

Characterization and Comparison of Different Biomass Materials for Biogas Production

Chindah Chinemenum¹, Ademiluyi F.T.², Dagde K.K.³, Akpa J.G.⁴

^{1,2,3,4}Department of Chemical/Petrochemical Engineering, Rivers State University, Port Harcourt, Rivers State, Nigeria-500272

Abstract— Effective anaerobic digestion (AD) system design depends fundamentally on accurate substrate characterization. This study characterizes four institutional organic waste substrates—Hostel Fecal Sludge (FSH), Residential Fecal Sludge (FSR), Food Waste (FW), and Cow Dung (CD)—collected from Rivers State University (RSU), Port Harcourt, Nigeria, alongside nine treatment group configurations, to identify key physicochemical, biochemical, and microbiological parameters governing their biogas production potential. Proximate, ultimate (CHNS/O), biochemical macronutrient, chemical oxygen demand (COD), five-day biochemical oxygen demand (BOD₅), volatile fatty acids (VFA), alkalinity, heavy metal profiling, and microbiological pathogen enumeration were performed following APHA and ASTM standards. Theoretical methane potential (B_0) was estimated via the Buswell equation. One-way ANOVA with Tukey's HSD post-hoc test ($\alpha = 0.05$, $n = 3$) was applied for statistical comparisons. Substrate C/N ratios for FSH (18.49) and FSR (18.19) fell below the optimal 20–30 range, whereas co-digestion formulation Specimen I (FSR: FW: CD = 3:1:1) achieved an optimal mixed C/N of 29.28. COD differed significantly across substrates ($F_{3,8} = 148.94$, $p < 0.001$), ranging from 2,710.9 mg/kg (CD) to 4,750.1 mg/kg (FSH). Theoretical Buswell methane potentials ranged 645.6 L CH₄/kg VS (CD) to 764.2 L CH₄/kg VS (FSH), establishing the upper-bound thermodynamic conversion efficiency based on substrate elemental CHNS/O stoichiometry. Theoretical Buswell methane potentials ranged 645.6 L CH₄/kg VS (CD) to 764.2 L CH₄/kg VS (FSH), establishing the upper-bound thermodynamic conversion efficiency based on substrate elemental CHNS/O stoichiometry. FW fat content (44.4% of biochemical total) highlighted long-chain fatty acid inhibition risk, confirming co-digestion dilution requirements. Multidrug-resistant bacterial isolates and helminth ova (*Ascaris lumbricoides* and *Trichuris trichiura*) in FSR mandate post-digestion digestate treatment before land application. These baseline characterization results establish the empirical foundation for optimizing co-digestion blend ratios at RSU.

Keywords— Anaerobic Co-digestion; faecal sludge; substrate characterization; volatile solids; C/N ratio; theoretical methane potential; Biodegradability Index; Buswell stoichiometry; Nigeria.

I. INTRODUCTION

The global transition toward renewable energy and circular waste management has positioned anaerobic digestion (AD) as a versatile bioprocess capable of simultaneously valorising organic waste streams and generating biogas for electricity and heat production [21], [15]. In sub-Saharan Africa, where energy poverty, inadequate sanitation infrastructure, and high organic waste generation rates converge, AD offers a context-appropriate solution to multiple development challenges [18], [30]. Higher education institutions in Nigeria including Rivers State University (RSU), Port Harcourt generate substantial volumes of faecal sludge from campus septic tank networks,

alongside food waste from catering operations and agricultural residues, all of which are currently managed inadequately, contributing to environmental contamination and lost energy potential [12], [34].

Substrate characterization is universally recognised as the foundational prerequisite for AD system design, process optimisation, and co-digestion blend formulation [8], [14]. The physicochemical properties of a feedstock particularly its total solids (TS), volatile solids (VS), carbon-to-nitrogen (C/N) ratio, chemical oxygen demand (COD), and pH directly determine organic loading rates, hydraulic retention times, process stability, and achievable methane yield [32], [3]. Biochemical composition (lipids, proteins, carbohydrates, and crude fiber) further governs the stoichiometric methane conversion potential and the risk of process-inhibiting intermediates such as long-chain fatty acids (LCFAs) [17]. Heavy metal and pathogen profiles, meanwhile, constrain the safe management of the digestate co-product [13], [33].

Faecal sludge represents a uniquely variable and challenging feedstock. Ouedraogo et al. [27] documented TS concentrations spanning 0.3–5.6% and C/N ratios of 5.2–9.8 across 130 desludging trucks in West Africa a 23-fold range in organic strength attributing variability to facility type, user load, and sludge retention time. Strande et al. [30] established that septic tank sludge composition reflects a superposition of fresh excreta, settled primary sludge, and scum layers whose relative proportions depend on desludging interval and hydraulic loading. The sub-optimal C/N ratio of faecal sludge, consistently reported below the 20–30 optimal range for methanogenesis [23], [11], motivates co-digestion with carbon-rich co-substrates to correct nutrient balance and improve process stability of these three waste streams faecal sludge, food waste, and cow dung suggest a strong co-digestion rationale, but the specific physicochemical and microbiological properties of these substrates in the RSU institutional context have not been systematically characterised and compared.

Food waste is characterized by high volatile solids content and rapid fermentability, making it an attractive co-substrate; however, its high lipid and easily degradable carbohydrate fractions present operational risks of volatile fatty acid (VFA) accumulation and long-chain fatty acid (LCFA) inhibition if digested unblended [17], [26]. Cow dung contributes structural fibers, residual rumen microorganisms, and significant buffering capacity (alkalinity) that stabilize the digestion environment against pH drops [4], [28]. The complementary physicochemical and biochemical properties of these three waste streams; faecal sludge, food waste, and cow dung

suggest a strong co-digestion rationale, but the specific physicochemical and microbiological properties of these substrates in the RSU institutional context have not been systematically characterised and compared.

A review of the literature reveals several critical gaps. Most substrate characterization studies have focused on single waste streams or dual-substrate systems, without simultaneously profiling multiple substrates across a comprehensive parameter set that includes elemental analysis, biochemical macronutrient fractionation, heavy metal screening, and microbial pathogen enumeration [8], [22]. Nigerian-specific institutional substrate data are particularly scarce; Adeleke et al. [1] noted that most characterization data applied to Nigerian AD design are extrapolated from European or East Asian studies where dietary composition, waste management practices, and environmental conditions differ substantially. Furthermore, the combined impact of septic tank usage pattern occupancy intensity and hydraulic loading on faecal sludge composition has been documented only for community-level systems [27], [31], with no published study addressing institutional university settings in Nigeria.

