

VARIATION EFFECTS OF FOOD-WASTE SUBSTRATE COMPOSITION ON ANAEROBIC CO-DIGESTION UNDER MESOPHILIC CONDITIONS

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ABSTRACT: Food waste (FW) is increasingly recognized as an environmental challenge due to its high moisture content, rapid perishability, and heterogeneous composition, which render it unsuitable for landfilling, composting, or direct use as animal feed. This raises the need to identify and implement a treatment technique that can significantly reduce FW. Anaerobic digestion (AD) offers an effective biological route to stabilize FW while producing renewable biogas. This study investigates the interactive effects of three cooked FW substrates—cooked chicken (protein-rich), cooked rice (carbohydrate-rich), and cooked vegetables (fiber-rich)—on biogas generation under mesophilic conditions. Three reactors with varying substrate ratios were operated at 37 °C for 30 days. Reactor R3, containing the highest proportion of protein (40 %), produced the greatest average daily biogas yield (145.3 ± 5.36 mL d⁻¹) and cumulative production ($4,358 \pm 161$ mL), exceeding R1 and R2. Balanced protein levels enhanced buffering capacity and promoted stable methanogenesis, whereas carbohydrate- and vegetable-dominant mixtures in R2 experienced early acidification, leading to markedly lower gas yields. The findings demonstrate that substrate composition strongly influences hydrolysis, pH stability, and methane potential during AD. These results provide practical guidance for optimizing FW mixtures in industrial-scale AD systems to maximize biogas output and improve waste-treatment efficiency.

KEY WORDS: Food Waste, Inoculum Sludge, Anaerobic Co-Digestion, Biogas Yield, and Mesophilic.

1. INTRODUCTION

The rapid growth of global populations and the intensification of agricultural and food-processing activities have resulted in a continual rise in organic waste generation. Food waste (FW), which represents a major portion of municipal solid waste, is characterized by high moisture levels, rapid decomposition, and heterogeneous biochemical composition, making it incompatible with composting, landfilling, or animal feed applications [1]. Consequently, the sustainable treatment of FW has become a priority for waste-management systems worldwide, as it is contributing significantly to greenhouse-gas emissions and resource depletion if not properly managed.

Anaerobic digestion (AD) is widely regarded as an eco-friendly technology and a viable solution for FW management due to its capacity to convert biodegradable material into biogas while simultaneously reducing the volume and environmental footprint of organic waste [2]. AD of FW has been extensively studied at laboratory scale and is increasingly implemented in full-scale industrial operations owing to its stability, environmental benefits, and ability to recover renewable energy [2-4]. It should be noted, however, that most previous studies have used either raw food waste alone or specific mixtures, whereas co-digestion of cooked food waste substrates has been less explored.

The biochemical composition of FW strongly influences AD performance. Substrates rich in proteins and lipids typically yield higher methane yields than carbohydrate-rich materials [4,5]. Maintaining a balanced microbial environment during anaerobic digestion can be achieved by incorporating adequate levels of lipids and proteins into the feedstock. This synergy enhances process stability and significantly boosts methane yield, up to 43% higher than typical expectations, by improving microbial adaptation and optimizing kinetic performance under mesophilic conditions [5]. However, high lipid concentrations may generate long-chain fatty acids (LCFAs), which can inhibit methanogens by adsorbing onto cell surfaces and disrupting mass transfer processes [6,7]. LCFA inhibition has been reported when lipid content exceeds 31–47 % on a COD basis or approximately 65 % on a VS basis [6,8]. Carbohydrate-rich materials hydrolyze rapidly, generating volatile fatty acids (VFAs) that may reduce pH and inhibit methanogenesis if not adequately buffered [9,10]. In contrast, protein-rich substrates release ammonia upon hydrolysis, which can stabilize pH but may cause inhibition at excessive concentrations [10]. The AD of cooked vegetable-dominant food waste FW presents unique challenges, primarily due to its high moisture content, rapid hydrolysis, and tendency toward acidification, which can inhibit methanogenic microbes [11-12]. Cooked vegetable waste degrades rapidly, leading to high accumulation of volatile fatty acids (VFAs), which can cause "acidification" and system failure [13-14].

