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Studies on the Effect of Anaerobic Digestion on the Microbial Flora of Animal Wastes 2: Digestion and Modelling of Process Parameters

[1]A.U. Ofoefule, [1]E.O. Uzodinma and [2]C.N. Anyanwu
[1]Biomass Unit,
[2]Fossil Unit, National Center for Energy Research and Development,
University of Nigeria, Nsukka, Enugu State, Nigeria

Abstract: The effect of anaerobic digestion on the microbial flora and modeling of process parameters in the digestion of some animal dung like cow (CD), swine (SD), rabbit (RD), poultry (PD) and goat dung (GD) were verified. The digestion study was batch operated within a 98 days retention period using 50 L capacity metallic prototype digester. Average daily volume of gas production for each of the systems was 44, 40, 37, 33 and 31 dm^3/total mass of slurry (TMS), respectively. Microbial analyses of the different dung before the digestion indicated the presence of microbes such as *Proteus* sp. *Salmonella typhosa*, *Aerobacter cloacae*, *E. coli*, *B. subtilis,* while isolation and identification of the microbes at the end of digestion showed that some of the initial microbes died during digestion giving way to other species of microbes like *Clostridium perfringes* and *Salmonella typhimorium*. Mathematical modells derived using computer aided regression analysis also indicated that biogas production of animal wastes can be predicted based on digestion time and total microbial viable count (TVC). Overall results indicate that anaerobic digestion does not completely destroy the pathogens found in animal wastes but reduces them to a safe level for handling and use. The results further show that cow, swine and rabbit dung are better starters or blending wastes for the low-yielding ones.

Key words: Anaerobic digestion, animal dung, microbial flora, biogas yield, biogas production, regression equation

INTRODUCTION

Biogas production is a complex biochemical reaction found to take place under the action of delicately pH sensitive microbes mainly bacteria in the presence of little or no oxygen. Three major groups of bacteria (hydrolytic, acidogens/acetogens and methanogens) are responsible for breaking down the complex polymers in biomass wastes to form biogas at anaerobic conditions and animal manures have been established as major sources of this gas (Bori *et al*., 2007). The fermentative bacteria convert the complex polymers into alcohols, acetic acid, other Volatile Fatty Acids (VFAs) and off-gas containing H_2, CO_2. These intermediate products are metabolized primarily into CH_4 (60-70%), CO_2 (30-40%) and other associated gases by methanogens. This methanogenic biogas production rate is sensitive to changes in influent materials like pH, temperature, Organic Loading Rate (OLR) and Hydraulic Retention Time (HRT). Thus, for effective anaerobic digestion operation for biogas

Corresponding Author: Akuzuo U. Ofoefule, Biomass Unit, National Center for Energy Research and Development, University of Nigeria, Nsukka Enugu State, Nigeria
Tel: +234-8036798570, +234-8058341164

production, a balance among the acidogens/acetogens and methanogens is crucial (Cantrell *et al*., 2008). During the digestion, most of the free energy present in the biomass substrate is found in the terminal product methane (CH_4) which arises from the greater degree of metabolic specialization existing among anaerobic microbes. Consequently, through the interactions of the microbes, a lot of diversity exists in the biogas system just as in the digestive system of ruminant animals (Igoni *et al*., 2008).

It has been reported that seventeen fermentative bacteria species have played important role in the production of biogas (Nagamani and Ramasamy, 1999). However, the nature of the feedstock determines the type and extent of fermentative bacteria present in the digester. Bori *et al*. (2007) reported the population distributions of the microflora in anaerobic digestion of banana and plantain peels as consisting of mainly *Micrococcus luteus, Bacillus subtilis, Escherichia coli* and *Clostridium perfringens* while the methanogens identified belonged to the genera, *Methanobacterium, Methanococcus* and *Desulfovibrio*. Some researchers also observed higher amylolytic microorganisms in cow dung digester system but found higher proteolytic population in poultry dung-fed digester systems. Further observation indicated a clear distinction that existed in the type of cellulolytic bacterial distribution in rumen and biogas systems. The observations indicated that while in rumen, *Ruminococcus* sp., alone accounted for 60% of the total population, the predominant species belonging to genera *Lacteriodes* and *Clostridium* were found in biogas system (Ramasamy *et al*., 1991).

