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Biomethane Potential of Cassava Leaves: Effect of co-digestion with cattle manure and Evaluation of Kinetic Parameters

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Abstract
Cassava leaves (CL) in Nigeria are largely a wasted biomass despite their huge mass flow, making it a potential substrate for biogas production. This paper examines the kinetics of the biomethane potential (BMP) of this readily available biomass. In doing so, 2.5 gVS of cattle manure (CM) was co-digested with 2.5, 3.75, 5.0, 6.25 and 7.5 gVS of CL and labelled R1, R2, R3, R4, and R5 respectively. Seven kinetic models were used to fit the experimental data of the BMP of the substrates. These models were evaluated using four statistical tools; coefficient of determination (R^2), root mean square error (RMSE), Akaike information criterion (AIC) and the Bayesian information criterion (BIC). Results show that co-digestion with CM improved the BMP of CL, with the highest BMP of 262 mL/gVS obtained from R5. Although all the investigated models provided reasonable fits to the measured BMP of the substrates judging from the high coefficients of determination ($R^2 \geq 0.8877$), the two-steps two-fraction first-order kinetic model (TSTF) and the one-step-two fraction first-order kinetic model (OSTF) gave the best prediction for the BMP of the substrates.

Keywords: *Waste biomass, Kinetic models, Anaerobic digestion*

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1. Introduction

In 2019, Nigeria produced 59.19 million tons of cassava (FAOSTAT, 2021), accounting for 19.47 % of the global cassava production in that year. Mainly produced in the southern and North-central part of the country with the root as the product of interest, one of the wastes generated from the production of cassava is cassava leaves (CL), some of which usually contain a high volume of toxic heavy metals due to environmental pollution (Harrison et al. 2018) and are therefore discarded as waste, while some varieties with low concentration of cyanide are used as animal feed. With a protein, lipid and carbohydrate content in the range of 19.7 - 38, 6.6 – 7.3 and 31.9 – 45.8 g/100 g dry matter respectively (Oni et al., 2011; Ravindran and Ravindran, 1988) depending on the specie and age, CL possess similar characteristics to vegetable kitchen wastes that has been reported to be a good substrate for biogas production (Morales-Polo et al., 2018). According to Lansche et al. (2020), a mechanised farming of cassava can produce a root to leaves ratio of about 8.7 ton/ha. With Nigeria producing about 0.82 tons/ha of cassava in 2019 (FAOSTAT, 2021), about 0.1 ton of CL is left as waste on every hectare of land after harvest, making it a readily available substrate for biogas production.

In order to obtain as much biomethane as possible from the anaerobic digestion (AD) of CL, co-digestion with other readily available substrates such as cattle manure (CM) is nessasary so as to avoid process inhibition that could occur as a result of unbalanced carbon-to-nitrogen ratio (Xing et al., 2020). Although the literature is extensive as far as co-digestion of agricultural residues with CM is concern, there is no evidence that co-digestion of CL and CM has been investigated for biogas production.

Kinetic models play very important roles in the design and operation of AD processes (Pramanik et al., 2019) because they are used to predict the performance of reactors. Kinetic parameters obtained from kinetic models are used to determine reactor volumes for a specified AD process (Fogler, 2016). However, despite their importance, the use of kinetic models to predict biogas production from CL is scarcely available in literature. Therefore, the purpose of this study was to (i) investigate the biomethane potential (BMP) of CL, (ii) investigate the effect of co-digesting CL with CM on BMP (iii) perform modelling studies of the AD process and (iv) estimate the kinetic parameters of the selected models.

2. Materials and methods

2.1 Substrates and inoculum collection

CL (Tropical Manioc Selection 30572) were collected from a local farm in Ogoja, South-Southern region of Nigeria after the harvesting of cassava plants that were 9 months old from the date of planting. The leaves were then washed and grinded with a local grinding machine to about 1 mm size. About 300 g of substrates were then packed into vacuum sealer bags of size 203 by 305 mm (Weston pro 2300 vacuum sealer) in triplicates. The filled bags were then sealed under vacuum using a vacuum sealer machine (Mueller Austria) and stored at ambient temperature for 280 days. Fresh CM was collected from a local farm in Obudu, South-Southern Nigeria and stored at 0 °C until required. The inoculum (total solids, TS = 2.64 %; volatile solids, VS = 71.87%TS; pH = 7.64) used for the BMP tests was obtained from an active continuous-stirred tank reactor (CSTR). The CSTR is specifically operated for methane yield tests, and runs on a maize silage-cow dung-sunflower oil mixture with a hydraulic retention time of 100 days and an organic loading rate of 0.5 g VS/L/d.

