)	Kjeltech block digestion and steam distillation unit	[67]
Phosphorus (P) Potassium (K)	Hewlett-Packard 8452A Diode Array Spectrophotometer	[67]
Total solids (TS) and Volatile solids	Oven method, desiccator	[68]
Carbon (C)	Block digester tubes and the concentration	[67]
PH	Digital pH meter	[64]

2.4 Experimental setup

A 2×4 factorial treatment structure was used to determine the best formulation for the AD of FVW. The two factors were the mixing ratio and temperature. The substrate mix ratios (FVW: cow dung) had four levels: 100:0% (control), 75:25%, 50:50%, and 25:75%. The temperature had two levels: ambient (25°C) and mesophilic (35 °C). The ambient condition corresponded to average daily temperatures during most of the year in the experimental area and regions of SSA, thereby simulating a no-heating operational advantage for the rural scenario. The mesophilic condition served as a benchmark to assess the trade-offs in biogas yield, digestate quality, and energy recovery. The mesophilic digestibility tests were performed at a constant controlled temperature of 35 °C. Specifically, the temperature was maintained using a water-jacketed heating system fitted with an industrial temperature detector (PR-10-2-100-100-A-STAT), programmed with a setpoint of 35 °C and a hysteresis of ±0.1 °C, such that the heater is switched ON when the measured temperature fell below

34.9 °C and OFF when it exceeded 35.1 °C. The experiments were conducted at a substrate-to-inoculum (S/I) ratio of 0.25, as it is recommended to be < 0.5 [37].

Laboratory-scale batch reactors in triplicates with a total volume of 20 L and a working volume of 18 L were used. Each reactor had suitable arrangements for feeding, stirring, gas collection, and draining digestate. Upon loading the reactors with substrates in their desired mixing ratios, they were sealed with a rubber stopper and an aluminum cap. Manual stirring was done daily for an average of 210 s [69], [70] using an in-built stirrer to prevent phase formation.

The biogas yield and quality resulting from the anaerobic digestion of the wastes in each reactor were measured daily until the production curve of cumulative biogas yield against days plateaued. All treatments reached >95% of their maximum cumulative biogas yield by day 47, which corresponds to the hydraulic retention time (HRT) for the batch tests [71], [72]. Biogas production was measured using the liquid displacement method, where the volume of water displaced from the reactor corresponded to the volume of gas generated. The biogas volume was then adjusted to standard temperature and pressure (STP) conditions as it was done by Filer et al. [37] and Banks et al. [64]. The specific biogas yield (L/VS added) was determined by dividing the biogas volume by the quantity of volatile solids in each treatment [37]. The quality of biogas was determined by analyzing daily gas samples taken from the gas holder for methane and carbon dioxide content using a GA2000 portable infrared gas analyzer (Geotechnical Instruments, Leamington Spa, UK).

2.5 Kinetic Modelling of Biogas Production

To provide a quantitative description of the anaerobic digestion process and enable comparison of digestion performance among the different substrate mixtures, the cumulative biogas production data were analyzed using the modified Gompertz kinetic model (Equation 2.1), which is widely applied for describing batch biogas production behavior.

$$B(t) = P \exp\left\{-\exp\left[\frac{R_{\max}}{P} \left(\frac{1}{e} - t\right)\right]\right\} + \frac{P}{e} \quad (2.1)$$

Where,

$B(t)$ represents the cumulative biogas production at digestion time t (mL/g⁻¹VS)

P is the maximum cumulative biogas yield or biogas production potential (mLg⁻¹ VS)

$R_{\max}$ is the maximum biogas production rate (mLg⁻¹ VS day⁻¹)

$1/e$ represents the lag phase duration (days)

e -Euler's number (2.71828)

The modified Gompertz model was fitted to the cumulative biogas production data obtained from the different substrate treatments using nonlinear regression analysis. The performance of the model was evaluated using the coefficient of determination (R^2) and root

mean square error (RMSE). The estimated kinetic parameters provide insight into the biodegradability of the substrates, microbial adaptation period, and overall digestion efficiency.

2.6 Specific Methane Yield

To enable standardized evaluation of anaerobic digestion performance, the specific methane yield (SMY) was calculated and reported as the volume of methane produced per unit of volatile solids added ($\text{mL CH}_4 \text{ g}^{-1} \text{ VS added}$). This parameter represents the energy-producing fraction of the biogas and is widely used as a key indicator of substrate biodegradability and digestion efficiency. The specific methane yield was determined using the cumulative biogas production data and the average measured methane concentration obtained during the experimental period. The cumulative methane volume generated during digestion was calculated by multiplying the cumulative biogas production by the corresponding methane fraction in the produced biogas. The resulting methane volume was then normalized by the amount of volatile solids added to the digester. The calculation was performed using Equation 2.2:

$$SMY = \frac{V_{\text{biogas}} \cdot CH_4}{VS_{\text{added}}} \quad (2.2)$$

Where

SMY is the specific methane yield ($\text{mL CH}_4 \text{ g}^{-1} \text{ VS added}$)

V_{biogas} is the cumulative biogas production (mL)

CH_4 is the methane fraction in the produced biogas (% v/v) and

VS_{added} is the mass of volatile solids added to the digester (g)

The calculated specific methane yields were used to compare the performance of the different substrate mixtures and to evaluate the energy recovery potential of the anaerobic digestion process.

2.7 Digestate characterization

Samples of solid digestate were analyzed for nutrient parameters, including nitrogen, phosphorus, and potassium, following procedures already highlighted (**Table 2**). The levels of selected heavy metals in the digestate, including Zn, Cd, Cu, Fe, Ni, Cr, and Pb, were determined as indicated in **Table 3**.

Table 3: Heavy metals analyzed in the solid digestate in this study

Parameters	Technique and equipment	Reference
Copper (Cu)	Atomic absorption spectrophotometer	[73],[74]
Iron (Fe)	X-ray fluorescence Spectrophotometer	[73],[74]
Lead (Pb)	Atomic absorption spectrophotometer	[74]
Zinc (Zn)	Atomic absorption spectrophotometer	[74]
Nickel (Ni)	Atomic absorption spectrophotometer	[74]
Manganese (Mn)	Atomic absorption spectrophotometer	[74]

2.8 Energy analysis

The energy efficiency of the anaerobic digestion (AD) system was assessed using the Energy Sustainability Index (ESI), which compares the energy output from methane production to the energy input required to maintain optimal operating conditions in the digester [27]. The ESI analysis was conducted by integrating experimentally derived methane yield data using MATLAB R2021b. Specifically, the laboratory experiments were performed using 20 L batch reactors with a working volume of 18 L. Among the tested substrate combinations, the 50:50 mixture of FVW and cow dung operated under mesophilic conditions produced the highest methane yield. Therefore, the experimentally measured specific methane yield (SMY) obtained from this treatment was used as the reference parameter for estimating methane production and energy recovery in the scale-up simulation.

Figure 1 presents a schematic representation of the AD system in two configurations, illustrating how heat is transferred, retained, and lost within the system.

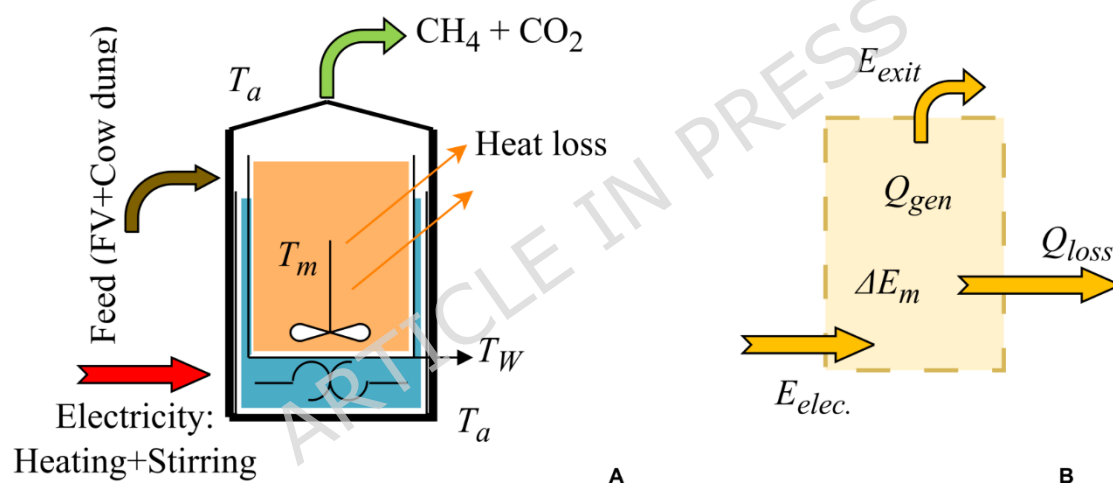


Figure 1: A- AD heated by water jacket (mesophilic condition), heat is supplied externally to maintain the optimal temperature range for the digestion process, and B- thermodynamic view of the AD system, showing the internal energy flows, heat exchange processes, and temperature gradients within the system, adapted from [27].

