

## EFFECT OF INOCULUM TO SUBSTRATE RATIO ON BIOGAS PRODUCTION FROM SHEEP PAUNCH MANURE DIGESTED IN BATCH BIODIGESTERS

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### ABSTRACT

In this study sheep paunch manure (SPM) was anaerobically digested under mesophilic condition to determine its biogas potential. Three inoculums to substrate (I/S) ratios 1.3, 1.9 and 3.8 were digested in biodigesters labeled R1, R2 and R3 respectively to determine the effect of substrate concentration on biogas yield. Results showed that I/S ratio had significant effect on biogas production rate and accumulation. Biogas production rate increased to their peak in the order of R3 ( $0.30526 \text{ Nm}^3/\text{kgVSd}$ ), R2 ( $0.15308 \text{ Nm}^3/\text{kgVSd}$ ) and R1 ( $0.11009 \text{ Nm}^3/\text{kgVSd}$ ) on day 5 and decreased to zero on day 27, 29 and 31 of the digestion period respectively. Biogas production accumulation increased from 0.57195 to  $1.46784 \text{ Nm}^3/\text{kgVS}$  as I/S ratio increased from 1.3 to 3.8. Linear and exponential regression equations were used to simulate biogas production rate while biogas production accumulation was simulated using first order kinetic and modified Gompertz equations. Coefficient of determination ( $R^2$ ) of linear regression equation of biogas production rate ranged from 0.707 to 0.797 while exponential regression equation had  $R^2$  values that ranged from 0.7718 to 0.9929 showing better simulation.  $R^2$  of first order kinetic equation ranged from 0.9769 to 0.9827 while  $R^2$  for modified Gompertz ranged from 0.9965 to 0.999. Thus modified Gompertz equation showed better simulation of biogas production accumulation.

**KEYWORDS:** Manure, anaerobic digestion, I/S ratio, biogas production rate and accumulation, simulation.

### 1. INTRODUCTION

Biomass is one of the renewable sources of bioenergy capable of significant contribution to the global future energy supply (WEC, 2004). Bioenergy generated from diverse sources provides local energy needs, reduce dependence on fossil fuel and help mitigates greenhouse gas effect. The main concerns limiting bioenergy utilization are relative inefficiency and cost of conversion technologies. However, anaerobic digestion of residue/waste materials from agricultural crops, animal rising and agro-processing industries could be of simple, efficient and low cost 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 to design, development and process control and optimization of biodigesters. But to fully utilize biogas potentials from biomass materials, biogas production behavior and potentials of more organic residue/waste materials need to be determined.

Researches to determine biogas potential of several substrates have been conducted for similar purpose. Buswell and Mueller (1952), Baserga (1998) and Raposo et al. (2011) proposed empirical relationship that utilizes the elemental or organic chemical compositions of biomass to estimate its theoretical maximum biogas yield. Researchers (Chynoweth et al., 1993; Hansen et al., 2004; Wymyslowski et al., 2010; Feng et al., 2013; Monch-Tegeder et al., 2013; Zhang et al., 2013) have investigated the biochemical methane potential of several substrates and co-substrates using batch method. They determined physicochemical compositions of substrate and developed anaerobic assay for biogas production. The effects of operating parameters on biogas potential have been investigated. Researchers (Chynoweth et al., 1993; Labatut et al., 2008; Feng et al., 2013; Kheiredine et al., 2014) investigated the

influence of inoculums to substrate ratio by varying the amount of substrate added to inoculums. The effect of temperature and pH were also investigated (Hashimoto et al., 1981). Simulation studies on biogas production accumulation and conversion kinetics of several substrates have been conducted (Raghunathan et al., 2008; Yusuf et al., 2011; Adiga et al., 2012; Feng et al., 2013) and results showed that different substrates have different potential and conversion kinetics.

The objectives of this study were to evaluate the effect of inoculums to substrate ration on biogas production of sheep paunch manure (SPM) under mesophilic condition and to simulate the biogas production rates and accumulation.

## **2. MATERIALS AND METHOD**

### **2.1 Sample Collection, Conditioning and Characterization**

#### **2.1.1 Inoculums**

For the purpose of this study cow dung was used as inoculum. Sample of fresh cow dung, 2 kg, were collected at the animal farm University of Maiduguri, Nigeria and taken to Agricultural and Environmental Resources laboratory of same institution for experiment. In order to adapt the inoculums to mesophilic condition, cow dung were diluted in distilled water to 10% dry matter (DM) and transferred into a 4 liter glass bottle. The headspace of the 4 liter glass bottle was flushed with a gas mixture of 80% N<sub>2</sub> and 20% CO<sub>2</sub> and closed with a thick rubber septum which was held tight by a resin. The inoculums solution was then incubated in a water bath at 35±1°C. During incubation, the inoculums solution was degassed completely by allowing gas build-up in the headspace to escape via a valve controlled tube.

