



Kinetics of Diauxic Biogas Production from Energy Crops: Sunflower (*Helianthus annuus*) and Napier Grass (*Pennisetum purpureum*) with Animal Manure

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Authors' contributions

This work was carried out in collaboration among all authors. Author CCO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CON and CEN managed the analyses of the study. Author NAN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluated the kinetics of diauxic-like pattern of biogas production from energy crops, Sunflower (SF) and Napier grass (NG) with cow dung (CD). The tests were performed in a batch reactor (R) operation for 60 days in R1 - R4 and 53 days in R5 - R8 under mesophilic conditions (24 - 36°C). The characteristics of the tested energy crops suggest that they hold prospects for bioenergy production. The cumulative biogas yield/gVS showed that the best performance was R1 with a biogas yield of 15.17 dm³ (0.046 dm³/gVS) followed by R3, 13.90 dm³ (0.041 dm³/gVS) and R2, 11.01dm³ (0.032 dm³/gVS). A significant difference ($P \leq 0.05$) in biogas yield was found in the reactors charged with SF/CD as against SF only. In the reactors that exhibited biphasic biogas production profile, two (2) kinetic parameters, K1 and K2 were determined by the bi-logistic function model. It was observed that the predicted values in the second phase (K2) of biogas production were considerably higher than the first phase (K1) in R2 - R5 as opposed to R6 - R8, which implies

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more biogas yield in phase 2 than phase 1. The results indicate that anaerobic digestion of SF and NG had a strong positive influence on biogas yield, BP, PR and λ_1 but not for λ_2 . The bi-logistic function model suitably fitted the experimental data with a high correlation coefficient (R^2) in the range of 0.986 - 0.997. Based on the kinetic parameters, the bi-logistic function model is well suited for the simulation of diauxic-like biogas production process.

Keywords: *Biogas yield; cow manure; diauxic growth; energy crops; kinetic study.*

1. INTRODUCTION

Energy is the key instrument for the acceleration of economic growth, poverty alleviation, and creation of employment opportunities. The steadily increasing global energy demand, fossil fuel depletion, hikes in oil prices, and the growing concerns on environmental issues have prompted intensive researches into new technological methods of obtaining clean and sustainable energy from renewable sources of energy [1]. Besides residues and waste-derived feedstocks for bioenergy production, a wide range of energy crops can be used in bioenergy production [2]. Currently, biogas produced from agricultural wastes and energy crops constitutes a very small fraction of the energy balance, but according to independent forecasts, its production in the nearest decade will develop dynamically at the rate of even tens of percent per year and it will become one of the largest in the so-called "green cart of energy" [3].

Biomass cultivation for the sole purpose of bioenergy, especially for biogas production through anaerobic digestion has significantly increased. Methane-rich biogas is produced from a wide variety of biomass types like manure, by-products, and organic waste but mainly from cultivated field crops, so-called biogas crops [4]. Grass crops including the wild types have been reported to be among the most promising energy crops for biofuel production. Organic materials such as animal manure, food waste, sewage sludge, industrial organic waste, and energy crops have been widely used as substrates in biogas production via anaerobic digestion. For biogas production, high biomass and methane yield per hectare are important requirements in the breeding of energy crops. Sunflower meets these requirements as a biomass yield of up to 20t/ha can be achieved [5]. Among the energy crops, grass has the greatest potential for biogas production (587.5m³ methane production/tonne of dry organic matter) [6]. Napier grass (*Pennisetum purpureum*) is among the most popular used energy crop for biogas production. Its growth requires very little additional nutrients,

and can easily be grown and harvested up to four times a year, thus making this grass one of the most promising crops for bioenergy production [7].

In the anaerobic digestion of Sunflower biomass for biogas production, several pretreatment methods have been evaluated to increase biogas yield because of the lignocellulosic nature of the biomass. However, some of the pretreatment methods such as alkaline pretreatment showed a negative effect on biogas production [8, 9]. In another pretreatment approach, Sunflower stalks were exposed to commercial-scale pretreatment by hot-water maceration and steam explosion technology to enhance biogas production. It was found that at optimal settings from both the macerator and steam-explosion subunits various synergistic effects may be achieved [10]. Olugbemide *et al* [11] co-digested fresh maize leaves with Elephant grass under anaerobic conditions in digesters labeled A - E. Digesters B and E had synergistic effects on biogas production, while digesters C and D had negative effects on biogas yield. Although organic materials such as Napier grass and Sunflower can be used as the sole feedstock in biogas production via anaerobic digestion, the process may tend to fail without supplementation with external nutrients and buffering agents. Co-digestion of biodegradable plant biomass with animal manure that has high buffering capacity (alkalinity) could be a better approach to energy recovery and effective treatment of the waste. In co-digestion of plant biomass with animal manure, the manure provides buffering capacity besides various other nutrients, while the plant biomass provides the high carbon content. More so, the addition of readily biodegradable organic material into animal manure could significantly enhance biogas production [12].