Due to these contrasting behaviors, the overall effectiveness of FW digestion is highly sensitive to substrate composition and synergistic interactions among FW fractions [15–16]. Anaerobic co-digestion (AcoD), the simultaneous digestion of multiple substrates, enhances biogas yields, reduces lag phases, and improves microbial stability by balancing nutrient availability and diluting inhibitory compounds [3,15–17].

Accordingly, this study explores the mesophilic anaerobic co-digestion of three commonly consumed cooked food substrates—chicken as a protein source, rice as a carbohydrate source, and mixed vegetables as a fiber source—in combination with inoculum sludge. The investigation focuses on how variations in substrate composition affect biogas generation dynamics and examines key physicochemical parameters, including pH, total solids (TS), total volatile solids (TVS), ash content, and moisture content. By understanding these interactions, the research aims to provide insights to guide the optimization of substrate ratios in industrial-scale food-waste digestion systems, thereby enhancing energy recovery and ensuring process stability.

2. MATERIALS AND METHODS

2.1. Materials

The equipment used in this study includes ceramic crucibles, 500 ml bioreactor flasks, a water bath, a pH meter, an S316 multi-gas detector, an analytical balance, 500 cm³ graduated cylinders, an oven, and a muffle furnace.

2.2. Inoculum and substrates

Anaerobic inoculum sludge (IS) was collected from an anaerobic treatment pond at Sime Darby Plantation, Bestari Jaya, Selangor, Malaysia. Cooked chicken (CC), cooked rice (CR), and cooked vegetables (CV: cucumber, cauliflower, and cabbage) were obtained from the cafeteria at International Islamic University Malaysia (IIUM), Gombak.

Each substrate was homogenized in a domestic blender with distilled water at a 1:2 (v/v) ratio (substrate:water), which is the dilution typically used for laboratory digestion. The mixtures (Fig. 1) were stored at approximately 6 °C until used. The characteristics of these substrates are shown in Table 2.

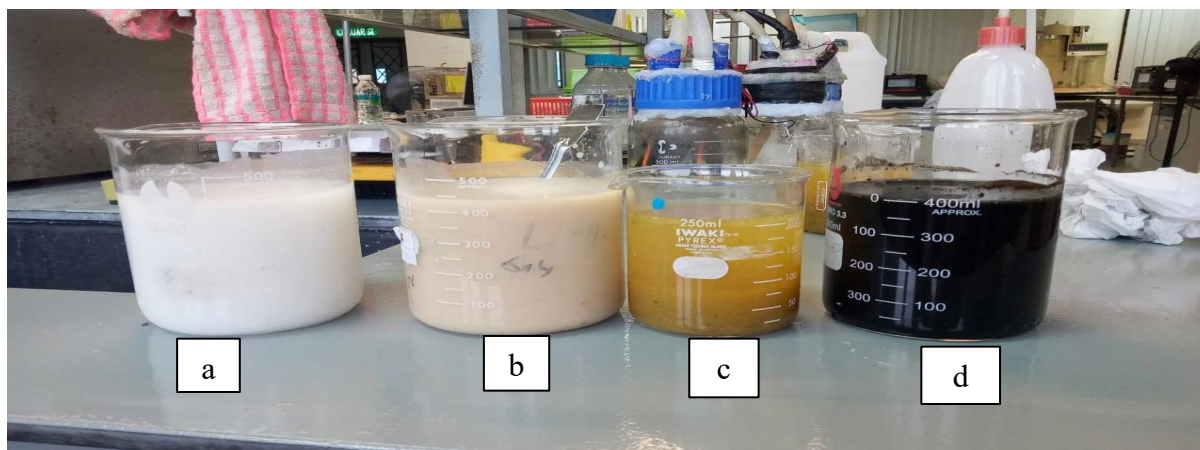


Fig. 1. Appearance of diluted substrates: (a) cooked rice, (b) cooked chicken, (c) cooked vegetables, and (d) inoculum sludge.

The FW mixtures were fed into three 500 mL anaerobic reactors (R1–R3) according to Table 1, maintaining a total of 400 mL of FW and 100 mL of inoculum (20% of the working volume).

