

Effect of inoculum to substrate ratio on biogas production of sheep paunch manure

A.A. LAWAL, A.U. DZIVAMA, M.K. WASINDA

Department of Agricultural and Environmental Resources Engineering, Faculty of Engineering, University of Maiduguri, Maiduguri, Nigeria

Abstract

LAWAL A.A., DZIVAMA A.U., WASINDA M.K. (2016): **Effect of inoculum to substrate ratio on biogas production of sheep paunch manure.** Res. Agr. Eng., 62: 8–14.

Sheep paunch manure was anaerobically digested to study the effect of inoculum to substrate ratio on biogas production rates and accumulation. Inoculum to substrate ratios of 1.37, 2.05 and 4.1 were digested in biodigesters labelled R1, R2 and R3 respectively. Results showed that inoculum to substrate ratio had a significant effect on biogas production rates and accumulation. Biogas production rates increased to peak in the order of R3 (0.30526 Nm³/kg volatile solids (VS) days), R2 (0.15308 Nm³/kg VS d) and R1 (0.11009 Nm³/kg VS d) on the 5th day. The biogas production accumulation increased from 0.57195 to 1.46784 Nm³/kg VS as the inoculum to substrate ratio increased. The result of regression showed that coefficient of determination values for the linear equation ranged from 0.707 to 0.797, while the exponential equation had higher values that ranged from 0.7718 to 0.9929 showing better simulation. The modified Gompertz equation showed better simulation of the biogas production accumulation than the first order kinetic equation due to its higher coefficient of determination values.

Keywords: volatile solids; biogas production rate; biogas accumulation; modified Gompertz; first order kinetic, simulation

Biomass is one of the renewable sources of bio-energy capable of significant contribution to the global future energy supply (WEC 2004). Bioenergy generated from diverse sources provides local energy needs, reduces dependence on fossil fuel and help mitigate greenhouse gas effect. The main concern limiting bioenergy utilisation is its relative inefficient conversion technologies. However, anaerobic digestion of residue/waste materials from agricultural crops, animal production and agro-processing industries could be a simple, efficient and low cost conversion technology.

Anaerobic digestion occurs when organic material is converted biologically in the absence of oxygen to gaseous product called biogas (ANGELIDAKI 2002). Studies on anaerobic digestion process of

several organic residues/wastes have led to the understanding of their inherent conversion potentials and kinetics that resulted in the design, development, process control and optimisation of biodigesters. But to fully utilise biogas potentials from biomass materials, biogas production behaviour and potentials of more organic residue/waste materials are needed to be determined.

Several researches have been conducted to determine biogas potential of different organic substrates. BUSWELL and MUELLER (1952), BASERGA (1998) and RAPOSO et al. (2011) proposed an empirical relationship that utilises the elemental or organic chemical compositions of biomass to estimate its theoretical maximum biogas yield. HANSEN et al. (2004), WYMYSLOWSKI et al. (2010), FENG

et al. (2013), MONCH-TEGEDER et al. (2013) and ZHANG et al. (2013) investigated the biochemical methane potential of solid organic waste, poultry slaughterhouse waste, vinegar residue, horse manure and goat manure with crop residue using batch method, respectively. They determined the physicochemical compositions of substrate and developed an anaerobic assay for the biogas production. CHYNOWETH et al. (1993), LABATUT et al. (2008), FENG et al. (2013), KHEIRELINE et al. (2014) also investigated the influence of inoculum to substrate (I/S) ratio on biogas production by varying the amount of substrate added to inoculum. Effects of temperature and pH were also investigated (HASHIMOTO et al. 1981). Simulation studies on biogas production and conversion kinetics for gelatin solid waste, co-digested horse and cow dung, water hyacinth and vinegar residue were carried out by RAGHUNATHAN et al. (2008), YUSUF et al. (2011), ADIGA et al. (2012) and FENG et al. (2013), respectively, and their results showed that different substrates have different potentials and conversion kinetics. The objectives of this study were to evaluate the effect of inoculum to substrate ratio on biogas production of sheep paunch manure (SPM) under mesophilic conditions and to simulate the biogas production rates and accumulation.