Given the complex nature of the biomass that serves as substrates in anaerobic digestion (AD), biogas production profile may sometimes exhibit a diauxic or biphasic pattern. Diauxic-like biofuel production pattern from complex organic substrates has been documented in the literature

[13, 14]. Depending on the relative composition of the major components (carbohydrates, proteins, fats, moisture, crude fiber, etc.) of the organic material used as a substrate, biogas production performance and pattern varies considerably [14]. However, there is a scarcity of report on the biphasic or diauxic response in biogas production from energy crops such as Sunflower (*Helianthus annuus*) and Napier grass (*Pennisetum purpureum*), and anaerobic digestion of these energy crops with cow dung could improve the biodegradability of the substrates and enhance energy recovery. This paper, therefore, presents results of diauxic-like or biphasic biogas production from Sunflower (*Helianthus annuus*) and Napier grass (*Pennisetum purpureum*) with cow dung, and the kinetic study of the process using logistic and bi-logistic function models.

2. MATERIALS AND METHODS

2.1 Collection, Preparation, and Characterization of Reactor Feeds

The fresh leaves and stalks of Sunflower (*Helianthus annuus*) and Napier grass (*Pennisetum purpureum*) were both harvested in the rainy season (June) from an arable farm. The samples were separately shredded before sun-drying for a period of 7 days to a moisture content of 10.01 and 10.8%, respectively. They were milled to further reduce the particle size, manually sieved with a sieve mesh of 0.2mm diameter, and thereafter stored in airtight polyethylene bags prior to analysis.

The cow dung was collected from an abattoir. The sample was sun-dried for a period of 10 days to a moisture content of 9.55%, milled and sieved with the same sieve mesh of 0.2mm diameter, then stored in an airtight bag accordingly. By adopting standard methods [15], substrate characteristics such as percentage moisture, Ash, total solids (TS), volatile solids (VS), total nitrogen, C/N ratio, total organic carbon content, etc. were determined.

2.2 Inoculum Preparation

The fresh cow rumen waste was collected from the evisceration unit with an airtight 2L conical flask. Triple-layered cheesecloth was used to strain the rumen waste. The strained rumen

liquor was stored in a clean airtight container to ensure anaerobiosis.

2.3 Experimental Design and Reactor Set-up

The experiment was designed in eight batch system reactors of 8L working volume (total reactor volume of 10L) labeled R1- R8 and operated under an ambient temperature range of 24 - 36°C for 60 days in R1 - R4 and 53 days in R5 - R8 (Table 1). Slurry of the reactor feeds was prepared in accordance with the experimental design and fed into the labeled reactors. A designated volume of prepared active inoculum was pitched into the charged reactors and the pH adjusted in the range of 7.20 -7.80 with NaOH before sealing and maintained throughout the study period. Each of the reactors has a fitted thermometer for temperature monitoring, an outlet with a regulator at the base of the reactor for sample collection, and a regulated gas out-let hose which was connected to a custom-built gas collecting system designed to be operated by the downward liquid displacement method. Each system has an inverted three (3) liter capacity transparent vessel made of plastic material in which the produced biogas was trapped. An outlet gas hose was fitted at the top of the inverted vessel and connected to a burner to monitor the flammability of the produced gas. Agitation of the reactors was done manually three times daily for approximately 30s, especially before measurement of the displaced water which was taken to be equivalent to the volume of the biogas produced. The performance of the test parameters was determined by the maximum cumulative biogas production.

2.4 Kinetics of Biogas Production

Bi-logistic function equation was used to fit cumulative biogas production data obtained from each of the batch experiments because of the observed diauxic-like anaerobic digestion pattern (Eq. 1). However, in the experiment that showed normal growth pattern, the logistic function model (Eq. 2) was used in the simulation of the obtained cumulative biogas production. This model (the logistic function model) has been used in several studies for describing biogas production in batch anaerobic fermentation experiment [16].