#### **2.1.2 Substrate**

Approximately 2 kg of fresh SPM was collected at animal slaughter house, Maiduguri. Sample collected was stored over ice and delivered to the laboratory for experiment and analysis. Sub-sample of SPM was collected and diluted to 15%, 10% and 5% (DM) and transferred separately into 2 liter glass bottles and stored at 5°C.

#### **2.1.3 Nutrient medium**

A nutrient medium containing the following groups of nutrients and vitamins was prepared:

- a. NaCl, MgCl<sub>2</sub>.6H<sub>2</sub>O, CaCl<sub>2</sub>.2H<sub>2</sub>O
- b. FeCl<sub>2</sub>.4H<sub>2</sub>O, ZnCl<sub>2</sub>, MnCl<sub>2</sub>.4H<sub>2</sub>O, (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>
- c. Folic acid and riboflavin

Stock solutions were prepared by dissolving specific quantities (g) of the nutrients from a group in 1 liter distilled water. 10, 1 and 1 ml of a, b and c stock solutions were then added to 988 ml distilled water to obtain the nutrient medium used for the experiment.

## **2.2 Physicochemical Composition Analysis**

Fresh samples of the inoculums and SPM were analyzed for total solids (TS) and volatile solids (VS) contents according to the standard method of American Public Health Association (APHA, 1992). The method involved TS determination by oven drying sample at 95°C until weight was constant and subsequent oven drying TS for 1 hour at approximately 550°C to determine proportion of matter lost in the dried sample. To determine the carbohydrate, crude protein, crude fat, crude fiber and ash content, SPM sample was analyzed at Soil Science Laboratory, University of Maiduguri.

## 2.3 Batch Digestion Test

### *Biogas unit*

The biogas unit consisted of the following equipment:

- A unit comprises of 200 ml glass bottle and a thick rubber septum with a flexible rubber tube fixed on the rubber septum through an opening was used as biodigester.
- A thermostatically controlled water bath with a plastic rack used for agitating and keeping biodigesters in place.
- A 100 and 10 ml plastic syringe and gas pressure gauge.
- 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.

## 2.4 Experimental Procedure

In this experiment 60 ml of the degassed inoculums (10% DM) was collected after shaking using 100 ml plastic syringe and transferred into a biodigester unit. 1 ml of nutrient medium and 30 ml of a SPM substrate solution were collected and added to the biodigester unit containing inoculums. This procedure was carried out in 3 biodigesters labeled R1, R2 and R3 containing inoculum to substrate ratios of 1.3, 1.9 and 3.8 respectively and control (biodigester containing only inoculums). Biodigesters were flushed with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture and transferred into water bath preset at 35±1°C. The entire biodigester unit was agitated twice a day. Biogas produced was measured using 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 of biogas accumulation over time biogas was allowed to escape in order to avoid pressure build up that would exceed pressure gauge capacity. This experiment was repeated 3 times and average was reported as biogas production.

## 2.5 Simulation of Biogas Production Rate and Accumulation

Biogas production rate of SPM was simulated using linear and exponential plots. Equation 1 was used for linear simulation with the assumption that biogas production rate increased linearly to its peak and then decreased linearly to zero. Exponential equation (equation 2) also was used to simulate the increasing and decreasing of stages biogas production rate under the assumption that production rate increased exponentially to peak and then decreased exponentially.

$$B_l = a_l + b_l \times t \quad (1)$$

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

B<sub>l</sub> and B<sub>e</sub> are biogas production rates for linear and exponential estimation (Nm<sup>3</sup>/kgVSd) at time (day), t is the time (day) of digestion period, a<sub>l</sub> is intercept (Nm<sup>3</sup>/kgVSd) and b<sub>l</sub> is slope (Nm<sup>3</sup>/kgVSd<sup>2</sup>), a<sub>e</sub> and b<sub>e</sub> are constants (Nm<sup>3</sup>/kgVSd) and c<sub>e</sub> is constant (d<sup>-1</sup>) and is negative for decreasing stage. Finally biogas production accumulation was simulated using the first order kinetic and modified Gompertz equations:

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

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

where B<sub>f</sub> and B<sub>G</sub> are biogas production accumulation for first order kinetic and modified Gompertz equations respectively at time (day), B<sub>Of</sub> and B<sub>OG</sub> are maximum biogas production for first order kinetic and modified Gompertz equations (Nm<sup>3</sup>/kgVS), k is first order kinetic constant, μ<sub>m</sub> is maximum biogas production rate (Nm<sup>3</sup>/kgVSd), λ duration of lag phase and e is equal to 2.718282.