MATERIAL AND METHODS

Sample collection, conditioning and characterisation. *Inoculum.* Two kilograms of fresh cow dung sample was collected at the Animal Farm, University of Maiduguri, Nigeria and taken to the Agricultural and Environmental Resources laboratory of the same institution for experiment. To adapt the inoculum to mesophilic conditions, 200 g of the cow dung was diluted in distilled water to 10% dry matter (d.m.) and transferred into a 4 l glass bottle. The headspace of the 4 l glass bottle was flushed with a gas mixture of 80% N₂ and 20% CO₂ and closed with a thick rubber septum which was held tight by a resin. The inoculum solution was then incubated in a water bath at 35 ± 1°C. During incubation, the inoculum solution was degassed completely by allowing gas build-up in the headspace to escape via a valve controlled tube.

Substrate. Two kilograms of fresh SPM was collected at an Animal Slaughter House, Maiduguri, Nigeria. The sample collected was stored over ice and

delivered to the laboratory for experiment and analysis. Three sub-samples of 100 g of SPM were collected and diluted to 15%, 10% and 5% d.m. and transferred separately into 2 l glass bottles and stored at 5°C.

Nutrient medium. A nutrient medium containing the following nutrients and vitamins was prepared: (a) NaCl, MgCl₂·6H₂O, CaCl₂·2H₂O; (b) FeCl₂·4H₂O, ZnCl₂, MnCl₂·4H₂O, (NH₄)₆Mo₇O₂₄; (c) folic acid and riboflavin.

Stock solutions were prepared based on the recommendation of ANGELIDAKI (2002). This involves dissolving certain quantities of the chemicals in group (a), (b) and (c) separately in 1 l of distilled water. Samples of 10, 1 and 1 ml were collected from stock solutions (a), (b) and (c), respectively, and then added to 988 ml distilled water to obtain a nutrient medium used for the experiment.

Physicochemical composition analysis. Fresh samples of inoculum and SPM were analysed for total solids (TS) and volatile solids (VS) content according to the standard method of the American Public Health Association (APHA 1992). TS was determined by oven drying the sample at 95°C until there was no change in weight. The TS was further oven dried for 1 h at 550°C to determine the proportion of organic matter (VS) lost in the dried sample. The proportions of carbohydrate, crude protein, crude fat, crude fibre and ash content of SPM substrate were determined by proximate analysis at the Soil Science Laboratory, University of Maiduguri, Nigeria.

Batch digestion test. The biogas unit consisted of the following:

- 200 ml glass bottle and a thick rubber septum with a flexible rubber tube fixed on the rubber septum through an opening used as biodigester,
- a thermostatically controlled water bath with a plastic rack used for agitating and keeping biodigesters in place,
- 100 and 10 ml plastic syringes and gas pressure gauge,
- 80% N₂ and 20% CO₂ gas mixture.

Experimental procedure. Sixty millilitres (5.91 g VS) of the degassed inoculum was collected after it was shaken and transferred into a biodigester unit using the 100 ml plastic syringe. Thirty millilitres of the solution of SPM substrate and 1 ml of the nutrient medium were collected and added to the inoculum in the biodigester unit. The combination of the inoculum, substrate and nutrient medium was prepared for the 15% (4.32 g VS), 10% (2.88 g VS) and 5% (1.44 g VS) concentrations of SPM substrate

doi: 10.17221/30/2014-RAE

in bioreactors labelled R1, R2 and R3. These contained inoculum to substrate ratios of 1.37, 2.05 and 4.1, respectively, while the control bioreactor contained only inoculum. The bioreactors were flushed with the 80% N₂ and 20% CO₂ gas mixture and transferred into the water bath at 35 ± 1 °C. The entire bioreactor units were agitated for 5 min twice a day. The biogas produced was measured using the gas pressure gauge twice daily at the initial stage and once daily toward the final stage of the process until no more biogas was produced. After every measurement, biogas accumulated was allowed to escape in order to avoid pressure build up that would exceed the pressure gauge capacity. This experiment was repeated 3 times and the average was calculated and recorded as the average biogas produced.