Table 1. Batch mode experimental design and reactor content

Treatment Ratio (%)		% TS	% VS
R1	SF/CD 50:50	5.91	4.42
R2	SF/CD 75:25	5.55	4.25
R3	SF/CD 85:15	5.51	4.30
R4	SF 100	5.39	4.43
R5	NG/CD 50:50	5.84	4.52
R6	NG/CD 75:25	5.83	4.73
R7	NG/CD 85:15	5.82	4.83
R8	NG 100	5.80	5.15

$$Gy = \frac{B_{p1}}{1 + \exp\left[\frac{4P_{R1}(\lambda_1 - t)}{B_{p1}} + 2\right]} + \frac{B_{p2} - B_{p1}}{1 + \exp\left[\frac{4P_{R2}(\lambda_2 - t)}{B_{p2} - B_{p1}} + 2\right]} \quad (1)$$

Where:

Gy - biogas yield (dm³) with respect to time t (days)

B_{p1} - maximum biogas potential of the substrate (dm³) before the second lag

P_{R1} - maximum biogas production rate (dm³.d) before the second lag

B_{p2} - maximum biogas potential of the substrate (dm³) in the second phase

P_{R2} - maximum biogas production rate (dm³.d) in the second phase

λ_1 - first lag phase (days)

λ_2 - second lag phase (days)

t - time (days).

$$Gy = \frac{P_b}{1 + \exp\left[\frac{4P_R(\lambda - t)}{P_b} + 2\right]} \quad (2)$$

Where:

Gy - cumulative biogas yield (dm³) with respect to time t (days)

P_b - maximum biogas potential of the substrate (dm³)

P_R - maximum biogas production rate (dm³.d)

t - time (days)

λ - lag period (days).

The biogas yield from the reactors was also evaluated statistically in 2 sets, R1 - R4 and R5 - R8 using analysis of variance (ANOVA) implemented in IBM SPSS version 20.0

3. RESULTS AND DISCUSSION

3.1 Feedstock Characteristics

The results of the chemical characteristics of the feedstocks are summarized in Table 2. The

percentage TS of the entire reactor feeds was very high and falls within the range of 89.2 - 90.9 %. However, the VS content of the energy crops, SF and NG were higher than the livestock manure, CD, indicating that the energy crops used in the experiment contain more digestible organic materials than CD and are essentially prospective organics for bioenergy production. The high TS and VS values of the energy crops (SF and NG) in this study are similar to that reported by Feng *et al* [17] and Zhurka *et al* [9] for Sunflower and Haryanto *et al* [18] for Napier grass.

The carbon-to-nitrogen ratio (C/N) ratio of NG (20.0) was within the optimal range (20 - 30) for anaerobic digestion (AD) [19], the CD (37.0) was slightly above, and the SF (11.5) below the required optimal range. Similarly, the percentage nitrogen content of SF and NG is 1.8-2.3 times higher than the CD. The pH of the reactor feeds was also within the optimal range (6.5-7.5) for AD [20, 21]. Compared to the pH of the raw Elephant grass reported by Mbachu *et al* [22], the Napier grass (NG) used in this study has a higher pH more suitable for biogas production.

3.2 Daily Biogas Production Profile

Biogas production profiles from mixtures of the reactor feeds are shown in Figs. 1 and 2. Biogas production started on the first day in all the reactors fed with mixtures SF/CD while in SF only gas production started on the 5th day. It is worthy to note that the treatment did not only improve biogas yield but significantly reduced the lag phase period. Anaerobic digestion and biogas production profile were biphasic (diauxic) in all the reactors except R1 which showed a

Table 2. Characteristics of the Feedstock

Parameters (%)	SF	NG	CD
Moisture content (MC)	9.10	10.8	9.6
Ash content	14.8	10.0	30.7
Fibre content	28.6	36.1	30.3
Nitrogen	3.4	2.5	1.5
Fat content	2.1	2.9	2.9
Crude protein	21.5	15.7	9.1
Organic Carbon	39.4	50.2	54.3
Total solid (TS)	90.9	89.2	90.5
Volatile solid (VS)	76.1	79.2	59.8
C/N ratio	11.5	20.0	37.0
pH	6.6	6.9	7.1

