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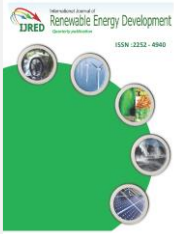

Research Article

Modeling anaerobic co-digestion of water hyacinth with ruminal slaughterhouse waste for first order, modified gompertz and logistic kinetic models

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Abstract. Water hyacinth (*Eichhornia crassipes*), an invasive aquatic weed with large biomass production is of socio-economic and environmental concern in fresh water bodies such as the Lake Victoria in East Africa. Efforts towards its control and removal can be complemented by biogas production for use as energy source. The co-digestion of water hyacinth (WH) with ruminal slaughterhouse waste (RSW) has the potential to improve biogas production from WH through collation of processes parameters such as the C/N and C/P ratios, potassium concentration and buffering capacity. Knowledge of optimum proportion of the RSW as the minor substrate is of both process and operational importance. Moreover, efficient operation of the process requires an understanding of the relationship between the biogas production and the process parameters. Kinetic models can be useful tools for describing the biogas production process in batch reactors. While the first order kinetics models assume that the rate of biogas production is proportional to the concentration of the remaining substrates, other models such as the modified Gompertz and the Logistic models incorporate the lag phase, a key feature of the anaerobic digestion process. This study aimed to establish the optimum proportion of RSW in co-digestion with WH under mesophilic conditions, and apply kinetics models to describe the biogas production. The study conducted batch co-digestion of WH with 0, 10, 20 and 30% RSW proportions at mesophilic temperature of 32°C. Co-digestion of WH with 30% RSW proportion improved biogas yield by 113% from 19.15 to 40.85 CH_4 ml/(gVS) at 50 days of co-digestion. It also exhibited the most stable daily biogas production and the largest biogas yield. The biomethanation data were fitted with the first order kinetics, modified Gompertz and the Logistic models. Biogas production for co-digestion of WH with 30% RSW proportion was best described by the modified Gompertz model with a biogas yield potential, Mo, of 43.2 ml $(gVS)^{-1}d^{-1}$; maximum biogas production rate, Rm, of 1.50 ml $(gVS)^{-1}d^{-1}$; and duration of lag, λ, of 3.89 d.

Keywords: Kinetics, modified Gompertz model, logistic model, first order kinetic model, anaerobic digestion

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1. Introduction

Global concerns over depletion of fossil fuel sources coupled with the need to reduce greenhouse effects necessitates the search for unconventional energy sources such as waste biomass. Production of biofuel from waste biomass would also double up as a sustainable waste management strategy for the biomas (Mmusi *et al.*, 2021). Accordingly, there is increased interest in production of biofuels such as biogas, biodiesel and bioethanol that are relatively cheap, renewable and eco-friendly (Ehiri *et al.*, 2014). On the other hand water hyacinth (WH), a highly reproductive aquatic weed with a doubling period of 7 to 12 days (Degaga, 2018), is of environmental concern because its high density hinders penetration of light into water bodies adversely affecting the aquatic life (Mironga *et al.*, 2012). It also interferes with the use of water bodies for transportation (Honlah *et al.*, 2019). Harvesting of the plant for use as a feedstock in biogas production can reduce the associated environmental challenges and generate relatively cheap and renewable energy. Moreover, because the water hycinth grows on water, it does not compete with crops for agricultural land (Bett, 2012).

Anaerobic digestion of WH alone suffers from process instability and limiting substrate composition and nutrient imbalance. For example, the WH large concentrations of cellulose, hemicellulose and carbohydrates and lesser concentration of lignin (Omondi *et al.*, 2019a) does not sufficiently buffer the pH during the acidogenic stage (Omondi *et al.*, 2019b) leading to acidic pH that cause prolonged lag phase. Moreover, the WH carbon and nitrogen concentrations of 15,480 ± 350 and 1,650 ± 60 mg/kg, respectively result in a C/N ratio of 9.38 (Omondi *et al.*, 2019a), which is at the lower limit of the 8-20 optimal range for biogas production., which makes mono-digestion of WH susceptible to ammonia toxicity (Kossman *et al.*, 2007).