Simulation of biogas production rate and accumulation. The biogas production rates of SPM were simulated using linear and exponential plots. Eq. (1) was used for linear simulation under the assumption that biogas production rate increased linearly to its peak and then decreased linearly to zero. The exponential equation (Eq. 2) was also used to simulate increasing and decreasing stages of biogas production rate under the assumption that production rate increased exponentially to its peak and then decreased exponentially.

$$B_l = a_1 + b_1 \times t \quad (1)$$

$$B_e = a_e + b_e \times \exp(c_e t) \quad (2)$$

where:

B_l, B_e – biogas production rates for linear and exponential estimation (Nm³/kg VSd) at time (day), respectively

t – time of digestion period (day)

a_1 – intercept (Nm³/kg VSd)

b_1 – slope (Nm³/kg VSd²)

a_e – b_e are constants (Nm³/kg VSd)

c_e – constant (d⁻¹) and is negative for decreasing stage

Finally, the biogas production accumulation was simulated using first order kinetic and modified Gompertz equations (Eq. 3 and 4):

$$B_f = B_{Of} (1 - \exp(-k \times t)) \quad (3)$$

$$B_G = B_{OG} \times \exp\{-\exp[(\mu_m \times e/B_{OG})(\lambda - t) + 1]\} \quad (4)$$

where:

B_f, B_G – biogas production accumulation for first order kinetic and modified Gompertz equations, respectively, at time (day)

B_{Of}, B_{OG} – max. biogas production for first order kinetic and modified Gompertz equations, respectively (Nm³/kg VS)

k – first order kinetic constant

μ_m – max. biogas production rate (Nm³/kg VSd)

λ – duration of lag phase

e – constant that equals to 2.718282

Statistical analysis. Simple descriptive statistical analysis was used to report averages and standard deviations of the experimental data. ANOVA test was used to detect if there was a significant difference of biogas production rate and accumulation due to the effect of I/S ratio. Microsoft Excel software was used to determine equation parameters and to plot graphs.

RESULTS AND DISCUSSION

Characteristics of SPM and inoculum

The results of physicochemical composition analysis of SPM and inoculum are presented in Table 1. The ash content of SPM was 4.3% which resulted in a high VS/TS ratio of 93.7%. This ratio indicates that SPM could be a suitable substrate for anaerobic digestion. The pH level of SPM was within anaerobic digestion range of 6 to 8.3.

Table 1. Physicochemical compositions of SPM and inoculum

	Moisture content (%)	TS (% w.b.)	VS (% w.b.)	VS/TS ratio	pH	Carbohydrate (% TS)	Crude protein (% TS)	Crude fat (% TS)	Crude fiber (% TS)	Ash (% TS)
SPM	82.4 (2.43)	17.4 (1.76)	16.3 (2.44)	0.937	7.8 (0.518)	36 (2.91)	9.5 (0.97)	1.1 (0.44)	46 (1.85)	4.3 (0.84)
Inoculum	24.04 (3.13)	75.9 (3.51)	67.6 (4.67)	0.89	ND	ND	ND	ND	ND	ND

numbers in parentheses are standard deviations; ND – not determined; VS – volatile solids; TS – total solids