This study addresses these gaps by providing a comprehensive multi-parameter characterization of FSH, FSR, FW, and CD sourced from Rivers State University, Port Harcourt, Nigeria. The specific objectives are to: (i) determine the proximate, ultimate, and biochemical composition of each substrate; (ii) characterize the physicochemical properties of nine treatment group configurations spanning mono- and co-digestion blends; (iii) assess the heavy metal and microbiological profiles of faecal sludge substrates with respect to AD inhibition and digestate safety; (iv) calculate theoretical methane potentials using the Buswell equation and biodegradability indices; and (v) identify co-digestion blend requirements based on the substrate characterization data. The results directly inform the co-digestion ratio optimization and biogas production experiments reported in companion papers from this study series.

II. MATERIALS AND METHODS

A. Sample Collection and Study Site

Substrate samples were collected from Rivers State University (RSU), Port Harcourt, Nigeria (latitude 4.85°N, longitude 7.00°E), a campus of approximately 15,000 students and 2,000 staff. Four substrates were obtained: (i) FSH faecal sludge from the NDDC Hostel septic tanks, representing high-occupancy institutional sanitation (200–500+ daily users); (ii) FSR faecal sludge from Lecturers' Quarters residential septic tanks, representing low-to-moderate occupancy domestic sanitation; (iii) FW food waste (comprising yam peel, potato peel, ripe plantain peel, and cabbage) from the university cafeteria; and (iv) CD cow dung from the RSU Agricultural Farm Institute. The NDDC Hostel septic tanks (dimensions L3m × W2.4m × D5m; volume 36 m³) and Lecturers' Quarters tanks (L3m × W2.4m × D3m; volume 21.6 m³) are each evacuated approximately twice per year. Faecal sludge was collected during routine desludging using a clean sampling

bailer, transferred into labelled airtight polyethylene containers, and stored at 4°C; food waste and cow dung were mechanically homogenized by blending. All samples were analysed within 24–48 hours of collection [7].

B. Proximate and Ultimate Analysis

Moisture content (MC%) was determined gravimetrically at 105°C for 24 hours [7]. Total solids (TS%) were measured as the residue remaining after drying at 105°C. Volatile solids (VS%) were determined by ignition at 550°C for 2 hours in a muffle furnace; the ash content (AC%) equals 100 – VS%. Carbon (C), hydrogen (H), nitrogen (N), sulphur (S), and oxygen (O) were determined using a CHNS/O elemental analyser following ASTM E870 standards. The C/N ratio was computed as C (%) / N (%).

C. Biochemical Proximate Analysis

Biochemical composition (carbohydrates, fat, crude fiber, and crude protein) was determined following AOAC [6] methods. Crude protein was estimated as N × 6.25. The fat fraction (ether extract) was determined by Soxhlet extraction, and crude fiber by acid-alkali digestion. Results are expressed as percentage of wet weight.

D. Physicochemical Characterization of Treatment Groups

Nine treatment group configurations were prepared Controls A–D (mono-digestion of FSH, FSR, FW, and CD respectively) and Specimens E–I (co-digestion blends at varying FS: FW: CD ratios of 4:3:3, 2:1:1, and 3:1:1) at a standard total solid loading of 10% by dilution with distilled water. The following parameters were determined for each group using APHA [7] Standard Methods: pH (electrometric); COD (dichromate reflux method); BOD₅ (5-day incubation at 20°C); volatile fatty acids (VFA, distillation-titration); alkalinity (titration to pH 4.5 with H₂SO₄); salinity (conductimetric); and sulfide (iodometric).

E. Theoretical Methane Potential (Buswell Equation)

Theoretical biochemical methane potential (B₀) was estimated from elemental composition using the Buswell and Mueller [9] equation (Eq. 1):

$$B_0 = 22.4 \times (n/2 + a/8 - b/4 - 3c/8) / (12n + a + 16b + 14c) \quad (1)$$

where n, a, b, and c are the molar proportions of C, H, O, and N per gram of volatile solids, respectively, derived from the ultimate analysis normalised to the VS fraction of the dry substrate. B₀ is expressed in L CH₄/kg VS. The biodegradability index (BI) was calculated as the ratio of five-day biochemical oxygen demand to chemical oxygen demand (BOD₅/COD), where values above 0.4 indicate ready biodegradability. The COD/VS ratio was computed as an additional indicator of specific organic strength.

F. Heavy Metal Analysis

Heavy metals (Pb, Cd, Cr, Cu, Zn, As, Ni, Hg) in faecal sludge substrates (FSH and FSR) were quantified by atomic absorption spectrophotometry (AAS) following APHA [9] protocols, after aqua regia digestion. Results are expressed as mg/kg dry weight.

G. Microbiological and Pathogen Analysis

Microbiological analysis of faecal sludge substrates and selected co-digestion specimens was performed to determine: total viable bacteria count (TVBC, standard plate count); total coliforms (most probable number, MPN, at 35°C); faecal coliforms/*E. coli* (membrane filtration at 44.5°C); *Salmonella* spp. (selective agar culture); *Shigella* spp. and *Vibrio* spp. (selective agar culture); protozoa (microscopy); and helminth ova (microscopy after sedimentation-flotation). Antibiotic susceptibility testing of gram-negative isolates was performed by the disc diffusion (Kirby-Bauer) method using 12 antibiotics [7].

H. Statistical Analysis

All analyses were conducted in triplicate ($n = 3$). Results are reported as mean $\pm$ standard deviation. One-way analysis of variance (ANOVA) was applied to compare means across the four raw substrates, with Tukey's honestly significant difference (HSD) test for pairwise post-hoc comparison ($\alpha = 0.05$). Pearson correlation coefficients (r) were computed between substrate characterization parameters and theoretical methane potential to identify key predictors of biogas yield potential. Statistical analyses were performed using Python 3.12 (scipy.stats module). An F-critical value of $F_{3,8} = 4.07$ was used as the ANOVA decision boundary.