Table 1: Composition of reactors used for anaerobic co-digestion experiments

Reactor	Carbohydrates (CR) [mL]	Proteins (CC) [mL]	Vegetables (CV)[mL]	Total FW [mL]	Inoculum [mL]
R1	200	120	80	400	100
R2	120	80	200	400	100
R3	80	200	120	400	100

2.3. Experimental Design

Batch anaerobic digestion was performed in triplicate using 500 mL Schott-bottle reactors placed in a thermostatic water bath maintained at 37 ± 1 °C for 30 days, representing

mesophilic conditions (Fig. 2). Each reactor was sealed with a rubber stopper and equipped with a gas-collection line leading to a water-displacement system for volumetric biogas measurement.



Fig. 2. Experimental anaerobic digestion setup maintained at 37 °C.

2.4. Process-Parameter Measurements

Analyses of total solids (TS), total volatile solids (TVS), ash content and moisture content were determined gravimetrically following APHA Standard Methods (2017, 23rd edition). pH was measured using a calibrated handheld digital pH meter [18]. Biogas volume was recorded daily via the water-displacement method. Gas composition was determined using an S316 Multi-Gas Detector (Henan Zhongan, China) [19].

2.5. Biogas-Production Kinetics

The kinetics of biogas generation were evaluated using the Modified Gompertz Equation (Eq. (1)), which describes cumulative gas production as a function of digestion time [19,20]:

$$M_{(t)} = M_0 \cdot \exp \left\{ -\exp \left[\frac{R_{max} \cdot e}{M_0} (\lambda - t) + 1 \right] \right\} \quad [1]$$

Where:

$M_{(t)}$: the cumulative methane yield (mL/g VS) at the anaerobic digestion time t (d);

M_0 : the methane potential maximum production (mL/g VS);

R_{max} : the maximum methane production rate (mL/g VS^{-d});

λ : the lag phase (d);

t : the duration of the assay (d); and

e : the Euler's number (2.71828).

This model enables assessment of microbial adaptation, maximum production potential, and specific growth rate of methanogens during AcoD.

3. RESULTS AND DISCUSSION

3.1. Substrate Characterization

The physicochemical characteristics of cooked chicken (CC), cooked rice (CR), cooked vegetables (CV) and inoculum sludge (IS) are summarized in Table 2. These properties determine the initial conditions under which hydrolysis, acidogenesis, and methanogenesis occur. CR and CV exhibited notably low initial pH values (4.66 and 3.69), indicating a

tendency toward rapid acidification upon hydrolysis, whereas CC, with a pH of 6.20, provided a more neutral starting condition. The TS and TVS values also varied considerably, with CR having the highest TS (682 g L⁻¹), and CV the lowest (297 g L⁻¹). Protein-rich CC presented elevated TVS levels (499 g L⁻¹), reflecting its high organic content. These differences in substrate characteristics explain, at least in part, the subsequent variations in biogas production and stability observed across reactors. High-moisture CV, for example, is associated with rapid VFA release, whereas CC tends to generate ammonia during hydrolysis, which helps buffer the digestion environment [9,10].

Table 2: Physicochemical characteristics of substrates

Parameter	CC	CR	CV	IS
TS (g L ⁻¹)	219.60	272.80	118.80	170
TVS (g L ⁻¹)	199.60	262.40	108.80	165.60
Moisture content (%)	16.22	10.74	28.80	23.77
Ash (%)	23.93	29.26	9.28	16.22
pH	6.20	4.66	3.69	7.39
Density (g L ⁻¹)	1072.52	995.34	1024.93	939.56

(CC = cooked chicken; CR = cooked rice; CV = cooked vegetables; IS = inoculum sludge)

The results confirm that FW composition was chemically diverse, ensuring a range of substrates for hydrolysis and subsequent methanogenesis. The inoculum/substrate ratio (ISR) is equal to 0.2899 (< 1). This result is adaptable to laboratory-scale experiments.

3.2. Biogas Production

Daily biogas production patterns for the three reactors are shown in Fig. 3, demonstrating that substrate composition had a pronounced influence on system performance.