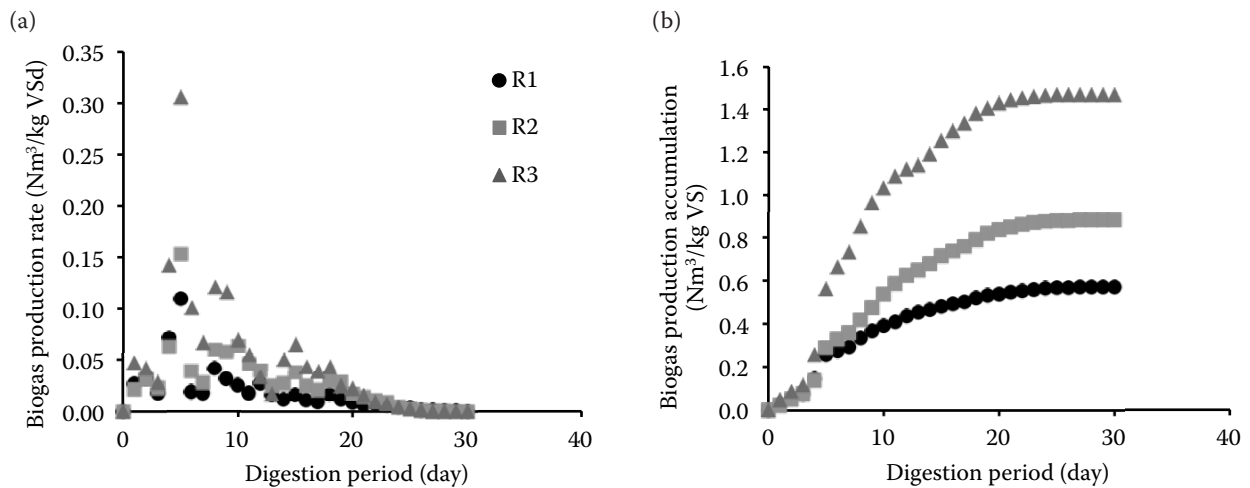


Fig. 1. Experimental data plots of biogas production (a) rate and (b) accumulation in R1, R2 and R3 biodigesters VS – volatile solids; VSd – volatile solids/day; R1, R2, R3 – biodigesters

Biogas production rate and accumulation

The result showed no biogas produced from the controlled biodigester over the entire digestion period. Fig. 1 (a and b) show biogas production rates and accumulation plots of SPM over the digestion period, respectively. It can be seen from Fig. 1a that R1, R2 and R3 biodigesters needed 30, 29 and 27 days to complete digestion, respectively. It appeared that as I/S ratio increased, digestion period decreased and biogas production rates increased. It can also be observed that curves of biogas production rates for R1, R2 and R3 biodigesters exhibited similar pattern over the entire digestion

period; R3 biodigester exhibited the max. biogas production rates followed by R2 and R1 biodigesters (Fig. 1b). The peak (maximum) biogas production rate occurred on the 5th day for all biodigesters in the order of R3 (0.30526 $\text{Nm}^3/\text{kg VS days}$), R2 (0.15308 $\text{Nm}^3/\text{kg VS days}$) and R1 (0.11009 $\text{Nm}^3/\text{kg VS days}$). ANOVA showed that inoculum:substrate (I/S) ratio had a significant effect ($P < 0.0372$) on biogas production rates.

The total accumulated biogas produced was found to be in the order of 1.46784, 0.88177 and 0.57195 $\text{Nm}^3/\text{kg VS}$ in R3, R2 and R1 biodigesters, respectively (Fig. 1b). RAGHUNATHAN et al. (2008) reported similar biogas production accumulation