2.2 Experimental procedures

Five samples of CL consisting of 2.5, 3.75, 5.0, 6.25 and 7.5 gVS were mixed with an equal mass of 2.5 gVS of CM, corresponding to a co-digestion ratio of 1:1, 1.5:1, 2:1, 2.5:1, and 3:1, and used for BMP determination using the AMPTS II (Bioprocess Control, Lund, Sweden). These five samples were prepared in triplicates and labelled R1, R2, R3, R4, and R5 respectively. A sample consisting of only CL was also prepared and labelled R0. The inoculum was degassed by incubation at 37 °C for 7 days to minimize the generation of non-specific gases. Each reactor had an inoculum to substrate ratio of 3:1. Additionally, a reactor consisting of 4.26 g of microcrystalline cellulose (MCC) was used to monitor the quality of the inoculum. The headspace of each reactor was flushed with nitrogen for about 2 minutes to ensure anaerobic conditions. Daily methane production of each reactor was recorded using the Bioprocess Control software and corrected to standard conditions (273.15 K and 101.325 kPa).

2.3 Physicochemical analysis

The total solids (TS), volatile solids (VS), organic acids, ethanol and elemental (hydrogen, carbon, nitogen and sulphur) composition of the substrates and inoculum were determined using standard methods as previously described (Undiandeye et al., 2022b).

2.4 Kinetic study

Seven kinetic models were used to fit the experimental BMP of the substrates. These models are presented in Table 1 (Brulé et al., 2014).

2.5 Model evaluation and statistical analysis

Parameters from the models were determined using the Solver add-in program in Microsoft Excel, where values of the models were adjusted to minimize the sum of squares of differences between experimental and estimated values. OriginPro software (OriginLab Corporation, USA) was used to plot all curves using the data obtained from the models. The statistical indicators that were used to determine the more appropriate model that predicted the experimental BMP were the coefficient of determination, R^2; the root mean square error, RMSE (Equation 1); the Bayesian information criterion, BIC (Equation 2); and the Akaike information criterion, AIC (Equation 3).

$$RMSE = \sqrt{\frac{ss}{n}} \tag{1}$$

$$BIC = n ln\left(\frac{ss}{n}\right) + k ln(n) \tag{2}$$

$$AIC = n \times \ln\left(\frac{ss}{n}\right) + 2k + \frac{2k(k+1)}{n-k-1} \tag{3}$$

where n is the number of experimental data, ss is the squared sum of residuals, and k is the number of parameters in the model.

Table 1: Selected models to describe biomethne production

Kinetic Model	Equation of the Kinetic Model
OSOF	$G_{(t)} = G_0(1 - e^{-kt})$
TSOF	$G_{(t)} = G_{(0)} \times (1 + \frac{Ke^{-k_{VFA}.t} - k_{VFA}.e^{-kt}}{K_{VFA} - k})$
OSTF	$G_{(t)} = G_{(0)} \times [1 - \propto.(e^{-k_F t}) - (1 - \propto).(e^{-k_L t})]$
TSTF	$G_{(t)} = G_{(0)} \times [\propto.\left(1 + \frac{k_F.e^{-k_{VFA}.t} - k_{VFA}.e^{-k_F t}}{k_{VFA} - k_F}\right) + (1 - \propto).\left(1 + \frac{k_L e^{-k_{VFA}.t} - k_{VFA}.e^{-k_L.t}}{k_{VFA} - k_L}\right)]$
DFKM	$G_{(t)} = G_{(0)} \times (1 - e^{-k.(t-\lambda)})$
MGKM	$G_{(t)} = G_{(0)} \times e^{-e(\frac{R_{max}.e}{G_{(0)}}.(\lambda - t) + 1)}$
MTKM	$G_{(t)} = G_{(0)} \times (\frac{k.t}{1 + k.t})$

