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A Taguchi fractional factorial design approach to assessing Cattle-Poultry-Hog manure mix ratio influences on biogas yield

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Abstract

Methane production from co-substrate (CS) bio-digestion of cow, poultry and hog manure (CM: PM: HM) can be a potential energy alternative supply. The effect of the co-digestion of these CSs on biogas and methane yield was investigated in this study. Using the Taguchi fractional factorial design (TFFD) method, twenty-eight experiments with varying CSs mix ratios (CMR) were prepared including three experiments made up of the three individual substrates (ISs). Under mesophilic temperature conditions, controlled volatile solid concentration, and a thirty-day hydraulic retention time with all other physicochemical conditions kept unconstrained, the daily cumulative biogas and methane yields were collected and evaluated. Using these, the ultimate methane yields ($V^p_{mi\infty}$) were predicted using Richard's kinetic model. The relative $V^p_{mi\infty}$ of the CSs with those of the ISs was compared to establish the synergistic properties of the CSs. The effects of the CSs interactions were also assessed using a quartic polynomial regression model. Furthermore, the optimal CMR necessary for producing the maximum methane yield was also predicted using the TFFD analysis. The $V^p_{mi\infty}$ range for the CMRs and ISs was (0.93-1.97) and (0.55-1.31) litres respectively indicating that higher methane yield production is possible with the use of CSs. However, to achieve this, the CMRs have to be carefully chosen. The relative $V^p_{mi\infty}$ was also found to be higher for all the CMRs further indicating the superiority of co-digesting the substrate rather than mono-digestion. The CSs interactions model showed that the addition of HM and PM produced the best and worst synergistic effects respectively. The TFFD analysis revealed an optimal CMR for CM: PM: HM to be 5:1:5.

Keywords: Co-digestion, Biogas, Cow manure, Poultry manure, Hog manure, Substrate mix ratio, Taguchi fractional factorial design

1. Introduction

Energy is regarded as one of the key essentials of human needs. Given its strategic significance in the effective running of domestic, commercial and industrial activities, the importance of sustained energy availability cannot be overemphasized. Africa, with a population value in the excess of one billion [1], has huge energy demands. Based on the prediction by the International Energy Agency (IEA) [2] the continent's energy demand which is experiencing a fast growth is currently estimated to be about 784 Terawatts-Hour (TWh) with a projected exponential increase to 2300 TWh by next two decades.

Unfortunately, the energy needs in many of these countries, which is hugely dependent on hydrocarbon-based fuels for energy generation [3, 4], has been in very short supply [5, 6]. This has resulted in just about one-quarter of the population having access to such energy sources [7]. Areas most hit by this supply problem are the rural communities where over 50 percent of the total population reside [8]. As such, most members of this group resort to satisfying their energy needs through the use of fuel wood resources [9]. However, this type of energy utilisation comes with various health and environmental consequences [10]. Energy generation using renewable sources including bio-energy from biogas production has been suggested as a much better alternative to fossil and wood-based fuels [11, 12].

Biogas is obtained from the anaerobic digestion [13, 14] of suitable organic materials such as animal waste, industrial waste, and plant residues [15, 16] in suitable digesters or plants, under different physical and environmental conditions [17, 18]. The small-scale biogas generation option for households in rural communities has been adjudged to be the most affordable and implementable due to its lower cost and decentralization potential [19, 20]. This is further backed up by the availability of biogas substrates given that a large percentage of dwellers in these communities keep livestock such as Cattle, Poultry, and Pigs [21, 22]. The waste products from these animals are considered suitable for biogas generation [23, 24] and have produced encouraging results [25, 26].

However, the effort to harness this potential has been very little [27]. Some of the factors responsible for this is the practice of open range grazing [28] and the low number of livestock type owned by each household [29, 30]. These factors limit the amount of biogas substrate per type of animal available. As such, for the households to be able to effectively address their energy needs, improve digester management efficiency and sustain bioenergy generation, the co-digestion of readily available substrates such as cow manure (CM), poultry manure (PM), and hog manure (HM) as co-substrates seems the way forward. Co-digestion is a process of producing biogas

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from a mixture of complementary substrates. The co-digestion process aids in increased biogas production, substrate reduction [31, 32] and encourages greener environments [33, 34].

An important factor that affects co-digestion biogas yield is that of the co-substrate mix ratio (CMR). It has been reported that physicochemical properties such as the Carbon-Nitrogen (CN) ratio and effective volatile solid (VS) removal changes with the CMR [35-37]. This is particularly true for cattle, poultry, and swine dungs which have been reported to exhibit poor digestion efficiencies when used in mono-substrate digestion [38, 39]. In that regard, the conduction of co-digestion experiments to cover a wide range of co-substrate mixing ratios has been advised [40].

One of the ways of determining the CMRs that ensure output quality and productivity of experiments is through the use of experimental design array (EDA) models. EDAs refer to the number of experiment runs (number of CMRs) desired by the analyst that takes cognizance of the number of factors (substrates and digestion conditions) and desired number of levels for each factor (maximum proportion of substrate split). Various objective and validated EDA models such as factorial, response surface, and orthogonal arrays, are available for adoption or adaptation in the literature [41, 42].

However, their use can have a huge bearing on results and resource costs depending on the EDA model used, the number of factors used in the experiments, and the expected outcomes [42]. The Taguchi fractional factorial EDA otherwise called the robust design technique is an EDA model that has gained popularity due to its ability to recommend relatively small EDAs towards a more efficient determination of optimal responses [41] and identification of key factors that have the most effect on performance characteristics [43].

It involves the use of orthogonal arrays to analysis of process parameters at different variation levels. It is distinct from other EDAs because rather than test all possible combinations as applicable in factorial design, its orthogonal arrays are chosen based on tested pairs of combination [43] leading to the lesser experiments without compromising the balance of the factor levels. The goal of the approach is to minimise loss function, that is, the deviation between the process characteristic performance and its corresponding target value. This is achieved through the signal to noise (S/N) ratio, main effects and ANOVA as evaluation methods [44].

The method has found well application in different fields of research. For example it has been applied in the health sector for tumour identification and treatment conditioning [45, 46], heat exchanger optimisation [47], mechanical polishing processes [48] and plant growth conditioning [49]. Although it has been suggested that the method could be quite useful in the field of biotechnology, few works exist in this area. Some of these include the optimisation of Hydrazine bio sorption [50] and the effect of some physicochemical properties on methane yield using mono substrates [51]. It's Application for the in biogas co-substrate mix ratio influences on biogas yield is sparse.

Previous investigations regarding the co-digestion of CM, PM, and CM have been done. For example, the assessment of the effect of the co-digestion of CM-PM [33, 52], PM-HM [38, 53], and CM-PM [54-56] on biogas has been carried out at different substrate physicochemical characteristics, digester and environmental conditions. Results obtained from the reviewed cases show that the substrate pairs used complemented each other to produce biogas yield volumes that were superior to those obtained from digesting each substrate independently. Regarding the co-digestion CM-PM-HM, some attempts have been made [57, 58]. The studies also revealed similar results to those of the two substrate co-digestion.

Regarding the previously highlighted studies of Olanrewaju and Olubanjo [57] and Belaid et al. [59], only a single scenario of substrate mixing was considered, while Samuel et al. [58] considered four types of co-substrate ratios. Since these co-substrate ratio choices were not reported as being made based on any EDA method, it is unclear whether the co-substrate ratios used in the previous studies were adequate in determining the best yield capability of the cattle-poultry-hog co-substrate. The gap observed therein the literature indicates that more information is still required towards understanding the effect of the interactions of CM-PM-HM on biogas yield. Also, little attempt has been made in the use of standard techniques such as the Taguchi Experimental Design Array (EDA) in the estimation of Cow, Poultry and Hog manure (CM-PM-HM) co-substrate mix influences on biogas yield.