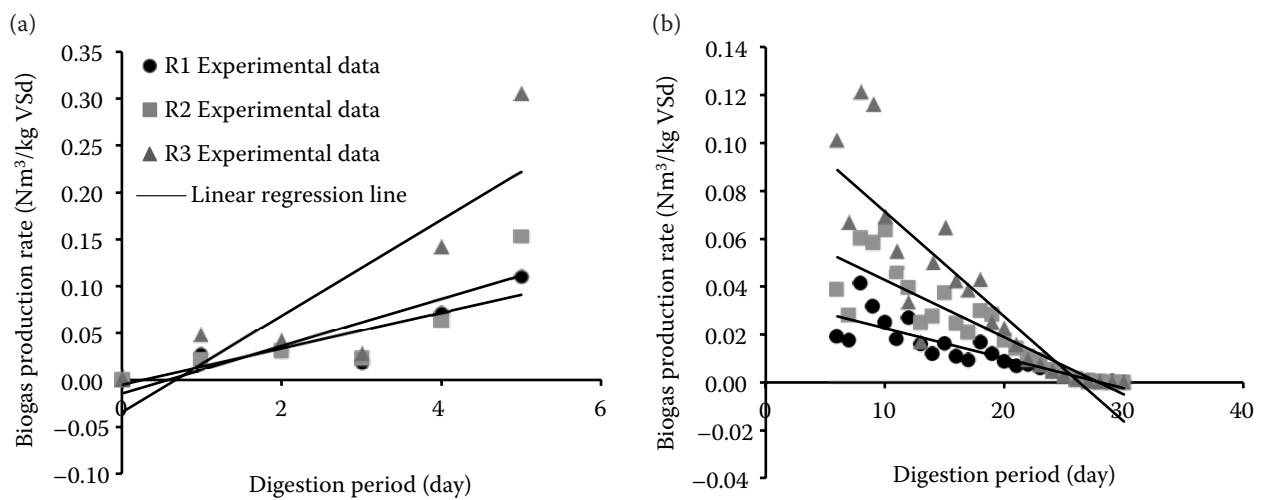


Fig. 2. Linear regression plots fitted in to experimental data plots of biogas production rates of (a) rising limb and (b) falling limb in R1, R2 and R3 biodigesters

VSd – volatile solids/day; R1, R2, R3 – biodigesters

doi: 10.17221/30/2014-RAE

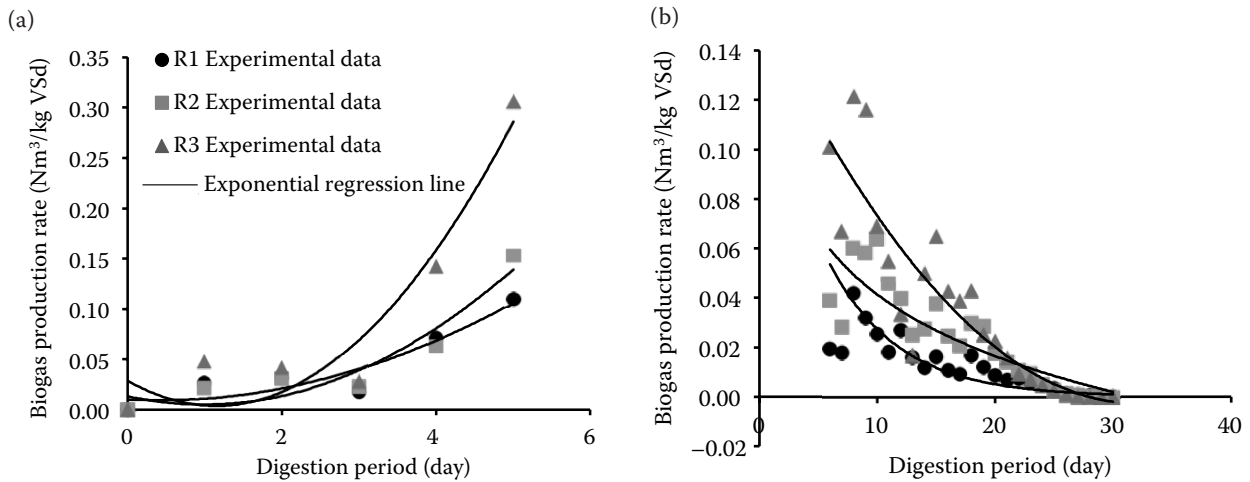


Fig. 3. Exponential regression plots fitted into experimental data plots of biogas production rates of (a) rising limb and (b) of falling limb in R1, R2 and R3 biodigesters
VSd – volatile solids/day; R1, R2, R3 – biodigesters

values (1.1 to 0.382 Nm³/kg VS) for gelatin solid waste. Cumulative analysis of the results showed that 80% of biogas produced in biodigesters R1, R2 and R3 accumulated on the 14th, 15th and 14th day of digestion period, respectively.