OSOF - one-step-one-fraction first-order model; TSOF - two-steps-one-fraction first-order model; OSTF - one-step-two-fractions first-order model; TSTF - two-steps-two-fractions first-order model; DFKM - delayed first-order model; MGKM - Modified Gompertz model; MTKM - Monod-type model; $G_{(t)}$ - biomethane potential; $G_{(0)}$, maximum possible biomethane potential; k - first-order kinetic constant; k_{VFA}- first-order kinetic constant of volatile fatty acids; k_F - first-order kinetic constant of fast degradable component; k_L - first-order kinetic constant of slow degradable component; α - fraction of readily degradable component; λ - lag phase; R_{max}, maximum biomethane production rate; t - digestion time; e - Euler's constant.

3. Results and discussion

3.1 Physicochemical parameters of substrates

The physicochemical parameters of the substrates are shown in Table 2. TS and VS content of CL were consistent with the values reported by Alfa et al. (2021), compares with those of vegetable food wastes (Morales-Polo et al., 2018) but higher than those of sugar beet leaves (Undiandeye et al., 2022a). The organic acids in CL were mainly acetic acid. Lactic acid was below detectable limit and was therefore not included in the table. Butyric acid was present in the samples, an indication that clostridial activities was favoured probably as a result of the absence of lactic acid (Borreani et al., 2018).

Although higher than that of Jatropha seed cake (Raheman and Mondal, 2012), the ratio of carbon to nitrogen content in CL was far below the reported optimum of 30 (Okonkwo et al., 2018), but was however improved by co-digestion with CM whose C/N was higher and comparable to the reports of Almomani and Bhosale (2020) and Meng et al. (2019). A lower C/N value of 5.1 (Yusuf et al., 2011) as well as a higher value of 64.67 (Baniviga et al., 2019) has also been reported for cattle manure.

Table 2: Physicochemical parameters (± standard deviation) of the substrates

Physicochemical Parameters	CM	CL
Total solids(%)	19.86 (±0.02)	25.82 (±0.03)
Volatile solids (%TS)	83.17 (±0.05)	91.82 (±0.09)
Acetic acid (g/l)	ND	2.83 (±0.01)
Butyric acid (g/l)	ND	0.66 (±0.08)
Ethanol(g/l)	ND	1.90 (±0.00)
Carbon(%TS)	39.70	49.35
Hydrogen (%TS)	4.08	6.10
Nitrogen(%TS)	1.7	4.21
Sulphur(%TS)	5.42	0.40
Carbon/Nitrogen ratio	23.35	11.72

CM - cattle manure; CL - cassava leaves; ND - not determined

3.2 Biomethane potential results

Fig. 1 shows the cumulative BMP in the reactors. The duration of digestion was 39 days for all substrates. R0 had the lowest BMP of 139 mL/gVS. Co-digestion with CM significantly increased ($p<0.05$) the BMP of CL by at least 62 % with the maximum increase of 88 % obtained in R5. There was a significant difference ($p<0.05$) between the BMP in R1 and R5, but the BMP in R2, R3, R4 and R5 were not significantly different ($p<0.05$). CM has also been reported to improve the BMP of subsrates like vegetable peel (Lahbab et al., 2021) and food waste (Xing et al., 2020). Since sulphur content is strongly negatively correlated to BMP (Barrera et al., 2013), the addition of sulphur-removing agents that are commonly available such as mill scale can improve the BMP of the process (Ahn et al., 2021). In addition, if process conditions like CL to CM ratio, inoculum to substrate ratio and pH are optimised, the BMP of the substrates may be enhanced.