Reactor R3, which contained the highest proportion of protein, delivered the most consistent output, peaking above 350–400 ± 5.36 mL during the first few days and maintaining relatively high levels until around day 10. After this point, production gradually declined, yet small amounts of gas continued to be generated throughout the 30-day period, indicating strong process stability. In contrast, Reactor R1 began with a sharp initial surge of approximately 360 ± 6.16 mL on day 1, but its output dropped steadily after day 4 and slowed markedly by day 10. By day 18, biogas generation had fallen to minimal levels, suggesting early depletion of easily degradable components and reduced microbial activity. Reactor R2 showed the weakest performance overall; although its first-day peak was similar to the others at about 369 ± 3.09 mL, production collapsed almost immediately. By day 4, gas generation had nearly ceased, and for the remainder of the digestion period, output was negligible—a clear indication of rapid acidification and methanogen inhibition caused by insufficient buffering capacity.

The average daily gas production varied among the three reactors. Reactor R3, containing the highest protein proportion (40 %), produced substantially higher daily biogas compared with R1 and R2, reaching an average of 145.3 ± 5.36 mL day⁻¹. This enhanced performance can be attributed to protein hydrolysis, which increases buffer capacity through ammonia release, thereby preventing excessive acidification and promoting methanogenic activity [9]. Reactor R1, with a moderate protein content (24 %), exhibited a correspondingly moderate biogas profile, averaging 84.4 ± 6.16 mL day⁻¹. In contrast, R2

produced the lowest biogas volume, at only $36.8 \pm 3.09 \text{ mL day}^{-1}$, primarily due to its high vegetable fraction and low buffering capacity. The dominance of CV and CR in R2 likely led to rapid VFA accumulation during the early stages of digestion, thereby lowering pH and inhibiting methanogenesis. This behavior aligns with previous reports indicating that carbohydrate- and fiber-rich mixtures tend to acidify quickly, delaying methane production and reducing total yield [11,13].

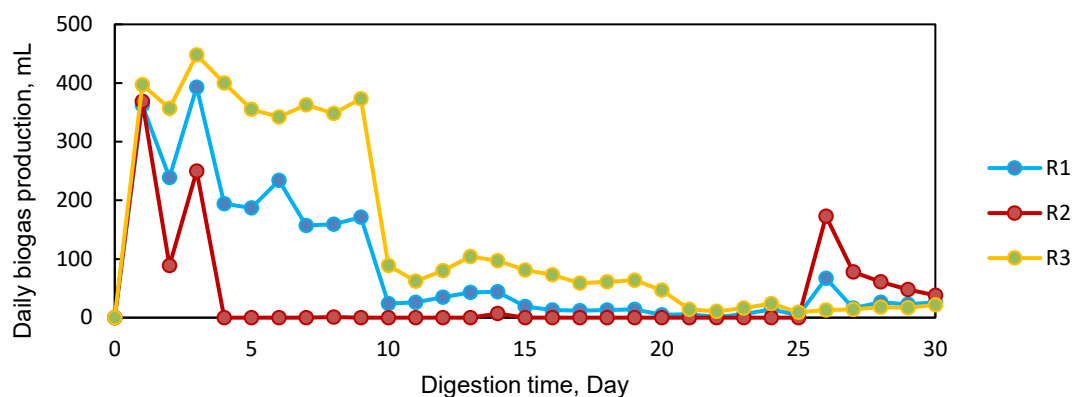


Fig. 3. The production of biogas from each reactor.

The cumulative biogas production shown in Fig. 4 highlights that reactor R3, which contained the highest proportion of protein (40 % v/v), achieved the greatest performance, with an average daily biogas output of $145.3 \pm 5.36 \text{ mL d}^{-1}$ and a cumulative yield of $4,358 \pm 161 \text{ mL g}^{-1} \text{ VS}$ over 30 days. This superior performance is attributed to the balanced nutrient availability and buffering capacity of protein-rich substrates, which enhance methanogenic stability and prevent excessive acidification. Reactor R1, containing a moderate protein fraction (24 %), recorded a lower average daily yield of $84.4 \pm 6.16 \text{ mL d}^{-1}$ and a cumulative total of $2,533 \pm 184 \text{ mL g}^{-1} \text{ VS}$, suggesting that reduced nitrogen content limited buffering capacity and slowed methanogenic activity. In contrast, Reactor R2, with the lowest protein proportion (16 %), exhibited the poorest performance, producing only $36.8 \pm 3.09 \text{ mL d}^{-1}$ on average and $1,104 \pm 92.79 \text{ mL g}^{-1} \text{ VS}$ cumulatively. The low protein content in R2 likely resulted in insufficient nitrogen for microbial growth and limited alkalinity to counteract volatile fatty acid accumulation, leading to partial acid inhibition. These findings confirm that protein-enriched substrates promote higher biogas yields within the safe concentration range, consistent with the synergistic effects reported in [8,14].