The research is thus concerned with the problems of (1) Identifying the synergistic effect of cow, poultry and hog manure co-substrate ratio mix on biogas yield and (2) Utilising a standard Taguchi EDA as a standard approach in the determination of cow, poultry and hog manure co-substrate ratio mix

The goal of this research revolves around addressing these problems by firstly investigating the synergistic interactions that exist between CM-PM-HM and their corresponding effects on biogas yield. Then, utilizing the Taguchi EDA as the analytical tool to determine CM-PM-HM substrate mix ratio influences on biogas yield using a laboratory-scale batch reactor. The study objectives are to (i) Evaluate the anaerobic co-digestion efficiency of CM-PM-HM based on CMR choices made using the Taguchi fractional factorial EDA (ii) Validate the cumulative methane yield produced from the co-digestion process and determine the corresponding and related parameters using kinetic models (iii) Determine the CMR that can produce the maximum methane yield from the co-digestion process.

It is hoped that this contribution will add value to the literature as well as provide stakeholders with the information needed to trigger the biofuel-based alternative energy industry for rural communities.

2. Methodology

2.1 Design of experiment

Four variables CM, PM, HM, and the EDA retention time interval (RTI) [t^*] were adopted as controllable factors for the design of the laboratory-scale experiment. t^* constitutes different intervals within the hydraulic retention time HRT used specifically for the EDA analysis. Due to the need to optimise result quality and deployed resources, five levels of each factor were used to create the Taguchi fractional factorial EDA ($R_4 - R_{28}$) using Minitab 19.

This was achieved using L25 orthogonal array in Minitab 19 which corresponds to the number of runs required for a 3 factor 5 Level or a 4 factor 5 Level experiment from the "Create Taguchi Design" feature in Minitab 19. This resulted in Twenty five CMRs (Table 1). In addition, three control runs ($R_1 - R_3$) each made up of pure forms of CM, PM, HM, and t^* were added to the experiment runs.

Table 1 Co-substrate ratios for different experiment runs

Run(i)	x_{i1}	x_{i2}	x_{i3}	$x_{i4}\,(t^*)$
1	1	0	0	5
2	0	1	0	5
3	0	0	1	5
4	1	1	1	1
5	1	2	2	2
6	1	3	3	3
7	1	4	4	4
8	1	5	5	5
9	2	2	1	3
10	2	3	2	4
11	2	4	3	5
12	2	5	4	1
13	2	1	5	2
14	3	3	1	5
15	3	4	2	1
16	3	5	3	2
17	3	1	4	3
18	3	2	5	4
19	4	4	1	2
20	4	5	2	3
21	4	1	3	4
22	4	2	4	5
23	4	3	5	1
24	5	5	1	4
25	5	1	2	5
26	5	2	3	1
27	5	3	4	2
28	5	4	5	3

2.2 Experiment materials, substrate collection, and characterisation

The main apparatus and materials used for the experiments included twenty-eight 5 litre plastic digesters, each tightly fitted with a 10 mm diameter delivery tube, mini-sized water baths, measuring cylinders, electronic weighing scale, thermometer, and a pH meter.

CM, PM, and HM stored under aerobic conditions were obtained from the respective pens of a selected farm located within a rural community in South-Western Nigeria. The experiment was set up such that for each run ($i\ \{i = 1, 2, 3, \ldots, 28\}$), $40g\ TS$ of the mixed pulverised manure types was fixed as the dry matter (DM) weight (M^{cs}) of the co-substrate and the mass content of the CMR substrate component ($j = 1,2,3$) for all runs summed up to M^{cs}(Equation 1).

Next, the masses of the wet matter (WM) weights of the individual substrates (IS) were determined (Equation 2), measured, and then added to obtain the co-substrate (CS) wet matter weight $\left(W_i^T\right)$ (Equation 3). The starting total solids (TS) , volatile solids (VS) and chemical-oxygen demand (COD) concentration of each IS $\{[TS]_{i0}\ (\%),\ [VS]_{i0}\ (\%), [COD]_{i0}\ (g/L): i = 26, 27, 28\}$ were determined as described by Mahmoodi et al. [60] (Table 2). The $[TS]_{i0}$, $[VS]_{i0}$, and $[COD]_{i0}$ for each of the CS were then calculated (Equation 4).

$$M_{ij} = \delta_j M^{cs} \tag{1}$$

$$W_{ij} = \frac{M_{ij}}{[TS]_{ij0}} \tag{2}$$

$$W_i^{cs} = \sum_{j=1}^{3} \delta_j W_{ij} \tag{3}$$

$$Z_{i0} = \left(1/\sum_{j=1}^{3} x_{ij}\right)\left(x_{i1}Z_{26,0} + x_{i2}Z_{27,0} + x_{i3}Z_{28,0}\right) \quad \{i \neq 26, 27, 28\} \tag{4}$$

The digestate volume $\left(V_i^d\right)$ was determined (Equation 5) by fixing M_i^T as 9% of the diluted co-substrate weight [61]. The volume of water required to make up each digestate (V_i^w) was then determined (Equation 6) measured and added to a 1.5 litre plastic digester along with M^{cs}. The digester volume was carefully selected to also accommodate the biogas that was generated.

$$V_i^d = W_i^{cs} \rho_i^{cs-1} + V_i^w \tag{5}$$

$$V_i^w = (11.11M^{cs} - W_i^{cs})\rho_w^{-1} \tag{6}$$

Where,

$$\rho_i^{cs} = \rho^w + \sum_{j=1}^{3} \delta_j \rho_j\ , \tag{7}$$

$$\delta_j = x_{ij} / \sum_{j=1}^{3} x_{ij} \tag{8}$$

$j \neq 4$, Z_{i0}: $[TS]_{i0}\ \vee\ [VS]_{i0} \vee\ [COD]_{i0}$

(δ_j is the fraction of substrate $j.\rho_{ij}^{cs}, \rho^w, \rho_j$ are the densities (g/L) of the diluted co-substrate, water and individual substrates respectively. The values of ρ_j used were those proposed by Houkom et al. [62], Chen [63], and Landry et al. [64] in situations where the required input parameters fell within the respective function range. Otherwise, the density function of Lorimor and Sutton [65] were employed).

Three replications of the experiment were conducted at ambient temperature and normal pressure conditions within a 30 day HRT. At thirty different RTIs (t), that is every day after the bio-digestion process had begun, the cumulative biogas yield (V_{Bit}) and methane yield (V_{Mit}) produced from each run were recorded. For each interval, V_{Bit} was collected by a 0.5 molar HCL solution displacement, using a calibrated inverted cylinder that was placed in a water bath filled with the same solution. By basifying the resulting water bath using concentrated NaOH solution until a pH of at least 9 [60] was attained, the methane yield volume was read off as the decreased biogas volume in the cylinder. The pH and temperatures of the digesters were also recorded at the start and within the RTIs. On the final day of each experiment, samples of the digestate were collected and analysed for their TS, VS, and COD properties.

2.3 Theoretical and experimental bio-Methane potential analysis

To have a better understanding of the nature of the ISs employed for the study, an adaptation of the Hill and Chen and Hashimoto mono-digestion models [66-68] was used in the estimation of the theoretical bio-methane potential (TBMP) of the CM, PM, and HM (Equation 9).

$$V_{Mj\infty}^T = \frac{B_j[\phi_j\mu - 1 + K_j]}{\theta\mu - 1} \tag{9}$$

$$B_j = \frac{\phi_j\theta}{S_{0j}} \tag{10}$$

$$\mu = 0.13T_d - 0.129 \tag{11}$$

$$K_j = 0.6 + 0.0206e^{0.051S_0} \tag{12}$$

$$\phi_j = 0.5C_{ai}\left[\frac{S_0}{\theta}\right]_j \left(0.5 + \left(ATAN\left[\frac{\left(C_{ai} - \left[\frac{S_0}{\theta}\right]_j\right)}{0.211}\right]\right)\Big/2.95\right) \tag{13}$$

$$C_{aj}, C_{bj} = \begin{cases} 0.48, 9.21 & \{i = 1\} \\ 0.63, 4{,}89 & \{i = 2\} \\ 0.63, 6.69 & \{i = 3\} \end{cases} \tag{14}$$

$$S_{oj} = \frac{M_j\rho^w\rho_j}{M_j\rho^{w-1} + M^w\rho_j^{-1}} \tag{15}$$

Where,

$V_{Mj\infty}^T$: Theoretical bio-methane methane yield (L). B_j: Yield of substrate per VS-added. ϕ_j: Methane productivity (L/L-day). θ: HRT (day). S_{0j}: Specific TS of the feed (g/L). μ: Digestion growth rate operated at mesophilic conditions. T_d: Average digestion temperature at mesophilic conditions taken as 30°C. M^W: mass of water. K: Kinetic constant for digester operation at mesophilic conditions. C_{aj}: Unstressed (maximum) VS reduction index. C_{bj}: Loading rate stress index for $j = 1,2, and$ 3 for CM, PM and HM respectively.