3.3 Evaluation of the tested kinetic models and validation

All kinetic models predicted the experimental BMP to some good degree as seen from the high values of R^2 (Table 4). Kinetic parameters from the OSOF and TSOF were similar in all reactors probably because of the high values of k_{VFA} compared to k in the TSOF. Brulé et al. (2014) also reported a similarity in the values of G_0 and k from the OSOF and TSOF when k_{VFA} was more than 14 times the value of k in the TSOF during the AD of hay. When values of k_{VFA} far exceed the values of k, the TSOF reduces to the OSOF as seen in the equations (Table 2). Parameters from the DFKM were similar to the parameters from both the OSOF and TSOF probably because of the absence of a lag phase in the reactors. In the absence of a lag phase, the DFKM reduces to the OSOF. Values of k from the MTKM were significantly higher ($p<0.05$) than values of k from the OSOF, TSOF and DFKM. Nguyen et al. (2019) has also reported more than 45 % increase in the value of k from the Cone model compared to k value from the OSOF. Co-digestion of CL with CM increased the hydrolysis constant (k) as seen in the OSOF, TSOF and DFKM. Almomani and Bhosale (2020) have also reported an increase in k values when CM was co-digested with pretreated solid agricultural waste. The values of k obtained in the present study are higher than 0.26 /d reported during the co-digestion of untreated rice straw and pig manure (Zhong et al., 2021) but lower than the values obtained by Almomani and Bhosale (2020), probably because of the chemical pretreatment of the agricultural wastes by the authors. Higher values of k is an indication of increased biodegradability (Nguyen et al., 2019). Values of k were not significantly different in R1-R5, probably because the reactors were operated at the same temperature, as factors like concentration of organic acids have been reported to have little effect on k for a given substrate (Fogler, 2016).

The models that gave the best fit of the experimental BMP in all reactors were the TSTF and the OSTF, probably because these models were derived on the assumption that complex substrates like CL used for AD contain both fast and slow degradable components (Brulé et al., 2014). The TSTF and OSTF have also been reported to give a better fit to the BMP from a mixed silage of *Elodea* and wheat straw than the OSOF and MGKM (Gallegos et al., 2018). The fitness of the TSTF and

OSTF models to the experimental BMP is shown in Figure 2. The hydrolysis constant of fast degradable components (k_F) were at least fives times higher than the hydrolysis constant of slow degradable components (k_L) in all reactors, which is consistent with the reports of Brulé et al. (2014) and Gallegos et al. (2018), indicating that the substrates contain both fast and slow degradable components. Co-digestion of CL with CM significantly increased ($p<0.05$) the fraction of biodegradable components (α) in all reactors from 40 % to a minimum of 50 %, indicating why there was an increase in BMP in the reactors containing CM compared to R0. Since both the TSTF and OSTF fitted the experimental data better than other models, a comparison of one-stage and two-stage digestion in co-digestion systems of CL and CM could be explored.

The lag phase (λ) of the reaction in each reactor was estimated from the DFKM and the MGKM. In both models, λ was zero in all reactors except in R3 where it was 0.09 and 0.14 days in the DFKM and MGKM respectively, an indication of a fast bacterial acclimatization to the system (Mao et al., 2017) and therefore faster biomethane production. A high lag phase may have been obtained if CM was used as a sole inoculum (Janke et al., 2016). Depending on the particle size of some agricultural silages, Szlachta et al. (2018) has reported a lag phase of between 2.33 – 12.19 days using an inoculum with similar TS, VS and pH as the inoculum used in the present study. Differences in lag phase during AD could occur as a result of (i) a difference in the feed composition of the reactors from which an inoculum was harvested (Rajput and Sheikh 2019) (ii) a difference in the inoculum to substrate ratio (Córdoba et al., 2018) and (iii) differences in the nature of substrates (Li et al., 2019). The absence of a lag phase in the present study could be the reason why the prediction by the MGKM was the least fitted among the models (Shen and Zhu, 2017). The maximum rate of methane production, R_{max}, could only be predicted from the MGKM, and was observed to be lowest in R0, an indication of a lower methanogenesis rate in that reactor compared to the reactors containing both CL and CM. Estimated Values of R_{max} were not significantly different ($p<0.05$) from the measured values in R0 and R5 unlike in other reactors where the estimated values and measured values were significantly different, an indication that substrate characteristics could determine how well a model could predict its BMP (Brulé et al., 2014). Using Equation (1), the time, t_{max}, at which the maximum rate of methane was obtained was calculated to be 2.32, 2.43, 1.39, 1.37, 1.73 and 1.56 in reactors R0, R1, R2, R3, R4 and R5 respectively, similar to the t_{max} of other vegetable crop residue (Li et al., 2019) and are fairly consistent with the experimental values as shown in Fig. 3. The low t_{max} values are an indication of a fast start-up, ease of degradability and rapid rate of biomethane production (Li et al., 2019) that has been associated with substrates that contain a high carbohydrate content (Wang et al., 2017) such as CL. The predicted BMP, G_o, varied between all the models. Brulé et al. (2014) and Nguyen et al. (2019) have also reported a variation in the maximum predicted BMP depending on the model used.

