

# Influence of inoculum/substrate ratio on biogas yield and kinetics from the anaerobic co-digestion of food waste and maize husk

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## ABSTRACT

The study reports for the first time, the influence of inoculum/substrate (I/S) ratios on biogas yield and kinetics of food waste (FW) and maize husk (MH) anaerobic co-digestion. The study was investigated in six anaerobic digesters, D-0, D-0.25, D-0.5, D-1, D-2, and D-4 at  $36 \pm 2$  °C. D-1 with I/S ratio of 1 had the largest cumulative biogas yield of 9.99 L/gVS. Results from the modified Gompertz model revealed a decrease in lag phase ( $\lambda$ ) by 20.4% at I/S ratio 1, hence, shows that the amount of time needed for active methanogenesis to commence was hugely decreased. Maximum specific biogas production ( $R_m$ ) and maximum biogas production potential (A) increased with increase in I/S ratio. Also, D-1 yielded the highest values of 0.37 L/gVS/day and 9.99 L/gVS for  $R_m$  and A, respectively. A decrease in biogas yield at D-0.25 and D-0.5 was however obtained with respect to the control experiment. The study recommends confirmation of optimum I/S ratio prior to establishment of commercial biogas plants.

## 1. Introduction

Nations of the world are faced with the challenge of meeting their fast-growing energy demand without damaging the environment (Samuel and Gulum, 2019a; Samuel and Okwu, 2019b). This has necessitated the exploration and exploitation of renewable energy alternatives (Owamah, 2020; Owamah et al., 2020; Onochie et al., 2021). Zhang et al. (2016) and Okwu et al. (2020) opined that the production of biogas from waste organic substances is a viable renewable energy alternative as resulting digestates are also utilized as compost for farming. Anaerobic digestion improves agricultural productivity, generates bio-fuel, and reduces environmental pollution (Almomani et al., 2019; Almomani and Bhosale, 2020). Notwithstanding these great potentials, development of industrial anaerobic plants have been confronted with many sustainability challenges such as cost-effectiveness and optimization of process factors and substrates. Researchers have

however, used anaerobic co-digestion of two or more organic substances in a bioreactor to optimize the biogas yield of various substrates (El-Mashad and Zhang, 2010).

Maize husk is a plant biomass produced as a by-product of intensive and widespread maize cultivation in Nigeria and many other African countries (Owamah and Izinyon, 2015a). According to the official statistics of the Food and Agricultural Organization of the United Nations (FAO, 2017), maize production in the world, Africa, and Nigeria was estimated as 1130, 84, and 11 million metric tonnes (MMT), respectively. The Federal Ministry of Agriculture, Water Resources, and Rural Development (FMAWRD, 1988) reported that out of the 1.37 MMT of annual maize production in Nigeria, about 4.11 MMT residual wastes of husks, cobs, straws, skin and trimmings are generated. Not only that these huge maize residues are discarded as wastes, the current disposal technique of uncontrolled burning cause serious environmental health hazard and global warming. Anaerobic digestion for biogas production could be an alternative for managing this agricultural waste while

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### Nomenclature

FW	Food waste
MH	Maize husk
$\lambda$	Lag phase
$R_m$	Maximum specific biogas production
A	Maximum biogas production potential
I-S	Inoculum-substrate
C/N	Carbon/Nitrogen
VS	Volatile solid

**Table 1**

Properties of mixed substrates fed into respective digesters.

Digester	<sup>1</sup> FW: MH (%) (w/w, based on total weight (g))	<sup>2</sup> Total weight of sample (g)	<sup>2</sup> wt of FW (g)	<sup>2</sup> wt of MH (g)	<sup>2</sup> Influent VS(g/L)	C/N ratio	pH	<sup>2</sup> I/S ratio
Dig. D-0.25	75:25	30	22.55	7.5	3.5	19.6	6.8	0.25
Dig. D-0.5	75:25	30	22.55	7.5	3.3	19.3	6.6	0.5
Dig. D-1	75:25	30	22.55	7.5	4.2	20.7	7.1	1
Dig. D-2	75:25	30	22.55	7.5	3.7	23.4	7.2	2
Dig. D-4	75:25	30	22.55	7.5	3.9	22.6	7.3	4
Dig. D-0	75:25	30	22.55	7.5	3.6	20.2	6.9	0

<sup>1</sup> based on fresh weight;

<sup>2</sup> based on volatile solid

harnessing the energy potentials. On the other hand, in Nigeria, there is an alarming increase in food waste (FW) generation from both residential and non-residential outlets owing to rapid urbanization and population explosion (Owamah and Izinyon, 2015a). At the global level, literature shows that about 30% processed food for human consumption eventually turns out as waste, hence resulting in about 1300 billion kilograms (Nathan and Pragasen, 2012). Presently, one of the perturbing environmental challenges to governments of developing nations is the effective management of municipal solid waste, mainly food waste (Owamah et al., 2017).

An earlier study by Owamah and Izinyon (2015a) showed that co-digestion of FW and MH performed optimally at ratio 3:1 (w/w), respectively. Boulanger et al. (2012) reported that biogas yield from substrates could be further improved by the addition of inoculum to give appropriate microbial profile. However, the influence of inoculum on biogas yield is dependent on the nature of biomass as well the inoculum/substrate ratio among other factors (Raposo et al., 2009). Proper addition of inoculum helps overcome inhibition of digesters and reduce the time for the beginning of active methanogenesis (Raposo et al. (2009). Moreno-Andrade and Buitrón (2003) reported an increase in methane content of biogas with inoculum-substrate ratio ranging from 1 to 4. The S/I ratio is the initial ratio of VS in the feedstock (FW and MH) to the VS in the inoculum during the start of a batch digestion experiment (Feng et al., 2013). It involves the addition of some amount of inoculum to the substrate for provision of the needed microorganisms to start the reaction. The S/I ratio was reported as a very important parameter in batch anaerobic digestion (Li et al., 2018). Every substrate has its appropriate S/I ratio because of the quantity of VFA and the capacity to buffer the accumulated VFA during the digestion process. It was reported that while higher S/I ratios could be toxic, lower S/I ratios could prevent induction of the enzyme essential for anaerobic digestion (Feng et al., 2013). S/I ratio could also affect the lag phase of anaerobic digestion (Lavagnolo et al., 2018).