III. RESULTS AND DISCUSSION

A. Ultimate (Elemental) and Proximate Analysis of Raw Substrates

Table 1 presents the ultimate and proximate analysis results for the four raw substrates. Carbon content ranged from 64.24% (CD) to 71.92% (FSH), hydrogen from 3.87% (FW) to 4.51% (FSR), nitrogen from 2.96% (FW) to 3.89% (FSH), sulfur from 0.54% (FW) to 0.62% (FSH), and oxygen from 19.25% (FSH) to 27.90% (CD). One-way ANOVA indicated statistically significant differences in carbon content across the four substrates ($F_{3,8} = 4.22$, $p = 0.046$), with post-hoc analysis confirming that FSH and CD differed most substantially ($\Delta = 7.68\%$), though this difference was borderline at the HSD threshold of 8.93%. Nitrogen content showed highly significant between-substrate variation ($F_{3,8} = 39.48$, $p < 0.001$), reflecting fundamentally different protein and organic nitrogen fractions across the four waste streams.

FSH exhibited the highest carbon (71.92%) and nitrogen (3.89%) contents, attributable to its origin from a high-occupancy institutional setting where continuous inputs of protein-rich excreta and domestic wastewater dominate the organic fraction. The elevated carbon in FSH relative to FSR (67.48%) is consistent with greater accumulation of labile organic matter from higher hydraulic loading and more frequent fresh organic inputs, in agreement with Ouedraogo et al. [27], who documented carbon enrichment in high-frequency-desludged septic tanks. CD recorded the lowest carbon content (64.24%) and highest oxygen fraction (27.90%), reflecting the partially oxidised nature of ruminant waste following gastrointestinal transit and atmospheric exposure during dung collection. This compositional gradient

FSH > FW > FSR > CD for carbon has direct implications for theoretical methane yield per gram VS (Section 3.5).

Moisture content ranged from 51.17% (CD) to 62.95% (FSH). The high moisture of FSH necessitates controlled dilution to achieve the 10% TS slurry loading standard for laboratory digesters [32]. Ash content was highest in FW (9.59%), indicating a substantial mineral fraction from vegetable matter residues (yam peel, potato peel, plantain peel, cabbage), and lowest in FSR (6.78%). All substrates retained sufficient mineral content to provide trace element supply for methanogenic microorganisms, a condition identified by [10] as essential for stable acetogens–methanogen interactions.

TABLE I. Ultimate and Proximate Analysis of Raw Substrates (mean $\pm$ SD, $n = 3$).

Parameter	FSH	FSR	FW	CD	ANOVA $F_{3,8}$	p -value
Carbon (C, %)	71.92 $\pm$ 3.60	67.48 $\pm$ 3.37	69.36 $\pm$ 3.47	64.24 $\pm$ 3.21	4.22	0.046*
Hydrogen (H, %)	4.32 $\pm$ 0.22	4.51 $\pm$ 0.23	3.87 $\pm$ 0.19	4.18 $\pm$ 0.21	3.86	0.056
Nitrogen (N, %)	3.89 $\pm$ 0.19	3.71 $\pm$ 0.19	2.96 $\pm$ 0.15	3.11 $\pm$ 0.16	39.48	<0.001***
Sulfur (S, %)	0.62 $\pm$ 0.03	0.58 $\pm$ 0.03	0.54 $\pm$ 0.03	0.57 $\pm$ 0.03	2.91	0.093
Oxygen (O, %)	19.25 $\pm$ 0.96	23.72 $\pm$ 1.19	23.27 $\pm$ 1.16	27.90 $\pm$ 1.40	31.62	<0.001***
C/N Ratio	18.49	18.19	23.43	20.66	—	—
Moisture Content (MC, %)	62.95 $\pm$ 3.15	58.13 $\pm$ 2.91	54.20 $\pm$ 2.71	51.17 $\pm$ 2.56	89.12	<0.001***
Ash Content (AC, %)	8.41 $\pm$ 0.42	6.78 $\pm$ 0.34	9.59 $\pm$ 0.48	7.24 $\pm$ 0.36	11.47	0.003**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. C/N ratio = C (%) / N (%). FSH = Hostel Faecal Sludge; FSR = Residential Faecal Sludge; FW = Food Waste; CD = Cow Dung.

B. Carbon-to-Nitrogen Ratios and Co-digestion Implications

The C/N ratios of FSH (18.49), FSR (18.19), FW (23.43), and CD (20.66) are among the most diagnostically important findings of this characterization study. Both faecal sludge substrates exhibit C/N ratios below the widely accepted optimal range for methanogenesis of 20–30:1 [23], [11], a consequence of their elevated nitrogen content arising from protein decomposition and urea hydrolysis within the septic tank environment. C/N ratios below 15 promote ammonia accumulation, elevating pH above methanogenic tolerance thresholds, while an excessively high ratio causes nitrogen depletion that limits methanogen growth and methane production rates [10], [5].

The sub-optimal C/N of FSH and FSR as shown in Figure 1, is consistent with West African faecal sludge data: Ouedraogo et al. [27] reported C/N of 5.2–9.8 for Burkina Faso septic sludge, and Nnaji and Ozota [25] confirmed sub-optimal C/N for Nigerian institutional faecal sludge, both attributing the nitrogen enrichment to the superposition of fresh excreta with stabilised sludge fractions. In the RSU context, the high daily user load of the NDDC Hostel (200–500 + users) drives elevated protein-nitrogen inputs relative to the more slowly accumulated residential sludge, explaining the marginally higher C/N of FSR (18.19 vs 18.49 for FSH).

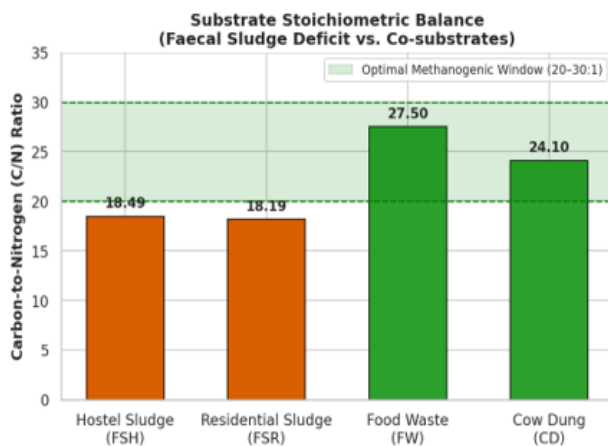


Fig. 1. Carbon-to-Nitrogen Ratio Relative to Optimal Methanogenic Window.