single-step lag phase. Biogas production in R1 was expectedly very low until day 5. Days 6 and 7 recorded an accelerated biogas production and thereafter gas production started to fluctuate until day 49. There was a sharp decrease in gas production on day 50, after which production became insignificant and finally stopped on day 60. There were four (4) major peak values of biogas production. The highest was on day 22 (1670ml), followed by day 23 (1560ml) then day 31(1260ml), and day 46 (980 ml). The reactor, R2 showed a different pattern of biogas production compared to R1. In R2, gas production was relatively high on day 2 and progressively increased until day 7. Biogas production abruptly stopped on day 8 and 9. It started again on day 10 and continued to increase and fluctuate until day 56 when gas production eventually ceased. Three (3) peaks of biogas production were observed in the plot. The highest was on day 32 (1180ml), followed by day 37 (1076ml) and day 28 (860ml). In R3, gas production started very low and slowly accelerated as it fluctuated until day 38 when gas production ceased. It commenced again on day 42 with a gradual increase in gas production which started declining on day 50 and finally leveled off on day 57. With the fluctuation in the daily rate of gas production, four (4) major peaks were recorded and are thus in increasing order: day 32 (1720ml), day 32 (1680ml), day 34 (1340ml), and day 36 (910ml). In R4, a four (4) day lag phase duration was observed. Gas production started on day 5 and gradually began to decline after day 6. No biogas production was recorded between days 18 and 24. Gas production started again on day 25, attained its peak value on day 49, and gradually decelerated and leveled off on day 58.

In the reactors fed with NG/CD (R5-8), gas production was within 24hr in R5, day 5 in R6, day 3, and 2 in R7 and R8, respectively. After the initial rise in biogas production in all the reactors which occurred between day 4-7, gas production declined abruptly and continued to fluctuate until day 29 which recorded an increased gas production in R5 and R7 on day 37. Thereafter gas production decreased and finally ceased on day 53.

Biogas production profile in all the reactors was biphasic with a second lag period except R1. This may be ascribed to the smaller quantity and lower fat content of the SF. But in other reactors with a higher quantity of SF, diauxic-like biogas production profile was observed.

Diauxic-like growth pattern in AD has been reported for several substrates such as those high in fat and lignocellulose [23]. This observation has been frequently ascribed to the exposure of microorganisms to two or more organic substrates which are consumed at different rates, resulting in a biphasic biochemical reaction pattern. Some diauxic-like biogas production patterns, however, maybe as a result of the presence of toxic substances that exert an inhibitory effect on biochemical steps. Kim and Kim [14] evaluated the efficiency of anaerobic digestion and biogas production from organic wastes with high-fat content based on carbon number and double bond count of long-chain fatty acids (LCFAs). The result showed that castor, safflower, and perilla oils (with double bond counts of ≤ 3) exhibited a single-step lag-phase, while salmon oil (with double bond counts of ≥ 4) exhibited a three-step lag-phase (i.e., three instances of a lag-phase). This indicates that LCFAs with double bonds ≤ 3 had a minimal influence on biogas production after the initial lag-phase.