The cumulative bio-methane produced during each experiment run was analysed to determine its experimental bio-methane potential (EBMP) [$V_{Mi\infty}$]. By assuming that the process followed a first-order non-linear characteristic and with considerations given to the biogas yield and the efficiency of the digestion process, the Richards model (Equation 16) was used both as a fitness function for the experimentally generated bio-methane data and in the estimation of $V_{Mi\infty}$ $\{\forall i\}$. Richard's kinetic model is one that can accurately describe methane production with methanogenic bacterial growth [69, 70] and was found to be the model which most fit the experimentally generated bio-methane data characteristics after its results were compared with those of the Gompertz, Modified-Gompertz and Logistic biogas growth models. The parameters of the kinetic model were determined using a non-linear curve fitting computer program developed in Python 3.7. Based on this, the root mean square error $(RMSE)$ and the predicted R-Squared values were also determined and used to evaluate the model fitness to the generated data.

$$V_{mit}^p = V_{Mi\infty}\left\{1 + \left(a_i exp[1 + a_i] exp\left[\left(\frac{P_i}{V_{Mi\infty}}\right)(1 + a_i)^{\left(\frac{1+a_i}{a_i}\right)}(\lambda_i - t)\right]\right)\right\}^{-\frac{1}{a_i}} \tag{16}$$

Where, V_{mit}^p: Predicted cumulative methane yield (L) at t; $V_{Mi\infty}$: methane potential (L) as $t \to \infty$; P: Maximum methane production rate (L/day); λ_i: Bio-digestion lag phase (day),a_i: Richard's shape parameter.

2.4 Performance measurement and Investigation of co-substrate interactions

The performances of the different bio-digestion processes were evaluated by comparing the biogas yields (V_{Bi30}) and methane yields (V_{Mi30}) produced by the co-substrate mixes (i) at maximum HRT. The digestion efficiencies were also determined by computing the percentage reduced fractions of TS, VS, and COD during the processes. TS and VS percentage reductions were computed using Koch model [71], while the reduced COD (COD_r) was obtained using equation 17. By adapting Adelard et al. approach [72], the co-substrate mixtures (CM-PM-HM) relative performances (ΔA) were also determined by comparing the respective yield and digestion efficiencies of i with those that could be obtained in a situation where the individual substrates were deployed in the same mix proportion [72] (Equation 18).

$$COD_r = 100([COD]_{i0} - [COD]_{i30}) / [COD]_{i30} \quad (17)$$

$$\Delta A = \left(A - \sum_{i=1}^{3} x_i A_i\right)/A \quad \{i = 1,2,3\} \quad (18)$$

$$A = V_{Mq30}\big|[TS]_{q30}\big|[VS]_{q30}\big|[COD]_{q30}\big|\lambda_q \quad \{i \le 3; q > 3\} \quad (19)$$

Further, regression analysis was used to study the interactions between the co-substrates as digestion took place daily. One of the usefulness of the regression model is that it can provide information on the strength of the effect of the predictors on the response when the conditions that set up the latter and the former are properly modelled. In our case, the model predictors were the CMRs (Table 1). In addition to defining $x_{ij}\{j \neq 4\}$ (Table 1), the EDA codes for factor $x_{i4}(t^*)$ were defined as five RTIs, chosen to cover the entire span of the HRT of the bio-digestion (Table 2). The quartic polynomial regression model was adapted to study the interactions between the substrate levels and the RTI levels (Equation 20).

The quartic model was employed for the analysis because during preliminary investigation, it was observed that the data exhibited sinusoidal characteristics with multiple inflexions and turning points. This supported the adoption of the quartic regression model for the analysis of such data characteristics [73, 74]. The response factor used was the methane volume (V_{Mit^*}) produced by each CMR co-digestion that corresponds to the $x_{ij}\{t^*: t^* = 1,2,3,4,5\}$. For example, for the CMR described by R_9 (Table 1), the methane volume obtained on day 18 [$x_{i4}(t^* = 3)$] of the co-substrate digestion of CM, PM, HM dry matter in the ratio 2:2:1 of gram equivalent mass 36:36:12 was used as the response factor for that CMR.

Table 2 EDA code definition for CMR factor x_{i4}

x_{i4} (EDA) Code	1	2	3	4	5
EDA RTI Code [t^*]	1	2	3	4	5
EDA RTI (day)	6	12	18	24	30

$$V_{Mit^*} = K + \sum_{i=1}^{N}\sum_{j=1}^{3} \alpha_{ij4} x_{ij} x_{i4}(t^*) + \sum_{i=1}^{N}\sum_{j=1}^{3}\sum_{m=1}^{3} \alpha_{ijm4} x_j^p x_m^q x_{i4}(t^*) + \sum_{i=1}^{N}\sum_{j=1}^{3}\sum_{m=1}^{3}\sum_{n}^{3} \alpha_{ijmn4} x_{ij} x_{im} x_{in} x_{i4}(t^*) \; \{p,q > 0; p + q < 4; j \neq m\} \quad (20)$$

The model was fitted to the data using the general regression analysis feature of Minitab 19. The final model fit was achieved by reducing the insignificant model terms by the use of a backward elimination procedure [75].

2.5 Optimal mix-ratio yield determination

The TFFD analysis was also done to determine the CMR that could potentially produce the maximum methane yield (CMR_{Max}). This was done using information from the signals-noise (SN) ratio and means plots of the CMRs and their corresponding response factors (V_{Mit^*}) using the analysis and prediction functions of the TFFD model in Minitab 19. The control runs were not used in the TFFD analysis. In addition (CMR_{Max}) was validated by investigating the predictive accuracy of the model. In achieving this, the "Predict Result" feature in the Minitab 19 was deployed in predicting V_{Mit^*} for all one hundred and twenty-five experiment points used. Evaluation to establish the predictive accuracy was done using the mean absolute percentage error (MAPE) and the root mean square error (RMSE).

3. Results and discussion

This section discusses the result of the experiments and analyses carried out on CM, HM, and PM as individual substrates and in their combined forms.

3.1 Individual substrate characterization

The pre-treatment characteristics of the individual substrates (Table 3) indicated that the substrates generally had high TS fractions at levels that could inhibit digestion efficiency and methanogenic activity. This necessitated the dilution of both the mono-substrate and co-substrate before bio-digestion to improve their yield potentials. With regards to the VS fractions, all control substrate values were low and existed outside the 70-90% range frequently reported [76, 77]. This indicated a low organic matter content [78] and by implication, a lowered biogas production potential of the substrates [79]. This may be attributed to the open-air storage conditions in which the substrates were subjected before collection [80].

Table 3 Characterisation of the individual cow, poultry, and hog substrates

Substrate	**Physicochemical Characteristics**						**Potential**	
	TS (%)	**TS (g/L)**	**VS (%)**	**VS (g/L)**	**COD (g/L)**	**pH**	**CH_4 Yield ($V_{Mi\infty}^T$) [L]**	**Yield per VS-added (B) (L/g)**
CM	38.19	88.16	59.78	52.70	68.93	6.7	5.73	0.272
PM	34.37	91.29	65.40	59.70	53.79	6.8	8.46	0.354
HM	26.09	92.74	62.25	57.73	87.52	6.9	8.34	0.361

3.2. Biogas and methane yield

On average, about 15% of the total methane yield from the respective ISs and CMRs was produced by the 7th day of digestion and more than 75% and 90% of the yield had been obtained by the 18th and 22nd day respectively. The volume of biogas and methane produced within the HRT varied from one form of combined substrate ratio to another (Table 4).