Previous studies showed co-digestion of WH with other substrates such as slaughterhouse waste stabilized the digestion

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process and increased biogas production (Omondi *et al*., 2019b). However, most of the slaughterhouse waste components with the exception of ruminal slaughterhouse waste (RSW), have large concentrations of proteins that make the digestion process susceptible to ammonia toxicity (Chen *et al*., 2008). The RSW is characterized by C/N ratio of 18.75 (Omondi *et al*., 2009a), that is the higher limit that can affect anaerobic digestion (AD) process through nitrogen deficiency. Co-digestion of WH with RSW as a minor substrate can potentially balance the C/N ratio for WH, to an optimal level and improve biogas production.

Biogas production in batch systems is affected by the type and characteristics of biomass, nutrient availability, and the biodigester conditions such as pH and temperature (Nguyen *et al*., 2021). The anaerobic digestion process is commonly characterized by instabilities from feedstock overload, presence of inhibitors and temperature fluctuations (Gavala *et al*., 2003; Rabii *et al*., 2019). Consequently, the design of efficient biogas generation system requires an understanding of the relationships between biogas production process, substrate characteristics and biodigester conditions. Kinetic models can be useful tools for describing these relationships.

Kinetic models are developed for specific objectives that may include the establishment of process parameters, process simulation, optimization, and control (Kim *et al*., 2018; Oyaro *et al*., 2021). Consequently, the models can be applied to observe, predict, simulate, and optimize the system kinetics or mechanisms at different operation conditions (de Oliveira, 2016; Pramanik *et al*., 2019). Historically, development of kinetic models was substrate specific with the aim of simulating the kinetics of substrate degradation and biogas production (Momodu *et al*., 2021; Wang *et al*., 2022). Presently, the development and study of kinetics of biodegradation primarily considers digestion parameters such as microbial growth rate, substrate utilization rate, bio-kinetic coefficients, and growth constants (Borja *et al*., 2003; Nguyen *et al*., 2021; Hadiyanto *et al* 2023). Fitting experimental data with kinetic models can assist estimate process parameters such as the initial conditions, stoichiometry, and kinetic parameters.

The first order kinetic model has previously been adopted in batch tests for modelling the rate of hydrolysis (Feng *et al*., 2017; Pramanik *et al*., 2019). However, the model is not well suited for describing the acclimatization processes that exhibit a lag in biogas production (Hassan *et al*., 2022). The lag phase is associated with the rapid acidogenic and acetogenic stages (Momodu & Adepoju, 2011; Lafratta *et al*., 2021) that depress the pH before development of sufficient methane formers to consume the acids (Omondi *et al*., 2020). To overcome this challenge and to describe substrate consumption under the AD process, other kinetic models such as the Modified Gompertz and the Logistic models include the duration of the lag phase. The Logistics model assumes that the rate of biomethanation is proportional to the size of the microbial population as indicated by biogas production rate, and the concentration of digestible substrate that is indicated by the maximum biogas yield potential (Rabii *et al*., 2019). The modified Gompertz model, on the other hand, assumes that the rate of biomethanation is proportional to the microbial activity; however, the proportionality decreases with the solids retention time, which can be interpreted as loss of the efficiency of substrate conversion with time (Donoso-Bravo *et al*., 2010).

The Modified Gompertz model is one of the most utilized models for the anaerobic digestion process. Donoso-Bravo *et al.* (2010) and Nguyen *et al.* (2016) found that the model closely correlated biogas production with four biochemical reaction parameters; namely, biogas yield potential M_o, maximum biogas production rate R_m, methane production rate constant k, and the duration of lag λ. Other kinetic studies under mesophilic conditions have recommended the use of the modified Gompertz equation for design of continuous stirred tank reactor (CSTR) in digestion of organic wastes originating from pulp and paper industries, food processing industries and wastewater treatment plants (Linke, 2006; Bakraoui *et al*., 2019). However, the model is associated with several drawbacks that affect the prediction of methane production (Zhu *et al*., 2019). For example, Donoso-Bravo *et al.* (2010) found that the model tends to give higher values for negative lag phase without an objective biological explanation. Similarly, Li *et al.* (2019) observed that the model was prone to errors where substrate to inoculum ratio (S/I) exceeded 0.7.

This study evaluated the biogas production for various proportions of RSW in co-digestion with WH in a single-stage batch reactor. It then compared the application of three kinetic models; namely, first order kinetic, modified Gompertz, and the logistic models in describing the experimental data.