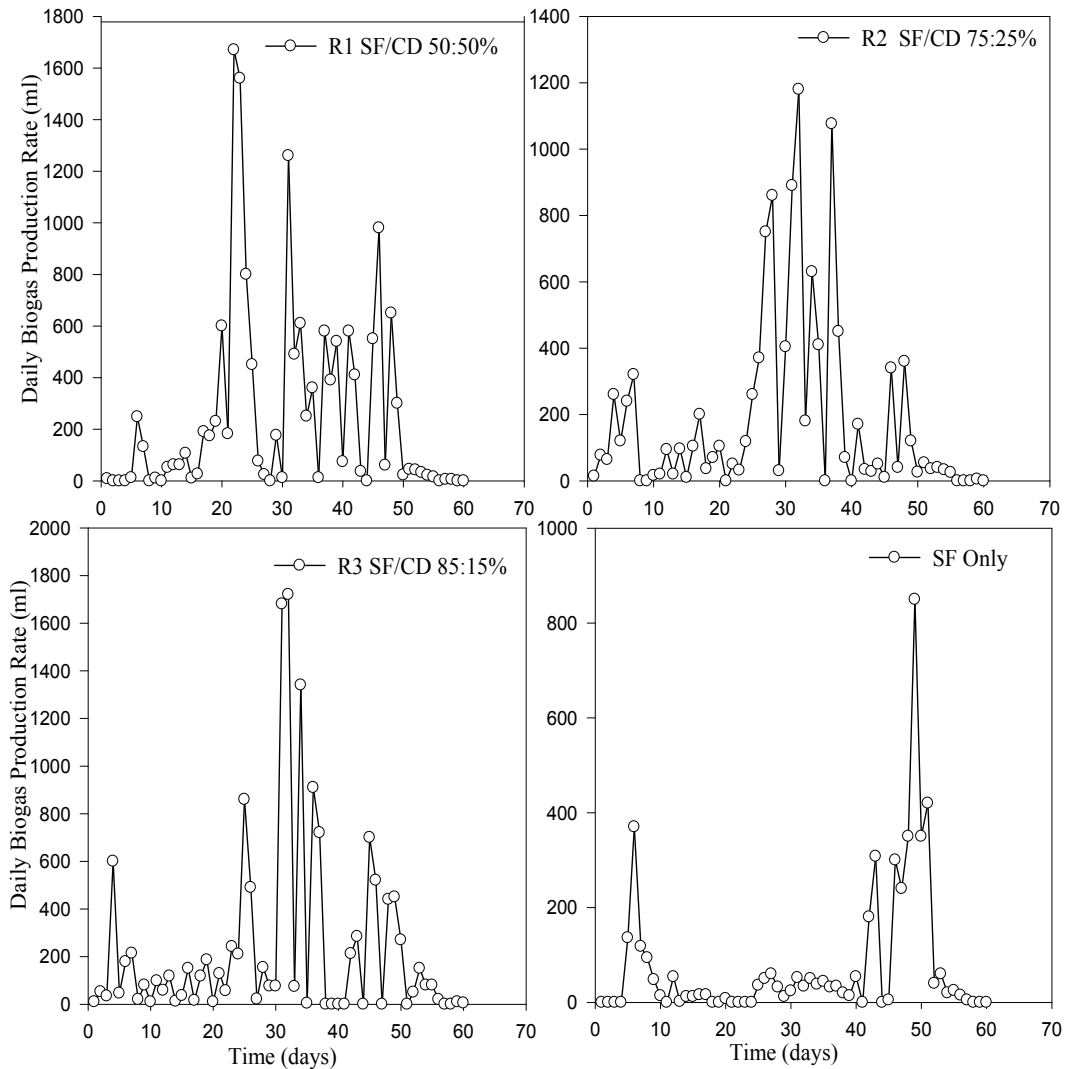


Fig. 1. Daily biogas production Profile from Mixtures of SF/CD

3.3 Biogas Yield

The results of maximum cumulative biogas and yield per gram VS are shown in Table 3. The best performance was R1 with a biogas yield of 15.17 dm³ and 0.046 dm³/gVS. This was followed by R3 (13.90 dm³ and 0.041dm³/gVS) and R2 (11.01 dm³ and 0.032 dm³/gVS). In the reactors fed with NG/CD, the best performance was recorded in R5 (8.66 dm³ and 0.024 dm³/gVS). Analysis of variance (ANOVA) revealed a significant difference ($P \leq 0.05$) in biogas yield in the reactors charged with SF/CD as against SF only. Similarly, a significant

difference in gas yield was also observed in all the reactors fed with NG/CD except R6 (NG/CD 75/25%). A similar result on the co-digestion of Napier grass with animal manure (Chicken manure) for biogas production showed an improved yield [7]. In contrast to our experimental result, the biogas yield reported by Haryanto *et al.* [18], from the co-digestion of cow dung and Elephant grass was much lower compared to that of control. The biogas yield from Sunflower only (SF) was 45.02% higher than Napier grass (NG), suggesting that Sunflower (SF) has a higher biogas production potential than Napier grass (NG).