Table 4 Biogas and methane results obtained from experiments and kinetic modelling

i	$[TS]_{i0}$ (%)	$[VS]_{i0}$ (%)	$[COD]_{i0}$ (gL^{-1})	V_i^d (L)	Biogas					Methane					$[TS]_{i30}$ (g)	$[VS]_{i30}$ (%)	$[COD]_{i30}$ (gL^{-1})
					V_{Bi30} (L)	EBGP (L)	λ_{Bi} (day)	RMSE	RSqr (%)	V_{Mi30} (L)	EBMP (L)	λ_{Mi} (day)	RMSE	RSqr (%)			
1	38.19	59.78	68.93	0.454	2.187	2.379	4.18	42.20	90.83	1.122	1.224	5.98	38.47	74.51	30.89	44.41	31.38
2	34.37	65.40	53.79	0.438	1.258	1.264	1.61	16.51	96.49	0.545	0.548	2.93	9.67	94.85	30.48	58.67	45.03
3	26.09	62.25	87.52	0.431	2.445	2.564	6.30	51.02	90.65	1.311	1.377	6.88	36.62	83.85	19.63	45.44	55.12
4	32.05	62.48	70.08	0.441	2.344	2.373	3.79	33.49	95.11	1.318	1.336	4.41	22.69	93.28	24.99	46.88	35.38
5	31.62	61.89	73.34	0.442	2.186	2.235	3.40	27.53	95.90	1.242	1.274	4.39	17.40	95.34	25.15	47.55	45.28
6	31.44	61.64	74.73	0.442	2.197	2.257	3.39	23.04	97.15	1.231	1.270	4.28	14.88	96.47	24.94	47.06	44.27
7	31.34	61.50	75.51	0.442	2.277	2.326	3.60	13.78	99.07	1.283	1.315	4.48	9.33	98.75	24.61	46.16	40.91
8	31.28	61.41	76.00	0.442	2.270	2.334	3.45	16.59	98.59	1.291	1.336	4.34	10.87	98.25	24.58	46.12	40.99
9	33.58	62.52	66.59	0.443	1.929	1.952	2.74	17.73	97.74	1.065	1.078	3.75	10.89	97.46	27.70	50.54	42.68
10	32.80	62.09	69.92	0.443	2.099	2.226	3.18	18.37	97.93	1.185	1.261	4.40	12.31	97.33	26.47	48.59	41.32
11	32.38	61.85	71.76	0.443	2.124	2.166	2.92	31.05	94.37	1.173	1.198	4.02	18.00	94.36	25.99	47.97	41.30
12	32.12	61.70	72.94	0.443	2.126	2.174	3.28	15.49	98.61	1.186	1.215	4.42	11.51	97.74	25.77	47.78	41.61
13	28.98	62.73	76.76	0.436	3.363	3.427	5.30	37.51	97.15	1.971	2.017	5.78	23.35	96.88	19.18	35.89	31.22
14	34.29	62.54	65.10	0.444	1.892	1.932	3.47	14.97	98.39	1.044	1.069	4.83	6.84	99.01	28.46	50.87	41.51
15	33.50	62.20	68.02	0.444	1.827	1.861	3.45	12.94	98.73	1.024	1.045	4.37	6.16	99.15	27.98	50.99	43.55
16	33.01	61.99	69.87	0.443	2.005	2.061	3.50	17.91	97.94	1.132	1.166	4.43	12.56	97.03	26.97	49.26	41.86
17	29.99	63.12	72.55	0.437	3.213	3.611	4.44	29.22	97.87	1.856	2.110	5.65	26.68	95.01	20.43	38.47	32.75
18	30.18	62.70	73.68	0.438	2.492	2.663	4.61	34.67	95.24	1.416	1.526	5.55	21.48	94.62	22.93	45.80	43.42
19	34.69	62.55	64.27	0.444	1.791	1.896	2.98	30.95	92.00	0.994	1.059	4.34	20.85	89.25	29.18	51.73	41.93
20	33.95	62.27	66.81	0.444	1.768	1.843	3.37	18.85	97.02	0.997	1.044	4.44	9.61	92.60	28.58	51.52	43.31
21	31.06	63.52	68.33	0.438	3.010	3.297	5.10	50.99	93.12	1.746	1.929	6.11	40.33	87.90	21.94	41.51	34.80
22	31.05	63.02	70.31	0.439	2.704	2.883	3.75	13.40	99.35	1.565	1.670	4.80	14.70	97.84	22.99	44.21	38.75
23	31.04	62.68	71.63	0.439	2.648	2.786	3.99	52.45	90.45	1.521	1.603	5.39	46.38	79.78	23.15	44.23	38.78
24	34.95	62.56	63.74	0.445	1.731	1.743	2.34	10.32	99.00	0.931	0.938	3.95	9.01	97.78	29.58	52.11	41.92
25	32.22	63.91	64.12	0.438	2.865	3.025	4.34	46.05	93.78	1.639	1.739	5.24	34.07	90.18	23.39	43.82	35.74
26	31.97	63.33	66.94	0.439	2.775	2.954	3.62	22.38	98.29	1.569	1.685	4.87	20.12	96.00	23.47	43.83	36.22
27	31.80	62.95	68.82	0.440	2.694	2.734	4.13	24.13	98.03	1.509	1.535	4.96	10.17	98.95	23.60	44.05	36.84
28	31.68	62.67	70.16	0.440	2.560	2.643	3.79	17.74	98.80	1.454	1.506	4.64	15.66	97.27	23.97	45.09	38.61

For the individual substrates ($R_1 - R_3$), 2.18, 1.26, and 2.45 litres of biogas were generated for CM, PM, and HM respectively. Similarly, 1.12, 0.55, and 1.31L of methane were produced. These yields were much less their theoretical equivalents of 5.73, 8.46 and 8.34 for CM, PM and HM respectively (Table 3).

Measured in terms of the amount of VS added, the methane yields generated from the experiment (0.047, 0.021, and 0.053 L/VSadded) within the HRT adopted, were much lower than the computed theoretical methane yields of the corresponding individual substrates (0.272, 0.354, 0.361 L/VSadded). The result could be attributed to the substrates' poor VS composition as the amount of volatile solids in a substrate directly impact the potential for higher methane yields [81, 82].

Another reason that could be attributed to this is that the physicochemical mechanisms that lead to improved VS reduction and in effect improved methane yield may have been hampered by the uncontrolled physicochemical conditions in which the experiments were subjected. A glaring example of this is seen in the PM (R_2) digestion (Figure 1) where the biogas yield rate which was high in the first week, quickly decelerated by the end of the second week and stopped altogether by the third week. The R_2 effluent COD value of 58.03g/L indicated the substrate still had a high methane potential. However, digestion may have stalled due to negative interference in the methanogenic pathway of the process by some of the mentioned conditions [18, 38].

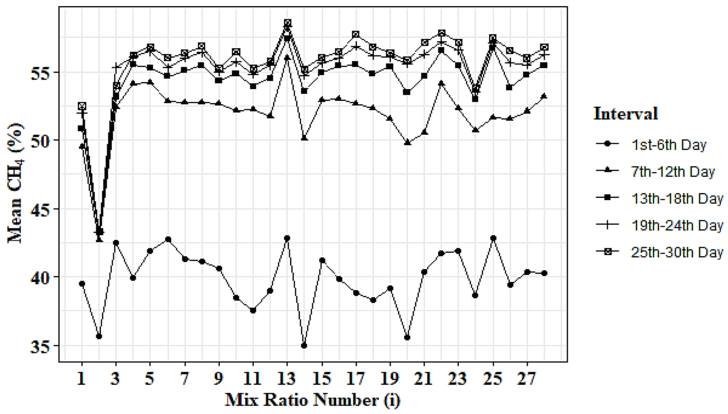

Figure 1 Mean periodic methane yield from bio-digestion of control and various co-substrate mix

The lowest amount of biogas and methane produced by the co-substrates was 1.73 and 0.93L respectively, while the highest amount produced was 3.36L and 1.97L respectively. The highest biogas yields were generated by R_{13} (3.36L), R_{17} (3.21L), and R_{21} (3.01L) corresponding to 1.97L, 1.86L, and 1.72L of methane produced respectively. The least biogas producing co-substrates were R_{24} (1.73L), R_{20} (1.77L), and R_{19} (1.79L) with 0.93L, 0.99L, and 1.00L of methane produced respectively. The methane proportion of the biogas produced was observed to be low in the first six days of production, but quickly rose and stabilised as the HRT increased to 12, 18, 24, and 30 days except for R_2 (Figure 1) whose methane production may have likely been stalled by the uncontrolled temperature, pH, digesting organism, VFA formation, and free ammonia formation conditions in which the experiments were subjected [83].