Fig. 2 shows linear regression lines fitted to rising and falling limbs plots of the experimental data of biogas production rates in R1, R2 and R3 biodigesters, respectively. Eqs. (5) to (10) present the linear relationship between biogas production rates and digestion period:

$$R1_A: B_1 = 0.019t - 0.005, R^2 = 0.784 \quad (5)$$

$$R2_A: B_1 = 0.025t - 0.014, R^2 = 0.731 \quad (6)$$

$$R3_A: B_1 = 0.051t - 0.034, R^2 = 0.707 \quad (7)$$

$$R1_D: B_1 = 0.001t - 0.035, R^2 = 0.757 \quad (8)$$

$$R2_D: B_1 = 0.002t - 0.066, R^2 = 0.797 \quad (9)$$

$$R3_D: B_1 = 0.004t - 0.115, R^2 = 0.766 \quad (10)$$

where:

A, D – subscripts represent rising and falling limbs

B₁ – biogas production rates for linear estimation (Nm³/kg VSd)

t – time of digestion period (days)

The coefficient of determination (*R*²) values of falling limbs showed better linear simulation than rising limbs except for R1 biodigester. Exponential regression lines simulating biogas production rates for both limbs are presented in Fig. 3. The following

equations (Eqs. 11 to 16) express the exponential relationship between biogas production rates and digestion period:

$$R1_A: B_e = 0.00533 + 0.00491\exp(0.6144t), R^2 = 0.9065 \quad (11)$$

$$R2_A: B_e = 0.0118 + 0.00087\exp(1.0175t), R^2 = 0.9822 \quad (12)$$

$$R3_A: B_e = 0.0154 + 0.00236\exp(0.9642t), R^2 = 0.9749 \quad (13)$$

$$R1_D: B_e = -0.0733 + 0.1111\exp(-0.0146t), R^2 = 0.9929 \quad (14)$$

$$R2_D: B_e = 0.0236 - 1.14 \times 10^{-7}\exp(-220.52t), R^2 = 0.9075 \quad (15)$$

$$R3_D: B_e = 0.0362 - 7.8 \times 10^{-8}\exp(-107.82t), R^2 = 0.7718 \quad (16)$$

where:

B_e – biogas production rates exponential estimation (Nm³/kg VS)

*R*² of the exponential equation of biogas production rates ranged from 0.7718 to 0.9929, where rising limbs showed better simulation than falling limbs except for R1 biodigester. The exponential regression appeared to show better simulation of biogas production rates than the linear regression except for falling limb of R3 biodigester.

First order kinetic and modified Gompertz plots of the biogas production accumulation are present-