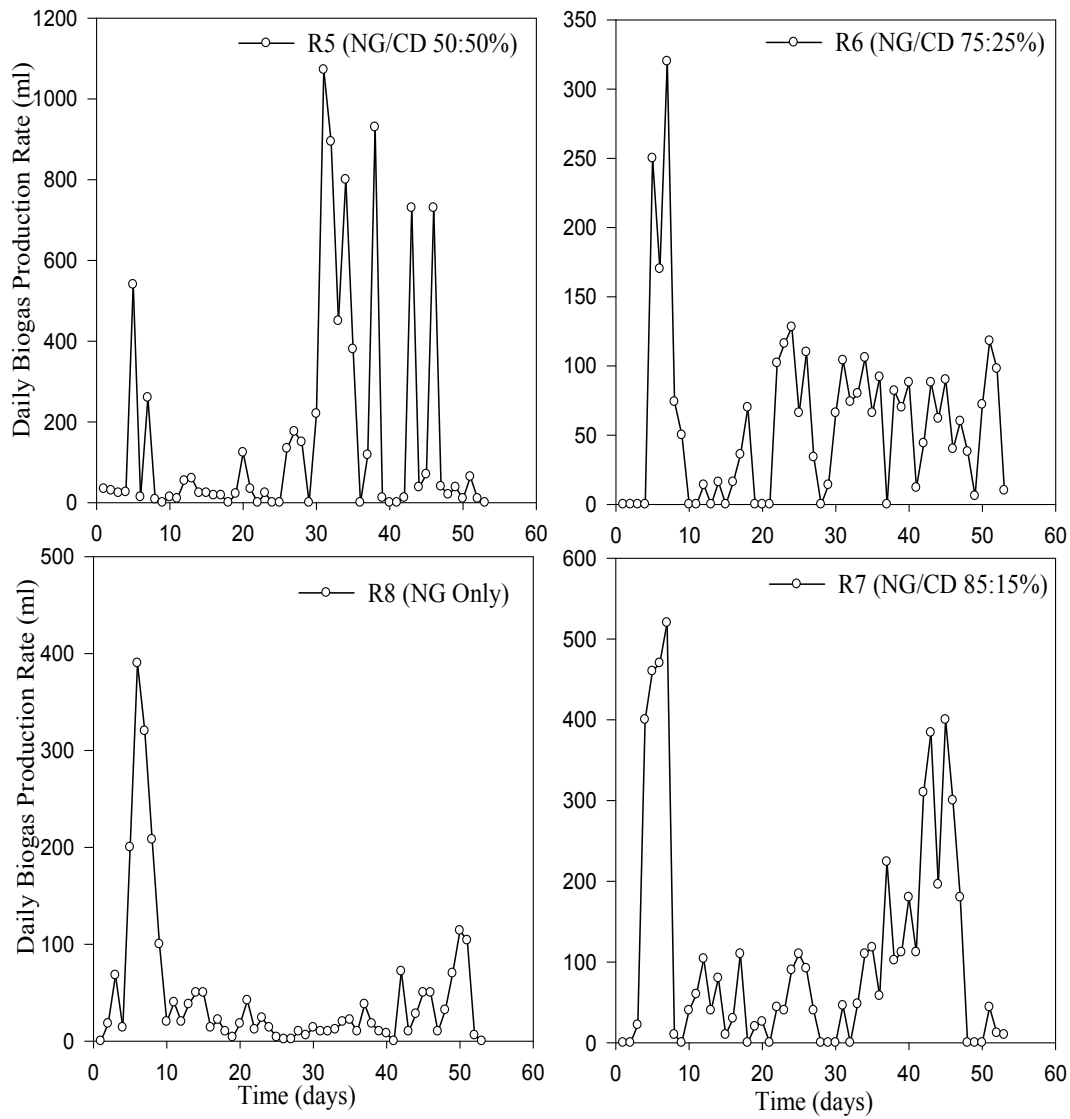


Fig. 2. Daily biogas production Profile from Mixtures of NG/CD.

Table 3. Cumulative biogas yield from the treatments

Reactors/Treatments (% Feeds Content)	Cumulative Biogas Yield (dm ³)	Yield Per gram VS (dm ³ /gVS)
R1. SF/CD 50:50	15.17	0.046
R2. SF/CD 75:25	11.01	0.032
R3. SF/CD 85:15	13.90	0.041
R4. SF only	4.62	0.013
R5. NG/CD 50:50	8.66	0.024
R6. NG/CD 75:25	3.15	0.0083
R7. NG/CD 85:15	5.75	0.015
R8. NG only	2.54	0.0062

3.4 Kinetic Study

Plots of the experimental data and model simulation are shown in Figs. 3 and 4. The Kinetic parameters estimated by using non-linear regression are summarized in Table 4. In the reactors that exhibited diauxic growth and biphasic biogas production profile, two (2) kinetic parameters, K_1 (Bp_1 , PR^1 , and λ_1) and K_2 (Bp_2 , PR_2 , and λ_2) were determined by the bi-logistic model. It could be observed that the predicted values in the second phase (K_2) of biogas production were higher than the first phase (K_1) in R2 - R5 as opposed to R6 - R8. This implies that there was more biogas yield in phase 2 than phase 1. This observation could be attributed to the increased microbial population after the initial lag phase and the availability of the necessary intermediates for methanogenic activity and bioconversion to biogas.

These results suggest that anaerobic digestion of SF and NG had a strong positive influence on biogas yield, BP, PR, and the λ_1 (lag phase

duration or minimum time to produce biogas) but not for λ_2 . The reactor, R1 had the highest values of Bp and PR which were 15.46 dm^3 and $0.544 \text{ dm}^3/\text{d}$, respectively. This implies that the ratio, SF/CD 50:50% had the optimum ratio that exhibited good conditions suitable for bacterial growth in the reactor, thus biogas maximally generated.