Where; Q_{Elec} is the external energy input that ensures the internal environment of the tank remains optimal for microbial activity. This includes the stirring energy, and the heat provided by the electric heater, which is used to maintain the desired thermal conditions inside the water jacket surrounding the digesting material. Simultaneously, Q_{gen} represents the heat that is generated internally as a result of the chemical decomposition of organic matter during the digestion process. On the other side of the equation, Q_{loss} accounts for the heat lost from the system to the surroundings, due to conduction through the tank walls and insulation. This term represents unavoidable thermal losses that reduce the overall energy

efficiency. The term ΔE_m captures the change in internal energy of the reacting mass; this reflects the energy absorbed or released as the temperature and state of the material change over time. Meanwhile, E_{exit} represents the total energy carried out of the system by the produced gases, which include both methane (CH_4) and carbon dioxide (CO_2).

Figure 2 illustrates energy balance and heat transfer considerations for the AD system. Part A (Left) illustrates the energy balance, detailing the relationship between energy inputs, such as electrical energy for heating and stirring, and energy outputs, such as the chemical energy contained in the produced biogas. The stirring power requirement used in the analysis was 80 W m^{-3} of digester volume [75]. However, this value may underestimate the stirring power consumption of AD systems with volumes smaller than 0.5 m^3 , as mechanical losses in the stirring system may become comparable to the required mixing power. In small-scale experimental reactors such as those used in this study, mixing was performed manually. To estimate the stirring energy consumption, the average daily stirring time of 210 s observed during the experiment was used, as recommended Zhai et al., [69], and [70].

In the laboratory experiment, mixing was performed manually for approximately 210 s day^{-1} , and therefore no direct electrical energy was consumed for stirring. However, for the MATLAB-based scaling simulations, an equivalent mechanical stirring energy was estimated using a power density of 80 W m^{-3} , which is commonly reported for anaerobic digestion mixing systems [75]. This power density was combined with the experimentally observed mixing duration of 210 s day^{-1} to estimate the electrical energy required for stirring in the simulated reactors. It should be noted that for very small digesters (diameter $< 1 \text{ m}$), the 80 W m^{-3} assumption may underestimate actual mechanical mixing requirements due to mechanical inefficiencies. However, such small digesters in decentralized systems are often manually mixed, meaning that the adopted assumption likely represents a conservative estimate of the electrical energy required for stirring. Consequently, at the lower end of the simulated reactor size range (diameter $< 1 \text{ m}$), the assumed stirring energy may slightly influence the calculated ESI values because the relative contribution of stirring energy to the total energy balance becomes more significant. However, in decentralized systems where manual mixing is commonly practiced, the actual electrical energy consumption for mixing would be minimal, meaning that the ESI values presented in this study represent a conservative estimate of system sustainability. Other auxiliary power needs, such as feeding and pumping, have been neglected. They account for a small quantity of the overall energy, as they are not continuous processes.

To evaluate the potential influence of these excluded energy inputs, a sensitivity analysis was performed using literature-based estimates for auxiliary operations and construction material energy intensities. The results of this analysis, presented in Supplementary **Table S4**, indicate that although inclusion of auxiliary energy inputs slightly reduces the calculated

ESI values, the overall trends and conclusions of the study remain unchanged, confirming the robustness of the energy sustainability assessment.

It is important to distinguish between E_{exit} and E_{out} . While E_{exit} represents the combined chemical energy carried by both methane (CH_4) and carbon dioxide (CO_2), only methane contributes to usable energy because CO_2 has no calorific value. Therefore, E_{out} represents the useful energy extracted from methane and is calculated based on the lower heating value of methane (LHV_{CH_4}). The useful energy output from methane production is estimated using Equation 2.3.

$$E_{\text{out}} = V_{\text{CH}_4} \cdot \text{LHV}_{\text{CH}_4} \quad (2.3)$$

Where

V_{CH_4} is the methane volume produced (m^3)

LHV_{CH_4} is the lowest heating value of methane (35.8 MJ m^{-3})

The methane volume generated in the simulation was estimated using the experimentally determined specific methane yield using Equation 2.4.

$$V_{\text{CH}_4} = \text{SMY} \cdot \text{VS} \quad (2.4)$$

Where

SMY is the specific methane yield obtained from the experiment ($\text{m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{VS}$)

VS is the mass of volatile solids introduced into the digester

To evaluate the overall energy efficiency and sustainability of the system, the Energy Sustainability Index (ESI) was defined as the ratio between useful methane energy output and the electrical energy input required to maintain the digester operation as indicated by Equation 2.5.

$$ESI = \frac{E_{\text{out}}}{E_{\text{elec}}} \quad (2.5)$$

Where

E_{out} is the useful methane energy output

E_{elec} is the electrical energy input required for heating and mixing

A higher value of the ESI indicates a more sustainable and energy-efficient system, where the energy recovered from methane production exceeds the energy required to operate the digester.

Part B (Right) of Figure 2 illustrates the heat transfer view of the AD system, showing the temperature distribution within the digester and the surrounding insulation layers. Key parameters influencing the thermal performance include the ambient temperature (T_a), the mesophilic operating temperature (T_m), the internal reactor radius (r_1), and the insulation thickness of the digester walls and jacket. These parameters influence the thermal mass of

the reactor and the heat transfer surface area, thereby affecting heat loss and the efficiency of the heating system.

To evaluate the impact of reactor size on energy sustainability, different digester geometries were simulated in the MATLAB model. The digester diameter (D_1) was varied from 0.5 m to 4 m, corresponding to reactor volumes ranging approximately from 0.1 m³ to 52 m³. For each reactor size, the corresponding tank volume and volatile solids (VS) mass were computed and used to estimate methane production and energy output using the experimentally derived methane yield coefficient.

The MATLAB simulations assumed batch reactor operation, consistent with the experimental digestion system. The hydraulic retention time (HRT) was fixed at 47 days, corresponding to the digestion duration used in the laboratory experiments. The organic loading rate (OLR) applied in the simulation was derived from the experimental substrate loading and expressed as the ratio of volatile solids added to the reactor working volume. These parameters were used to estimate methane generation and energy output for each simulated digester size. The analysis assumed batch operation, consistent with the experimental digestion system, and the digestion duration used in the simulation corresponded to the experimental digestion period. Additionally, the thickness of the insulation walls, including the top and bottom insulation layers, was varied between 10 mm and 100 mm in order to evaluate its effect on the overall heat transfer efficiency of the system.

The detailed modelling assumptions used in the ESI computation, including insulation material properties and associated costs (Supplementary Table S1), flow-type heat transfer coefficients (Supplementary Table S2), steel thermal conductivity (Supplementary Table S3), and sensitivity analysis results (Supplementary Table S4), are provided in the Supplementary Material.

(A)

$$E_{Elec.} + Q_{gen} = Q_{loss} + \Delta E_m + E_{exit}$$

$$E_{exit} \neq E_{out}$$

$$E_{out} = f(LHV_{CH_4})$$

$$E_{exit} = E_{exit,CH_4} + E_{exit,CO_2}$$

$$Sus_index = \frac{E_{out}}{E_{Elec.}}$$

$$E_{Elec.} = E_{Heating} + E_{Stirring}.$$

(B)

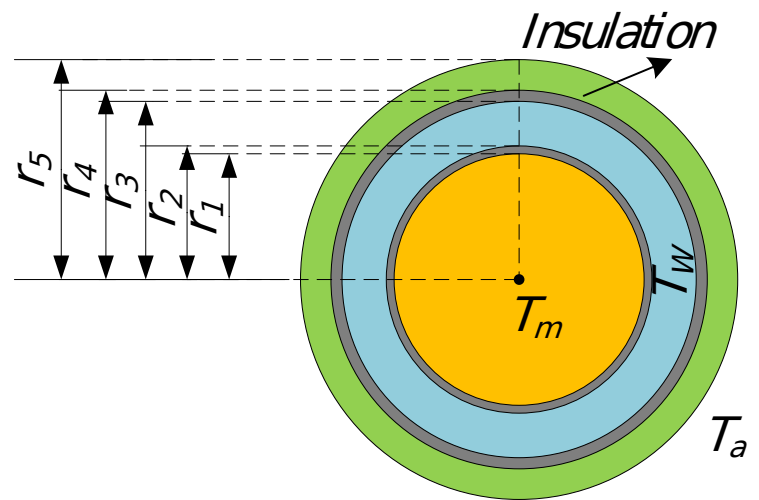


Figure 2: A (Left)- Energy balance and Sustainability index computation, B (Right)- Heat Transfer view, where T_a is the ambient temperature, T_m is the mesophilic temperature, and r_1 is the Internal radius [m] of the reactor.