2. Materials and Methods

2.1 Overview of Methods

Co-digestion of WH with various proportions of RSW was conducted in a single - stage batch reactor. The biogas output was measured by displacement method. Biomethanation was carried out for a retention time of 60 days in a controlled mesophilic condition of 32°C. Cumulative biogas production, slurry temperatures and pH were monitored throughout the study. Fig. 1 shows the schematic of the substrate preparation and biogas production and collection

2.2 Sample Collection and Preparation of Substrates

Water hyacinth samples were obtained from the shores of Winam Gulf in Lake Victoria, Kisumu City, in Kenya at coordinates 0° 5'39.71" S, 34045'2.44" E. The ruminal slaughterhouse waste was obtained from the Nairobi Dagoretti Slaughterhouse at coordinates 1°17'3.71" S, 36°41'1.98" E. Sampling for WH selected fresh, healthy and mature plants that were then transported in sampling bags to the laboratory awaiting substrate preparation. Similarly, RSW was transported in plastic sample buckets. Whole WH plants including leaves,

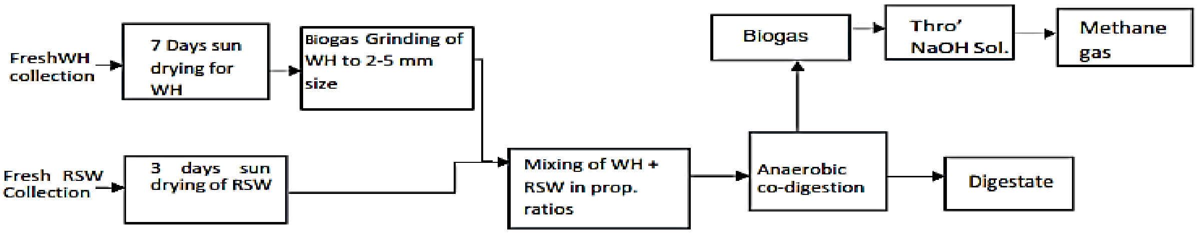

Fig. 1. Schematic of the experimental procedure

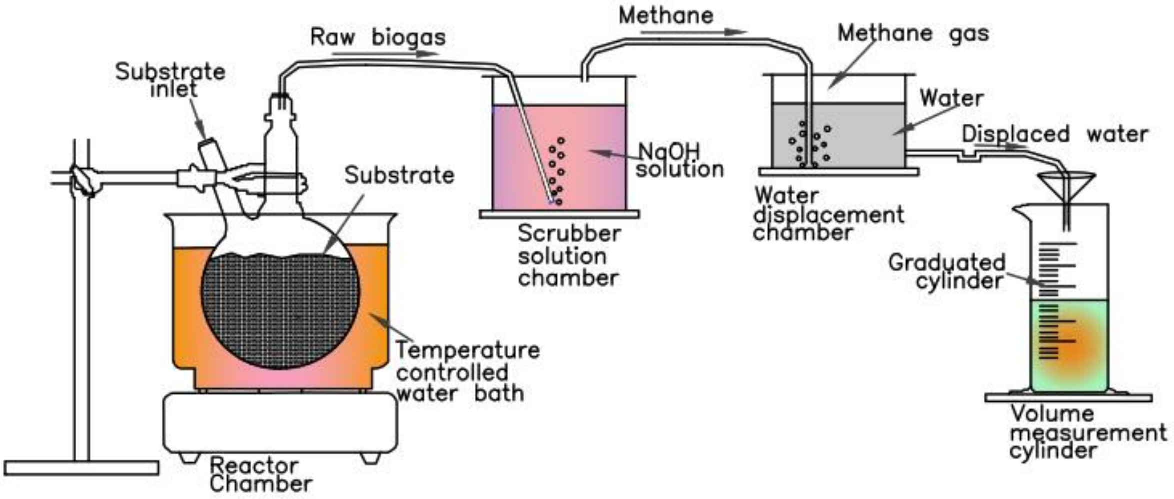

Fig. 2. Biomethanation Experimental set-up

stems and roots, were cut into small sizes of about 2 cm and dried under the sun for a period of 7 days for ease of storage and handling. The sun-dried WH was ground to fine particles by the use of mortar and pestle to form feedstock that was placed in plastic bags and stored in a refrigerator at 4°C. Similarly, fresh RSW was dried in the sun for a period of 3 days. The dried samples were kept in plastic bags and stored for biogas production as a co-substrate.