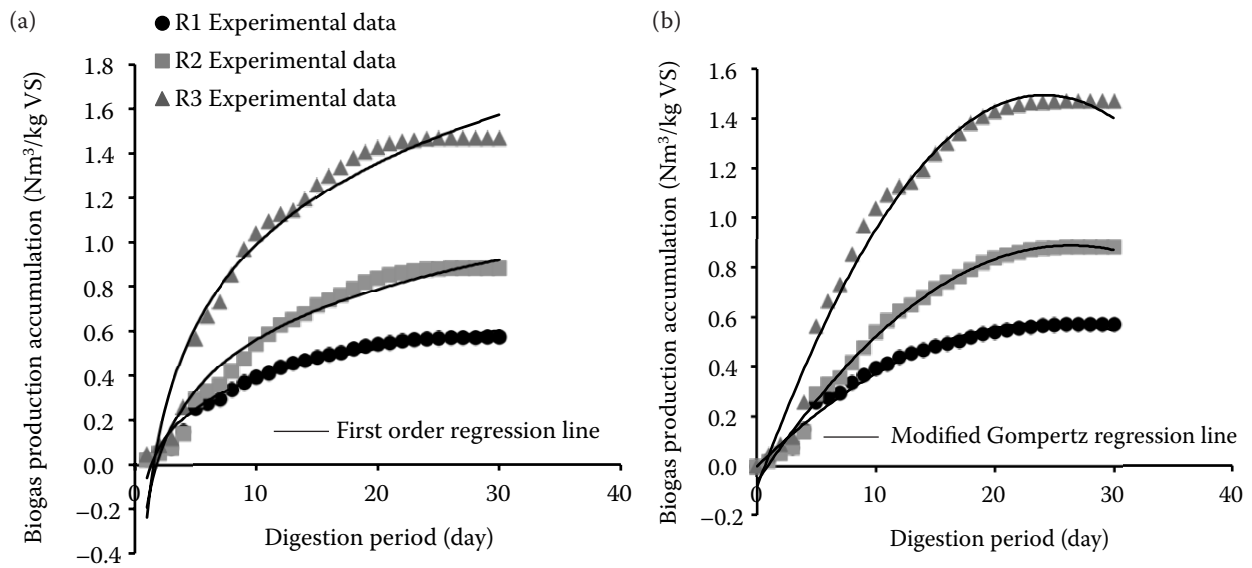


Fig. 4. The first order kinetic plots (a) and modified Gompertz plots (b) fitted into experimental data plots of biogas production accumulation
 VS – volatile solids; R1, R2, R3 – biodigesters

ed in Fig. 4. First order kinetic constants (k) were found to be in the order R1 (0.0926), R3 (0.0858) and R2 (0.0649), while the estimated biogas potential increased as I/S ratio decreased (Eqs 17–19). For the modified Gompertz equation, R3 biodigester had the max. biogas production rate (μ_m) followed by R2 and R1 (Eqs 20–22). Simulation results of biogas production accumulation for SPM showed that modified Gompertz equation had higher R^2 values that ranged from 0.9965 to 0.999 compared to R^2 values of first order kinetic equation that ranged from 0.9769 to 0.9827.

$$R1: B_f = 0.6308(1 - \exp(-0.0926t)), R^2 = 0.9827 \quad (17)$$

$$R2: B_f = 1.0985(1 - \exp(-0.0649t)), R^2 = 0.9812 \quad (18)$$

$$R3: B_f = 1.6796(1 - \exp(-0.0858t)), R^2 = 0.9769 \quad (19)$$

$$R1: B_G = 0.5677 \exp\{-\exp[(0.0434e/0.5677) \times (0.4 - t) + 1]\}, R^2 = 0.999 \quad (20)$$

$$R2: B_G = 0.8965 \exp\{-\exp[(0.0631e/0.8965) \times (1.4 - t) + 1]\}, R^2 = 0.999 \quad (21)$$

$$R3: B_G = 1.4639 \exp\{-\exp[(0.1257e/1.4639) \times (1.4 - t) + 1]\}, R^2 = 0.9965 \quad (22)$$

where:

B_f – biogas production accumulation for first order kinetic equations

B_G – biogas production accumulation for modified Gompertz equations
 t – time of digestion period (days)
 e – constant (2.718282)

CONCLUSION

From the study carried out, the following conclusions can be made:

- The SPM had high volatile solids/total solids ratio.
- Biogas production rates and accumulation values increased as I/S ratio from 1.37 to 4.1.
- The exponential plot generally simulated biogas production rates better than the linear plot due to its higher value of coefficient of determination.
- The modified Gompertz plot better simulated biogas production accumulation than the first order kinetic plot.

References

Adiga S., Ramya R., Shankar B.B., Jagadish H.P., Geetha C.R. (2012): Kinetics of anaerobic digestion of water hyacinth, poultry litter, cow manure and primary sludge: A comprehensive study. In: International Conference on Biotechnology and Environment Management. Singapore: IACSIT, 42: 73–78.