2.9 Data analysis

The substrates were characterized before and after the process to analyze the compositional differences resulting from the AD. All experiments and analyses were performed in triplicate, and the results were presented as means with standard deviations. Biogas yield readings were adjusted to STP conditions of 273.15 K and 101.325 kPa to ensure standardized results. Total biogas yield curves were generated by dividing the cumulative biogas volume (adjusted to STP) by the quantity of volatile solids added in each treatment [37]. A two-way analysis of variance (ANOVA) was performed at a 5% significance level using R Studio software version 4.13 to statistically analyze biogas yields from each of the different substrate mixtures. Significant means were compared using Tukey's Honestly Significant Difference (HSD) Test at $p < 0.05$. This was done to determine if the main effects (mixing ratio and temperature) and their interaction significantly affected biogas yield. Using MATLAB version R2021b with the Refprop 10.0 library for thermodynamic properties, the experimental data obtained were simulated to examine the energy balance as a function of reactor diameter for scaling up purposes and associated sustainability index. These tools were used to analyze the relationship between system scaling and energy efficiency, and to assess the associated sustainability index, thereby understanding the system's performance under varying conditions.

3. Results and discussion

3.1 Characterization of feedstock

Table 4 shows the physicochemical properties of FVW, cow dung, and the inoculum. The feedstock's total solids (TS) varied from 8.92% to 15.53% on a Dry Basis (% DB). The TS of 11.24% of FVWs is relatively lower than the 15% DB reported by Dhanalakshmi et al.[76], 19.54% reported by Pavi et. al [77] and the 27% reported by Salehiyoun et al.[78], but greater than the 8.3% reported by Bouallagui et al. [79]. The TS of FVW, however, is close to 10.89% reported by Miramontes-Martínez et al.[72]. This slight variation in the results could be due to the different fruit and vegetable cultivars employed in these experiments. Based on these results, the substrates were suitable for biogas production since the TS content ranged from 5 to 25% DB [80] and [81].

The methane yield is highly related to the amount of biodegradable volatile solids (VS) in a substrate [77]. The VS of all substrates was relatively high (79.63-88.13% DB), indicating a high fraction of organic matter that can be converted into biogas by the microorganisms, as also postulated by Asquer et al.[82] and [83]. The higher VS concentration in cow dung is

likely due to its enrichment with microbial seed due to partial digestion in the rumen [84], [85], [86].

Regarding pH, FVW showed the highest acidity (4.57), compared to 6.88 for cow dung, but was close to 4.66 for FVW in Brazil [77]. The recommended pH range for anaerobic digestion is 6.8–7.2, and the growth rate of methanogens is significantly reduced below pH 6.6 [87]. Conversely, an excessively alkaline pH can lead to the disintegration of microbial granules and subsequent process failure [87], [88]. The low pH of FVW in this study suggests that the anaerobic digestion of FVW alone may result in rapid acidification, potentially suppressing and inhibiting the activity of methanogenic bacteria [79]. Therefore, co-digestion with cow dung can contribute to increasing alkalinity and controlling a potential acidification during FVW digestion. Co-digestion can also help provide essential nutrients, improving AD kinetics, and removing the inhibitory effects of ammonia and long-chain acids [89], [90].

The carbon-to-nitrogen (C/N) ratio of mixed FVW (34:1) was highest, a result similar to the 34:1 obtained by Ohenewa [91] but higher than the 22:1 reported by Salehiyoun et al. [78] in Iran and Asquer et al. [82] in Italy. This variation could be explained by the inclusion of a variety of fruits and vegetables in their study, such as cauliflower, celery, eggplant, fennel, leeks, melons, peaches, pears, peppers, pineapples, and both American and Italian potatoes. The C/N ratio of the substrates is an essential factor in the anaerobic digestion process. Bouallagui et al. [79]; Ahmad et al., [90]; Dadaser-Celik et al. [92] reported that the optimum C/N ratio for biogas production should range from 25 to 30. In this study, the C/N ratio of the mixed FVWs was higher than 24:1 for cow dung. It is, therefore, expected that the co-anaerobic digestion of FVW and cow dung could improve the physicochemical properties (C/N balance) and may give a synergistic effect that can result in a higher production rate of the biogas [93], [94].

Table 4: Physicochemical properties of substrates

Parameter*	Mixed FVW	Cow dung	Inoculum
Total solids (% DB)	11.24[0.86 ^b	15.53[0.86 ^{ba}	8.92[0.51 ^a
Volatile solids (% DB)	79.63[0.67 ^a	88.13[0.78 ^b	82.44[1.31 ^{ac}
pH	4.57[0.82 ^b	6.88[0.10 ^b	7.26[0.29 ^{ab}
Carbon content (% DB)	34.58[2.81 ^a	54.05[1.97 ^b	49.45[0.64 ^c
Nitrogen (% DB)	1.01[0.43 ^b	2.27[0.26 ^b	3.25[0.43 ^b
Phosphorous (% DB)	0.22[0.03 ^a	0.62[0.01 ^b	0.53[0.21 ^{ba}
Potassium (% DB)	1.01[0.08 ^a	0.89[0.06 ^b	0.72[0.02 ^{ba}
C/N	34.09	23.81	15.21

Values are mean $\pm$ SD of triplicates on each sample. Means in the same row with different superscripts are significantly different at $p \leq 0.05$

*DB refers to dry matter basis

3.2 Biogas production

Figure 3a-b shows the cumulative biogas profiles for the substrate combinations evaluated over 47 days under mesophilic and ambient conditions. Initially, no biogas was produced in any of the batch treatments, likely due to the acclimatization of microbes to the new environment, a characteristic of the lag phase. This is attributed to the balance between the amounts of bacteria present and essential nutrients available, such as proteins and trace elements [37], [95].

Treatments with only FVW had the lowest cumulative biogas yields, at $79.4 \text{ mLg}^{-1}\text{VS}$ during the first 15 days. This could be associated with the accumulation of volatile fatty acids (VFAs), which likely caused the partial inhibition of methanogens as proposed by Li et al. [96]. In contrast, co-digestion of FVW with cow dung significantly improved biogas yields across all batch treatments, suggesting improved nutrient balance upon co-digestion of these wastes, which enhances microbial activity.