2.3 Biomethanation Experimental Set-up

The biomethanation set-up comprised four sets of batch test apparatus. Each set consisted of a constant temperature water bath, a digester, an alkaline scrubber solution chamber, a water displacement chamber and a graduated cylinder for collection of the water displaced by the gas (Fig. 2). The digester comprised a two neck round bottom flask. One neck was used as an inlet for the substrate and the other one as the gas outlet. After feeding the substrate, the inlet was sealed for the duration of the test. The second chamber comprised a 1,000 ml cylindrical vessel with a gas inlet pipe immersed in an alkaline scrubber solution for CO_2 and other minor gases. The solution was prepared from 1 molar sodium hydroxide solution containing 40 g sodium hydroxide per 1 L of water. Three drops of phenolphthalein indicator were added for monitoring pH variation; the scrubber solution was replaced when the pink/violet color of the indicator turned colorless. The scrubber solution chamber was fitted with a methane gas outlet pipe leading to a 1,000 ml water displacement chamber that was covered with an aluminum foil to prevent loss of water by evaporation. The final unit consisted of a 1,000 ml graduated cylinder for measurement of the volume of water displaced by the gas.

2.4 Anaerobic Digestion Tests

Batch anaerobic digestion tests were conducted for co-digestion of WH with 0, 10, 20 and 30% RSW proportions at a mesophilic temperature of 32°C. Biomass, 150 g, was fed into each reactor and the biomethanation monitored for 60 days. The mix proportions were prepared on the basis of weight/weight of the substrates as illustrated in Table 1. Daily biogas production was recorded as the volume of water displaced by the scrubbed gas and converted to biogas yield per gram of volatile solids.

2.5. Biomethanation Kinetics and Data Analysis

The growth functions for anaerobic co-digestion of WH with RSW were fitted with models by the use of non-linear regression analysis curve-fitting tool in IBM SPSS software. The tool computed the correlation coefficient R^2. It also computed the root mean square error (RMSE) of the differences between the predicted values and the experiment data as expressed in Equation 1.

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}(P_i - O_i)^2}{n}} \qquad (1)$$

where; P_i is the model result, O_i is the experimental result and n is the number of data.

The studied kinetic models; namely, First order kinetic, modified Gompertz and Logistic models have previously been used to describe the kinetic methane production for co-digestion of sewage sludge with food waste (Sulaiman & Seswoya, 2012); water hyacinth with poultry liter (Patil *et al.*,

Table 1
Mix Proportions of Dried Substrates

Digester	Water Hyacinth (g)	Slaughter-house waste (g)	Percent of co-substrate (%)
RSW-0	150	Nil	0
RSW-10	135	15	10
RSW-20	120	30	20
RSW-30	105	45	30

2012); water hyacinth with poultry liter, cow manure and primary sludge (Adiga *et al.*, 2012); banana peels with poultry manure (Nwosu-obieogu *et al.*, 2020); and different agricultural wastes (Zhang *et al.*, 2021). The models are expressed in Equations 2, 3 and 4.

First order : $M = Mo\,(1 - exp(-kt))$ (2)

Modified Gompertz: $M = Mo.\exp\{-exp\,[\frac{Rm.e}{Mo}(\lambda - t) + 1]\}$(3)

Modified Logistic: $M = \frac{Mo}{\{\,1+exp[\frac{4Rm}{Mo}+(t-\lambda)+2]\}}$ (4)

Where: M is the cumulative biogas production, in ml (gVS) $^{-1}$ at any time t in days, M_o is the biogas yield potential in ml (gVS) $^{-1}$, R_m is the maximum biogas production rate in ml (gVS) $^{-1}$, k is the first order model constant in l/day, λ is the duration of lag phase in days, e is Euler's constant (2.7183), and k is the methane production rate constant in day^{-1}.

3. Results and Discussions

The following sub-sections present the daily and cumulative biogas production for co-digestion of WH with 0, 10, 20 and 30% RSW proportions, compare the First order kinetics, modified Gompertz and Logistic models fitting of the experimental data and establish the kinetic parameters.