The application of inoculum for starting anaerobic digestion has been arbitrary. There is a need therefore to determine the optimum quantity of inoculum that will be required for optimum biogas yield of various substrates. Furthermore, literature shows that scientific information on the best inoculum/substrate ratio for optimum biogas

production from co-digestion of FW and MH is scarce or non-existent. This research was therefore targeted at establishing the optimum I/S ratio for enhanced biogas yield, using FW and MH as co-substrates. It also applied the modified Gompertz model to determine the influence of these I/S ratios on the biogas yield kinetics.

## 2. Materials and methods

### 2.1. Collection and preparation of substrates

The FW and MH used as substrates in this study were collected from the restaurant of Landmark University, Nigeria. They were crushed and coalesced following the procedures outlined by El-Mashad and Zhang

(2010). The FW and MH were combined in the ratio 3:1 based on previous findings (Owamah and Izinyon, 2015a).

### 2.2. Physicochemical analysis of substrates

Physicochemical characterization of the substrates was carried out three times following the guidelines stipulated in APHA (2012). Laboratory oven, model DHG-9053A, was used to determine the total solids (TS) and the volatile solids (VS) of the substrates. Similarly, direct-reading photometer model 7100 was used to determine the ammonium nitrogen ( $\text{NH}_4^+\text{N}$ ), total kjeldahl nitrogen (TKN), chlorine (Cl), potassium (K), sulphur (S), sodium (Na), calcium (Ca) and magnesium (Mg). A pH meter, model PHS-3C was used to measure the initial pH values. The physicochemical properties of the prepared substrates are presented in Table S1, and measured based on the wet weight samples.

### 2.3. Collection of inoculum and experiment for biogas production

Effluent from an anaerobic reactor digesting similar substrates was collected and utilized as the inoculum. The impact of inoculum-substrate ratios on the kinetics and yield of biogas production through six experimental designs was investigated using 10-liter computer controlled anaerobic digesters (Fig. S1) at a temperature of  $36 \pm 2^\circ\text{C}$ . The reactors were labeled as D-0.25, D-0.5, D-1, D-2, and D-4 and D-0 for 0.25, 0.5, 1, 2, 4 and 0 inoculum/substrate ratios, respectively. The percentage mix ratios of FW to MH and other determined physicochemical properties of the respective digesters' contents are shown in Table 1. A batch flow anaerobic digestion process was utilized following similar procedures by Owamah and Izinyon (2015a).

The digestion ran for 44 days until biogas yield became insignificant. The total biogas production for each day was measured daily from the digester's calibrated receptacle using water displacement approach. The batch anaerobic digestions were carried out twice and the mean values of daily biogas production recorded. The analysis of percentage methane was done with a gas chromatograph (BUCK GC122, China).

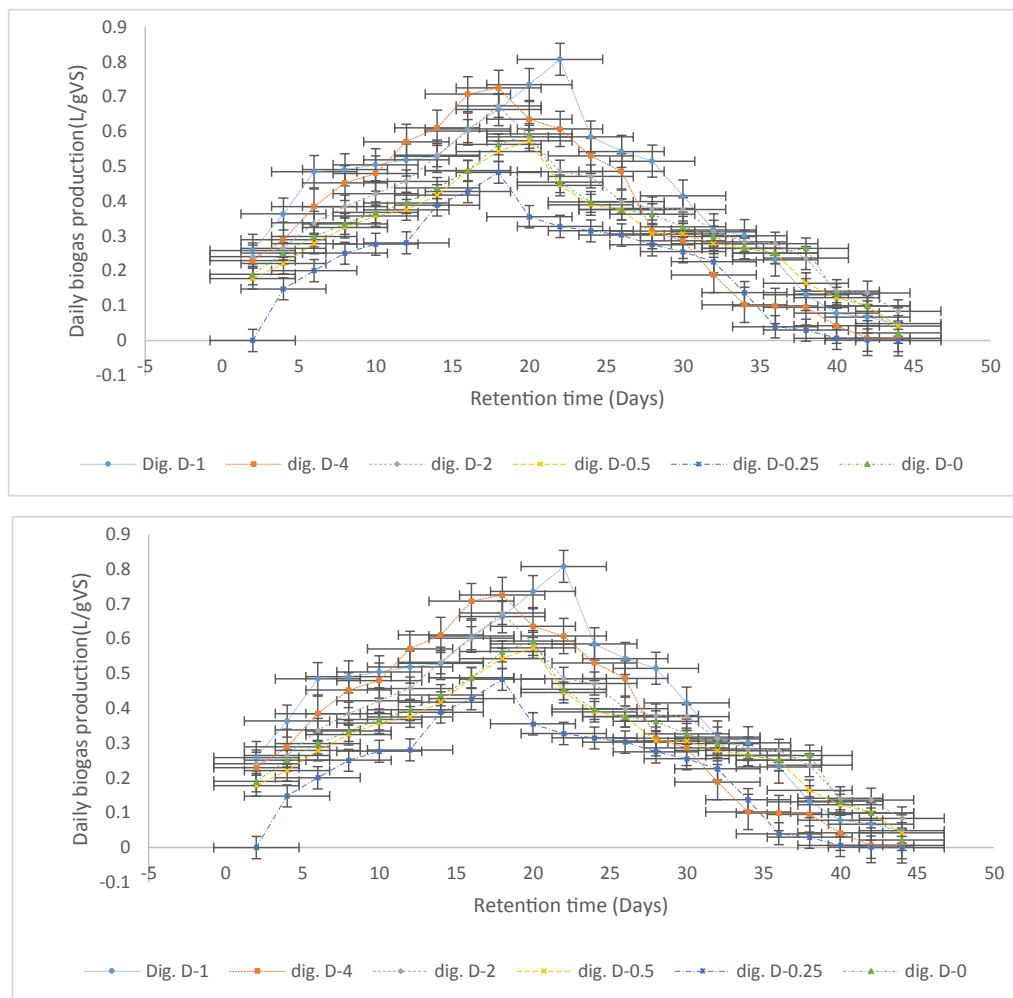


Fig. 1. Daily biogas yield at varied inoculum- substrate ratio.