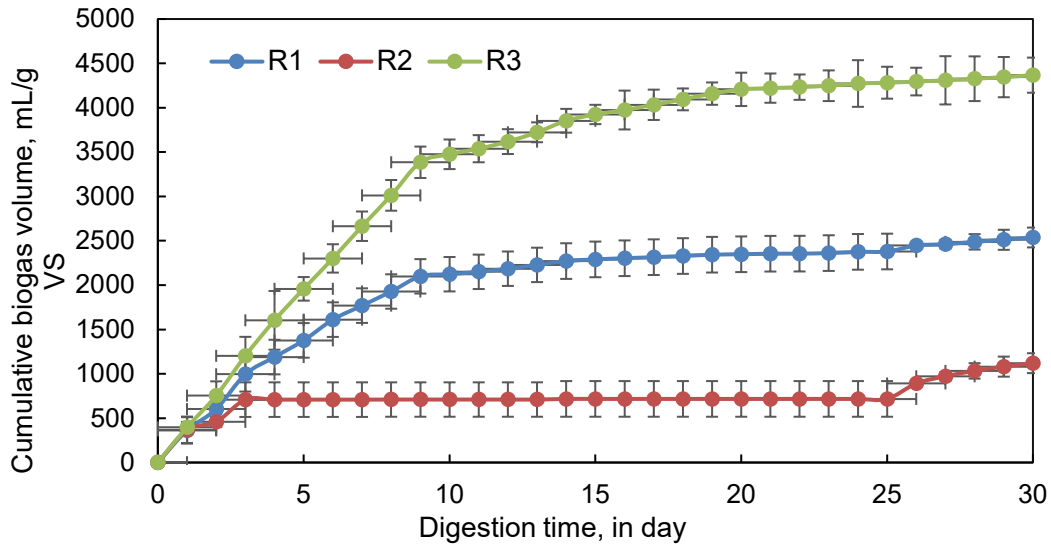


Fig. 4. Cumulative biogas production.

3.3. Digestion Kinetics via Modified Gompertz Model

The Modified Gompertz model was applied to describe the dynamic behavior of biogas generation in the three reactors. Fig. 4 illustrates typical sigmoidal curves characterized by an initial lag, an exponential production phase, and a stationary phase. Reactor R3 exhibited the most favorable kinetic parameters, with a maximum production rate ($R_{\max}$) of 448 ± 5.36 mL g⁻¹ VS day⁻¹, a biogas potential (M_0) of $4,358 \pm 161$ mL g⁻¹ VS, and the shortest lag phase of approximately 2 days. Its coefficient of determination R^2 is 0.99879 (Table 3). These values indicate rapid microbial adaptation, likely supported by effective buffering by protein hydrolysis, thereby enabling stable methanogenesis at an early stage [10,15]. Reactor R1 showed a slightly longer adaptation period ($\lambda = 3$ days), a lower $R_{\max}$ (392 ± 6.16 mL g⁻¹ vs. day⁻¹), and a lower coefficient of determination, $R^2 = 0.9704$, compared to reactor R3, reflecting its reduced protein content. Reactor R2 exhibited the least favorable kinetic performance, with the longest lag phase (5 days), the lowest maximum production rate, and the lowest coefficient of determination ($R^2 = 0.5348$). This outcome suggests that early acidification suppressed methanogenic activity, delaying the transition from acidogenesis to methanogenesis. Such inhibition is consistent with findings that carbohydrate-dominant substrates can cause accumulation of volatile fatty acids (VFAs) when buffering is insufficient [11].

$R_{\max}$ represents the maximum methane production rate (mL/g VS^{-d}); M_0 represents the methane potential maximum production (mL/g VS); λ represents the lag phase (d).