3.1 Experimental Methane Yield

The four studied combinations of WH and RSW substrates; namely, WH with 0, 10, 20 and 30% RSW proportions exhibited biogas production with a lag between day 3 and day 10. It was followed by rapid gas production up to day 20 to day 30 and after which there was a gradual decline to almost zero production after day 49 to day 53 (Fig. 3). Biogas production for WH alone (0% RSW) was characterized by large fluctuation with nil productions on some days including after the lag period. The instability was attributed to large concentration of carbohydrates, cellulose and hemicellulose in WH biomass without corresponding concentration of lignin (Omondi *et al.*, 2019a) that can cause a mismatch of hydrolysis and acidogenesis on one hand and biomethanation on the other. Introduction of the lignin rich RSW (Omondi *et al.*, 2019a) in proportions of 10, 20 and 30% as co-substrates progressively stabilized the biogas production. The 30% RSW proportion substrate demonstrated the most stable production throughout the digestion period. Previous work by Omondi *et al.* (2019b), indicated no significant improvement of the cumulative production beyond the 30% RSW proportion.

The cumulative biogas production of the studied substrates combinations (Fig. 4) exhibited an initial fast biogas production up to day 3 followed by a lag period of up to about day 10. The lag period is associated with acidification of the substrate during the acidogenic process, which affects the subsequent methanogenesis process. Co-digestion of the WH with RSW is considered to reduce the acidification by buffering the pH. The 30% RSW proportion substrate exhibited the shortest lag period and also the largest overall biogas yield.

3.2 Analysis of Kinetics Data

The fitting of the experimental biogas gas production data with kinetics models was by the IBM SPSS Software, which searched for biogas yield potential (M_o), and the first order reactions constant (k) for the First order kinetic model at the minimum residual sum of squares (RMSE) and their 95% confidence

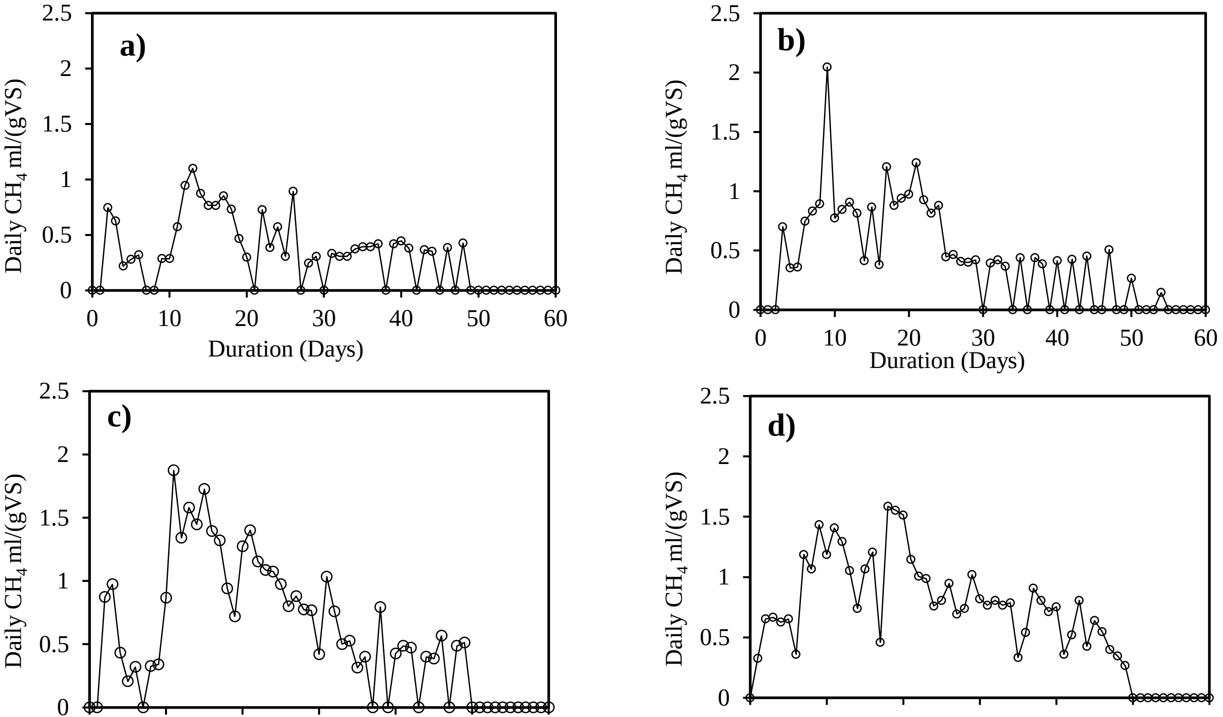

Fig. 3. Daily methane gas production for **a)** WH-0% RSW, and **b)** 10% RSW, **c)** 20% RSW and **d)** 30% RSW at 32°C