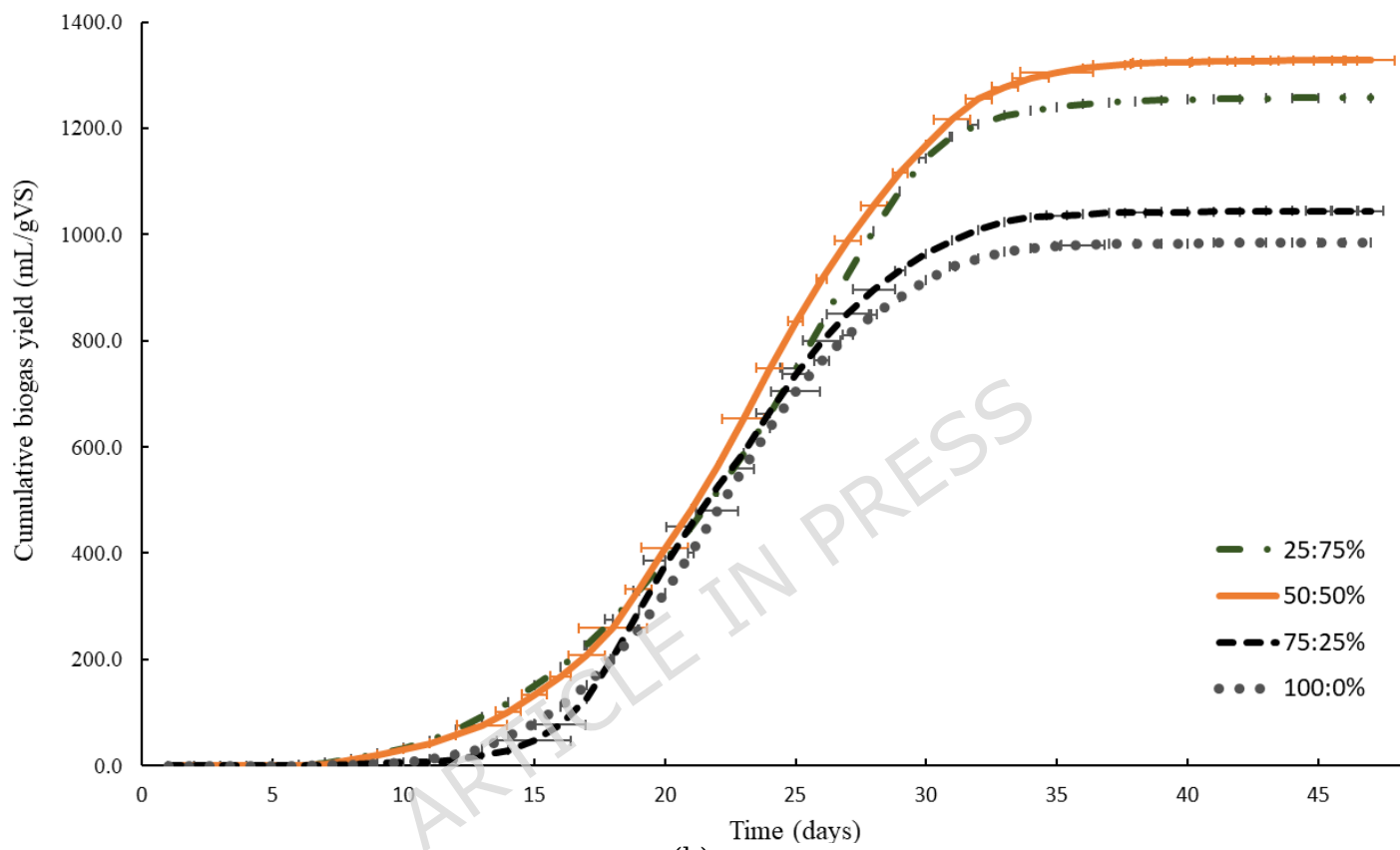
The mixing ratios of FVW and temperature showed a significant effect on biogas yield ($p < 0.05$). The co-digestion ratio of 50%:50% of FVW to cow dung yielded the highest cumulative biogas yield of $1328.4 \text{ mLg}^{-1}\text{VS}$ and $1125.6 \text{ mLg}^{-1}\text{VS}$ at mesophilic and ambient temperatures respectively. Regarding temperature, the two-way ANOVA revealed a significant interaction effect between temperature and mixing ratio on cumulative biogas yield ($F(3,16) = 5.87$, $p \leq 0.05$), suggesting that the response of biogas production to substrate composition varied under different temperature regimes. Total biogas production was greatest at mesophilic temperature, with a statistically significant difference, as illustrated in Figure 3a-b. This can be attributed to the improved nutrient balance, a synergistic effect of microorganisms, and an increased organic matter load [72],[97]. From a practical perspective, this indicates that while mesophilic conditions maximize biogas yield, the 50:50 mixing ratio remains effective under ambient conditions. This is particularly relevant for low-resource settings where temperature control is limited, supporting the feasibility of ambient anaerobic digestion systems with optimized substrate combinations.

The superior performance of the 50:50 (FVW: cow dung) mixture at mesophilic temperatures underscores the importance of co-digestion, as well as the critical need to maintain proper temperature. Nevertheless, the biogas yields observed in this study are from laboratory-scale experiments, reflecting the maximum methane potential under controlled conditions. In real-world applications, lower yields are expected due to operational challenges such as feedstock variability, mixing inefficiencies, and environmental fluctuations. Therefore, while the batch results offer valuable insights for feedstock selection and formulation as well as operational temperature, continuous operation at pilot or full scale will require further validation.

The results obtained in this study for FVW mono-digestion are comparable with previous findings, which have reported values ranging from 160 to $954 \text{ mLg}^{-1}\text{VS}$ [79], [98], [99], [72].

This is mainly attributed to factors such as waste composition, reactor design, and operating temperature [79], [98]. Similarly, the co-digestion results are comparable with the 1294 and 1352 mLg⁻¹VS range reported by Miramontes-Martínez et al. [72] for FVW co-digested with slaughter house manure.

(a)



(b)

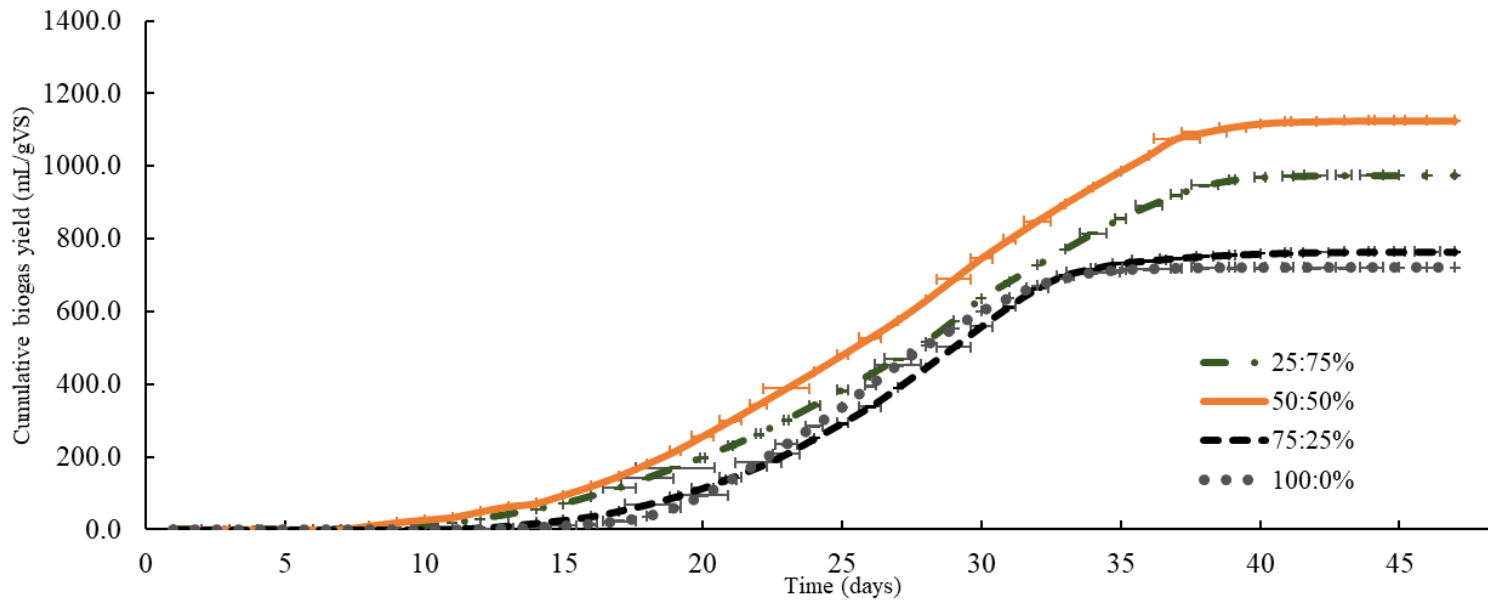


Figure 3: Cumulative biogas profiles for the substrate combinations under (a) mesophilic conditions and (b) ambient conditions

3.3 Kinetic Modelling of Biogas Production

The kinetics of cumulative biogas production under ambient (Table 5) and mesophilic (Table 6) temperature conditions were analyzed using the modified Gompertz model, which is widely used to describe microbial growth and gas production dynamics in batch anaerobic digestion systems. The model allows estimation of key kinetic parameters including the maximum cumulative biogas production potential (P), the maximum biogas production rate (R_{max}), and the lag phase duration (λ). These parameters provide insight into the digestion performance and microbial adaptation under different substrate mixing ratios and temperature regimes.

The modified Gompertz model provided an excellent fit to the experimental cumulative biogas production data under both temperature conditions. The coefficients of determination (R^2) ranged from 0.995 to 0.998 under ambient conditions and 0.994 to 0.999 under mesophilic conditions, indicating that the model adequately captured the typical sigmoidal pattern of cumulative biogas production in batch anaerobic digestion processes. The relatively low root mean square error (RMSE) values obtained for both temperature regimes further confirmed the reliability of the model in predicting the experimental data.