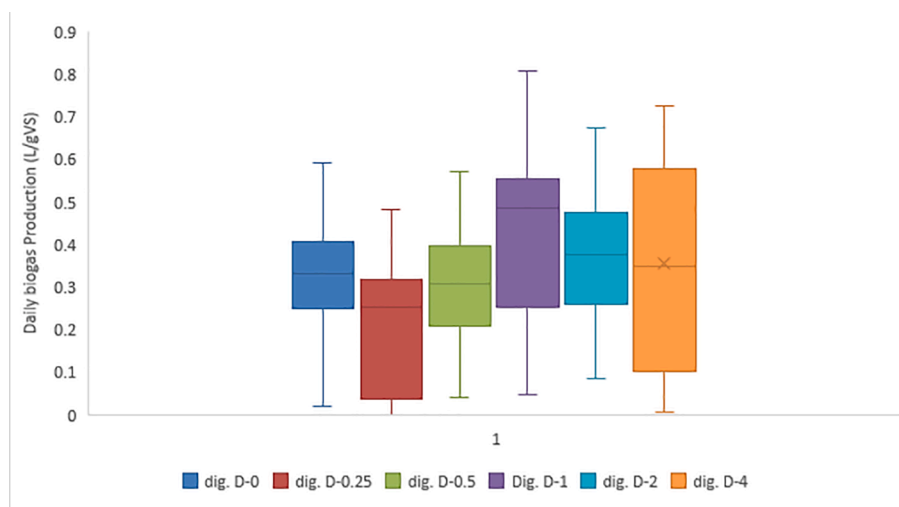


Fig. 2. Box Plot of daily biogas yield at varied inoculum- substrate ratio.

#### 2.4. Determination of biogas yield kinetics using the modified Gompertz model

The daily cumulative biogas yields for the respective experimental designs were fit into the modified Gompertz model expressed by Eq. (1)

to determine respective biogas production kinetics: the maximum biogas production potential,  $A$  (L/gVS); maximum specific biogas yield;  $R_m$  (L/gVS/day); and lag phase,  $\lambda$  (days) (Owamah et al., 2020)

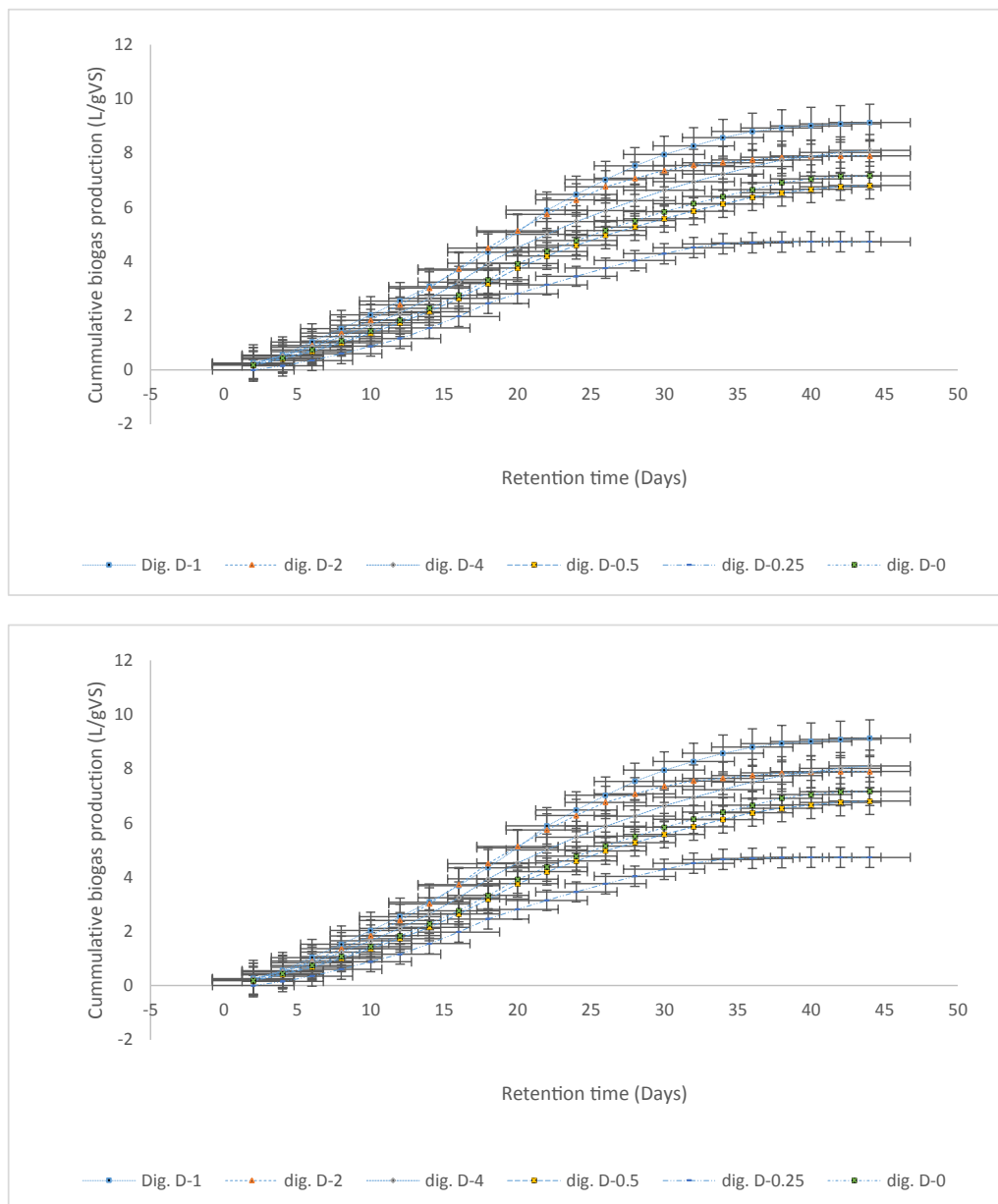


Fig. 3. Cumulative biogas yield at varied inoculum-substrate ratio.

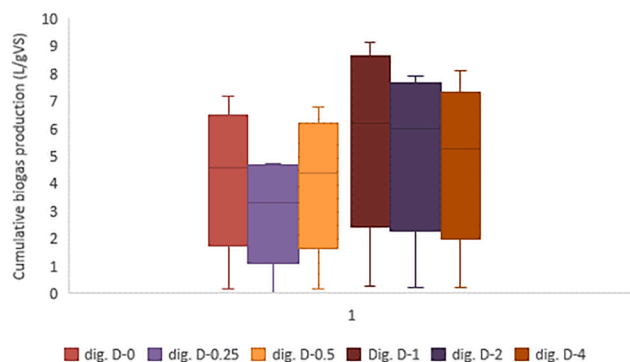


Fig. 4. Box Plot of Cumulative biogas yield at varied inoculum- substrate ratio.

$$A_t = A \exp \left\{ - \exp \left[ \frac{R_m \times e}{A} (\lambda - t) + 1 \right] \right\} \quad (1)$$

where  $t$  is time of biogas yield (days),  $A_t$  is cumulative biogas yield (L/gVS). The values of  $A$ ,  $R_m$  and  $\lambda$  were determined using the optimization tools of Microsoft Excel 2010 version.