Table 2
Methane production kinetic parameters for First order kinetic, modified Gompertz and Logistic models

Substrate	Maximum biogas yield (M), l (gVS)-1	Model parameters			R^2	RMSE
		M_o, ml $(gVS)^{-1}$	k, R_m (d^{-1}), ml $(gVS)^{-1}d^{-1}$	λ *d*		
(a) First order kinetic Model (M, M_o, k)						
WH	18.89	20.52	0.988	N/A	0.977	2.087
10%RSW	25.59	27.41	0.970	N/A	0.971	2.283
20%RSW	35.35	38.75	0.988	N/A	0.963	5.942
30%RSW	40.66	44.37	0.992	N/A	0.983	3.363
(b) Modified Gompertz Model (M, M_o, R_m, λ)						
WH	18.89	19.67	0.738	3.912	0.994	0.017
10%RSW	25.59	25.69	1.218	4.100	0.997	0.020
20%RSW	35.35	36.05	1.606	5.969	0.999	0.020
30%RSW	40.66	43.21	1.496	3.892	0.998	0.021
(c) Logistic Model (M, M_o, R_m, λ)						
WH	18.89	18.842	0.594	4.730	0.988	0.021
10%RSW	25.59	24.97	0.984	4.922	0.991	0.031
20%RSW	35.35	34.81	1.306	6.880	0.994	0.034
30%RSW	40.66	41.04	1.222	5.014	0.992	0.042

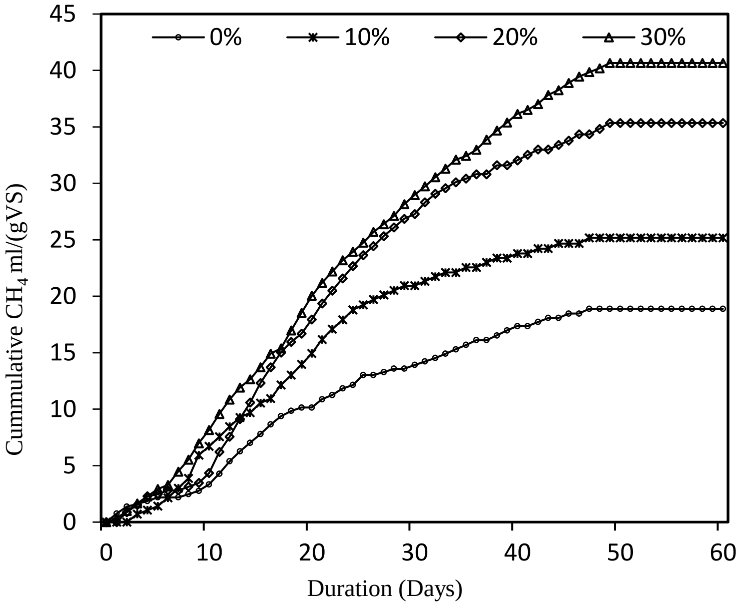

Fig. 4. Cumulative methane gas production for WH, 10, 20 and 30% RSW at 32°C

(Table 2). For the modified Gompertz and Logistic models, the Software searched for biogas yield potential (M_o), maximum biogas production rate (R_m), and duration of lag phase (λ). Fitting of the models with the experimental results generated correlation coefficient, R^2, values for cumulative methane yields. The fitted curves for first order kinetics, modified Gompertz and Logistic models for different substrate mixes are illustrated in Fig. 5. The curves obtained by the modified Gompertz and logistic models closely related with experimental curves with a typical S-shape signifying a relatively slow upward trend (lag phase) followed by a steady biomethanation. However, the curve for first order kinetic model did not depict the S-curve shape. Additionally, it had a large departure from the experimental data as also confirmed by RMSE values that were 2 orders of magnitude greater than those for the other two models (Table 2).