## 2.6 Statistical Analysis

Simple descriptive statistical analysis was used to report averages and standard deviations of experimental data. ANOVA test was used to detect if there was significant difference on biogas production rate and accumulation due to the effect of I/S ratios. Statistix version 9 software was used to determine equation parameters while Microsoft excel was used to plot graphs.

## 3. RESULTS AND DISCUSSION

### 3.1 Characteristics of SPM and Inoculums

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

Table 1: Physicochemical Compositions of SPM and Inoculum

	Moisture content (%)	Total solids% (w.b)	Volatile solids% (w.b)	VS/T S ratio	pH	Carbo hydrate % (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

### 3.2 Biogas Production Rate and Accumulation

Experimental result showed no biogas produced from controlled biodigester over the digestion period. Figure 1 (a) and (b) presents SPM biogas production rate and accumulation plots. Results showed that it took biodigesters R1, R2 and R3 31, 29 and 27 days to complete digestion respectively. It appeared that as I/S ratio increased from digestion period decreased and biogas production rate increased from. It can be observed from Figure 1 (a) that the biogas production rate curves of all biodigesters exhibited similar pattern over the entire digestion period where R3 biodigester exhibited maximum biogas production rates followed by R2 and R1 biodigesters respectively. The peak (maximum) biogas production rate occurred on the 5<sup>th</sup> day for all biodigesters in the order of R3 (0.30526 Nm<sup>3</sup>/kgVSd), R2 (0.15308 Nm<sup>3</sup>/kgVSd) and R1 (0.11009 Nm<sup>3</sup>/kgVSd). It is apparent that I/S ratio had significant effect ( $p < 0.0372$ ) on biogas production rate. Total accumulated biogas produced was found to be in the order of 1.46784, 0.88177 and 0.57195 Nm<sup>3</sup>/kgVS in R3, R2 and R1 biodigesters respectively. Raghunathan et al., (2008) reported closely similar biogas production accumulation values (1.1 to 0.382 Nm<sup>3</sup>/kg VS). Experimental results showed that 80% of maximum biogas production in biodigesters R1, R2 and R3 had accumulated on the 14<sup>th</sup>, 15<sup>th</sup> and 14<sup>th</sup> day of digestion period respectively.

Figure 2(a and b) shows the linear regression line fitted to rising and falling limbs plots of experimental data of biogas production rate in R1, R2 and R3 biodigesters. Equations 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  $B_i$  is linear estimation of biogas production rate ( $\text{Nm}^3/\text{kgVSd}$ ), subscripts A and D represent rising and falling limbs, and  $t$  is time (day). The coefficient of determination of falling limbs showed better linear simulation than rising limbs except in R1 biodigester. The exponential regression line simulating biogas production rate for both limbs are presented in Figure 3(a and b). The following equations (equations 11-16) expressed the exponential relationship between biogas production rates and digestion period:

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

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

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

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

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

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

where  $B_e$  is exponential estimation of biogas production rate ( $\text{Nm}^3/\text{kgVSd}$ ).  $R^2$  of exponential equation of biogas production rates ranged from 0.7718 to 0.9929, where rising limbs showed better simulation than falling limbs except R1 biodigester. Exponential regression appeared to show better simulation of biogas production rates than linear regression except for falling limb of R3 biodigester.