Another important parameter that is often used to predict the duration and effectiveness of AD z\zprocesses is the effective methane production time (T_{90}), which is the time, beyond the lag phase, for 90% of the accumulated methane to be produced. T_{90} was calculated to be 7.54, 7.91, 4.51, 4.47, 5.62, and 5.09 days in R0, R1, R2, R3, R4 and R5 respectively, indicating that co-digestion with CM reduces the T_{90} of CL. T_{90} of about 9 days has been reported during the co-digestion of rice straw and pig manure (Zhong et al., 2021) and up to 19.2 days during the co-digestion of corn straw and swine manure at a pH of 7.5 (Mao et al., 2017).

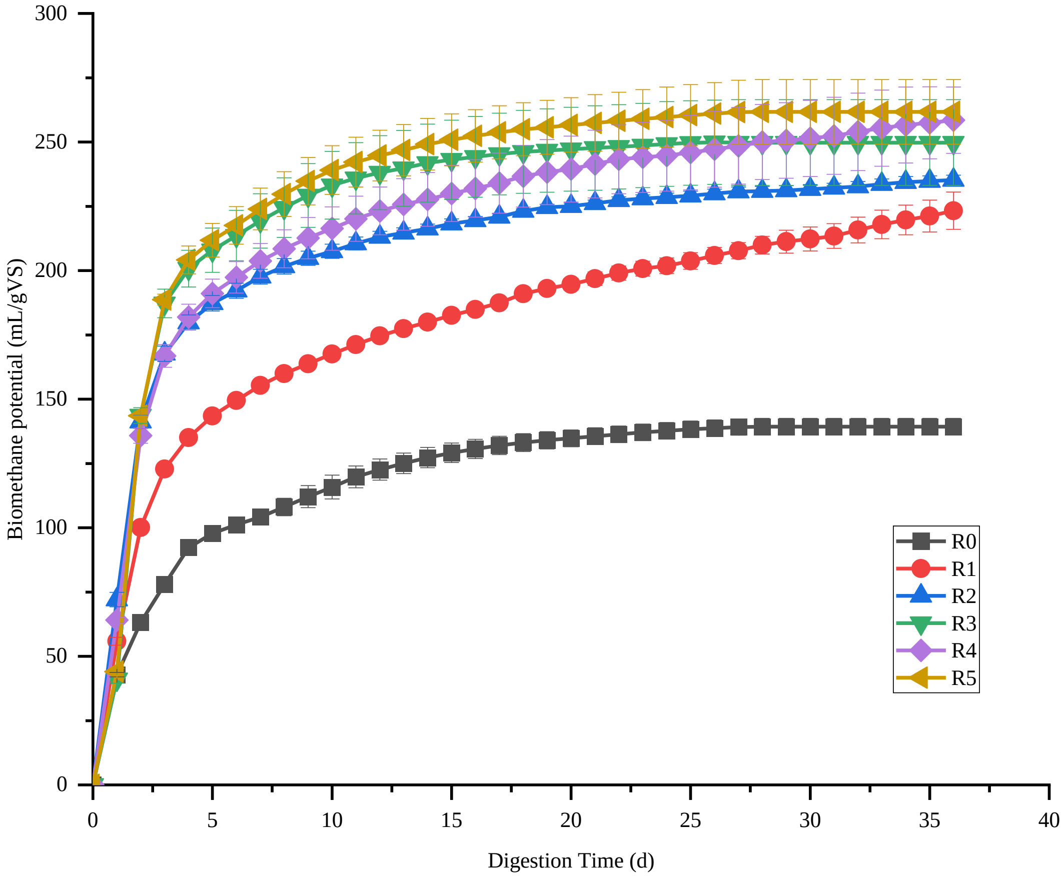

Fig. 1: Comparative plot of the cummulative biomethane potential. Error bars indicate standard deviation of the mean.