The results agreed with the findings that longer HRTs seemed to enhance better biogas quality in terms of increasing methane proportion [84]. Within the HRT considered, the average percentage methane constituent of biogas was highest for R_{13} (58.6%), R_{22} (57.9%), and R_{17} (57.8%) compared to the individually digested substrates R_2 (43.3%), R_1 (51.3%) and R_3 (53.6%). This showed that a mix of the substrates at certain ratios produced better biogas quality than digesting the substrates individually.

In terms of the relative methane yield of the CMR to the combined individual substrates (ΔV_{Mi30}) [Equation 14], all of the co-substrates mixes produced better relative methane yield than those of the individual substrates (Figure 2) with R_{13} (40.7%), R_{17} (38.3%) and R_{21} (35.8%) being the best performers and R_{24} (5.8%), R_{20} (10.3%) and R_{19} (10.8%) being the worst. However, ΔV_{Mi30} was significantly different among i ($p < 0.05$). This result showed that the methane yields produced by the CMRs were characterised by synergistic and non-synergistic properties which differed in magnitude according to the effect of the CMRs were affected.

3.3 Total solids, volatile solids, and chemical-oxygen demand reduction

The amount of TS, VS, and COD remaining in the reactor at the end of the HRT are shown in Table 4, while their corresponding percentage reductions are shown in Figure 3. It was observed that generally for all the CMRs, TS, VS, and COD exhibited similar reduction trends. However, COD percentage reductions were observed to be highest while those for TS were lowest.

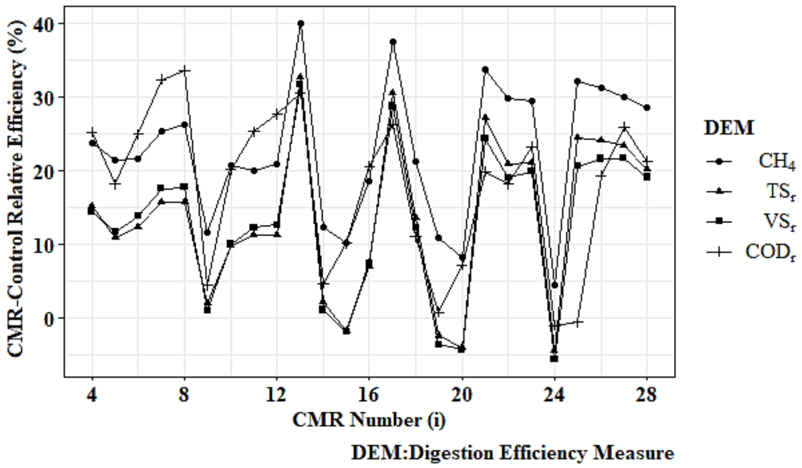

Figure 2 CMR-Control mix ratio relative performances in terms of methane yield, TS, VS and COD

The synergistic behaviour of reductions from CMR digestion showed that some co-substrate mixes had more materials reduced than others. For example, R_{13} showed a reduction of more than 30% in TS, VS, and COD respectively when compared to the reduction obtained from the separately digested control substrates measured in the same ratio as R_{13}. On the other hand, R_{24} relative reductions were -4, -5, and -1% for TS, VS, and COD respectively.

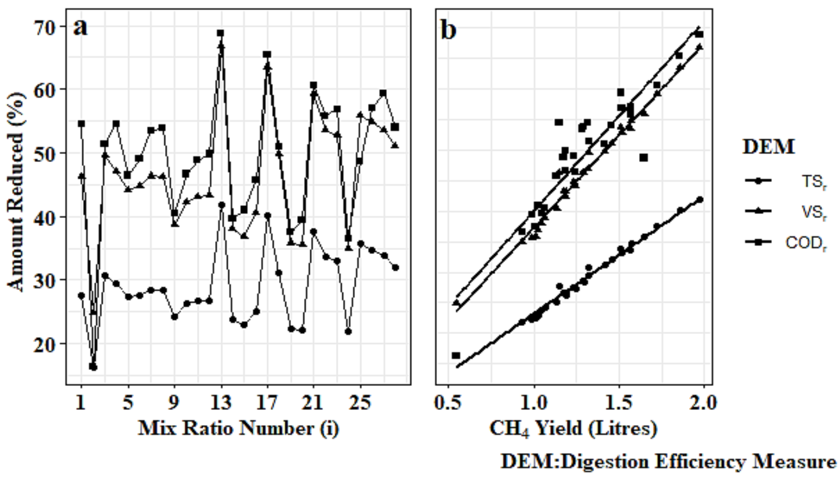

Figure 3 The plot of TS, VS, and COD reduced (a) for all CMR digested and (b) showing trend relationship with methane yield for all CMR

Among the CMRs R_{13}, R_{17}, and R_{21} were most reduced, while R_{19}, R_{20}, and R_{24} were least reduced. For each efficiency measure, the reductions significantly differed among treatment [$p(TS) = p(VS) = p(COD) = 0.0000$] indicating the importance of determining appropriate CMRs that guarantee optimum TS, VS, and COD reductions.

It was also observed that a direct relationship exists between the percentage reduction and methane yield for all the digestion efficiency measures considered (Figure 3b) implying that the higher the degree of reduction, the higher the amount of methane produced.

3.4 Potential biogas and methane yield

The parameter values of the kinetic modelling of the methane yield behaviour (V_{Mit} and λ_i) alongside the model fitness indicators ($RMSE$ and R^2) for the 28 substrate types are provided in Table 4, while the cumulative methane yields from the experiment and model predictions for the three best-performing, three worst-performing CMRs and ISs are shown in Figure 4.

The Richards model was able to accurately model the biogas and methane yield (V_{Mit}) profiles of the individual substrates and CMRs as can be observed in Figure 4. The respective mean, range and standard deviations for the RMSE and R^2 was [0.026L; (0.010, 0.052)L; 0.01L] and [96.34%, (90.45, 99.35)%, 2.72%] for V_{Bit} and [0.019L; (0.006, 0.046)L; 0.011L] and [93.98%, (74.51, 99.15)%, 5.94%] for V_{Mit}. Thus based on these results, $V_{mit}^{p}, V_{Mi\infty}$, and λ_i were inferred to be reliable individual substrates and CMR yield profile parameter estimates. The specific biogas potential and methane potential for the individual substrates ($i < 4$) was obtained as (0.099, 0.048, and 0.103) and (0.051, 0.021, and 0.055) L/VS$_{added}$ for CM, PM, and HM respectively. These results are quite low in comparison to the methane yield ranges of (0.13-0. 24), (0.02-0.39) and (0.12-0.19) for CM, PM and HM respectively obtained from previous studies [85-87]. Concerning the CMRs, the ultimate methane yield potential ranged from 0.938L (R_{24}) to 2.11L (R_{17}) which translates to a specific methane yield range of 0.037 and 0.084 L/VSadded respectively. This again indicated that co-substrate mix types affected yield synergistic and non-synergistic properties.

The lag times of the Individual substrate digestion as provided by Richard's model show that the times required by CM, PM, and HM to begin methane production were approximately 6, 3, and 7 days respectively. However, with the adoption of the co-substrate mixes, the methane production lag times were observed to be relatively lower than $\sum_{i=1}^{3} x_i\lambda_i$ in all CMR cases except for R_{14} and R_{21}. From this observation, it was clear that the adaptation time of the methane production process was shortened when the considered substrates were co-digested. This observation is also consistent with previous studies on co-substrate digestion of different biogas materials [87, 88].