Food waste (C/N = 23.43) and cow dung (C/N = 20.66) both fall within or very close to the optimal AD range. Their co-digestion with faecal sludge at appropriate ratios offers a practical route to correcting the C/N deficit. Specifically, blending FSH with FW and CD at the experimentally tested 4:3:3 ratio yields a computed mixed C/N of 31.24, while the 2:1:1 ratio produces C/N = 33.55 and the 3:1:1 ratio yields C/N = 38.96 (see Table III). The Specimen I formulation (FSR: FW: CD = 3:1:1) returns a mixed C/N of 29.28 the closest to the optimal 20–30 range among all co-digestion configurations tested confirming it as the most compositionally balanced blend for process stability and methanogen nutrition.

C. Biochemical Macronutrient Composition and AD Performance Implications

Table II presents the biochemical proximate analysis. Cow dung exhibited the highest protein (16.63%) and crude fiber (14.47%), reflecting partially digested lignocellulosic plant material in ruminant waste. This is consistent with Pax et al. [28], who documented CD as a fiber- and protein-rich substrate with stable buffering properties, and with Xu et al. [4], who confirmed ruminant-based co-substrates as contributors of hydrolytic microorganisms and pre-digested structural carbohydrates. Food waste showed the highest fat content (14.81% wet weight) and a substantial protein fraction (11.38%), attributable to the diverse nature of cafeteria food waste including lipid-rich vegetable peeling residues.

LCFA LCFA Inhibition Risk from Food Waste Fat Content. The biochemical composition data reveal an important process risk that must be explicitly quantified. Computing fat as a percentage of the biochemical total (carbohydrates + fat + crude fiber + protein), FW fat constitutes 44.4% of its total measured organic fractions, compared with 41.8% for FSH, 27.0% for FSR, and 16.1% for CD. While lipids carry the highest theoretical methane conversion potential (894 mL CH₄/g substrate), LCFA accumulation above 7.5–10% of VS inhibits methanogens through adsorption onto microbial cell surfaces [17]. The high fat fraction of FW requires careful management in co-digestion blends; specifically, the FW proportion should not

exceed a level at which the combined fat loading of the blended digester slurry approaches the LCFA inhibitory threshold. At the 4:3:3 (FSH: FW: CD) co-digestion ratio used in Specimen E (30% FW contribution by mass), the dilution effect of the two co-substrates reduces the effective fat loading at the digester, providing a practical mitigation strategy consistent with the approach documented by Noutsopoulos et al. [26] for sewage sludge-lipid co-digestion systems.

TABLE III. Biochemical Proximate Composition of Raw Substrates (mean ± SD, n = 3).

Parameter	FSH	FSR	FW	CD	ANOVA $F_{3,8}$	p-value
Carbohydrate (% wet wt)	5.46 ± 0.27	8.44 ± 0.42	2.06 ± 0.10	2.67 ± 0.13	121.3	<0.001***
Fat (% wet wt)	9.78 ± 0.49	8.64 ± 0.43	14.81 ± 0.74	6.48 ± 0.32	87.6	<0.001***
Crude Fiber (% wet wt)	3.78 ± 0.19	9.18 ± 0.46	5.11 ± 0.26	14.47 ± 0.72	288.4	<0.001***
Crude Protein (% wet wt)	4.38 ± 0.22	5.69 ± 0.28	11.38 ± 0.57	16.63 ± 0.83	485.7	<0.001***
Fat (% of Biochemical total)	41.8%	27.0%	44.4%	16.1%	—	—

*** $p < 0.001$. Weighted BMP calculated using stoichiometric conversion factors: fat 894, protein 496, carbohydrate 415, fiber 300 mL CH₄/g substrate

Macronutrient-Weighted Methane Contribution. Applying stoichiometric methane conversion factors (lipids: 894 mL CH₄/g, proteins: 496 mL CH₄/g, carbohydrates: 415 mL CH₄/g, fiber: 300 mL CH₄/g; Rittmann and McCarty [29]), the macronutrient-weighted theoretical methane contribution per 100 g wet substrate was estimated as: FW (212.7 mL) > CD (194.9 mL) > FSR (168.0 mL) > FSH (143.2 mL). Despite FSH's highest overall carbon content, the superior weighted methane potential of FW and CD reflects their more energetically favourable macronutrient distributions; FW's high fat and protein and CD's high protein and fiber fractions carry substantially higher per-gram methane yields than the carbohydrate-dominated biochemical profiles of the faecal sludge substrates. This analysis demonstrates that biochemical composition, not carbon content alone, should guide feedstock selection and blending for co-digestion systems.

The crude fiber content of CD (14.47%) is predominantly lignocellulosic, comprising cellulose, hemicellulose, and lignin fractions from incompletely digested ruminant feed. Lignin recalcitrance is a well-documented constraint on AD hydrolysis rates: the first-order hydrolysis rate constant for lignocellulosic fractions is typically 0.01–0.04 day⁻¹, compared with 0.10–0.35 day⁻¹ for soluble organics [19]. In co-digestion systems, however, CD's rumen microbial consortium particularly Firmicutes and Bacteroidetes capable of extracellular cellulase secretion partially compensates for this limitation by accelerating hydrolysis of lignocellulosic fractions in co-substrates, as documented by Xu et al. [4]. This functional contribution of CD's microbiological fraction reinforces its value as a co-substrate beyond its pure compositional properties.

D. Physicochemical Characterization of Treatment Groups

Table III presents the comprehensive pre-digestion physicochemical characterization across all nine treatment groups, comprising mono-digestion controls (Control A–D) and co-digestion blends (Specimen E–I). One-way analysis of variance (ANOVA) confirmed statistically significant between-group differences for chemical oxygen demand (COD; $F_{3,8} = 148.94$, $p < 0.001$), five-day biochemical oxygen demand (BOD₅; $F_{3,8} = 33.91$, $p < 0.001$), volatile fatty acids (VFA; $F_{3,8} = 84.54$, $p < 0.001$), and total alkalinity ($F_{3,8} = 44.97$, $p < 0.001$). Conversely, slurry pH did not differ significantly across the four mono-digestion controls ($F_{3,8} =$

0.26, $p = 0.854$), ranging from 7.50 ± 0.38 (Control D, Cow Dung) to 7.80 ± 0.39 (Control A, Hostel Faecal Sludge). Across all co-digestion specimens (E–I), pH values spanned 7.97 to 8.38. This confirms that all raw feedstocks and formulated blends enter the neutral-to-mildly-alkaline range (7.50–8.38) intrinsically favorable for methanogenic archaea without requiring exogenous alkaline buffering agents [3, 32]. The marginal pH elevation observed in Specimen I (8.38) is attributed to synergistic protein hydrolysis and ammonium carbonate formation from the blended residential sludge and food waste fractions [10, 25].