Table 5: Modified Gompertz kinetic parameters for cumulative biogas production under ambient conditions

Treatment	P (mL)	R_{max} (mLd ⁻¹)	λ (days)	R^2	RMSE
A (25:75)	7199.03	315.85	16.49	0.996	92.14
B (50:50)	8184.70	344.39	15.54	0.996	88.63
C (75:25)	5080.48	340.17	18.98	0.995	97.51
D (100:0)	4454.73	377.95	19.24	0.998	84.27

Under ambient temperature conditions, the estimated biogas production potential (P) ranged from 4454.73 mL to 8184.70 mL across the different substrate mixing ratios. The 50:50 FVW:cow dung mixture (Treatment B) exhibited the highest production potential (8184.70 mL), followed by Treatment A (7199.03 mL). Treatments with higher proportions of fruit and vegetable waste (Treatments C and D) produced comparatively lower cumulative biogas yields. The maximum biogas production rate ($R_{\max}$) varied between 315.85 and 377.95 mL d⁻¹, while the lag phase duration ranged from 15.54 to 19.24 days. The longer lag phases observed in Treatments C and D suggest slower microbial acclimation and possible inhibition effects associated with higher proportions of fruit and vegetable waste, which may lead to rapid acidification and reduced buffering capacity in the digestion system.

Table 6: Modified Gompertz kinetic parameters for cumulative biogas production under mesophilic conditions

Treatment	P (mL)	$R_{\max}$ (mLd ⁻¹)	/ (days)	R ²	RMSE
A (25:75)	8571.46	537.65	15.03	0.994	121.48
B (50:50)	8571.46	583.75	15.32	0.997	96.32
C (75:25)	6673.82	553.25	15.87	0.999	84.95
D (100:0)	6314.92	510.67	15.76	0.999	79.62

Under mesophilic temperature conditions, overall digestion performance improved across all substrate combinations. The estimated biogas production potential ranged from 6314.92 mL to 8817.59 mL, with the 50:50 mixture again producing the highest cumulative biogas potential (8817.59 mL). The maximum biogas production rates were also significantly higher under mesophilic conditions, ranging from 510.67 to 583.75 mL d⁻¹, reflecting enhanced microbial metabolic activity at elevated temperatures. In addition, the lag phase durations under mesophilic conditions were shorter and more consistent across treatments (15.03–15.87 days), indicating faster microbial adaptation compared with ambient digestion.

Comparison of the two temperature regimes shows that mesophilic digestion generally resulted in higher biogas production potentials, faster production rates, and shorter lag phases compared with ambient digestion. These findings highlight the beneficial role of controlled temperature in enhancing microbial activity and improving digestion efficiency. Nevertheless, the relatively strong performance observed under ambient conditions suggests that anaerobic co-digestion of fruit and vegetable waste with cow dung can still produce substantial biogas yields without external heating. This finding is particularly relevant for decentralized biogas systems in resource-limited settings where temperature control may not be feasible. Therefore, the kinetic modelling results confirm that the 50:50 mixing ratio of FVW and cow dung provides the most favorable digestion performance under both temperature regimes, likely due to improved nutrient balance, buffering capacity, and synergistic microbial activity between the two substrates.

3.4 Specific Methane Yield

The specific methane yield (SMY) of the fruit and vegetable waste (FWV)–cow dung mixtures was evaluated under both ambient (**Table 7**) and mesophilic (**Table 8**) digestion conditions to assess the influence of substrate composition and operating temperature on methane production efficiency. Under mesophilic conditions, the specific methane yield ranged from 162.4 to 255.0 mL CH₄ g⁻¹ VS across the different substrate mixtures. The highest methane yield was observed for the 50:50 FWV: cow dung mixture (Treatment B), which produced 255.0 mL CH₄ g⁻¹ VS, followed by the 25:75 mixture (Treatment A) with 237.0 mL CH₄ g⁻¹ VS. In contrast, the mono-digestion treatments, particularly the 100:0 mixture (Treatment D), produced the lowest methane yield (162.4 mL CH₄ g⁻¹ VS). The improved methane production observed in the co-digestion treatments can be attributed to the synergistic interaction between fruit and vegetable waste and cow dung, which improves nutrient balance, buffering capacity, and microbial activity in the digestion system. Cow dung provides essential nutrients, trace elements, and an active microbial inoculum that facilitate efficient degradation of the readily biodegradable organic matter present in fruit and vegetable waste.

Table 7: Specific methane yield of fruit and vegetable waste-cow dung mixtures under mesophilic conditions

Treatment	Mean Cumulative biogas (mL)	Methane (%)	VS added (g)	Methane Volume (mL)	Specific methane yield (mL CH ₄ g ⁻¹ VS)
A (25:75)	8172.9	64.5	20	5271.5	263.6
B (50:50)	8501	60.8	20	5168.6	258.4
C 75:25)	6402.3	59.7	20	3822.2	191.1
D (100:0)	6126.7	58.3	20	3571.9	178.6

VS-volatile solids

Table 8: Specific methane yield of fruit and vegetable waste-cow dung mixtures under ambient conditions

Treatment	Mean Cumulative biogas (mL)	Methane (%)	VS added (g)	Methane Volume (mL)	Specific methane yield (mL CH ₄ g ⁻¹ VS)
A (25:75)	7199	61.8	20	4449.0	222.4
B (50:50)	8184.7	60.5	20	4951.7	247.6
C 75:25)	5080.5	59.1	20	3002.6	150.1
D (100:0)	4454.7	55.8	20	2485.7	124.3

VS-volatile solids

A similar trend was observed under ambient temperature conditions (**Table 8**), although methane yields were generally lower than those obtained under mesophilic conditions. The specific methane yield under ambient conditions ranged from 111.4 to 233.3 mL CH₄ g⁻¹ VS, with the 50:50 substrate mixture again producing the highest methane yield (233.3 mL CH₄ g⁻¹ VS). The 100:0 treatment produced the lowest methane yield (111.4 mL CH₄ g⁻¹ VS), indicating that mono-digestion of fruit and vegetable waste may lead to reduced methane

production due to rapid acidification and limited buffering capacity in the absence of cow dung.

Comparison of the two operating conditions shows that mesophilic digestion improved methane production across all substrate mixtures, demonstrating the importance of temperature in enhancing microbial metabolic activity and substrate degradation efficiency. Nevertheless, the relatively high methane yields obtained under ambient conditions suggest that anaerobic co-digestion of fruit and vegetable waste with cow dung can still generate substantial methane without external heating. This observation is particularly relevant for decentralized and small-scale biogas systems in developing regions, where maintaining controlled mesophilic temperatures may be technically challenging or economically infeasible. Overall, the results indicate that the 50:50 mixing ratio of fruit and vegetable waste and cow dung provides the most favourable conditions for methane production under both temperature regimes. The balanced composition of this mixture likely promotes optimal microbial growth and stable digestion conditions, thereby enhancing methane generation and overall process performance.

3.5 Biogas composition

The percentage of methane represents the quality of biogas produced. **Figure 4a-b** shows the concentration of methane and other components, namely carbon dioxide, ammonia, and hydrogen sulfide, under mesophilic and ambient conditions. The methane content ranged from 55.8% to 64.5%, with significantly higher values ($p \leq 0.05$) observed in treatments with higher proportions of cow dung. On the other hand, when FVW was the predominant substrate, methane quality significantly lowered ($p < 0.05$). The lower methane content of FVW can be attributed to its relatively high C/N ratio of 34.09 (see **Table 4**), which hinders optimal microbial activity. This underscores the need for co-digestion of FVW with wastes such as cow dung with relatively lower C/N ratio, which can adjust nutrient content and methane quality, as based on other studies [28], [89], [100].

The variations in CO_2 and H_2S concentrations across treatments were not statistically significant ($p > 0.05$), indicating relatively stable levels regardless of substrate composition. The results for concentrations of CH_4 and CO_2 observed in this study fall within the ranges commonly reported for biogas (50 to 75% for methane and 25 to 45% for carbon dioxide)[101]. Levels of H_2S (11.2 ppm) on average in FVW alone at ambient conditions and 7.6 ppm on average at mesophilic conditions did not exceed the Environmental Health Hazard Assessment (EHHA) risk management trigger levels (22 ppm for H_2S and were still well below the lower action level (216 ppm for H_2S)) [102].

While no immediate risks were identified, routine monitoring of biogas hydrogen sulfide (H_2S) content is advisable, as elevated levels of H_2S and ammonia can compromise biogas quality, accelerate corrosion in Combined Heat and Power (CHP) systems, and increase the

demand for gas purification technologies such as desulphurization and ammonia stripping. Therefore, the scale-up of anaerobic digestion technologies using FVWs, particularly in Eastern Africa, necessitates targeted training and capacity-building for plant operators, engineers, and technical personnel. This ensures the proper management of feeding ratios, post-treatment, and supports the practical relevance and successful integration of these solutions into real-world biogas applications.