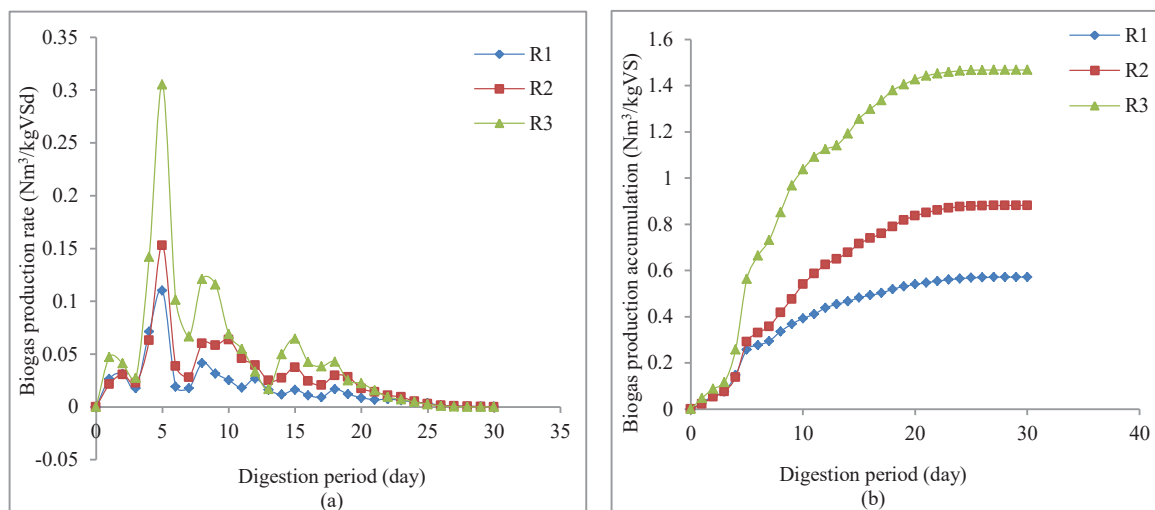


Figure 1. Biogas production rate (a) and accumulation (b) in R1, R2 and R3 biodigesters.

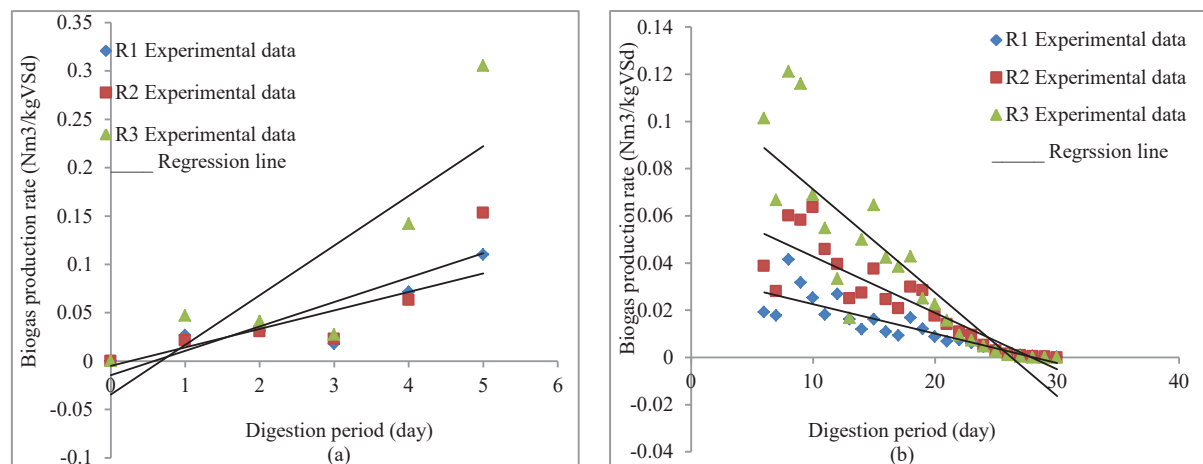


Figure 2. Linear plots of biogas production rates of rising (a) and falling (b) limb in R1, R2 and R3 biodigesters.

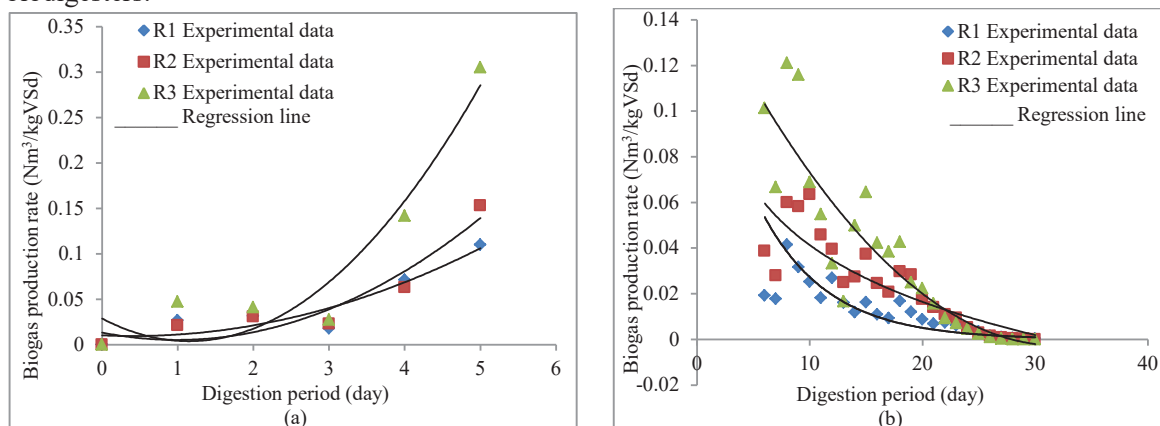


Figure 3. Exponential plots of biogas production rates of rising (a) and falling (b) limb in R1, R2 and R3 biodigesters.