3.3. Discussions

The largest biogas yield potential for the tested substrates was achieved for the 30% RSW substrate at 43.2 ml/(gVs) while the least was obtained for the 0% RSW at 19.7 ml/(gVs). The results show a 136% increase in biogas production for WH following co-digestion with ruminal slaughterhouse waste. The increase was attributed to synergies in the co-digestion of WH with RSW (Omondi *et al.*, 2019b).

All the three studied kinetic models showed that the 20% RSW substrate achieved the highest maximum biogas production rate and the 30% RSW substrate the largest maximum biogas yield potential. Both the Gompertz and the Logistic models described the lag phase whereby the 20% RSW had longer lag duration of 6.88 d compared to 5.04 d for 30% RSW. The longer lag duration for the 20% RSW substrate compared to the 30% RSW suggested a limited capacity of the 20% RSW substrate to buffer the pH coupled with slower development of methane formers (Omondi *et al.*, 2019b).

The highest biogas production rate (R_m) of 1.606 ml/(gVsd) was observed for the 20% RSW substrate compared with the lowest rate of 0.738 ml/(gVsd) for WH alone and 1.496 ml/(gVsd) for 30% RSW. The result suggested that the 20% substrate offered the optimum combination of process parameters for biomethanation after the lag phase. However, the maximum biomethanation rate could not be sustained with