The models well fitted the experimental data as indicated by the high correlation co-efficient (R^2) which is in the range of 0.986-0.997, which is in line with Parra-Orobio *et al* [24]. The correlation coefficient (R^2) obtained by Latinwo *et al* [25] in the kinetic study of biogas production from co-digestion of cow dung with plantain peels using logistic function model was in the range of 0.9775-0.9859, which is similar to the result of our study. Bi-logistic function model is well suited for anaerobic digestion and biogas production process that mimics diauxic-like curve. There is no literature report yet on the kinetic evaluation of biphasic or diauxic-like biogas production response using bi-logistic model.

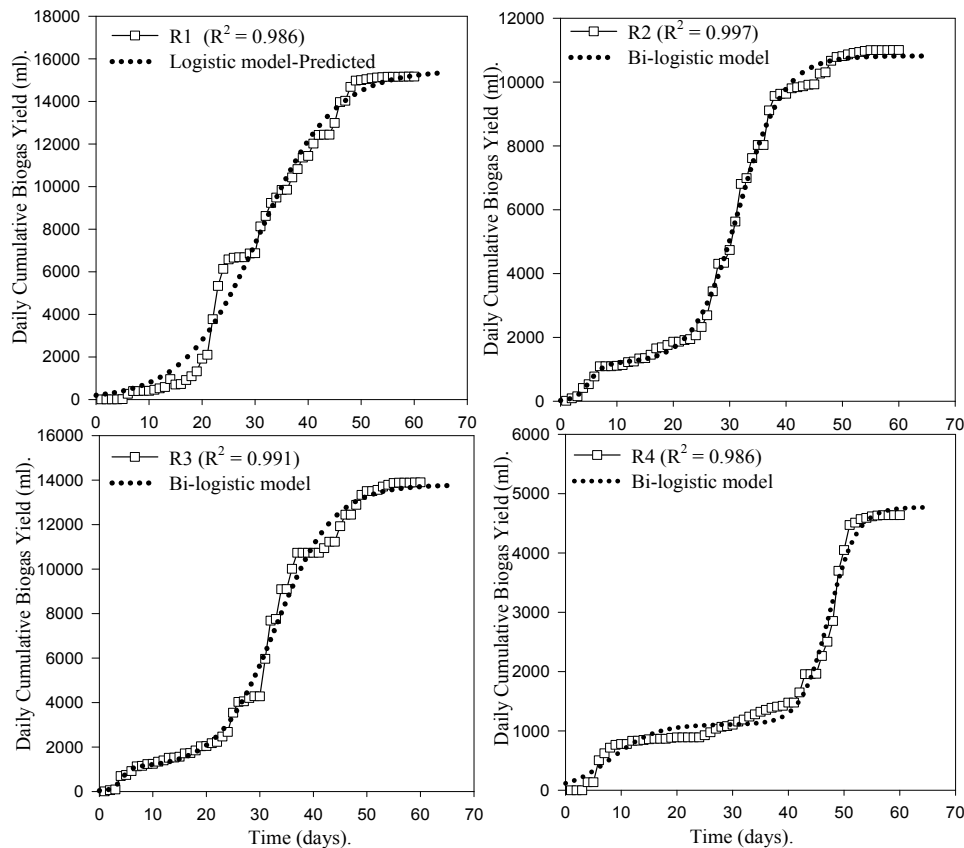


Fig. 3. Plots of Experimental data and Predicted Biogas Yield from SF/CD (R1-R4)