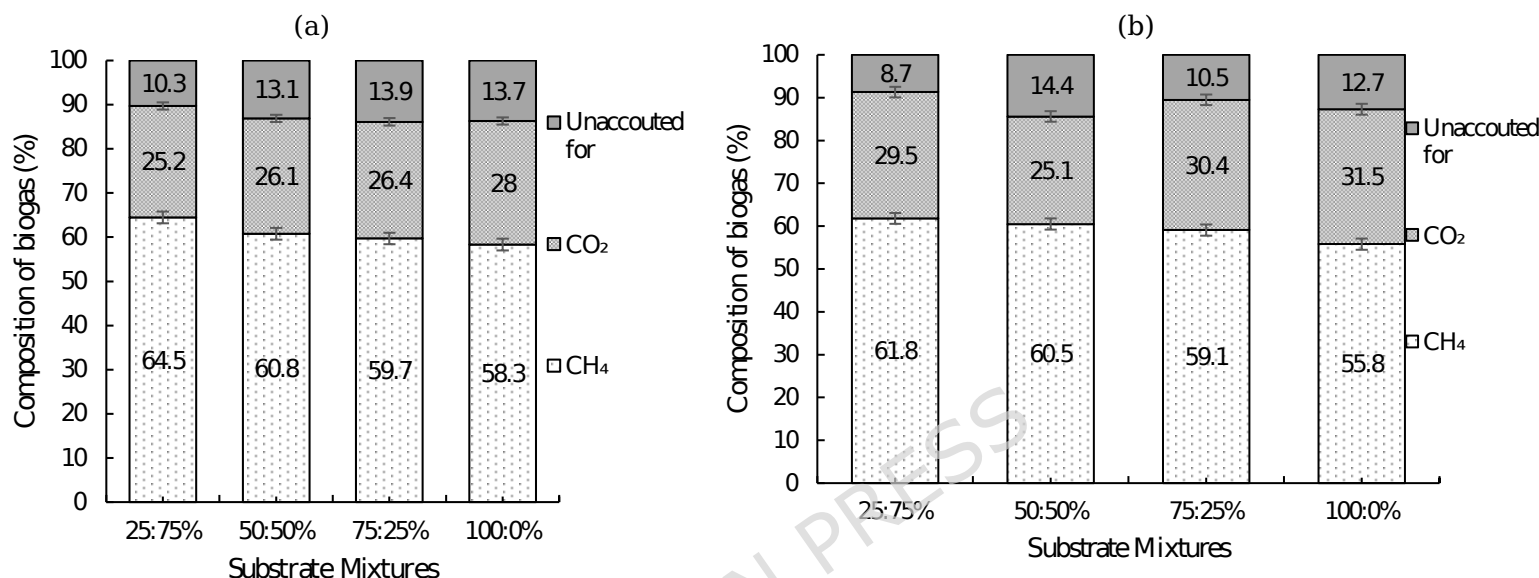


Figure 4: Major component concentrations by volume in biogas at (a) mesophilic conditions and (b) ambient conditions

3.6 Digestate characteristics

3.6.1 Macro-nutrient content in the digestate

The concentration of three macronutrients, namely NPK, could potentially influence the quality of the digestate as a soil amendment for commonly grown food crops. **Table 9** shows the average nutrient content (N-P-K) of the digestate. The results indicate a significant difference ($p < 0.05$) in the N content of the digestate samples, suggesting that the N composition of the digestate varies depending on both the feedstock and the digestion process. Thus, the judicious selection of feedstocks and careful control of digestion process is important to generate digestate with properties that could be tailored for useful application, including soil conditioning. Generally, FVW with more cow dung and at ambient temperature had higher nitrogen content. At ambient temperature, the mixture containing 25% FVW and 75% cow dung had the highest nitrogen concentration, measured at 79.46 g kg⁻¹. This concentration was significantly higher ($p < 0.05$) than the 57.34 g kg⁻¹ recorded when FVW was the main component of the mixture. The results of N content in the FVW digestate are comparable to those obtained by [103], where the authors reported N content ranged from 36 g Kg⁻¹ to about 70 g kg⁻¹.

Concerning the phosphorus content in the digestate samples, it varied from 13.42 g kg⁻¹, where FVW was the predominant substrate, to 15.34 g kg⁻¹, where cow dung was the primary substrate (6). Similar values, ranging from approximately 3.6 gkg⁻¹ to 31 g kg⁻¹, were reported in the digestate by Di et al. [103]. The statistical analysis, however, showed no significant differences ($p > 0.05$) among the mixing ratios under both ambient and mesophilic conditions. This suggests that phosphorus remained relatively stable in the digestate regardless of the fruit and vegetable waste to cow dung ratio and digestion conditions.

In contrast, higher K content was obtained in the proportions where cow dung was predominant (Table 6). The K content varied from 9.71 to 25.55 g kg⁻¹. Generally, the variation in the macronutrients (N, P, and K) reflects the nutrient content in the raw waste streams and the extent to which these nutrients remain in the residue during and at the end of the AD processes [104], [105].

Table 9: Nutrient (N-P-K) content of the digestate samples, (g kg⁻¹, dry matter basis)

Mixing ratio (FVW: Cow dung)	N (gkg ⁻¹)	P (gkg ⁻¹)	K (gkg ⁻¹)
Ambient Conditions			
100:0	57.34±2.40 ^a	13.42±0.93 ^c	9.71±0.76 ^b
75:25	61.23±1.65 ^b	14.50±0.03 ^c	11.01±0.66 ^b
50:50	73.23±3.00 ^c	15.34±1.08 ^c	20.32±0.85 ^c
25:75	79.46±2.98 ^d	13.68±1.00 ^c	25.55±0.05 ^d
Mesophilic Conditions			
100:0	60.86±1.28 ^{ae}	13.82±0.77 ^c	10.24±0.42 ^b
75:25	63.10±0.37 ^{bf}	15.17±0.23 ^c	11.68±0.19 ^b
50:50	64.56±0.90 ^{fg}	15.34±0.08 ^c	20.32±0.05 ^c
25:75	73.37±0.77 ^h	13.75±0.20 ^c	24.14±0.74 ^d

Means in the same column with different superscripts are significantly different at $p \leq 0.05$

3.6.2 Organic carbon (OC) (%).

Figure 5 shows the values for organic carbon in the digestate in this study. Organic carbon values ranged from 11.99% to 17.67%. There were no significant differences ($p < 0.05$) in mean OC recorded between all the digestate types (mixing ratios) for the temperature conditions studied at a 5% level of significance. Notably, there was a reduction in the carbon content of the feedstock and digestate. This reduction was probably due to the transformation of carbon into CO₂ and CH₄ during biogas production [106].

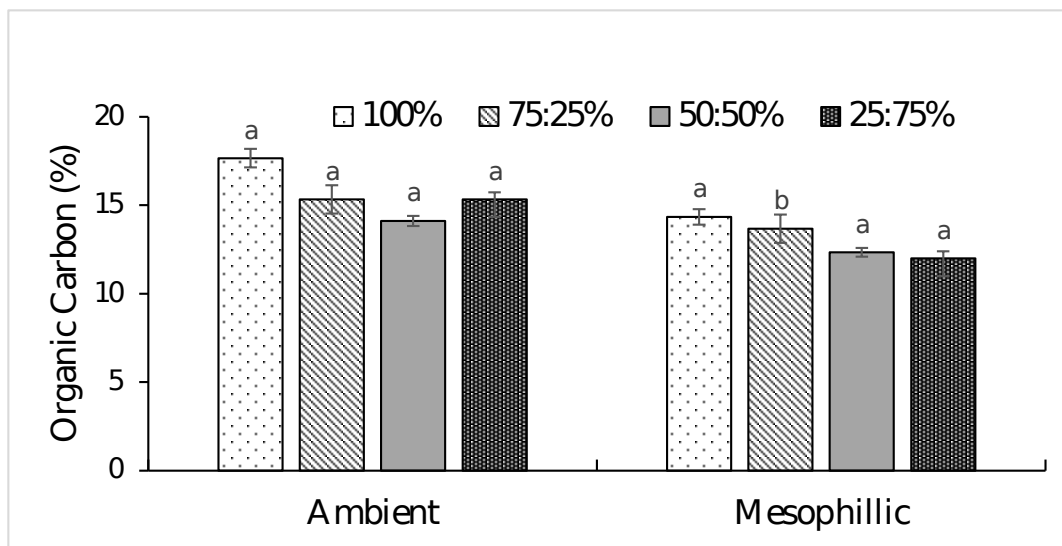


Figure 5: Organic Carbon content of the digestate

3.6.3 Heavy metal analysis

The heavy metal (Cu, Zn, Pb, Fe, Ni, and Mn) content of the solid digestate samples is shown in 7. Lead (Pb) concentrations were below the detection limit. Ni was detected in solid digestate samples where FVW was predominant, with a concentration of less than 0.5 mgkg^{-1} . The Cu content was detected in all the solid digestate samples, with a concentration below 0.33 mgkg^{-1} , which is lower than the 4.2 mgkg^{-1} reported by Di et al. [103]. According to Di et al.[103], as volatile solids degrade, they release various trace elements, such as Copper (Cu). Thus, the higher Cu content concentration in the FVW solid digestate can be attributed to the removal of volatile solids. It is essential to mention that the metal contents were below and within the range of compost quality guidelines and standards used by the Uganda National Bureau of Standards [59] (for agriculture), the European Union, and the USA (for biosolids) [60] and the Canadian Council of Ministers of the Environment [58] guidelines for compost quality for unrestricted use (**Table 1**).