## 2.5. Statistical analysis

One-way Analysis of Variance and the Fisher's LSD post hoc tests were used to compare the daily yield, cumulative yield and the maximum biogas potential from the respective inoculum-substrate ratios. The tests were conducted using STATA/SE statistical software version 13.1 at 95% confidence level.  $P$  value < 0.05 was considered statistically significant

**Table 2a**

ANOVA Test Results for comparison of daily biogas yield at varied inoculum/ substrate ratio.

Source of Variation	SS	df	MS	F	P-value	F crit	Remark
Treatment	0.517741	5	0.1035482	3.2873491	0.007981	2.2861844	Significant
Error	3.968874	126	0.031499				
Total	4.486615	131					

**Table 2b**

Fisher's LSD Post Hoc Test Results Matrix for comparison of daily biogas yield at varied inoculum/substrate ratio.

	Dig. D-0	Dig. D-0.25	Dig. D-0.5	Dig. D-1	Dig. D-2	Dig. D-4
Dig. D-0						
Dig. D-0.25	Not Significant					
Dig. D-0.5	Not Significant	Not Significant				
Dig. D-1	Significant	Not Significant	Not Significant			
Dig. D-2	Significant	Not Significant	Not Significant	Not Significant		
Dig. D-4	Significant	Significant	Significant	Significant	Not Significant	

**Table 3a**

ANOVA Test Results for comparison of cumulative biogas yield at varied inoculum/ substrate ratio.

Source of Variation	SS	df	MS	F	P-value	F crit	Remark
Treatment	94.412656	5	18.882531	2.8508056	0.017902	2.2861844	Difference is Significant
Error	834.57075	126	6.6235773				
Total	928.9834	131					

**Table 3b**

Fisher's LSD Post Hoc Test Results Matrix for comparison of cumulative biogas yield at varied inoculum/substrate ratio.

	Dig. D-0	Dig. D-0.25	Dig. D-0.5	Dig. D-1	Dig. D-2	Dig. D-4
Dig. D-0						
Dig. D-0.25	Not Significant					
Dig. D-0.5	Not Significant	Not Significant				
Dig. D-1	Significant	Not Significant	Not Significant			
Dig. D-2	Significant	Not Significant	Not Significant	Not Significant		
Dig. D-4	Significant	Significant	Significant	Not Significant	Significant	

**Table 4**

Performance of the digesters.

Digester	Inoculum-substrate ratio	Average methane content (%)	Peak biogas yield (L/ gVS)	Peak methane yield (L/ gVS)	Mean biogas yield (L/ gVS)	Standard deviation (SD)
D-0 (Control)	0	58.8	0.59	0.35	0.33	0.14
D-0.25	0.25	46.4	0.48	0.22	0.22	0.15
D-0.5	0.5	47.2	0.57	0.27	0.31	0.14
D-1	1	66.8	0.81	0.52	0.42	0.22
D-2	2	63.8	0.73	0.45	0.36	0.24
D-4	4	62.9	0.67	0.41	0.37	0.16

### 3. Results and discussion

#### 3.1. Performance of the digesters at different inoculum-substrate ratio

The combination of both FW and MH at ratio 3:1 helped adjust the C: N ratios as presented in Tables S1 and 1, which became fairly within the recommended range for optimum production of biogas (Owamah and Izinyon, 2015b). Co-digestion of the two substrates was also found to improve pH (Tables S1 & 1). Haider et al. (2015) observed an improvement in C/N ratio and pH through the co-digestion of food waste and rice husk. The results of the daily biogas yield at the different inoculum-substrate ratios (Fig. 1) reveal that the addition of appropriate inoculum dosage helped to activate microbial activities early in the digesters. There was a clear difference in the initial volume of biogas recorded on the first 6 days of digestion in digesters D-1, D-2 and D-4 when compared with digester D-0 with no inoculum (Figs. 1–4).

Digs. D-1, D-2 and D-4 with higher inoculum-substrate ratios exhibited better startup performances than Digs. D-0.25 and D-0.5 with lower inoculum-substrate ratios which is attributable to a consequent increase in active methanogen population occasioned by increased organic load. This increased organic load could have reduced the duration for the multiplication of the methanogens to the required population for biogas yield (Li et al., 2014; Zhu et al., 2014; Owamah et al., 2020). Raposo et al. (2009) reported that since the addition of inoculum to substrate enhances biogas production, ascertaining the optimum inoculum to substrate ratio is imperative for large scale anaerobic digestion operations. Furthermore, the results of the Analysis of Variance and the Fisher's Post Hoc test on both daily and cumulative biogas yield showed that the difference in yield from the respective inoculum ratios were statistically significant ( $p < 0.05$ ) (Tables 2a and b & Tables 3a and b).