Table 3: The parameters of the modified Gompertz model

	$R_{\max}$ [mL/g VS ^{-d}]	M_0 [mL/g VS]	λ [d]	R^2
R1	392	2,533	3	0.9704
R2	250	1,104	5	0.5348
R3	448	4,358	2	0.9988

3.4. pH Evolution and Process Stability

pH is recognized as a major indicator of system stability during anaerobic digestion, with optimal methanogenesis occurring at pH 6.5–8.0 [16]. The reactors exhibited distinct pH trajectories that directly correlated with their substrate compositions. R3 maintained a stable pH throughout the digestion period, reflecting sufficient ammonia release from protein degradation to neutralize volatile fatty acids (VFAs). This behavior has been well documented, with studies reporting improved methanogenic stability in moderately protein-rich mixtures [10,16]. R1 showed moderate pH stability, though occasional fluctuations were observed due to the combined influence of carbohydrates and proteins (Fig. 5). R2 experienced the most pronounced pH drop during the early digestion phase, confirming that carbohydrate- and vegetable-dominated substrates rapidly generate VFAs that exceed the buffering capacity of such mixtures [11,17]. This low-pH environment inhibited methanogens, consistent with the weak biogas production observed.

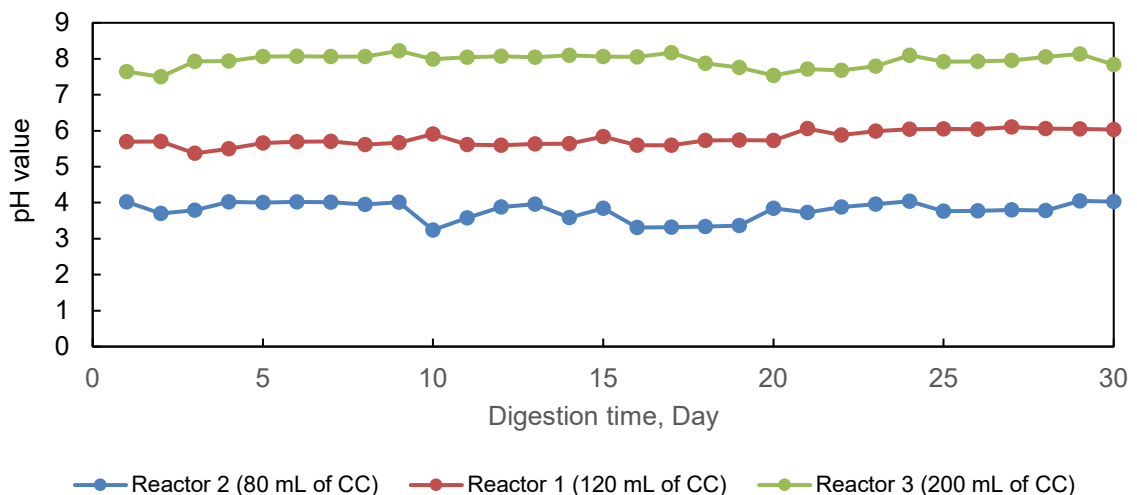


Fig. 5. pH evolution over digestion time.

3.5. Substrate Ratios and Interactive Effects

Although the total volatile solids (TVS) content across reactors did not differ dramatically, the proportion of each substrate contributed significantly to performance variations. The distribution of mass and solids in each reactor is summarized in Fig. 6. R3 had a substantially higher proportion of protein-derived VS than R1 and R2, and this difference directly correlated with its superior biogas output. The enhanced performance of R3 supports existing evidence that moderate protein enrichment improves system stability and methane potential, if ammonia concentrations remain below inhibitory thresholds [10,12,14]. At the same time, excessive carbohydrate or vegetable fractions—as in R2—tend to promote rapid acidification, destabilize pH, and impair methane production. This confirms that substrate interactions in anaerobic co-digestion are highly sensitive to biochemical composition and that balanced mixtures with a controlled proportion of protein provide the most favorable conditions for robust biogas generation [22,23].