However, a key limitation of this study is the absence of a complete heavy metal analysis, particularly for elements such as arsenic (As) and chromium (Cr), as well as a complete characterization of the microbial community, which is regulated under national and international compost and biofertilizer quality standards. As such, the current findings do not guarantee the hygienization of digestate and its long-term environmental safety when applied to agricultural land. **Therefore, compliance with international biofertilizer standards should be limited to the analyzed heavy metals.** Further studies of the FVW solid digestate could be characterized for a broader range of heavy metals and microbial communities, as specified in biofertilizer standards. This will help advance understanding of both the effectiveness and the potential challenges associated with using FVW digestate as a biofertilizer.

Table 10: Heavy metal content of the solid digestate samples

Mixing ratio (FVW: Cow dung)	Heavy metal content (mgkg ⁻¹)					
	Cu	Zn	Pb	Ni	Fe	Mn
Ambient Conditions						
100:0	0.25± 0.01 ^a	0.66± 0.03 ^a	nd	0.42± 0.10 ^a	49.52±0.51 ^a	7.56±1.94 ^a
75:25	0.22± 0.00 ^a	0.82± 0.01 ^b	nd	0.32± 0.02 ^b	45.41±0.99 ^a	5.03±0.21 ^a
50:50	0.02± 0.00 ^b	2.34± 0.26 ^c	nd	nd	88.54±0.97 ^b	4.09±0.47 ^{ab}
25:75	0.06± 0.02 ^c	3.47± 0.03 ^d	nd	nd	101.09±6.34 ^c	3.22±0.18 ^b
Mesophilic Conditions						
100:0	0.33± 0.09 ^a	0.72± 0.10 ^e	nd	0.42± 0.10 ^a	49.52±0.51 ^a	7.56±1.94 ^a
75:25	0.07± 0.03 ^b	0.96± 0.02 ^f	nd	0.61± 0.12 ^c	24.23±2.79 ^d	7.20±1.57 ^a
50:50	0.02± 0.00 ^c	0.51± 0.04 ^a	nd	nd	88.60±1.64 ^b	3.67±0.45 ^b
25:75	0.20± 0.03 ^a	3.30± 0.22 ^d	nd	nd	101.09±6.25 ^c	3.84±0.67 ^b

*nd stands for not detected. Means in the same column with different superscripts are significantly different at $p \leq 0.05$

3.7 Energy efficiency and sustainability analysis

Figure 6 shows the energy balance as a function of different tank volumes, ranging from 4 m³ to 52 m³. This volume range is a result of the tank diameters (0.5 to 4 m) selected for the parametric analysis, which was intended to explore scaling effects on system performance. **Figure 6** highlights the interplay between energy inputs and outputs as affected by system size. It can be noted that as the tank volume increased, both heat dissipation and biogas production were impacted. Larger tank sizes necessitate increased energy input to maintain mesophilic temperature conditions, yet the corresponding energy output in biogas is also increased [107], [108]. This creates a dynamic relationship where larger systems have a higher biogas yield but may also experience higher thermal losses, necessitating more energy input to compensate for heat dissipation. This observation is consistent with findings by Malave et al.[27], which highlighted the magnitude of such systems' energy efficiency and sustainability. Only 3 out of 15 cases in that study were energetically efficient, highlighting the critical importance of conducting such analyses and evaluating the sustainability index.

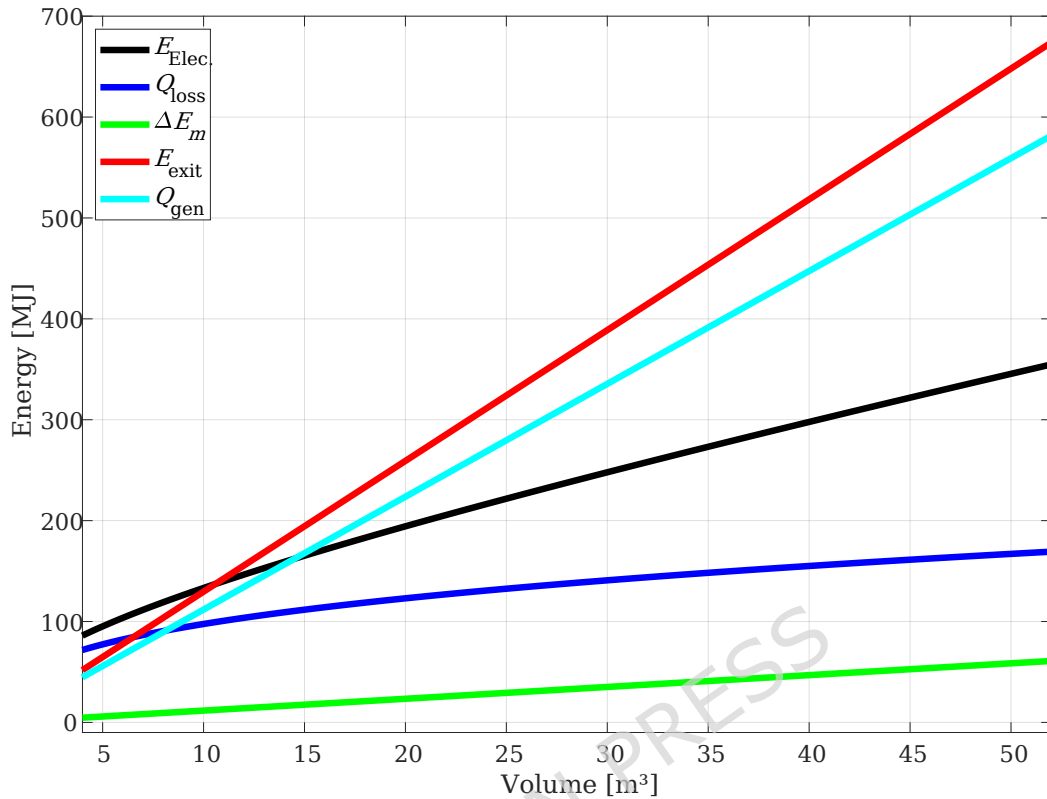


Figure 6: Energy balance as a function of the AD volume

Figure 7 shows the Sustainability Index as a function of tank volume, with and without insulation. It is important to note that a value greater than 1 indicates that the energy produced exceeds the energy consumed in digestion. From **Figure 7**, it can be evident that the inclusion of insulation is observed to significantly impact the system's thermal efficiency, enhancing heat retention and reducing energy losses during the AD process, **with ESI values exceeding unity ($ESI > 1$) under insulated conditions, indicating net positive energy output.** This observation is consistent with Godara et al. [109], who reported that techniques such as insulation can reduce heat loss by up to 82% and mitigate internal temperature fluctuations, thereby improving AD efficiency. The results of this study indicate that the system is thermodynamically favorable, with a net positive energy output. Such findings underscore the importance of closely examining the thermal balance of the AD process, as excessive energy production can have significant implications for process optimization and efficiency in large-scale implementations. However, it should be noted that the analysis relied on idealized assumptions including constant insulation properties (Table S1), and heat transfer coefficients (Table S2). In practice, insulation performance may degrade over time, while convective heat losses can vary with environmental conditions. Moreover, the embodied energy, construction energy, as well as auxiliary operation energy for feeding and

pumping were not accounted as explained in Section 2.6. Despite these limitations, which may overestimate net energy recovery, the consistent application of assumptions across the scenarios studied ensured that the relative comparison between insulated and non-insulated systems remains robust.