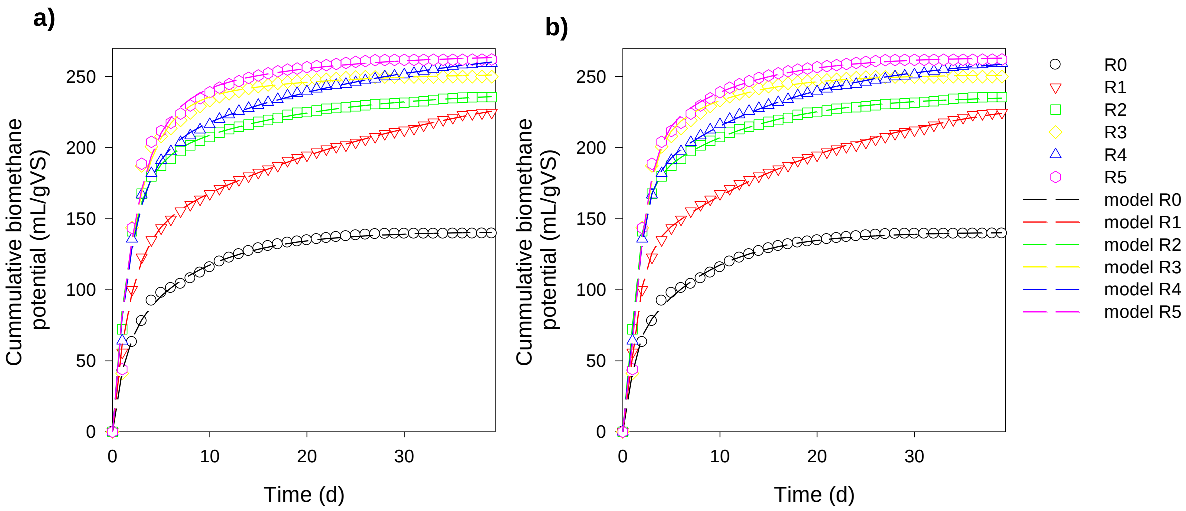

Fig. 2: Fitness of the (a) OSTF and (b) TSTF to the experimental BMP (symbols represent experimental measurements and lines represent model simulations)