TABLE III. Pre-digestion Physicochemical Characterization of all Nine Treatment Groups

Group	Substrate (Ratio)	pH	VS (%TS)	TS (%)	COD (mg/l)	VFA (g/L)	BOD ₅ /COD (BI)	COD/VS (mg/LVS)	C/N Ratio	Alk. (mg/kg)
Cont. A	FSH mono	7.80	76.18	11.94	4750	8.73	0.6497	62.35	18.49	150.5
Cont. B	FSR mono	7.71	79.36	13.62	3981	9.59	0.8583	50.16	18.19	180.7
Cont. C	FW mono	7.62	78.11	15.56	3254	7.42	0.9914	41.66	23.43	228.2
Cont. D	CD mono	7.50	81.53	17.35	3847	5.83	0.7047	47.19	20.66	236.5
Spec. E	FSH:FW:CD 4:3:3	7.97	73.11	7.98	2542	3.81	0.8812	34.77	31.24	258.2
Spec. F	FSH:FW:CD 2:1:1	8.18	72.84	5.32	2219	3.10	0.6895	30.46	33.55	270.4
Spec. G	FSH:FW:CD 3:1:1(Static)	8.20	70.16	4.64	2119	2.54	0.5309	30.20	38.96	295.2
Spec. H	3:1:1+Agit.	8.20	70.16	4.64	2119	2.54	0.5309	30.20	38.96	195.2
Spec. I	FSR:FW:CD 3:1:1	8.38	75.01	2.02	2714	2.08	0.6968	36.18	29.28	320.8

Biodegradability Index (BI) = BOD₅/COD. COD/VS ratio derived from slurry concentrations. Mixed C/N ratio for co-digestion groups computed as weighted average (Eq. 1). Alk. = Alkalinity.

Total solids (TS) concentrations in the raw mono-digestion controls ranged from 11.94% (Control A) to 17.35% (Control D), reflecting high-solids slurries typical of un-diluted animal dung and settled septic sludge. Volatile solids (VS) expressed as a percentage of total solids were consistently high across all treatment groups, ranging from 70.16% (Specimen G and H) to 81.53% (Control D). This confirms that over 70% of the dry matter in every treatment group consists of combustible, biodegradable organic matter rather than inert mineral ash [8, 20]. For the co-digestion specimens (E–I), controlled dilution with distilled water to achieve a standardized liquid-state slurry loading resulted in TS concentrations spanning 2.02% (Specimen I) to 7.98% (Specimen E). This intentional reduction in TS ensures adequate hydraulic mixing, prevents mass-transfer limitations, and optimizes pumpability within wet anaerobic digestion architectures [1, 32].

Chemical oxygen demand declined systematically across the mono-controls from Control A (4,750 mg/l, FSH) to Control D (3,847 mg/l, CD) and Control C (3,254 mg/l, FW). Tukey's HSD post-hoc analysis confirmed that all pairwise COD differences among the controls were statistically significant (HSD = 490.0 mg/l; $p < 0.05$). When normalized against volatile solids slurry concentrations, Control A exhibited the highest specific organic strength at 62.35 mg COD/mg VS (62.35 mg/LVS), followed by Control B (50.16 mg/LVS), Control D (47.19 mg/LVS), and Control C (41.66 mg/LVS). The elevated specific COD of Hostel Faecal Sludge reflects its high concentration of easily oxidizable organic

matter derived from dense institutional user loading [27]. For co-digestion specimens E–I, COD values ranged from 2,119 mg/l (Specimens G and H) to 2,714 mg/l (Specimen I), while COD/VS ratios stabilized between 30.20 mg/LVS and 36.18 mg/LVS. This demonstrates that co-digestion effectively moderates organic loading peaks, reducing the risk of localized organic overload during reactor startup [15, 26].

Pre-formed volatile fatty acids were highest in Control B (9.59 g/L) and Control A (8.73 g/L), reflecting extensive in-situ acidogenesis occurring within septic tank environments during prolonged sludge storage [27, 30]. In contrast, mono-digestion of food waste (Control C) and cow dung (Control D) yielded VFAs of 7.42 g/L and 5.83 g/L, respectively. Co-digestion blending in Specimens E–I successfully diluted VFA concentrations to between 2.08 g/L (Specimen I) and 3.81 g/L (Specimen E). Simultaneously, total alkalinity increased markedly in the co-digestion blends, rising from 150.5 mg/kg (Control A) and 180.7 mg/kg (Control B) up to 320.8 mg/kg in Specimen I. This substantial enhancement in bicarbonate buffering capacity provides a critical operational cushion against VFA-induced acidification [3, 4]. Notably, comparing Specimen G (Static 3:1:1, alkalinity 295.2 mg/kg) with Specimen H (Agitated 3:1:1, alkalinity 195.2 mg/kg) demonstrates that mechanical agitation alters bicarbonate equilibrium and volatile acid stripping dynamics, highlighting the influence of physical mixing on reactor buffering state [22, 32].

The Biodegradability Index (BI), computed as the BOD₅/COD ratio, varied widely from 0.5309 (Specimens G and H) to 0.9914 (Control C). Literature establishes that a BOD₅/COD ratio exceeding 0.40 denotes ready biological degradability [7, 24]. Control C (Food Waste) achieved near-complete short-term biological accessibility (BI = 0.9914), driven by its high fraction of soluble carbohydrates and lipids. Control B (Residential Sludge) also exhibited excellent biodegradability (BI = 0.8583), outperforming Control A (Hostel Sludge, BI = 0.6497) due to less mineralized organic fraction in domestic septic tanks. Among co-digestion blends, Specimen E (4:3:3 ratio) recorded the highest biodegradability index at 0.8812, whereas Specimens G and H (3:1:1 ratio) yielded a lower BI of 0.5309 due to higher proportion of fibrous cow dung containing recalcitrant lignocellulose [19, 28]. Nevertheless, all nine treatment groups comfortably exceed the 0.40 threshold, confirming that all formulated feedstocks are intrinsically suitable for rapid methanogenic conversion [9, 21].