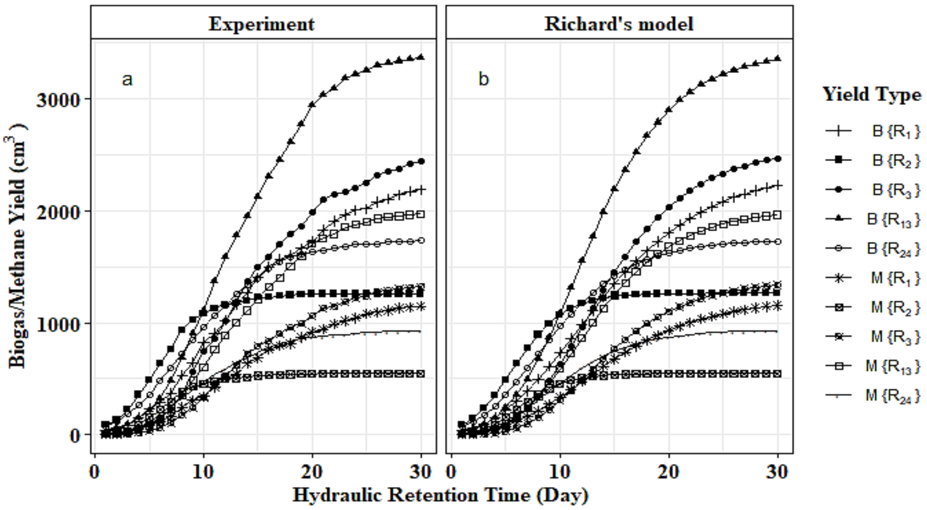

Figure 4 Cumulative methane yields for best and worst-performing CSs and all ISs

It was also observed from the kinetic model results that generally, the ultimate methane potential of substrates having longer lag periods tended to be higher than those with shorter lag periods.

Although this behaviour could be caused by the inclusion of other factors which may include the substrate quality and composition, microbial influences, and environmental conditions [89, 90], the observation nonetheless indicates that the lag time could be a potential control factor for optimising methane yield from the co-digestion of the considered ISs.

3.5 Substrate interaction analysis

The result of the evaluation of the effects of the co-substrate interactions on methane yield based on the significant factors determined at 95 % confidence interval from the quartic polynomial regression model analysis as produced from MINITAB 19 are shown in Figures 5 and 6. Specifically, the model summary and ANOVA outcomes are shown in Figure 5 and Equation 21.

From Figure 5, twelve out of the nineteen predictor variables initially considered in the model were significant ($pvalue \leq 0.05$). The high value of the adjusted R-Square (91.42) indicated that although the response variable exhibits a strong correlation with its predictor variables (R-Square =92.25%) and also exhibited good predictive capability (R-Square [Pred] = 89.50%).

$$\begin{aligned} V_{Mit^*} = &+10^{-3}x_{i4}(4.2x_{i4}^{-1} + 82.50x_{i1} - 104.10x_{i2} + 82.10x_{i3} - 11.83x_{i1}^2 + 17.86x_{i1}x_{i2} \\ &-23.75x_{i1}x_{i3} + 19.64x_{i2}x_{i3} - 10.45x_{i3}^2 + 1.15x_{i1}^2x_{i2} - 1.7283x_{i2}^2 \\ &+1.44x_{i1}x_{i2}x_{i3} - 0.90x_{i2}^2x_{i3} \end{aligned} \tag{21}$$

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
138.520	92.25	91.42%	89.50%

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	12	25578090	2131508	111.09	0.000
X1X4	1	779803	779803	40.64	0.000
X2X4	1	360359	360359	18.78	0.000
X3X4	1	791462	791462	41.25	0.000
X1^2X4	1	625050	625050	32.58	0.000
X1X2X4	1	180918	180918	9.43	0.003
X1X3X4	1	589331	589331	30.71	0.000
X2X3X4	1	233044	233044	12.15	0.001
X3^2X4	1	539384	539384	28.11	0.000
X1^2X2X4	1	424537	424537	22.13	0.000
X1X2^2X4	1	384563	384563	20.04	0.000
X1X2X3X4	1	676117	676117	35.24	0.000
X2^2X3X4	1	103116	103116	5.37	0.022
Error	112	2149029	19188		
Total	124	27727119			

Figure 5 Minitab 19 statistical evaluation indices obtained from quartic regression analysis in the determination of the methane yield-related significant causal factors

Regression Equation

CH4 = 4.2 + 82.5 X1X4 – 104.1 X2X4 + 82.1 X3X4 – 11.83 X1^2X4 + 17.86 X1X2X4 – 23.75 X1X3X4
+ 19.64 X2X3X4 – 10.45 X3^2X4 + 1.151 X1^2X2X4 – 1.728 X1X2^2X4 + 1.442 X1X2X3X4
– 0.897 X2^2X3X4

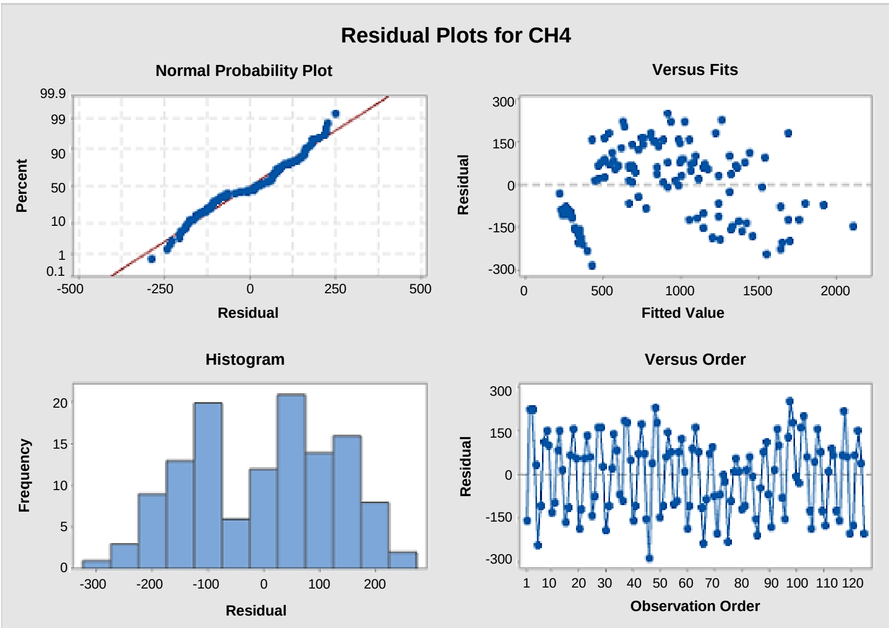

Figure 6 Regression Equation and Residual plot of the quartic regression model predictions

The probability plot of the predictions and residual-fit plot (Figure 6) indicated normally distributed predictions and randomly distributed residuals respectively. These further strengthen the adequacy of the developed predictive model. The predictive model (Equation 17) showed that the yield-related synergistic properties of the co-substrate digestion were stronger than the non-synergistic properties.

The synergistic co-digestion behaviours were linked to dominant CM and HM non-interactive composition properties and less dominant CM-PM, HM-PM, CM-PM-HM interactive composition properties. The results agree with previous findings that have established the significance in the synergistic influences of CM-PM and PM-HM co-substrates on biogas yield [52, 55].

On the other hand, PM and CM-HM compositions was observed to exhibit strong non-synergistic properties. Regarding the case of PM, the non-synergistic behavior may not be unconnected to its free Ammonia property. It is known that PM usually contains high free Ammonia at concentrations that can inhibit positive biogas production except carefully controlled [91, 92].

However, the non-synergistic behavior of CM-HM from the study appear to be at variance with previous studies which have reported that CM-HM digestion is synergistic [54, 56]. This behavior was not clearly understood given that firstly, HM main effect was synergistic and secondly, like PM, HM is characterized by high free Ammonia concentration [93] and as such CM-HM interactions should be synergistic. Although from the observation, it appears that their synergistic main effect contributions may be acting counteractively to the contribution of their interaction, this behavior requires further investigation.

The inferences derived from these observations are that a higher potential may exist for better methane production from the use of the synergistic co-substrates than the non-synergistic ones. Also, under the unconstrained physicochemical conditions considered in this work, PM used singularly, or CM-HM deployed as a mix may not serve as very useful substrates for methane production.