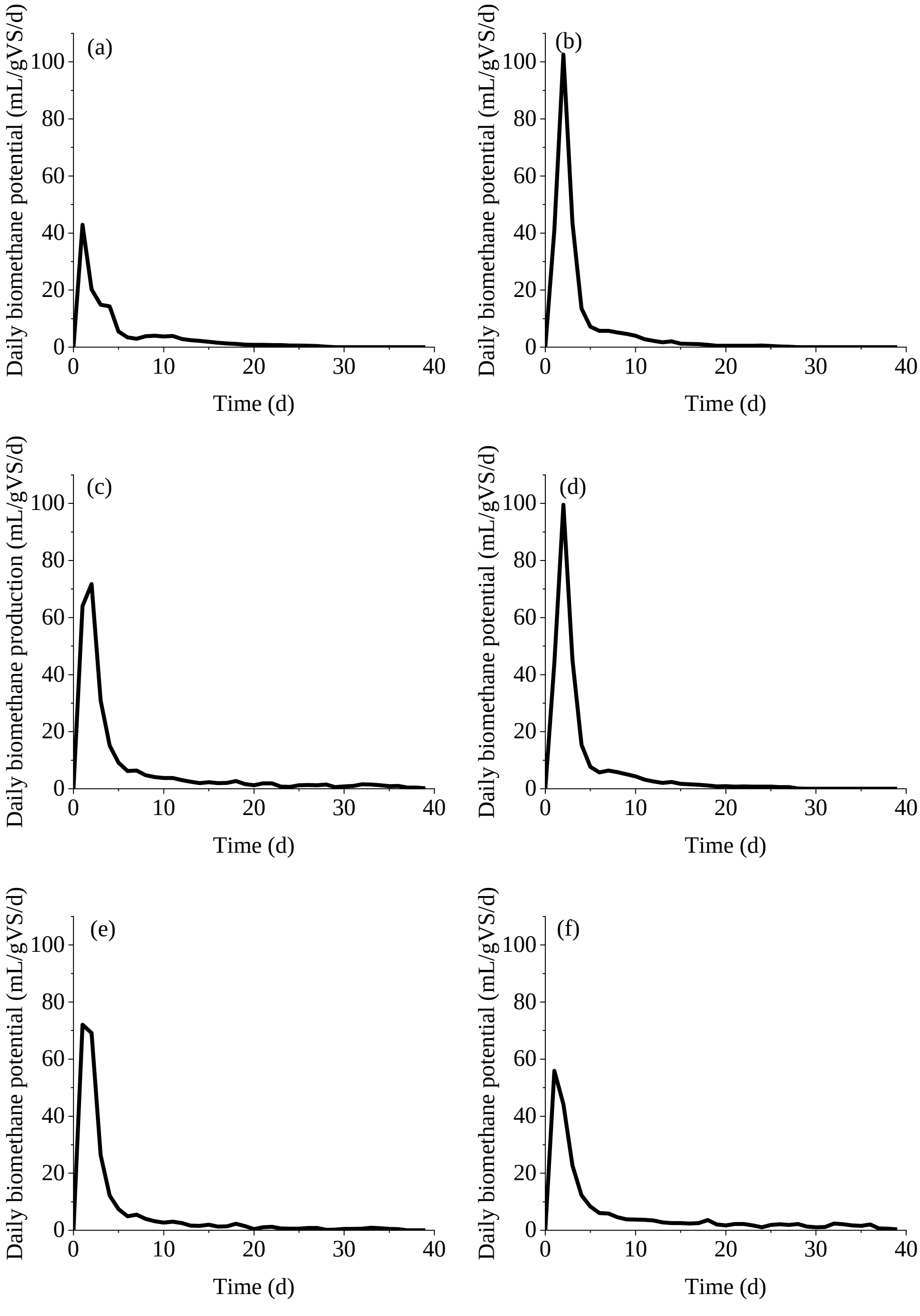

Fig. 3: Daily biomethane production from (a) R0, (b) R1, (c) R2, (d) R3, (e) R4 and (f) R5.

Table 3: Kinetic parameters estimated by modelling the BMP produced from the co-digestion of cassava leaves (CL) and cattle manure (CM)

		Reactor					
Model	**Parameter**	**R0**	**R1**	**R2**	**R3**	**R4**	**R5**
OSOF	G0 (ml/gVS)	136.65	206.85	226.04	247.00	244.67	257.37
	k (/d)	0.24	0.35	0.38	0.37	0.34	0.34
TSOF	G0 (ml/gVS)	136.64	206.85	226.04	246.99	244.64	256.64
	k (/d)	0.24	0.35	0.38	0.37	0.34	0.34
	kVFA (/d)	14799	25500.96	25500.92	14796.50	25500.34	4.38
OSTF	G0 (ml/gVS)	140.96	263.42	240.47	252.56	277.87	266.01
	kF (/d)	0.55	0.86	0.81	0.83	0.82	0.84
	kL (/d)	0.13	0.03	0.07	0.08	0.04	0.07
	A	0.40	0.69	0.75	0.88	0.68	0.83
TSTF	G0 (mL/gVS)	140.58	244.75	236.85	251.36	265.78	264.03
	kF (/d)	2.11	1.38	1.46	1.12	1.28	1.121
	kL (/d)	0.14	0.05	0.09	0.13	0.07	0.11
	kVFA (/d)	2.11	1.38	1.46	1.12	1.30	1.121
	A	0.39	0.71	0.70	0.76	0.75	0.73
DFKM	G0 (ml/gVS)	136.65	206.85	226.04	246.79	244.67	257.21
	k (/d)	0.24	0.35	0.38	0.37	0.34	0.35
	λ (d)	0.00	0.00	0.00	0.09	0.00	0.06
MGKM	G0 (ml/gVS)	134.30	201.37	223.46	244.16	240.77	254.33
	Rmax(ml/gVS/d)	21.29	30.44	59.27	65.36	51.23	59.76
	λ (d)	0.00	0.00	0.00	0.14	0.00	0.00
MTKM	G0 (ml/gVS)	152.13	234.11	244.55	267.66	269.45	280.45
	k (/d)	0.35	0.59	0.60	0.57	0.61	0.62