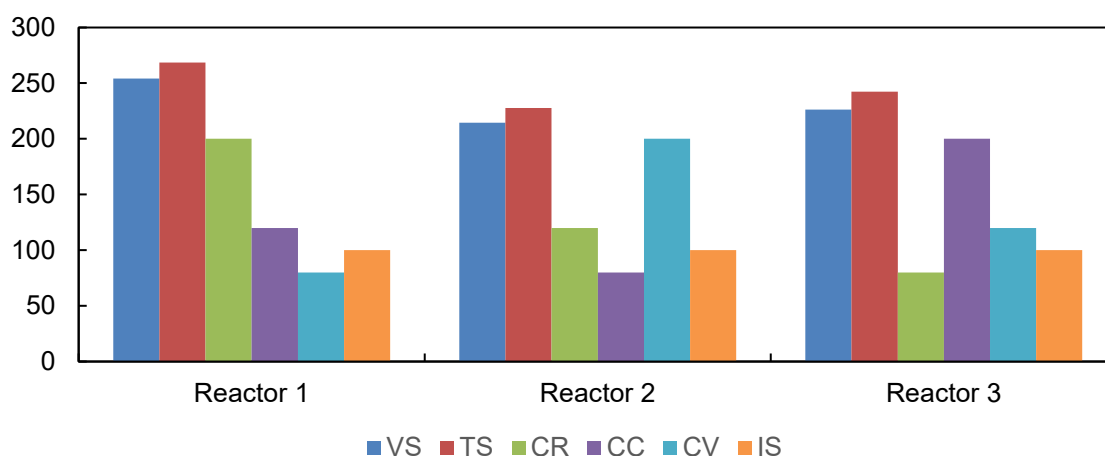


Fig. 6. Substrate ratios and interaction effects CR, CC, CV and IS in mL, TVS and TS in g/L.

The composition of biogas produced by AD of FW depends significantly on the proportions of substrates used. Higher substrate biodegradability leads to a higher percentage of methane obtained [24]. Studies indicate that optimal substrate ratios boost biogas quality [24-25]. The results presented in Table 5 below show a slight difference in the quality of the biogas collected in the reactors. However, reactor R3 produces biogas with a better composition than the other reactors. Therefore, R3 has the optimal substrate ratio for biogas production.

Table 5. Comparative biogas composition from R1, R2 and R3

	No of replication	Average of each replication			Average of each reactor		
		H ₂	CO ₂	CH ₄	H ₂	CO ₂	CH ₄
Reactor 1	Rep 1	0.50%	40.48%	59.02%	0.50%	40.25%	59.25%
	Rep 2	0.50%	41.00%	58.50%			
	Rep 3	0.50%	39.26%	60.24%			
Reactor 2	Rep 1	0.50%	42.55%	56.95%	0.50%	43.10%	56.40%
	Rep 2	0.50%	44.45%	55.05%			
	Rep 3	0.50%	42.30%	57.20%			
Reactor 3	Rep 1	0.50%	40.10%	59.40%	0.50%	39.33%	60.16%
	Rep 2	0.50%	39.50%	60.00%			
	Rep 3	0.50%	38.40%	61.10%			

4. CONCLUSIONS

The anaerobic co-digestion of FW is closely affected by the chemical composition of its substrates. The findings demonstrate that reactor R3 produces the most biogas due to its higher protein content, which represents 40% of its total volume, with a protein: vegetable: carbohydrate ratio of 40:24:16. Its kinetic insights are $R_{\max} = 448 \pm 5.36$ mL/g VS^{-d} and $\lambda = 2$ days with a pH stability between [7-8]. This result was consistent with other studies, which found that the protein substrate fraction in each reactor is closely correlated with the amount of biogas produced by that reactor. In conclusion, the proportion of R3 co-substrates was optimal, thus providing a favorable environment for protein hydrolysis and stabilizing the relationship between acidification and methanization. For future applications, the value of this experience lies in adjusting the composition and proportions of substrates used for

AD to optimize biogas production and degrade as much organic waste as possible. This study leads us to consider reusing digestate after AD. One might wonder if it could be used directly as a biofertilizer.

AUTHORS CONTRIBUTIONS

I.M.M. performed the research, analyzed data, and wrote the paper; M.R.F.A. designed the research, analyzed the data, and revised the paper; M.Z.A. performed the research; and A.A. performed the research. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

No conflict of interest exists in the submission of this manuscript, and the manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

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