3.6 Results from the Taguchi fractional factorial design analysis

The fitness capabilities of the TFFD model to the deployed data is described using the residual plots of the SN ration and the means (Figure 7) while the results of the ANOVA of the SN ratio and means that indicate the statistical significance of the control factors are presented in Figure 8. Also, Figure 9 shows the trend plot of the experimentally determined V_{Mit^*} and corresponding predictions obtained from the "Taguchi Predict Result" feature in Minitab 19. The response table (Figure 10) and the main effects plots (Figure 11) of the SN ratio and means provide information on the optimal substrate mix ratio determined from the TFFD analysis.

The TFFD approach was observed fit the deployed data based on the result of the residual plots (Figure 7). For example, the normal probability plot and histogram distribution plots of the indicate normality distribution of the residuals while the independence of the residuals is clearly observed in the residuals versus order plot. The ANOVA for the S/N ratio and means (Figure 8) produced a similar result trend with indication that all the main control factors had a statistically significant impact on methane production ($p < 0.05$). It also did reveal a few second order interactions CM, PM and HM with the HRT in each case. While the latter two were significant, CM-HRT interactions was observed to be as not being significant.

Although the CM-HRT significance appear to be in conflict with those obtained from those obtained in the regression analysis (Equation 17), it should be understood that while the TFFD is quite reliable at indicating factor main effects, one its drawback is that it is difficult to independently interaction effects independently from the main effects and using the approach [94, 95] and thus the need to investigate the interactions of the co-substrate through the use of the more reliable polynomial regression technique.

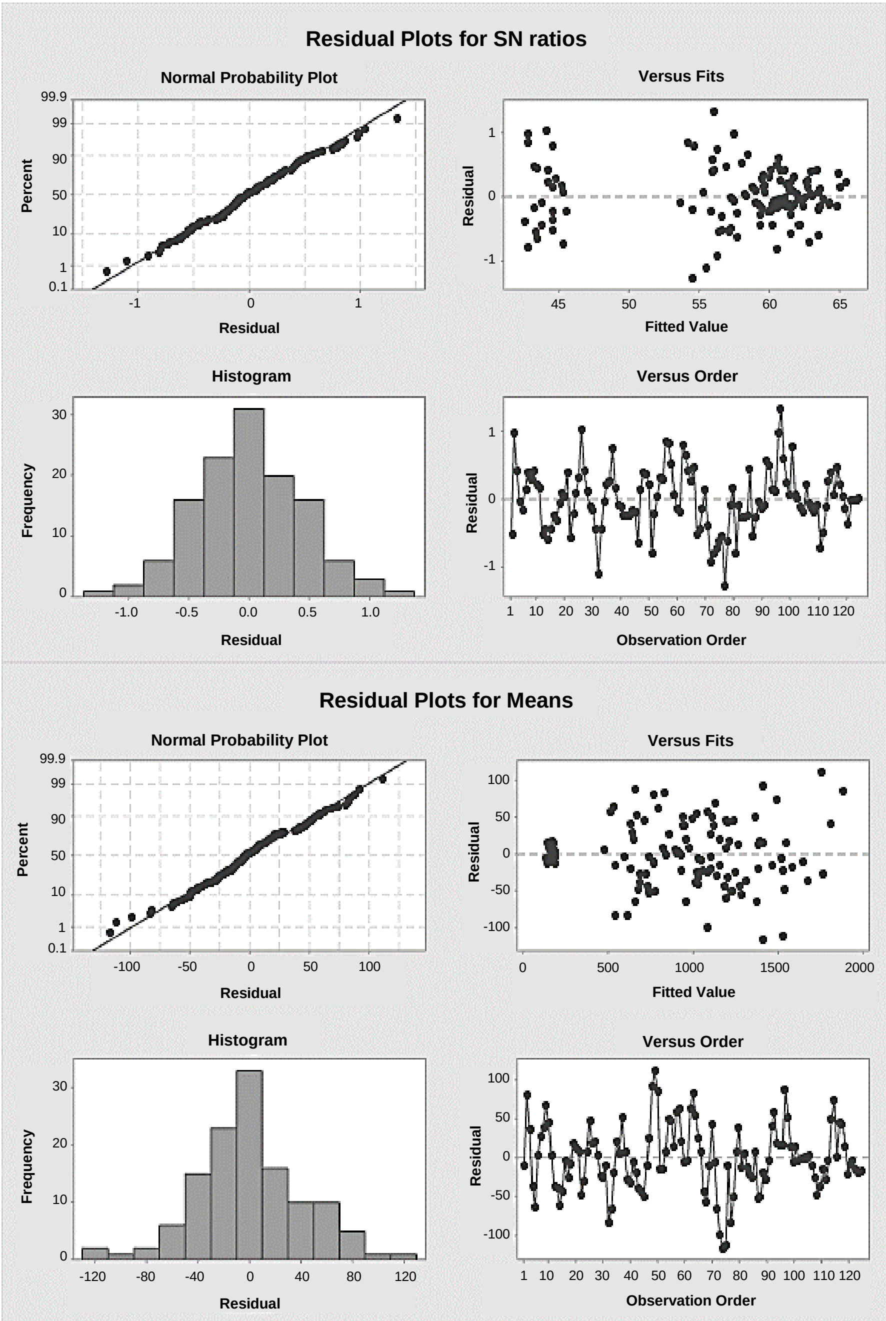

Figure 7 Residual plots the SN ratios and the test of means from the TFFD analysis

Model Summary

S	R-Sq	R-Sq(adj)
0.6570	99.57%	99.12%

Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
CM	4	25.00	25.00	6.25	14.48	0.000
PM	4	89.99	89.99	22.50	52.12	0.000
HM	4	70.32	70.32	17.58	40.73	0.000
HRT	4	5777.75	5777.75	1444.44	3346.18	0.000
CM*HRT	16	9.77	9.77	0.61	1.41	0.166
PM*HRT	16	30.60	30.60	1.91	4.43	0.000
HM*HRT	16	22.92	22.92	1.43	3.32	0.000
Residual Error	60	25.90	25.90	0.43		
Total	124	6052.26				

Model Summary

S	R-Sq	R-Sq(adj)
61.2360	99.19%	98.32%

Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
CM	4	161099	161099	40275	10.74	0.000
PM	4	1638337	1638337	409584	109.23	0.000
HM	4	1061793	1061793	265448	70.79	0.000
HRT	4	23299058	23299058	5824765	1553.33	0.000
CM*HRT	16	80573	80573	5036	1.34	0.202
PM*HRT	16	733680	733680	45855	12.23	0.000
HM*HRT	16	527587	527587	32974	8.79	0.000
Residual Error	60	224991	224991	3750		
Total	124	27727119				

Figure 8 Model summary and analysis of variance for signal to noise ratios and means

The accuracy of the TFFD analysis to predict the response methane of yield in terms of the root mean square error (RMSE) was 49.40 cm^3 with a corresponding mean absolute percentage error (MAPE) value of 6.54 cm^3 (Figure 9). This indicates excellent predictive capability of the model [96]. The response table (Figure 10) revealed that among the four control factors, the HRT exhibited the most impact on the S/N ratio. This result is expected as biogas formation is impossible without the HRT.

Besides, previous works have shown that depending on the physicochemical conditions in which the bio-digestion process is subjected, lowering or increasing the HRT significantly affects biogas yield and quality [97, 98]. For the substrates, PM exhibited the strongest effect on the S/N ratio followed by HM and CM respectively (Figure 10). Using the main effects plot for SN ratio (Figure 11), The PM effect on the S/N ratio was observed to be of the non-synergistic form, acting against the attainment of the maximum is better target value. This negative main effect study aligns with the observations from the results of the experiment runs where the bio-digestion of PM progressed normally as other substrate mixes, but digestion rate quickly decelerated and stopped altogether in the third week. The CM and HM main effect plots showed synergistic behaviour indicating that more methane could be produced at higher substrate compositions.

The results from the main effects plot for means (Figure 11) revealed that the co-substrate optimal mix ratio for maximum methane production was 5:1:5 for CM-PM-HM with a corresponding mean and standard deviation cumulative methane yield of 1964.71 and 130.39 cm^3 expected to be produced on the end of the HRT. In terms of percentage composition, this ratio corresponds to 45.5:9:45.5%.