OSOF - one-step-one-fraction model; TSOF - two-steps-two-fractions model; OSTF - one-step-two-fractions model; TSTF - two-steps-two-fractions model; DFKM - delayed first-order kinetic model; MGKM - modified Gompertz kinetic model; MTKM - Monod-type kinetic model; k - first-order kinetic constant (/day); k_F - first-order kinetic constant of fast degradable component (/day); kL - first-order kinetic constant for slow degradable component (/day); kVFA - first-order kinetic constant for organic acids (/day); α - fraction of biodegradable component; λ - lag phase (days); R_{max} - maximum rate of methane production (ml/gVS/day).

Table 4: Statistical data showing the fitness of measured data to the models

		Reactor					
Model	**Parameter**	**R0**	**R1**	**R2**	**R3**	**R4**	**R5**
OSOF	RMSE	5.01	12.84	8.38	8.14	11.09	8.59
	AIC	133.20	208.50	174.35	172.03	196.77	178.30
	BIC	136.26	211.55	177.40	175.08	199.83	182.70
	R^2	0.8980	0.8955	0.9837	0.9325	0.9702	0.9256
TSOF	RMSE	5.01	12.84	8.38	8.14	11.09	8.36
	AIC	135.55	210.84	176.69	174.37	199.12	176.50
	BIC	139.95	215.24	181.09	178.77	203.52	179.45
	R^2	0.8983	0.8955	0.9837	0.9325	0.9708	0.9607
OSTF	RMSE	1.34	1.38	2.77	7.47	3.17	7.13
	AIC	35.08	37.26	90.61	169.99	101.37	166.25

	BIC	41.76	43.94	96.22	175.60	106.98	171.86
	R^2	0.9984	0.9926	0.9758	0.9159	0.9738	0.9712
TSTF	RMSE	1.16	1.20	0.74	4.26	1.16	3.83
	AIC	21.05	23.88	-12.74	127.80	23.96	119.12
	BIC	26.66	29.50	-6.06	134.48	30.64	125.80
	R^2	1.0000	0.9942	0.9968	0.9763	0.9969	0.9756
DFKM	RMSE	5.01	12.84	8.38	8.00	11.09	8.55
	AIC	135.54	210.84	176.69	173.06	199.12	176.39
	BIC	139.94	215.24	181.09	177.46	203.52	180.90
	R^2	0.8980	0.8955	0.9837	0.9526	0.9702	0.9507
MGKM	RMSE	8.40	17.55	11.60	9.51	15.21	10.99
	AIC	174.56	233.51	200.42	184.49	222.08	196.12
	BIC	177.61	236.56	203.47	187.54	225.14	199.17
	R^2	0.9036	0.9489	0.9500	0.9134	0.9671	0.9035
MTKM	RMSE	1.87	6.51	4.08	10.39	5.10	9.50
	AIC	54.48	154.23	116.89	191.62	134.62	184.46
	BIC	57.53	157.28	119.94	194.68	137.67	187.51
	R^2	0.9927	0.9775	0.9699	0.8877	0.9750	0.9052

OSOF - one-step-one-fraction model; TSOF - two-steps-two-fractions model; OSTF - one-step-two-fractions model; TSTF - two-steps-two-fractions model; DFKM - delayed first-order kinetic model; MGKM - modified Gompertz kinetic model; MTKM - Monod-type kinetic model; RMSE - root mean square error; AIC - Akaike information criterion; BIC - Bayesian information criterion; R^2 - coefficient of determination.

4. Conclusion

This study filled the knowledge gap of the kinetics of the anaerobic co-digestion of cassava leaves and cattle manure. Co-digestion with cattle manure increased the hydrolytic constant, k, fraction of biodegradable components, α, and BMP of cassava leaves by up to 88 % at a CL:CM ratio of 3:1, as well as reduced the time for 90% of the measured biomethane to be produced from 8 to 5 days, indicating that the substrates have a potential as a source of biogas production. In practice, parameters from either the one-step-two-fractions or two-steps-two-fractions kinetic models can be used for the design, optimisation and operation of anaerobic digestion processes involving the co-digestion of cassava leaves and cattle manure.

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