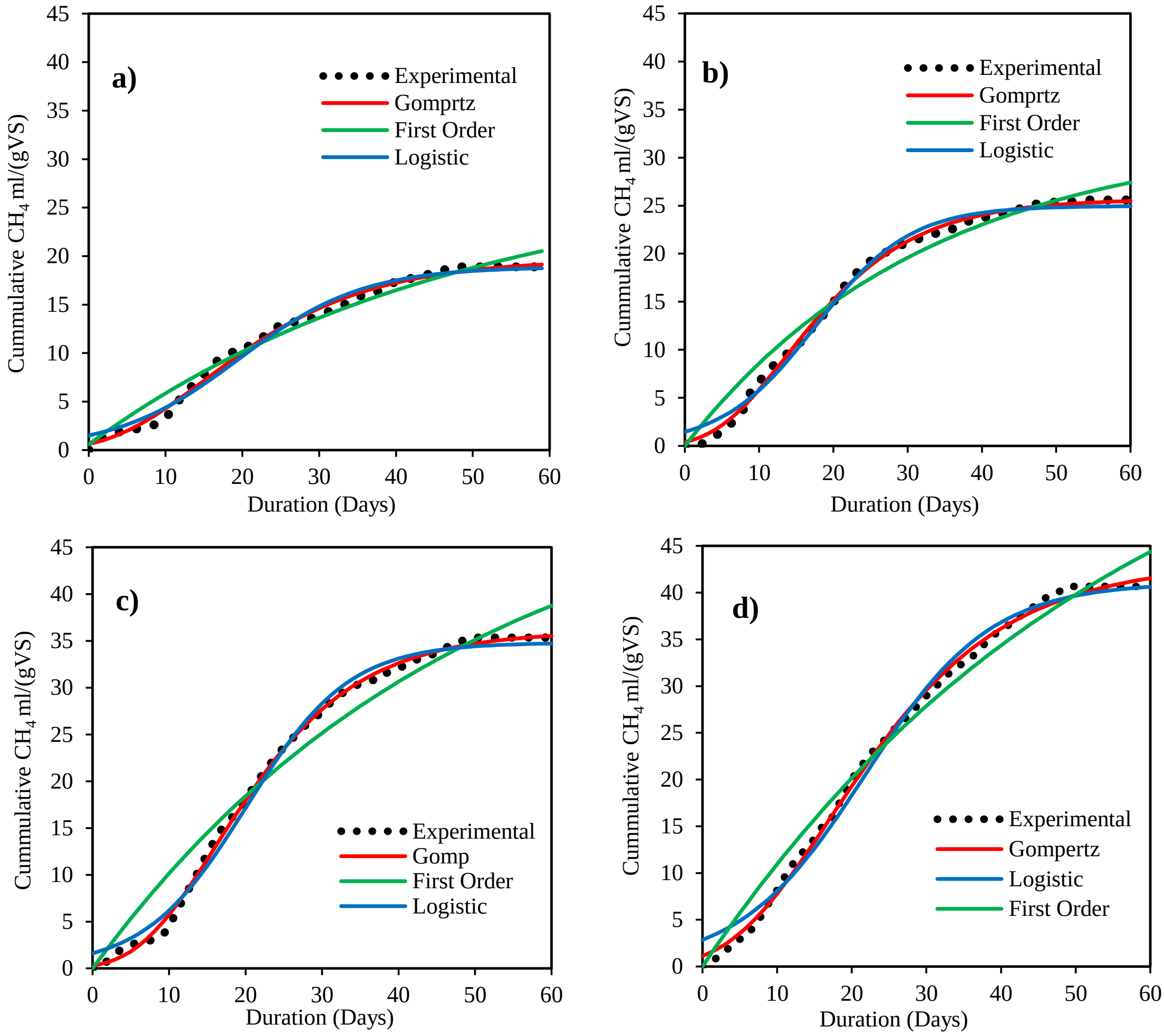

Fig. 5. Experimental, First order, Modified Gompertz and Logistic models biogas production potential for **a)** 0% RSW, **b)** 10% RSW, **c)** 20% RSW and **d)** 30% RSW proportions substrate at 32°C

the lesser RSW proportion, which could be caused by exhaustion of some key balancing ingredients such as the nutrients or lignin contributed by RSW. Similar results were obtained for co-digestion of WH with cattle dung, which failed to sustain maximum rate of biomethanationbecause of exhaustion of complementary nutrients derived from the cattle dung. (Ali *et al.*, 2022). Consequently, the substrate achieved a smaller biogas yield of 35.35 ml $(gVS)^{-1}$ compared to 40.66 ml $(gVS)^{-1}$ for the 30% RSW.

Theoretically, a continuous flow reactor may be designed to operate at the maximum biogas production rate (Balmat *et al.*, 2014; Sarker *et al.*, 2019). However, the operation would only occur over less than 13 days from day 10 to day 23 and would not consume all the prepared substrate (Camacho *et al.*, 2019). Consequently, it would fail to achieve the maximum biogas yield as well as complete the waste management of WH by digestion. For the studied substrates, the maximum biogas yield increased by 115% from 18.89 to 40.66 ml $(gVS)^{-1}d^{-1}$ for 0 and 30% RSW proportions, respectively. Consequently, co-digestion of WH with 30% RSW proportion provided the highest biogas production as well as the most effective digestion of WH as waste biomass.

The modified Gompertz model closely described the experimental data for the studied experimental substrates with correlation vectors (R^2) of 0.994 - 0.999 compared to 0.988 - 0.994, and 0.963 - 0.983 for the Logistic and First order kinetic models, respectively. These vectors demostrated that kinetic models closely fitted the experimental data for the anaerobic digestion (Bakraoui *et al.*, 2020; Tobo *et al.*, 2020; Hadiyanto *et al*, 2023). The RMSE parameter provided a more pronounced distinction between the kinetic models; the modified Gompertz model exhibited the least RMSE of 0.017 – 0.021, closely followed by the Logistic model with 0.021 – 0.034. Comparatively, the First order kinetic model had two orders of magnitude greater RSME of 2.087 - 5.942, which indicated its lesser suitability for describing the data.

5. Conclusion

The WH substrate with 30% RSW proportion exhibited the most stable daily biogas production and largest yield in co-digestion of WH with RSW. Introduction of RSW to WH substrate progressively improved biomethanation rate with the 20% RSW exhibiting the maximum biogas production rate. However, the 30% RSW presented the largest cumulative biogas production over the 60 days retention period.

The trend in the rate of biomethanation was similar for both Modified Gompertz and Logistic models. Similarly, the duration of lag for the two models followed a similar trend characterized by initial increase in biogas production followed by a lag phase that was attributed to lowering the pH by formation of acids, which suppressed methane formers. At 30% RSW proportion, the reactor pH was sufficiently buffered, which allowed uninterrupted growth of methane formers. The co-digestion was best described by the modified Gompertz model with an RMSE of 0.020 compared to 0.042 and 3.363 for the logistic and the First order kinetics models, respectively. The process kinetics parameters for the modified Gompertz model were: (1) Biogas yield potential, M_o, 43.2 ml $(gVS)^{-1}$; (2) Maximum biogas production rate, R_m, 1.50 ml (gVS)-1 d-1; (3) Lag phase function, λ, 3.89 d.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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