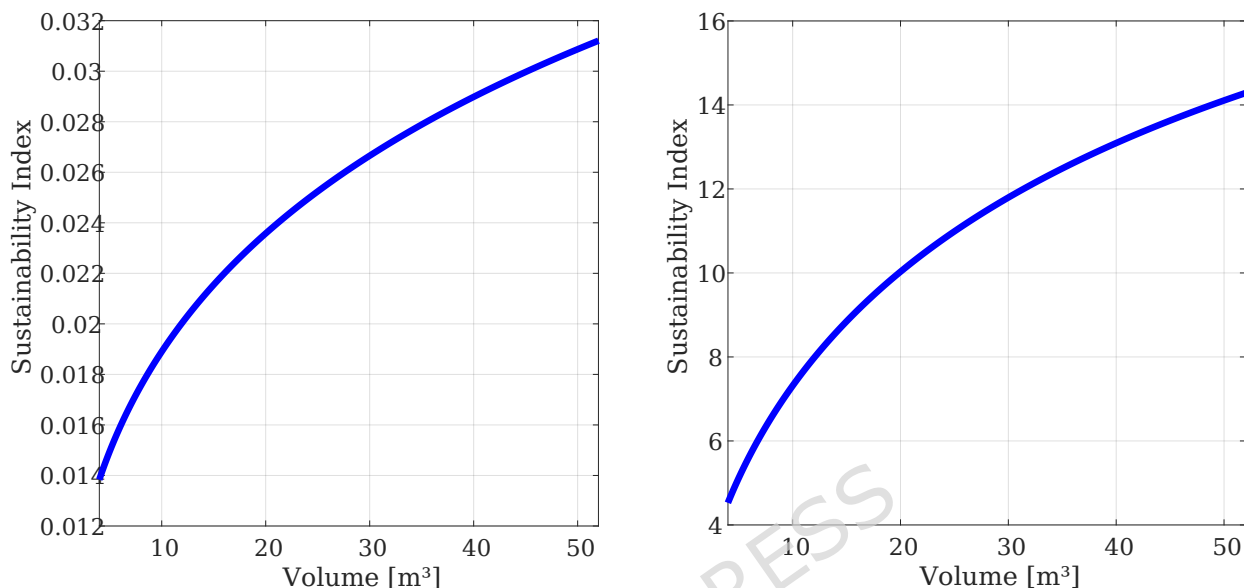


Figure 7: Sustainability Index as a function of AD volume with insulation (left) and without insulation (right)

From the energy recovery perspective, this study showed the importance of optimizing the thermal balance of the anaerobic digestion (AD) process, particularly for large-scale reactor applications. The integration of insulation and strategic tank sizing was found to improve both the energy yield and operational efficiency, thus enhancing the overall **thermodynamic efficiency** of the AD system. However, it is worth noting that the energy and sustainability analyses conducted in this study were intended to evaluate theoretical energy recovery and thermal behavior under idealized operating assumptions. While the modeled scenarios incorporate realistic parameters such as insulation thickness, reactor size, and stirring energy, they do not account for all operational inefficiencies encountered in field-scale systems. Consequently, the results should be interpreted as indicative of relative energy performance and design sensitivity, rather than as exact projections of field-scale energy output. Moreover, the sustainability index values presented here represent laboratory-informed, thermodynamically derived performance indicators. Actual field-scale performance will depend on site-specific factors including construction quality, operational control, feedstock variability, and maintenance practices.

Furthermore, this study did not explore potential regional variations and the economic feasibility assessment associated with scaling the AD system in different contexts. A pertinent case study in Ethiopia [110] demonstrated the financial and economic viability of a 10 m³ AD co-digesting cow dung, maize straw, and vegetables, reporting a benefit-cost

ratio of 1.30 to 1.38 and an economic benefit-cost ratio ranging from 5.49 to 6.01 over 15 years. Risk assessment results further confirmed the strong economic feasibility of a smallholder farm-based AD implementation. Future research should thus assess the financial feasibility of large-scale fruit and vegetable AD systems in East Africa, temperature adaptability experiments in different climate zones, and average seasonal temperatures, co-digestion with other organic wastes, and long-term operational stability testing at the pilot scale, including real-world energy demands and production such as continuous stirring, pumping, and feed handling. These additional parameters and precision will increase the Energy Sustainability Index (ESI) accuracy.

Although this study highlights the ESI, it should be noted that it is a decision tool, **not a comprehensive life-cycle assessment tool, as it does not take into account AD fabrication, end-of-life, or the source of energy and other resources. Further research** is needed to examine how these findings could influence policy development and contribute to global goals on waste reduction, renewable energy production, and environmental sustainability. Understanding the broader implications of FVW recycling practices could guide policymakers in creating frameworks that support sustainable waste management solutions. Additionally, this study did not include thermal or chemical pretreatment, reflecting realistic, energy-poor conditions in SSA. Future research could explore the potential of low-energy pretreatment methods such as mechanical shredding or enzymatic hydrolysis. This is because these techniques may improve substrate biodegradability and biogas yield while maintaining minimal energy input, thereby enhancing the Energy Sustainability Index (ESI) in decentralized applications.

Overall, the findings indicate that FVW generated during the market stage of the supply chain holds significant potential for anaerobic digestion, offering a sustainable solution for mitigating greenhouse gas emissions, producing renewable energy, and generating organic fertilizers. This aligns with the "zero waste" philosophy, emphasizing the "three Rs" rule: reduce, reuse, and recycle. These results have important implications for farmers, researchers, and policymakers, urging them to adopt FVW recycling practices as part of broader efforts to improve waste management and sustainable agricultural practices. Lastly, this work directly supports Sustainable Development Goal (SDG) 7: "Ensure access to affordable, reliable, sustainable, and modern energy for all" by demonstrating the energy recovery potential, particularly for the rural areas of sub-Saharan Africa, where biogas systems utilizing co-digested FVW can address pressing energy access challenges.

4. Conclusion and Limitations

This study investigated the optimal formulation of fruit and vegetable waste to maximize biogas yield and improve digestate quality, using four mixing ratios and two temperature conditions. The results demonstrate that anaerobic co-digestion of fruit and vegetable waste

with cow dung can deliver simultaneous energy recovery and nutrient recycling when evaluated through a system-level energy perspective relevant to energy-constrained contexts. Using batch anaerobic digestion as an upper-bound performance benchmark, the results show that a 50:50 FVW-cow dung mixture provides the most favorable balance between total biogas production, methane quality, and digestate nutrient content under both ambient and mesophilic conditions. At mesophilic temperature, this formulation achieved a total biogas yield of 1,328.4 mL g⁻¹ VS with a methane concentration of approximately 60.5%. The solid digestate from this combination also demonstrated valuable nutrient content, with nitrogen (N), phosphorus (P), and potassium (K) levels of 64.6, 15.3, and 20.3 gkg⁻¹, respectively and selected heavy metal content (Cu, Zn, Pb, Ni, Fe, Mn) in the digestate met biofertilizer quality standards set by the Uganda National Bureau of Standards. **However, because the study did not include microbial analysis and key heavy metal tests such as As, Hg, Se, and Cr, which are essential for biosafety, its suitability for field application remains preliminary and conditional, pending a thorough chemical and microbiological evaluation. Therefore, compliance with international biofertilizer standards should be limited to the analyzed heavy metals.**

Beyond biogas yield optimization, the study advances understanding of net energy sustainability index in anaerobic digestion by explicitly coupling biogas production with thermal balance, reactor sizing, and insulation effects. The energy sustainability index analysis highlights that system configuration, particularly insulation thickness and digester scale, plays a decisive role in determining whether mesophilic operation yields a net positive energy output. These findings underscore that temperature control alone does not guarantee energy efficiency; rather, an integrated thermal and geometric design approach is essential for achieving energetically viable digestion systems.

The results further indicate that ambient-temperature operation, while associated with lower absolute biogas yields, remains a technically viable option for decentralized AD systems where external heat input is limited or unavailable. In such contexts, co-digestion-driven improvements offer promising opportunities to enhance biogas production. However, this study did not evaluate how co-digestion affects microbial activity, buffering capacity, or degradation kinetics. Future research should examine these factors to better understand their impact on process stability and substrate conversion rates, offering valuable insights for scaling up anaerobic co-digestion.

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Competing Interests

The authors declare no conflict of interest in this work

Data Availability Statement

The authors declare that the data supporting the findings of this study are available within the article (however, datasets generated during computations, standardization and analysis are available from the corresponding author).

Author contributions

All authors contributed to the study conception and design. **Isa Kabenge:** Conceptualization, Methodology, Validation, Writing-original draft, Fund Acquisition and Project Administration. **Allan J. Komakech:** Conceptualization, Writing-original draft, Supervision, Review & editing. **Isaac Rubagumya:** Conceptualization, Experimentation, Writing-original draft, review & editing. **Simon Savio Kizito:** Conceptualization, Writing original draft, review & editing. **Hossein Ahmadisedigh:** Conceptualization, Supervision, Fund Acquisition, Project Administration, Writing - review & editing. **Emmanuel Menya:** Conceptualization, Writing-original draft, review & editing; **Julien Lepine:** Conceptualization, Writing original draft, review & editing and **Ahamada Zziwa:** Conceptualization, Writing-original draft, review & editing.

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