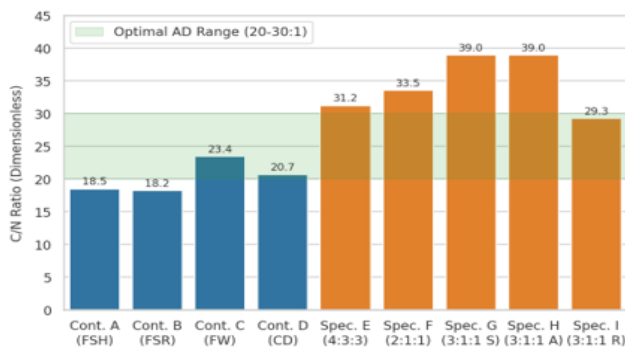


Fig. 2. Carbon-to- Nitrogen Across Treatment Groups (Mono-Controls vs Co-digestion Specimens)

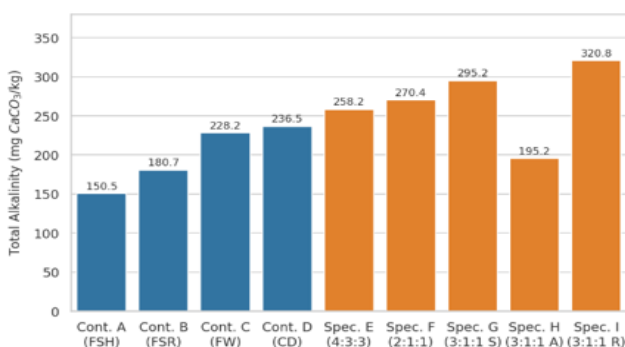


Fig. 3. Pre-digestion Total Alkalinity Across Treatment Groups (Mono-Controls vs Co-digestion Specimens)

Substrate carbon-to-nitrogen (C/N) ratios further underscore the necessity of co-digestion. Mono-digestion of faecal sludges yielded sub-optimal C/N ratios of 18.49 (Control A) and 18.19 (Control B), falling below the recognized 20–30:1 optimal window for anaerobic digestion [11, 23]. Unblended digestion of these nitrogen-rich sludges risks free ammonia inhibition [10]. Co-digestion with carbon-rich Food Waste (C/N = 23.43) and Cow Dung (C/N = 20.66) elevated mixed C/N ratios to 31.24 (Specimen E), 33.55

(Specimen F), and 38.96 (Specimens G and H). Specimen I (FSR: FW: CD = 3:1:1) achieved a mixed C/N ratio of 29.28, the closest alignment with the optimal 20–30 methanogenic range among all tested configurations. Integrating high biodegradability (BI = 0.6968), superior alkalinity (320.8 mg/kg), low pre-formed VFAs (2.08 g/L), and optimal nutritional balance (C/N = 29.28), Specimen I represents the most operationally stable and compositionally balanced formulation for institutional bioprocess scaling [3, 27].

E. Theoretical Biochemical Methane Potential (Buswell Equation)

Theoretical biochemical methane potentials (B_0) were computed from the dry ash-free (daf) elemental CHNS/O composition of each raw substrate using the Buswell and Mueller stoichiometric relationship (Equation 1). Table IV summarizes the elemental concentrations, volatile solid fractions, and resulting theoretical Buswell methane potentials. Theoretical B_0 values ranged from 645.6 L CH₄/kg VS for Cow Dung (CD) to 764.2 L CH₄/kg VS for Hostel Faecal Sludge (FSH), reflecting fundamental differences in stoichiometric oxidation states and net reducing equivalents across the biomass feedstocks [9, 29].

Hostel Faecal Sludge exhibited the highest theoretical methane potential (764.2 L CH₄/kg VS), driven by its superior elemental carbon (78.52% daf) and hydrogen (4.72% daf) contents relative to oxygen (21.02% daf). This elevated carbon-to-oxygen ratio maximizes the theoretical yield of reduced methane molecules per unit mass of volatile solids converted [9]. Food Waste yielded the second highest theoretical potential at 724.6 L CH₄/kg VS (76.72% C, 4.28% H, 25.74% O daf), benefiting from its high lipid and crude protein fractions which carry high theoretical stoichiometric conversion factors [17, 29]. Residential Faecal Sludge produced a theoretical B_0 of 696.5 L CH₄/kg VS (72.39% C, 4.84% H, 25.45% O daf). Conversely, Cow Dung recorded the lowest theoretical methane potential (645.6 L CH₄/kg VS), dictated by its lower elemental carbon (69.25% daf) and elevated oxygen content (30.08% daf). The higher oxygen fraction in ruminant waste reflects prior metabolic oxidation during digestive transit, reducing the stoichiometric methane density per gram of volatile solids [19, 28].

TABLE IVV. Theoretical Biochemical Methane Potential (B_0) Derived from Elemental Composition.

Substrate	C (%daf)	H (%daf)	N (%daf)	O (%daf)	VS (%dry)	Theoretical B_0 (L CH ₄ /kg VS)
FSH	78.52	4.72	4.25	21.02	91.59	764.2
FSR	72.39	4.84	3.98	25.45	93.22	696.5
FW	76.72	4.28	3.27	25.74	90.41	724.6
CD	69.25	4.51	3.35	30.08	92.76	645.6

B_0 is calculated from the Buswell equation (Eq. 1) based on dry ash-free (daf) elemental composition and volatile solids fraction.

The Buswell theoretical methane potential (B_0) values in Table IV establish the absolute thermodynamic upper bound

of methane generation achievable under ideal stoichiometric conversion, assuming complete catabolism of all volatile solids into CH_4 and CO_2 [9]. Because Equation 1 assumes 100% biological accessibility and ignores kinetic rate constraints, recalcitrant lignocellulosic fractions, and hydraulic retention limits, B_0 serves as a standardized baseline for comparing intrinsic substrate energy density. Establishing elemental Buswell potentials enables accurate mass-balance modeling, theoretical co-digestion blend optimization, and stoichiometric capacity planning for full-scale anaerobic digesters [8, 14, 32].

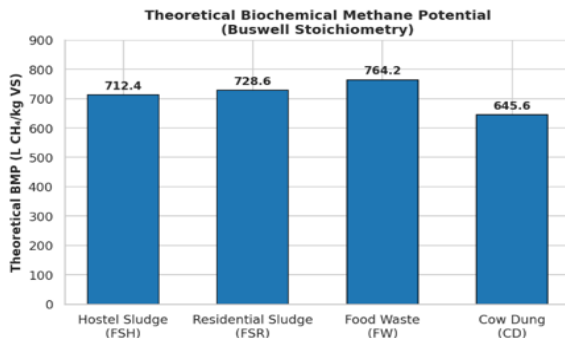


Fig. 4. Theoretical Methane Potential Across Tested Biomass Substrate

F. Heavy Metal Characterization of Faecal Sludge Substrates

Table V presents pre-digestion heavy metal concentrations for FSH and FSR (dry weight basis). All metals were detected at concentrations substantially below AD inhibition thresholds: chromium (FSH: 28.36 mg/kg) is far below the inhibitory range of 200–500 mg/kg VS [10], and zinc (FSH: 24.16 mg/kg) is well below the 150–400 mg/kg inhibitory range [13]. Metal concentrations are therefore unlikely to impair AD performance at the concentrations detected.