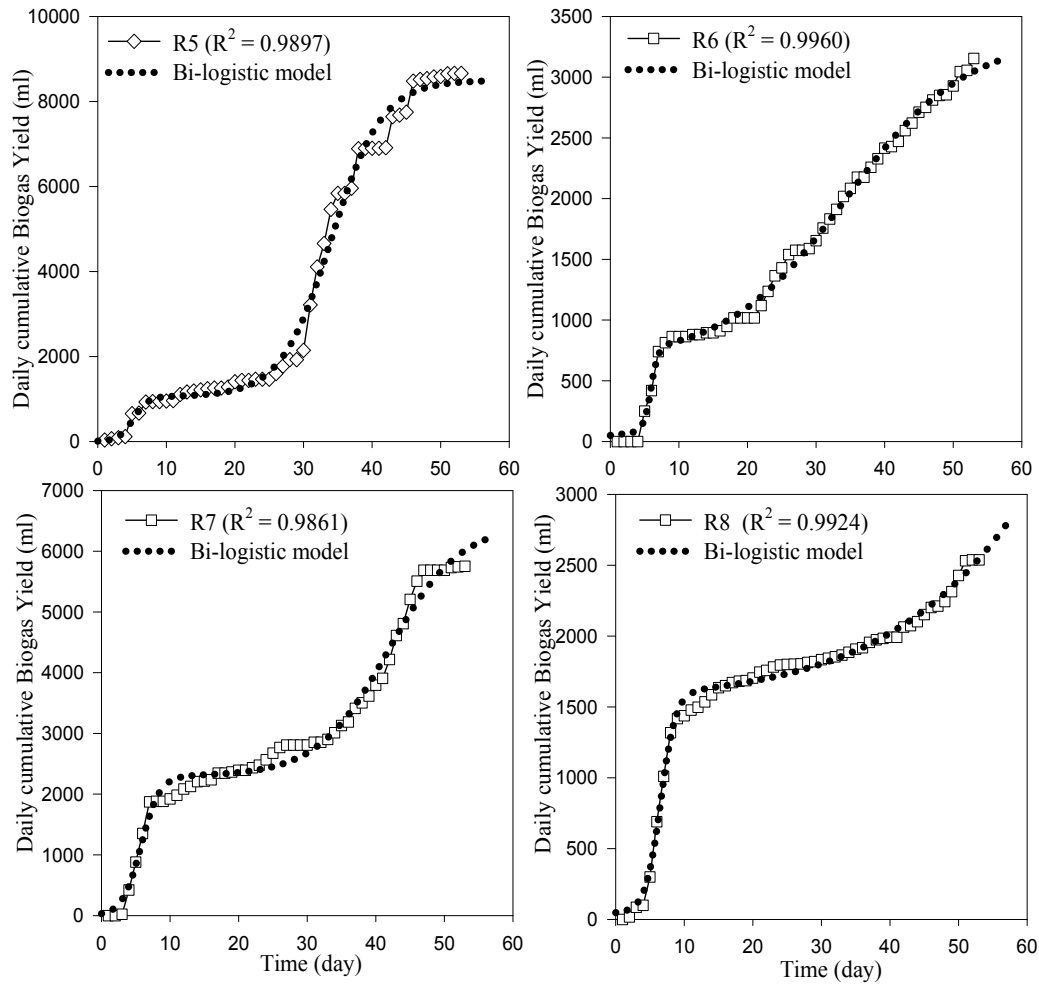


Fig. 4. Plots of Experimental data and Predicted Biogas Yield from NG/CD (R5-R8)

Table 4. Summary of the Estimated Kinetic parameters

Reactors	Modified Logistic model parameters					
	$B_{P1} (dm^3)$	$P_{R1}(dm^3.d)$	$\lambda_1(days)$	$P_{b2} (dm^3)$	$R_{m2} (dm^3.d)$	$\lambda_2(days)$
R1	15.46	0.544	16.56	-	-	-
R2	1.18	0.24	2.63	10.82	0.61	23.68
R3	1.04	0.32	2.5	13.78	0.59	22.27
R4	1.11	0.07	0.62	4.77	0.36	42.26
R5	1.06	0.25	3.08	8.49	0.506	26.74
R6	0.68	0.34	4.94	3.32	0.076	16.98
R7	2.30	0.43	3.11	6.45	0.20	31.51
R8	1.53	0.36	4.42	10.12	0.13	51.20

R1 - SF/CD 50:50, R2 - SF/CD 75:25, R3 - SF/CD 85:15, R4 - SF only,
 R5 - NG/CD 50:50, R6 - NG/CD 75:25, R7 - NG/CD 85:15, R8 - NG only

4. CONCLUSION

This study evaluates the anaerobic digestion of different biomaterials for the production of methane gas. Eight treatments were carried out, that is, 8 batches (R1 to R8) in a 10L total volume reactor: R1 to R4 with different proportions of Sunflower and Cow dung (SF / CD); R4 A R8 with different proportions of Napier grass and Cow dung (NG / CD). The results showed higher biogas production in R1 and better performance of Sunflower in relation to Napier grass. The biogas yield from mixed substrates was significantly higher than single substrate digestion except in R6 (NG/CD 75:25) with 24.02% increase in biogas yield.

As for the study of methane production accumulated over time, except R1 (SF/CD 50/50), an effect called "diauxic-like anaerobic digestion pattern" was noted (although all material is biodegradable, microorganisms give preference to one of them. Then, only after the end of it, do they attack the other material). The kinetics of this biphasic behavior was studied, finding an equation representative of the phenomenon of excellent fit, Bi-logistic function. This study has successfully demonstrated the suitable mixed ratios of Napier grass and Sunflower with cow dung for biogas production based on the kinetic parameters, and the bi-logistic function model is well suited for the simulation of diauxic-like biogas production process.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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