Angelidaki I. (2002): Anaerobic biodegradability of macropollutants. In: Ligthart J., Nieman H. (eds): Workshop on

doi: 10.17221/30/2014-RAE

- harmonisation of anaerobic biodegradation, activity and inhibition assays, June 7–8, 2002, Lago d'Orta, Italy: 13–29.
- APHA (1992): Standard Methods for the Examination of Water and Wastewater. Washington, DC, American Public Health Association.
- Baserga U (1998): Agricultural co-fermentation biogas plants: in biogas from organic wastes and energy grass. FAT Reports, 512: 1–11.
- Buswell A.M., Mueller H.F. (1952): Mechanism of methane fermentation. *Industrial and Engineering Chemistry*, 44: 550–552.
- Chynoweth D.P., Turick C.E., Owen J.M., Jerger D.E. (1993): Biochemical methane potential of biomass and waste feedstocks. *Biomass and Bioenergy*, 5: 95–111.
- Feng L., Li Y., Chen C., Liu X., Xiao X., Ma X., Zhang R., He Y., Liu G. (2013): Biochemical Methane potential (BPM) of vinegar residue and the influence of feed to inoculum ratios on biogas production. *Bioresources*, 8: 2487–2498.
- Hansen T.L., Schmidt J.E., Angelidaki I., Marca E. Jansen J.C., Mosbaek H., Christensen T. H. (2004): Method for determination of methane potentials of solid organic waste. *Waste Management*, 24: 393–400.
- Hashimoto A.G., Varel V.H. Chen Y.R. (1981): Ultimate methane yield from beef cattle manure: effect of temperature, ration constituents, antibiotics and manure age. *Agricultural Wastes*, 3: 241–256.
- Kheiredine B., Derbal K., Bencheikh-Lehocine M. (2014): Effect of inoculum to substrate ratio on thermophilic anaerobic digestion of the dairy wastewater. *Chemical Engineering Transactions*, 37: 865–870.
- Labatut R.A., Scott N.R. (2008): Experimental and predicted methane yields from the anaerobic co-digestion of animal manure with complex organic substrates. In: *Proceedings of the American Society of Agricultural and Biological Engineering*. St. Joseph.
- Monch-Tegeder M., Lemmer A., Oechsner H., Jungbluth T. (2013): Investigation of the methane potential of horse manure. *Agricultural Engineering International: CIGR Journal*, 15: 161–172.
- Raghunathan C., Velan M., Velmurugan B., Ramanujam R.A. (2008): Studies on batch kinetics of anaerobic digestion of solid waste (SINEWS) from gelatine industry. *Journal of Environmental Research and Development*, 3: 456–463.
- Raposo F. et al. (2011): Biochemical methane potential (BPM) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. *Journal of Chemical Technology and Biotechnology*, 86: 1088–1098.
- WEC (2004): Renewable energy projects handbook. London, World Energy Council.
- Wymyslowski M., Luczak M., Zawadzka A., Imbierowicz M. (2010): Methane fermentation of poultry slaughterhouse waste. *Polish Journal of Chemical Technology*, 12: 15–18.
- Yusuf M.O., Debora A., Ogheneruona (2011): Ambient temperature kinetic assessment of biogas production from co-digestion of horse and cow dung. *Research in Agricultural Engineering*, 57: 97–104.
- Zhang T., Liu L., Song Z., Ren G., Feng Y., Han X., Yang G. (2013): Biogas production by co-digestion of goat manure with three crop residues. *PLOS ONE*, 8: e66845.

Received for publication October 30, 2014

Accepted after corrections April 13, 2015

Corresponding author:

MR. ABUBAKAR A. LAWAL, University of Maiduguri, Faculty of Engineering, Department of Agricultural and Environmental Resources Engineering, Bama Road, P.M.B 1069, Maiduguri, Nigeria;
e-mail: abulawal2010@yahoo.com