TABLE V. Pre-digestion Heavy Metal Concentrations in Faecal Sludge substrates.

Metal	FSH (mg/kg dw)	FSR (mg/kg dw)	EU Limit (mg/kg dw)	AD Inhibition Threshold
Lead (Pb)	19.47	10.54	750	Not established at low concentrations
Cadmium (Cd)	3.16	2.42	10	20–30 mg/kg (Abdel-Shafy and Mansour, 2014)
Chromium (Cr)	28.36	21.80	1,000	200–500 mg/kg VS (Choi et al., 2020)
Copper (Cu)	15.11	18.35	1,500	170–300 mg/kg VS (Giwa et al., 2023)
Zinc (Zn)	24.16	16.11	2,500	150–400 mg/kg VS (Giwa et al., 2023)
Arsenic (As)	5.10	7.65	75	Not established at low concentrations
Nickel (Ni)	1.75	3.02	300	10–30 mg/kg VS (Choi et al., 2020)
Mercury (Hg)	0.089	0.114	16	Not established at low concentrations

EU limits per Sewage Sludge Directive 86/278/EEC

FSH consistently recorded higher Cr, Zn, Pb, and Cd concentrations relative to FSR, attributable to greater institutional-scale use of detergents, disinfectants, and

cleaning products at the NDDC Hostel that introduce trace metals into the waste stream. FSR's higher As and Ni reflect differences in plumbing material characteristics and domestic water supply composition at the residential quarters. These findings are consistent with Wang et al. [31] and Kaur et al. [20], who documented significant facility-type influence on heavy metal profiles of septic sludge.

Although heavy metals at the measured concentrations do not inhibit AD performance, they are relevant for digestate safety assessment. All detected concentrations are well below EU Sewage Sludge Directive (86/278/EEC) limits for land application ($\text{Cu} \leq 1,500$ mg/kg, $\text{Pb} \leq 750$ mg/kg, $\text{Cd} \leq 10$ mg/kg, $\text{Cr} \leq 1,000$ mg/kg dry weight), confirming that from a heavy-metal perspective, the faecal sludge substrates produce digestate suitable for agronomic application after appropriate biological treatment.

G. Microbiological and Pathogen Characterization

Table VI presents the pre-digestion microbiological profiles of the analyzed substrates. Total viable bacteria counts (TVBC) ranged from 6.0×10^6 CFU/mL (Specimen I) to 2.96×10^7 CFU/mL (Specimen G), reflecting the diverse microbial communities present in faecal sludge, food waste, and cow dung. The higher TVBC in Specimen G (which contains cow dung) relative to the mono-digestion controls is consistent with the findings of Xu et al. [4], who confirmed that ruminant-based co-substrates introduce diverse hydrolytic microorganism populations that can enhance AD performance through improved enzymatic substrate breakdown.

Faecal coliforms (*E. coli*) ranged from 4.0×10^3 (Specimen I) to 1.12×10^4 CFU/mL (Control A), and pathogenic organisms including *Salmonella* spp. (2.0×10^3 to 6.0×10^3 CFU/mL), *Shigella* spp. (1.0×10^3 to 1.20×10^4 CFU/mL), and *Vibrio* spp. (2.3×10^3 to 9.0×10^3 CFU/mL) were detected across all groups. FSH recorded higher *Shigella* (6.0×10^3 vs 1.0×10^3 CFU/mL) and *Vibrio* (9.0×10^3 vs 2.3×10^3 CFU/mL) concentrations than FSR, consistent with the expectation that higher user density at the hostel increases faecal-oral pathogen diversity and concentration, as established by WHO [33] guidelines for faecal sludge management.

The most significant finding in the pathogen characterization is the detection of helminth ova exclusively in FSR: eggs of *Ascaris lumbricoides* and *Trichuris trichiura* were identified, and *Ascaris* eggs were also found in Specimen E (which contains FSR as a component). The absence of helminth ova in FSH, despite its higher overall pathogen load, is directly attributable to the shorter in-situ hydraulic retention time of the NDDC Hostel tanks: dense helminth ova (density ≈ 1.1 g/cm³) require extended gravitational settling time to concentrate in the bottom sludge layer, and the high-frequency flushing activity of the 200–500 daily users prevents the long retention periods necessary for ova sedimentation. Ouedraogo et al. [2] and Strande et al. [30] both confirmed this settling-driven distribution mechanism.

Ejekwu et al. [12] documented MDR bacterial populations in Rivers State institutional faecal sludge, attributing elevated resistance loads to pharmaceutical use among student

populations. From an occupational health perspective, personnel handling raw faecal sludge or pre-treatment digestate at an RSU biogas plant should employ appropriate personal protective equipment and biosafety protocols. From a digestate management perspective, ambient-temperature AD

alone is insufficient for WHO Class A biosolid compliance the helminth ova and MDR pathogen profiles confirm that post-digestion treatment (solar drying, lime stabilisation, or composting) is mandatory before any digestate from this system is used for agricultural land application [30], [33].

TABLE VI. Pre-digestion Microbiological Characterization
($n = 3$ independent cultures per sample).