From Figs. 1 and 2 and Table 4, the peak biogas yield of reactor D-1

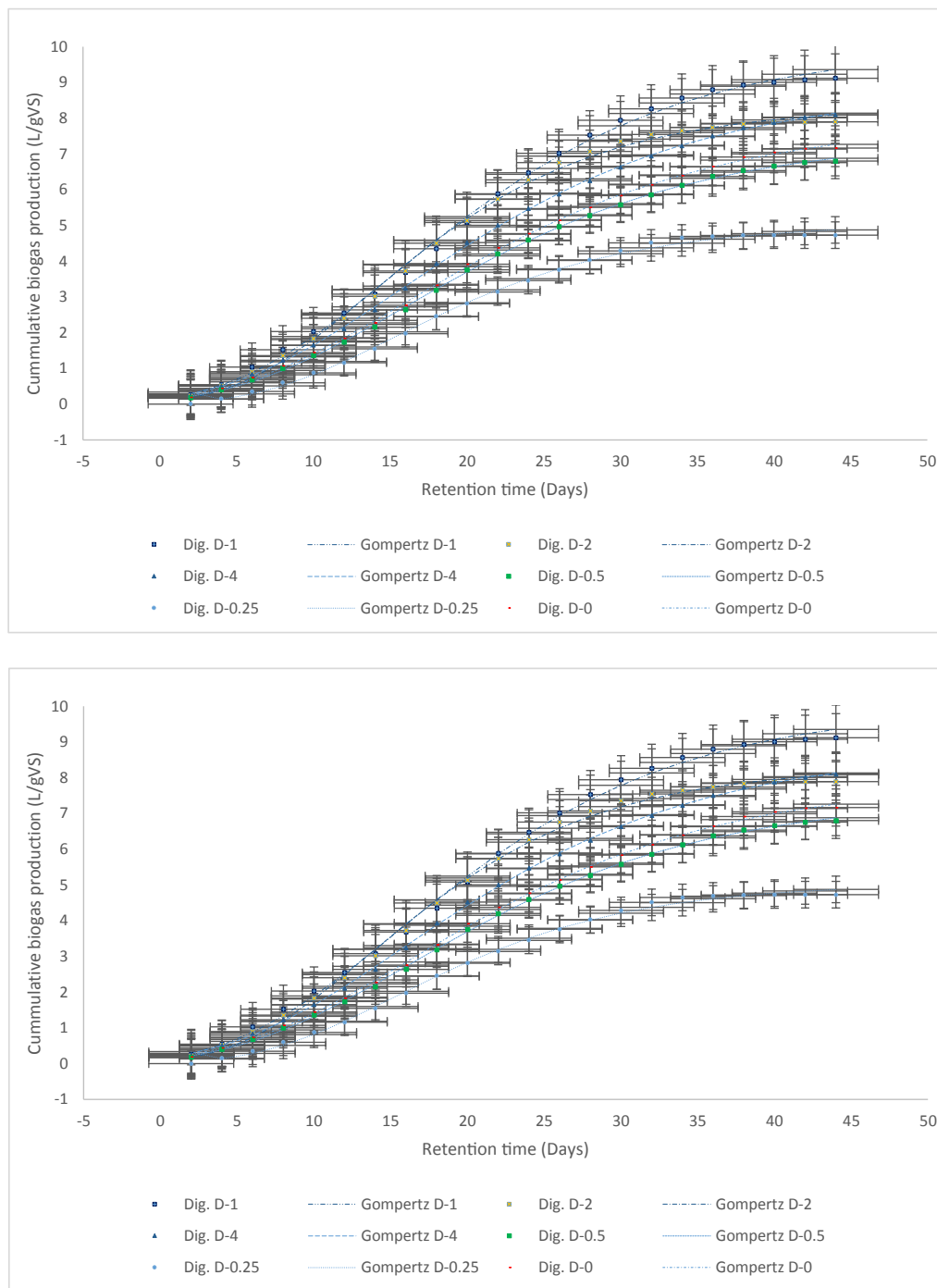


Fig. 5. Modified Gompertz model plots for the varied inoculum-substrate ratios.

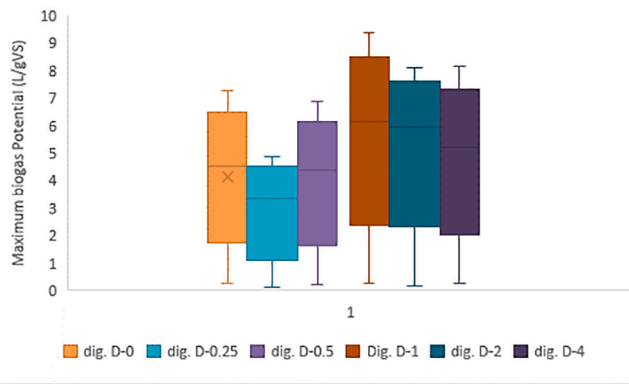
(0.81 L/gVS) was obtained on the 22nd day of digestion. Figs. 1 and 2 also show that the peak biogas yields of D-0.25, D-0.5, D-2, and D-4 are 0.334, 0.378, 0.478 and 0.432 L/gVS, respectively. These were respectively obtained on the 10th, 10th, 11th and 18th day of anaerobic digestion. Dig. D-0 with no inoculum (control experiment) recorded its maximum biogas yield (0.59 L/gVS) on the 20th day of digestion.

As observed, while Digs. D-1, D-2 and D-4 with higher inoculum/substrate ratios had better biogas yield than Dig. D-0 (control), such was not the case for Digs. D-0.5 and D-0.25 with lower inoculum/substrate ratios of 0.5 and 0.25, respectively. This implies that while the addition of inoculum boosts biogas production, attention must be paid to knowing and using the appropriate inoculum/substrate ratio for any particular substrate. Figs. 3 and 4 show that Digs. D-1 yielded the

highest cumulative biogas yield of 9.12 L/gVS. While Digs. D-0.25, D-0.5, D-2, and D-4 had respective cumulative biogas yields of 4.73, 6.80, 7.89 and 8.10 L/gVS, that of the control (Dig. D-0) was 7.17 L/gVS. The performance of Dig. D-0 was no surprise as food waste contains substantial amount of easily biodegradable matter; however, it is prone to early acidification. Furthermore, Figs. 3 and 4 show that the addition of appropriate amount of inoculum helped to increase biogas yield as revealed in Digs. D-1, D-2 and D-4.

Xu and Li (2012) reported through investigation into the influence of (I/S) ratio on methane content of biogas produced from the anaerobic co-digestion of expired dog food and corn stover, that there was a positive relationship between inoculum-substrate ratios and the maximum biogas production and methane percentage. Similarly, Raposo et al.





**Fig. 6.** Box Plot of the obtained maximum biogas potential at varied inoculum/substrate ratios.

**Table 5**  
Modified Gompertz model optimization result.

Parameter	Dig. D-0; I/S = 0	D-0.25; I/ S = 0.25	D-0.5; I/ S = 0.5	D-1; I/ S = 1	D-2; I/ S = 2	D-4; I/ S = 4
A	7.95	5.06	7.46	9.99	8.32	8.82
R <sub>m</sub>	0.25	0.21	0.25	0.37	0.36	0.29
λ	5.79	5.52	5.19	4.84	5.09	5.03

(2009) reported a positive outcome on the influence of inoculum/substrate ratio on the yield of biogas from municipal solid waste. In the same vein, the co-digestion of hay with soybean processing waste yielded a positive relationship with an increase in inoculum/substrate ratio (Zhu et al., 2014).