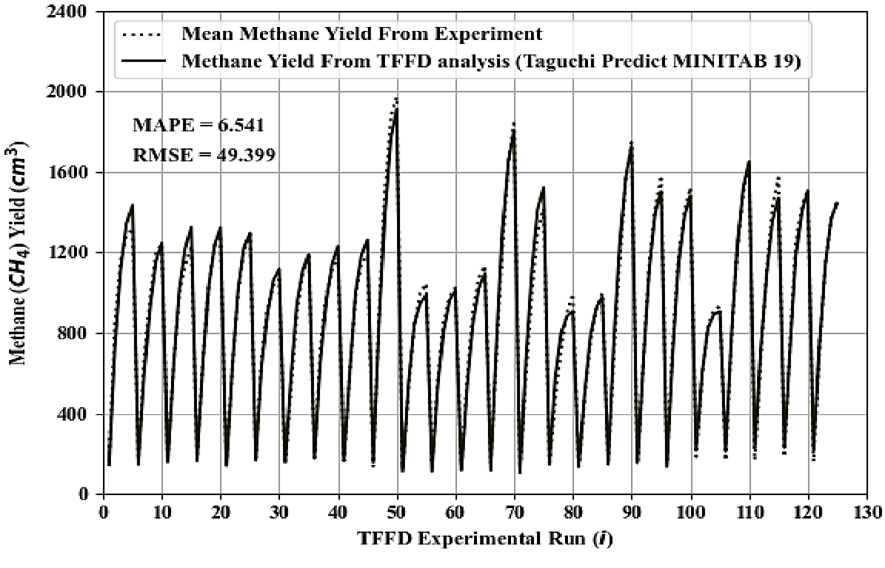

Figure 9 Methane Yield comparison between experimentally determined data and those predicted using the TFFD "Taguchi predict Result" feature in MINITAB 19.

Response Table for Signal to Noise Ratios

Larger is better

Level	CM	PM	HM	HRT
1	57.07	58.34	55.78	43.93
2	56.94	57.03	56.16	56.20
3	56.24	56.74	57.10	60.12
4	56.49	56.31	57.57	61.71
5	57.51	55.83	57.63	62.29
Delta	1.27	2.51	1.85	18.35
Rank	4	2	3	1

Response Table for Means

Level	CM	PM	HM	HRT
1	876.8	1092.8	753.6	159.1
2	893.9	898.8	809.2	656.0
3	837.8	867.0	899.7	1031.9
4	870.4	809.3	965.6	1245.2
5	947.1	758.1	997.9	1333.8
Delta	109.3	334.7	244.3	1174.6
Rank	4	2	3	1

Figure 10 Response Tables for SN ratios and test means for the different levels of variables investigated

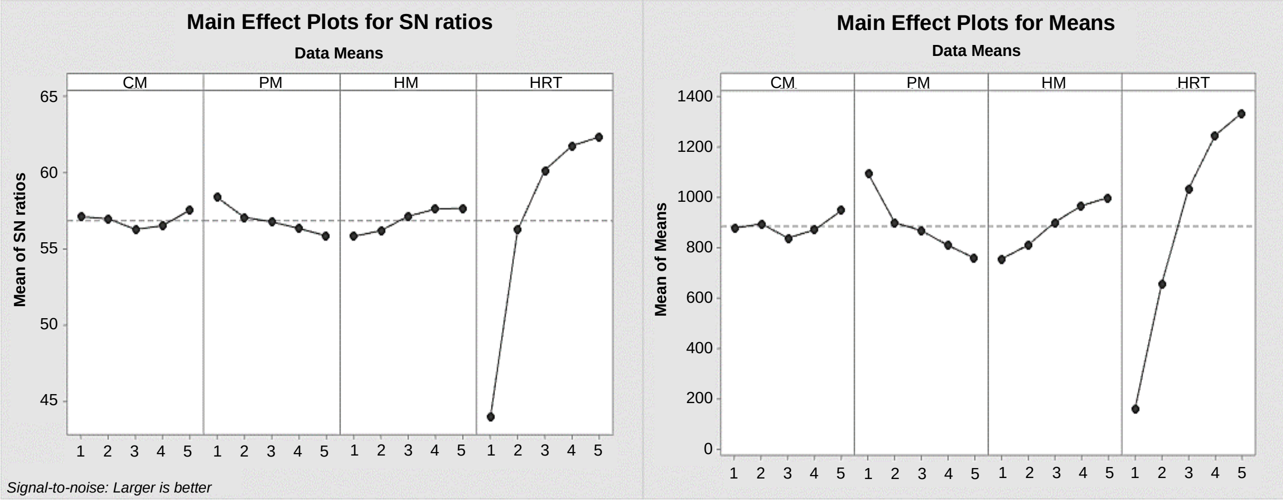

Figure 11 Main effect plots for the signal to noise ratio and means

4. Conclusion

In this study, the Taguchi fractional factorial design of experiments, kinetic modelling and regression analysis frameworks, were adopted to investigate the influence of co-digesting different mix ratios of cattle, poultry, and hog manure at unconstrained physicochemical conditions on methane yield was investigated**.** Based on findings from the analysis of the twenty-eight co-substrate mix ratios considered, the following conclusions are made.

(1) Increases or decreases in methane yield are dependent on the co-substrates composition which is directly affected by the ratio of co-substrates mix.
(2) The mono digestion of poultry manure or the co-digestion of cow and hog manure may not produce appreciable methane yield.
(3) The co-digestion of the substrates at certain mix ratios produced better methane yield volumes, VS reductions, and TS reductions than those of their mono-digested equivalents. Thus it may be possible to maximise the cumulative methane yield if the composition of the substrates is carefully selected.
(4) Generally, the co-digestion of the substrates led to a reduction in the digestion lag times and increases in biogas production rates and shorter retention times.
(5) CM, PM and HM main effects, and CM-PM and PM-HM, CM-HM interactions significantly influenced biogas yield, while CM-PM-HM interactions were non-significant
(6) CM and HM main effect as well as CM-PM, PM-HM and CM-PM-HM influences were synergistic while those for PM, CM-HM were non-synergistic.
(7) The optimal cow-poultry-hog co-substrate mix ratio was 5:1:5 with an expected yield volume of 1.96$\pm$ 0.13 Litres.

Although the effect of cow dung, poultry dung and hog dung (CM-PM-HM) co-substrate digestion on biogas yield has not been extensively studied, some of the findings from this study (conclusions 1-6) align with previous studies that investigated either CM-PM and PM-HM substrate co-digestion [33, 38, 52]. However, the non-synergistic behavior of CM-HM interactions as concluded in this study differ from previous studies as Kasisira and Muyiiya [56] and Li et al. [54] report on their synergistic behavior. However the basis of for this comparison may differ given that this study investigated CM-PM-HM co-substrate behavior while previous studies has so far been limited to CM-HM co-substrate digestions only. Thus, more study is required in this area.

This study has made the some contributions to the namely; (1) The provision of information on the synergistic interactions between CM-PM-HM co-substrate in triggering biogas yield and (ii) The utilization of the Taguchi fractional factorial design in the assessment and prediction of methane yield from CM-PM-HM co-substrate.

This research, however, was subject to one major limitation which is the adoption of unconstrained physicochemical conditions. It is established in the literature that biogas formation is majorly caused by microbial activities whose degree of performance could be affected by factors that include pH, temperature, carbon-nitrogen content, COD, VFA, and Nitrogen ammonia formation [18, 99]. As such, adopting the methodology reported in this paper but with consideration given to the effect of the changes in one or more of these physicochemical conditions can provide new insight into the study and is thus recommended as an area of consideration for future research. Also, the experiments done in this study were laboratory-scaled. Although the result trends such as methane yield and substrate reduction may apply to a large-scale system, the results obtained from this study may not directly apply. This is another area with future research potential. Furthermore, other EDA methods such as the method of Mixtures possess the potential to estimate properties from limited number of observations. However, the method o the best of the authors' knowledge, appears unutilized in biogas mixture. The application of such techniques for similar studies is also worth considering in future investigations.

5. References

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