The first order kinetic and modified Gompertz plots of biogas production accumulation are presented in Figure 4 (a and b). The first order kinetic constants ( $k$ ) were found to be in the order R1 (0.0926), R3 (0.0858) and R2 (0.0649), while estimated biogas potential increased as I/S ratio decreased (Equations 17 to 19). In modified Gompertz equation, R3 biodigester had the maximum biogas production rate ( $\mu_m$ ) followed by R2 and R1 respectively (Equations 20 to 22). Simulation results of SPM Biogas production accumulation showed that modified Gompertz equation had better  $R^2$  that ranged from 0.9965 to 0.999 than  $R^2$  for first order kinetic equation which ranged from 0.9769 to 0.9827.

R1 $B_f = 0.6308(1 - \exp(-0.0926t))$ ,	$R^2 = 0.9827$	(17)
R2 $B_f = 1.0985(1 - \exp(-0.0649t))$ ,	$R^2 = 0.9812$	(18)
R3 $B_f = 1.6796(1 - \exp(-0.0858t))$ ,	$R^2 = 0.9769$	(19)
R1 $B_G = 0.5677 \exp\{-\exp[(0.0434e/0.5677)(0.4-t)+1]\}$ ,	$R^2 = 0.999$	(20)
R2 $B_G = 0.8965 \exp\{-\exp[(0.0631e/0.8965)(1.4-t)+1]\}$ ,	$R^2 = 0.999$	(21)
R3 $B_G = 1.4639 \exp\{-\exp[(0.1257e/1.4639)(1.4-t)+1]\}$ ,	$R^2 = 0.9965$	(22)

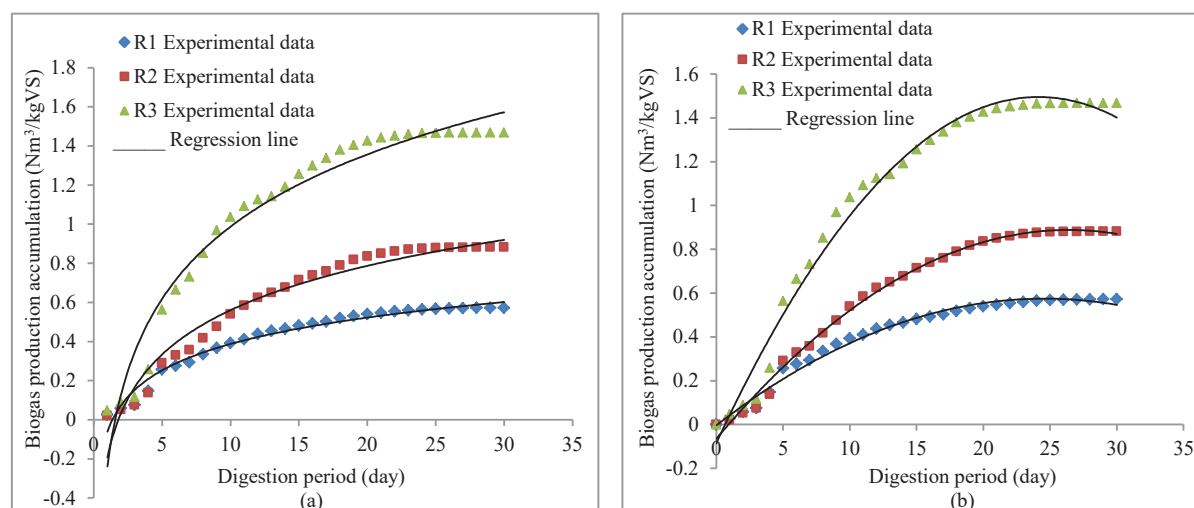


Figure 4. Biogas production as attested by digestion period

#### 4. CONCLUSION

The following conclusions follow, from the study:



SPM had high VS/TS ratio; Biogas production rate and accumulation increased with I/S ratio from 1.3 to 3.8; Exponential plot generally simulated biogas production rate better than linear plot; Modified Gompertz plot had better simulation than first order kinetic plot for biogas production accumulation.

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