The mean biogas yields for Digs. D-0.25, D-0.5, D-1, D-2 and D-4 were obtained as  $0.22 \pm 0.15$ ,  $0.31 \pm 0.14$ ,  $0.42 \pm 0.22$ ,  $0.36 \pm 0.24$  and  $0.37 \pm 0.16$  L/gVS, respectively. A comparison of these yields with that of Dig. D-0 ( $0.33 \pm 0.14$  L/gVS) further demonstrates that beyond the ability of inoculum addition to improve biogas production, the ratio of the inoculum to substrate is equally important. The addition of inoculum demonstrated inhibitive characteristics at inoculum/substrate ratio of 0.25 and 0.5. This was revealed by their respective lower biogas yield compared to that of Dig. D-0 without inoculum (Dig. D-0). This inhibition could be attributed to the release of intermediate products that are not appropriate for the conversion by methanogenic bacteria to methane and inadequate methanogen population. Inoculum are substrates that have previously undergone anaerobic digestion thus are rich in microbial population but with less methane yield. The biogas production comes primarily from the substrates.

**Table 6a**  
ANOVA Test Results for comparison of maximum biogas potential at varied inoculum/substrate ratio.

Source of Variation	SS	df	MS	F	P-value	F crit	Remark
Treatment	93.027531	5	18.605506	2.7998274	0.0196612	2.2861844	Difference is Significant
Error	837.2994	126	6.6452333				
Total	930.32693	131					

**Table 6b**  
Fisher's LSD Post Hoc Test Results Matrix for comparison of maximum biogas potential at varied inoculum/substrate ratio.

	Dig. D-0	Dig. D-0.25	Dig. D-0.5	Dig. D-1	Dig. D-2	Dig. D-4
Dig. D-0						
Dig. D-0.25	Not Significant					
Dig. D-0.5	Not Significant	Not Significant				
Dig. D-1	Significant	Not Significant	Not Significant			
Dig. D-2	Significant	Not Significant	Not Significant	Not Significant		
Dig. D-4	Significant	Significant	Significant	Not Significant	Significant	

The higher, the inoculum content above an optimum, the lower the substrate content and the lower the methane yield. This notwithstanding, better yields were obtained as inoculum/substrate ratio increased beyond 0.5 with best performance obtained at inoculum/substrate ratio 1. The availability of sufficient active methanogens could also be a possible explanation for the improved biogas yield at D-1, D-2 and D-4 (Boulanger et al., 2012). However, slight decrease in biogas yield was observed as inoculum/substrate ratio increased beyond 1, though the yields were better than that of D-0. A possible explanation for this may be characteristic changes of the substrate surface which during hydrolysis affects the bioavailability of digestible materials.

Parawira et al. (2004) had previously reported that the addition of inoculum increased the yield of biogas from solid potato waste. A similar study on the influence of inoculum/substrate ratio on biogas yield from co-digestion of maize and straw demonstrated that there was a significant decrease in the biogas yield at inoculum-substrate ratio < 0.25 gVM per gVM of the substrate (Raposo et al., 2009). The reason for an improvement in biogas yield at inoculum/substrate ratio > 0.2 was reported by Neves et al. (2004), who investigated granular inoculum on kitchen waste. It was concluded that the acidification of the digestion process was overcome at inoculum/substrate ratios between 0.2 and 4, which eventually, led to improved biogas yield. Also, Boulanger et al. (2012) reported the best production of biogas from municipal solid waste occurred at inoculum/substrate ratio 2. In this study, biogas yield increased with increase inoculum/substrate ratio and decreased slightly after inoculum-substrate ratio 1.

Moreover, Digs. D-0.25 and D-0.5 exhibited the least content of methane and recorded 46.4 and 47.2 %, respectively. Digs. D-1, D-2, and D-4 had higher percentage methane of 66.8, 63.8, and 62.9 % with inoculum/substrate ratio of 1, 2, and 4, respectively (Table 4). The reasons for an increase in the biogas yield with increasing inoculum/substrate ratio, as earlier adduced in this paper, could also be responsible for the higher methane content. Therefore, findings from this study are in agreement with the previous study in which FW and rice husk (RH) were co-digested at S/I ratios 0.25, 0.5, 1.0, 1.5, and 2.0 and yielded specific biogas of 557, 458, 267, 97, and 71 L/kg VS, respectively (Haider et al., 2015). The study further showed that specific biogas yield at S/I 0.25 (I/S = 4) was significantly higher ( $p < 0.05$ ) than what was obtained in other digesters except the digester with S/I ratio 0.5 (I/S = 2). Haider et al. (2015) also reported a positive influence on anaerobic co-digestion of FW and RH at substantial content of inoculum. In the same vein, Elbeshbishy et al. (2012) reported that the rate of biodegradation, lag time and chances of degradation of substrates depended on the concentration of microorganisms in a digester. The values of specific methane production obtained in digester D-1, D-2 and D-4 as shown in Tables 2 are in line with documented values in literature (Marañón et al., 2012).

**Table 7**

Present study compared with previous studies.