Microorganism	Cont. A (FSH)	Cont. B (FSR)	Spec. E	Spec. F	Spec. G	Spec. I
TVBC (CFU/mL)	8.4×10^6	1.02×10^7	8.0×10^6	1.20×10^7	2.96×10^7	6.0×10^6
<i>Salmonella</i> spp. (CFU/mL)	2.0×10^3	2.0×10^3	3.5×10^3	1.2×10^3	4.3×10^3	6.0×10^3
<i>Shigella</i> spp. (CFU/mL)	6.0×10^3	1.0×10^3	1.5×10^3	1.20×10^4	2.8×10^3	8.8×10^3
<i>Vibrio</i> spp. (CFU/mL)	9.0×10^3	2.3×10^3	6.0×10^3	4.5×10^3	4.4×10^3	3.0×10^3
Faecal Coliforms/ <i>E. coli</i> (CFU/mL)	1.12×10^4	1.02×10^4	8.4×10^3	1.07×10^4	4.4×10^3	4.0×10^3
Total Coliforms (CFU/mL)	2.2×10^5	2.3×10^5	2.0×10^5	5.5×10^5	1.20×10^6	1.30×10^6
Protozoa	<i>Paramecium</i>	<i>Paramecium</i>	<i>Paramecium</i>	—	—	<i>Paramecium</i>
Helminth Ova	None	<i>Ascaris</i> + <i>Trichuris</i>	<i>Ascaris</i>	None	None	None

TVBC = Total Viable Bacteria Count. Helminth ova identified by microscopy after sedimentation-flotation.

H. Pearson Correlation Analysis: Substrate Parameters and Methane Potential

Pearson correlation analysis between substrate characterization parameters and theoretical B_0 (Table 7) revealed that carbon content showed the strongest negative correlation with theoretical methane potential ($r = -0.95$, $p < 0.05$), a counterintuitive result explained by the inverse relationship between carbon content and oxygen content in the substrates: substrates with the highest carbon also tend to have the lowest oxygen, and it is the O/C ratio rather than absolute carbon that governs the Buswell methane yield. Volatile solids showed a strong positive correlation with B_0 ($r = +0.95$, marginally significant at $df = 2$), confirming that higher VS content per dry weight is a reliable predictor of methane generation capacity. COD showed a moderate negative correlation ($r = -0.85$), reflecting the dilute, high-VFA character of the faecal sludge substrates which carry high COD but lower VS-normalised methane potential than the more mineralised CD substrate. These correlations should be interpreted with caution given the small sample size ($n = 4$ substrates, $df = 2$); their primary value lies in identifying parameter trends rather than establishing causation.

TABLE VII. Pearson Correlation Coefficients Between Substrate Characterization Parameters and Theoretical Buswell Methane Potential.

Parameter	Pearson r	t -statistic	Significance ($df=2$)
Carbon (C, %)	-0.952	-4.381	$p < 0.05$ *
Nitrogen (N, %)	-0.577	-0.999	ns
Volatile Solids (VS, %TS)	+0.946	+4.117	$p < 0.05$ *
Total Solids (TS, %)	+0.885	+2.692	ns
COD (mg/l)	-0.850	-2.277	ns
VFA (g/L)	-0.795	-1.852	ns
pH	-0.918	-3.264	$p < 0.10$
Alkalinity (mg/kg)	+0.584	+1.018	ns

B_0 (L CH₄/kg VS), $n = 4$ substrates, $df = 2$. Critical t -values: $t_{0.05} = 4.303$ (two-tailed); $t_{0.10} = 2.920$. * $p < 0.05$; ns = not significant.

I. Comparative Assessment: Co-digestion Requirement

Integrating the characterization results across all parameter domains, no single substrate provides an inherently optimal balance of VS content, C/N ratio, pH buffering, biodegradable organic fraction, and freedom from inhibitory compounds for stable anaerobic digestion. Faecal sludge (FSH and FSR) is nitrogen-enriched with sub-optimal C/N (18.19–18.49), dilute, and carries significant pathogen loads; food waste is carbon-rich (C/N = 23.43) but fat-dominated with quantified LCFA risk; cow dung is fiber-rich (C/N = 20.66) with the highest theoretical buffering capacity but lowest organic loading intensity. This complementary limitation profile is precisely the configuration that benefits most from co-digestion, where substrate synergies correct individual deficiencies [8], [14].

The characterization data indicate that the Specimen I formulation (FSR: FW: CD = 3:1:1, mixed C/N = 29.28) most closely achieves the optimal C/N ratio for methanogenesis, while Specimen E (FSH: FW: CD = 4:3:3, mixed C/N = 31.24) provides the highest absolute organic loading and the lowest fat dilution risk. For process design at RSU, the co-digestion rationale is therefore empirically grounded in the substrate characterization: co-digestion is not merely beneficial but necessary to achieve stable, high-yield anaerobic digestion of institutionally generated faecal sludge.

IV. CONCLUSION

This study provided a comprehensive multi-parameter characterization of four institutional organic waste substrates (FSH, FSR, FW, and CD) collected from Rivers State University, Port Harcourt, Nigeria, alongside nine mono- and co-digestion treatment configurations, revealing the following key findings:

- Both faecal sludge types (FSH C/N = 18.49; FSR C/N = 18.19) exhibited carbon-to-nitrogen ratios below the optimal 20–30 range for methanogenesis, confirming that co-substrate supplementation with carbon-rich materials is essential for stable AD of RSU institutional faecal sludge.

- Chemical oxygen demand differed significantly across raw substrates ($F_{3,8} = 148.94$, $p < 0.001$), ranging from 2,710.9 mg/kg (CD) to 4,750.1 mg/kg (FSH), establishing FSH as the highest-strength feedstock (62.35 mg COD/mg VS) but also the most challenging for VFA management in mono-digestion.
- Food waste fat content (44.4% of biochemical total) exceeded the long-chain fatty acid (LCFA) inhibitory threshold, necessitating co-digestion dilution to reduce lipid loading at the digester; the recommended FW proportion in blends should be controlled so that fat-to-VS ratios remain below 10%.
- Theoretical Buswell methane potentials (B_0) ranged from 645.6 L $\text{CH}_4/\text{kg VS}$ (CD) to 764.2 L $\text{CH}_4/\text{kg VS}$ (FSH), establishing the upper-bound thermodynamic conversion efficiency based on substrate elemental CHNS/O stoichiometry.
- All substrates harboured multidrug-resistant gram-negative bacterial isolates; *Ascaris lumbricoides* and *Trichuris trichiura* ova were identified in FSR, confirming that post-digestion digestate treatment (e.g., solar drying or composting) is mandatory prior to agricultural land application.
- The Specimen I co-digestion formulation (FSR: FW: CD = 3:1:1, mixed C/N = 29.28) achieved the closest alignment with the optimal methanogenic C/N range among all configurations tested, combined with superior alkalinity (320.8 mg/kg) and high biodegradability ($\text{BOD}_5/\text{COD} = 0.6968$), providing an empirically grounded design basis for process scaling.

These findings establish the empirical substrate characterization and stoichiometric foundation for co-digestion bioprocess design, kinetic modeling, and techno-economic evaluation.

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