S/ N	Substrate	Mean biogas yield (mL/gVS)	Temperature /duration of digestion	Methane content	Digestion mode	Inoculum used	Reference
1	FW;80 g VS/L. day	448.9 ± 6.6	37 °C/60 days	61.1 ± 0.8	Semi- continuous	Seed Sludge	Zhang et al. (2020)
2	FW; 80 g VS/L. day + Trace Metal	484.6 ± 32.6	37 °C/60 days	64.2 ± 1.5	Semi- continuous	Seed Sludge	Zhang et al. (2020)
3	75% FW + 25% SS, 80 g VS/L	413.4 ± 29.3	37 °C/60 days	64.8 ± 2.0	Semi- continuous	Seed Sludge	Zhang et al. (2020)
4	75% FW + 25%SS, 80 g VS/L	413.4 ± 29.3	37 °C/60 days	64.8 ± 2.0	Semi- continuous	Seed Sludge	Zhang et al. (2020)
5	75% YW + 25% FW, 80 g VS/L	165.4 ± 15.6	37 °C/60 days	61.0 ± 2.0	Semi- continuous	Seed Sludge	Zhang et al. (2020)
6	56.3% YW + 18.7% FW + 25% SS, 80 g VS/L	164.7 ± 22.7	37 °C/60 days	61.3 ± 1.5	Semi- continuous	Seed Sludge	Zhang et al. (2020)
7	50% YW + 50%FW, 80 g VS/L	296.0 ± 19.9	37 °C/60 days	63.5 ± 1.3	Semi- continuous	Seed Sludge	Zhang et al. (2020)
8	37.5% YW + 37.5% FW + 25% SS, 80 g VS/L	232.4 ± 46.7	37 °C/60 days	63.9 ± 2.1	Semi- continuous	Seed Sludge	Zhang et al. (2020)
9	25% YW + 75%FW, 80 g VS/L	360.0 ± 30.2	37 °C/60 days	64.1 ± 1.5	Semi- continuous	Seed Sludge	Zhang et al. (2020)
10	18.7% YW + 56.3% FW + 25% SS, 80 g VS/L	314.9 ± 17.1	37 °C/60 days	64.4 ± 1.7	Semi- continuous	Seed Sludge	Zhang et al. (2020)
11	FW; Phase I (0–33 days)	387 ± 129	37 °C/60 days	Not stated	Semi- continuous	Anaerobic sludge collected from an anaerobic digester in the lab treating food waste	Shi et al. (2020)
12	FW; Phase II)	546 ± 52	35 ± 1 °C/(34–60 days	73.7 ± 2.6%	Semi- continuous	Seed Sludge	Shi et al. (2020)
13	FW; Phase III	531 ± 28	35 ± 1 °C/(61–92 days)	69.3 ± 2.6%	Semi- continuous	Seed Sludge	Shi et al. (2020)
14	FW; Phase IV	161 ± 119	35 ± 1 °C/(93–101 days)	Not stated		Seed Sludge	Shi et al. (2020)
15	Catering waste and treated parthenium biomass	559 ml L <sup>-1</sup> d <sup>-1</sup> ),	30 °C/60 days	Not stated	Semi- continuous	Cattle manure	Tayyab et al. (2019)
16	Catering waste and untreated parthenium biomass	386 ml L <sup>-1</sup> d <sup>-1</sup>	30 °C/60 days	Not stated	Semi- continuous	Cattle manure	Tayyab et al. (2019)
17	Food Waste and Switchgrass	205 to 345 ml/g VSadded	35 °C/30 days	50–80%	Batch	Anaerobic sludge	Uma et al. (2020)
18	Food Waste and Switchgrass	180 to 296 ml/g VSadded	55 °C/18 days	50–80%	Batch	Anaerobic sludge	Uma et al. (2020)
19	FW	520 L/kgVS (cumulative)	35 ± 1 °C /20 days	50–80%	Batch	Seed bacterial culture	El-Mashad & Zhang (2010)
20	FW	657 L/kgVS (cumulative)	35 ± 1 °C /30 days	53.7% (Average)	Batch	Seed bacterial culture	El-Mashad & Zhang (2010)
21	(32% of food waste + 68% manure)	411	35 ± 1 °C /20 days	53.7% (Average)	Batch	Seed bacterial culture	El-Mashad & Zhang (2010)
22	(32% of food waste + 68% manure)	455	35 ± 1 °C /30 days	53.7% (Average)	Batch	Seed bacterial culture	El-Mashad & Zhang (2010)
23	(48% of food waste + 52% manure)	504	35 ± 1 °C /20 days	53.7% (Average)	Batch	Seed bacterial culture	El-Mashad & Zhang (2010)
24	(48% of food waste + 52% manure)	531	35 ± 1 °C /30 days	53.7% (Average)	Batch	Seed bacterial culture	El-Mashad & Zhang (2010)
25	Food waste	445 ml/gVS added (cumulative)	50 ± 2 °C/28 days	73% (Average)	Batch	Anaerobic Sludge	Zhang et al. (2007)
26	FW at F/1 = 4	784 ml/gVS	50 ± 2 °C/28 days	66.1	Batch	Microbial Inoculum	Liu et al. (2009)
27	FW and <i>Phyllanthus emblica</i>	0.44 (L/g VS added)	25–30 °C/ at HRT 40 days	53.75	Semi-batch	Anaerobic mesophilic sludge	Panyadee et al. (2013)
28	FW	0.34 (L/g VS added)	25–30 °C/ at HRT 40 days	54.63	Semi-batch	Anaerobic mesophilic sludge	Panyadee et al. (2013)
29	<i>Phyllanthus emblica</i>	0.31 (L/g VS added)	25–30 °C/ at HRT 40 days	34.77	Semi-batch	Anaerobic mesophilic sludge	Panyadee et al. (2013)
30	FW and <i>Phyllanthus emblica</i>	0.39 (L/g VS added)	25–30 °C/ at HRT 30 days	54.52	Semi-batch	Anaerobic mesophilic sludge	Panyadee et al. (2013)
31	FW	0.31(L/g VS added)	25–30 °C/ at HRT 30 days	54.98	Semi-batch	Anaerobic mesophilic sludge	Panyadee et al. (2013)
32	Emblica	0.33 (L/g VS added)	25–30 °C/ at HRT 30 days	40.90	Semi-batch	Anaerobic mesophilic sludge	Panyadee et al. (2013)
33	FW and <i>Phyllanthus emblica</i>	0.30 (L/g VS added)	25–30 °C/ at HRT 20 days	53.78	Semi-batch	Anaerobic mesophilic sludge	Panyadee et al. (2013)
34	FW	0.30 (L/g VS added)	25–30 °C/ at HRT 20 days	54.68	Semi-batch	Anaerobic mesophilic sludge	Panyadee et al. (2013)
35	<i>Phyllanthus emblica</i>	0.29 (L/g VS added)		46.20	Semi-batch	Anaerobic mesophilic sludge	

(continued on next page)



Table 7 (continued)

S/ N	Substrate	Mean biogas yield (mL/gVS)	Temperature /duration of digestion	Methane content	Digestion mode	Inoculum used	Reference
36	Food waste and rice husk	Highest specific biogas yield of 584 L/kg VS at S/I ratio 0.25	25–30 °C/ at HRT 20 days 37 ± 1 °C/30 days	Not stated	Batch	Fresh dung	Panyadee et al. (2013) Haider et al. (2015)
37	75% FW and 25%Maize Husk	Peak daily 0.81 L/gVS; Average yield 0.42L/gVS at Inoculum/ Substrate ratio1	37 ± 1 °C/44 days	66.8	Batch	Anaerobic mesophilic effluent	This study

### 3.2. Modified Gompertz modelling of experimental data

#### 3.2.1. Effect of inoculum/substrate ratio on the maximum biogas production potential (A)

Fig. 5 shows the plots of the modified Gompertz model simulation of experimental data from digesters D-0 to D-4 while Fig. 6 presents the box plot of the maximum biogas production potential estimated using the modified Gompertz model. Table 5 shows the summary of the predicted kinetic parameters from the simulation curves.

The results presented in Table 5 reveal that inoculum/substrate ratio varied negatively with the maximum biogas production potential (A). In other words, an increase in inoculum/substrate ratio above the optimum, resulted in a corresponding decrease in the value of (A). There was however a slight decrease after inoculum/substrate ratio 1 to 2 and 4. Contrary to the expectations that the value of A and inoculum/substrate ratio should increase with increase in the amount and activity of methanogens, the value of A for ratios greater than 1 was surprisingly not as high as that of 1, underpinning the importance of determining the appropriate inoculum/substrate ratio before the establishment of large scale biogas plants.

The slight decrease in (A) at inoculum/substrate ratio greater than 1 is attributable to reduction in substrate quantity available for biodegradation since the quantity of inoculum was now greater than the substrates. According to Boulanger et al. (2012), it could be that the amount of active methanogens was gradually outweighing the available substrates for biodegradation. Dig. D-0 (control) had an (A) value of 7.95 L/gVS while Dig. D-0.25 and D-0.5 with lower inoculum-substrate ratios of 0.25 and 0.5, respectively, recorded (A) values of 5.06 L/gVS and 7.46 L/gVS, respectively. The highest value of A (9.99 L/gVS) was however obtained at inoculum-substrate ratio of 1. Although a slight decline was recorded at inoculum/substrate ratio of 2 and 4 as explained earlier, the maximum biogas potentials of 8.32 L/gVs and 8.82 L/gVs where still higher than the values of lower inoculum/substrate ratios. Compared with the control, D-1 with inoculum/substrate ratio of 1 produced the most significant maximum biogas production enhancement of 20.4 %. While the Analysis of Variance results for the maximum biogas potential (Table 6a) shows that the difference for the respective ratios is statistically significant, the post hoc test (Table 6b) showed that only ratios, 1, 2 and 3 were significantly different from that of D-0. However, the difference is more significant between the various ratios which buttresses our previous submissions. While D-1 was significantly higher compared to D-0, D-2 and D-4 were significantly lower.

#### 3.2.2. Effect of inoculum/substrate ratio on lag phase ( $\lambda$ )

From Tables 3, inoculum/substrate ratio showed a negative relationship with lag phase ( $\lambda$ ). As inoculum/substrate ratio increased,  $\lambda$  decreased. The highest  $\lambda$  of 5.79 days was recorded in Dig. D-0 (control) which had no inoculum. It was closely followed by  $\lambda$  of 5.52 days in Dig. D-0.25 with inoculum-substrate ratio of 0.25. The least  $\lambda$  of 4.84 days was recorded at inoculum-substrate ratio 1 (Dig. D-1). Lag phase ( $\lambda$ ) of 5.09 and 5.03 days was obtained from D-2 and D-4 with inoculum-substrate ratios of 2 and 4, respectively. A decrease in  $\lambda$  of 16.4% was obtained at inoculum/substrate ratio 1 in comparison with the performance of Dig. D-0, signifying a 16.4% reduction in the time taken for

microbial acclimatization. Lag phase ( $\lambda$ ) was also found to decrease with increase in inoculum/substrate ratio but for D-2 and D-4 (Tables 3). The increase in the population of active methanogens could be associated with a reduction in the lag phase with increase inoculum/substrate ratio (Boulanger et al., 2012).

#### 3.2.3. Influence of inoculum-substrate ratio on the maximum specific biogas production ( $R_m$ )

Tables 3 shows that inoculum/substrate ratio affected maximum specific biogas production ( $R_m$ ). Dig. D-0 (control) had  $R_m$  of 0.25 L/gVS/day. Dig. D-0.25 with inoculum-substrate ratio of 0.25 had  $R_m$  of 0.21 L/gVS/day showing a decrease in maximum specific biogas yield. Also, no noticeable change in  $R_m$  with increase in inoculum/substrate ratio to 0.5 as compared with the control was obtained. However, with an increase in inoculum/substrate ratio to 1, as obtained in Dig. D-1, a huge rise (32.4%) in  $R_m$  i.e. from 0.25 to 0.37 L/gVs/day was obtained. Improvement in  $R_m$  was also obtained at inoculum/substrate ratio of 2 and 4 when compared with the control experiment. The design of large-scale anaerobic plants requires the determination of  $R_m$  values of substrates to be digested. Moreover, the  $R_m$  values increased with increasing maximum biogas production potential (A), indicating an increase in the rate of attainment of substrate degradation. Furthermore, at inoculum/substrate ratio of 1, maximal rate of biogas yield and substrate consumption was obtained.

Digs. D-0.25 and D-0.5 with high lag phase ( $\lambda$ ), low ( $R_m$ ) and (A) values (Tables 3) could be categorized as failed or inhibited digesters. Inoculum/substrate ratios of 0.25 or 0.5 inhibited methanogenesis possibly as a result of reduced bioavailability and poor hydrolysis (Hashimoto, 1989). Considering the complex nature of the energy-economic considerations as well as growing global interest in the application of biogas for real life benefits, the authors hope to investigate the energy returned on energy invested in further studies. The comparison of the results of this present study with previous ones (Table 7) shows that the results of this study followed the trend of previous reports. Apart from the study of Zhang et al. (2007) in which methane content of over 73% was obtained, the purity of biogas obtained in the study is higher than most in the literature.

### 4. Conclusion

Buttressing the earlier reports that the addition of inoculum could enhance biogas production from various substrates, this study has demonstrated that inoculum/substrate ratio of 1 could greatly enhance the yield and methane content of biogas produced from the anaerobic co-digestion of FW and MH. Biogas yield was found to vary with inoculum/substrate ratios. While D-1, D-2 and D-4 experienced increased biogas yield with respect to D-0; D-0.25 and D-0.5 showed inhibitive tendencies. The hugest increase in biogas yield was observed at inoculum/substrate ratio 1. Furthermore, the inoculum/substrate ratio varied inversely with the lag phase for microbial acclimatization. Lower inoculum/substrate ratios of 0.25 and 0.5 had longer lag phase of 6.52 and 4.99 days respectively, while that of 1 was 4.84 days. The inoculum/substrate ratio of 1 also produced the highest maximum biogas production potential (A) and maximum specific biogas production rate

( $R_m$ ).

## 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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