Animal wastes often vary in chemical composition and physical forms mainly due to differences in the digestive physiology of the various species, the composition and form of diet, the stage of growth of the animal and management system of waste collection and storage (Anunputtikul and Rodtong, 2004). Thus, the composition, quantity and quality of biogas produced may vary. Itodo and Kucha (1998) derived an empirical relationship for predicting biogas yield from poultry waste slurry. A multiple regression analysis of the biogas yields from retention time and total solids was used to develop an equation for predicting biogas yield. They concluded that the equation can be used to fairly predict total biogas yield at any point during anaerobic digestion of poultry waste slurry. Grant and Marshalleck (2008) also predicted the biogas flow rates, methane production, temperature, pH, residual mass etc., from chicken manure combined with pig and cow dung using a 3^2 factorial design from which models were designed. Ojolo *et al*. (2008) derived a regression equation called the municipal solid wastes energy value model. The model estimated the biogas production from municipal solid wastes. The predictive model formulation showed a relationship between retention time and daily/total biogas yield.

Anaerobic digestion of these dung provides an alternative source of energy both for rural and urban populace and has been recognized globally as a major way in which the growing energy crisis could be reduced and partly alleviated. Thus, the microbial conversion of organic matter from biogenic wastes has become a method of waste treatment and resource recovery.

Nigeria is abundantly blessed with different types of energy resources. The climate permits average solar radiation as high as 5.538 $kWh/m^2/day$ (World Energy Council, 1993), making the country operate mainly under mesophilic temperature at ambient conditions. This energy needs to be tapped especially as the energy supply of the country is grossly inadequate. Consequently, biogas production via anaerobic digestion can be a good resource channel if properly harnessed as is the case of China and India. Moreover, the effluent of this process is a residue rich in essential inorganic elements like nitrogen and phosphorus needed for healthy plant growth known as biofertilizer which when applied to the soil, enriches it with no detrimental effects on the environment (Bhat *et al*., 2001). This will further augment the inadequate supply of chemical fertilizers which are very expensive in spite of the fact that the country is a net food importer.

This study investigated the effect of anaerobic digestion on the microbial flora of some animal dung such as cow (CD), swine (SD), goat (GD), rabbit (RD) and poultry dung (PD). It also modelled the digestion parameters that affect biogas yield as independent variables and derived regression equations for predicting gas yield. The study is also a continuation of an earlier investigation on isolation and identification of common pathogens found in these animal wastes (Ofoefule and Uzodinma, 2005).

MATERIALS AND METHODS

The cow dung used for this study was obtained from the abattoir in Nsukka town. The swine dung and poultry droppings were collected from the Veterinary and Animal farms respectively, University of Nigeria, Nsukka. The goat and rabbit dung were procured from local rears in Nsukka town. The study was carried out at the National Center for Energy Research and Development, University of Nigeria, Nsukka between July and December, 2006. The biodigesters used for the study were five identical metallic prototypes (50 L capacity each) constructed at the same Energy Research Center (Fig. 1). Other materials used include; Top loading balance (50 kg capacity Five goats model No. Z051599), thermometer (0-360°C), K-type thermocouple thermometer (Hanna HI 8757), digital pH meter (Jenway 3510), hose pipes, plastic water troughs, graduated buckets for measuring daily gas production and biogas burner fabricated locally for checking gas flammability.

Experimental Set-Up

The poultry and goat dung were charged in their respective biodigesters at the ratio of 1:3 of waste to water, the cow dung was charged in the ratio of 1:2, while the swine and rabbit dung were charged with water in the ratio of 1:1. The moisture content of the respective wastes determined the waste to water ratios used. The experiment was batch

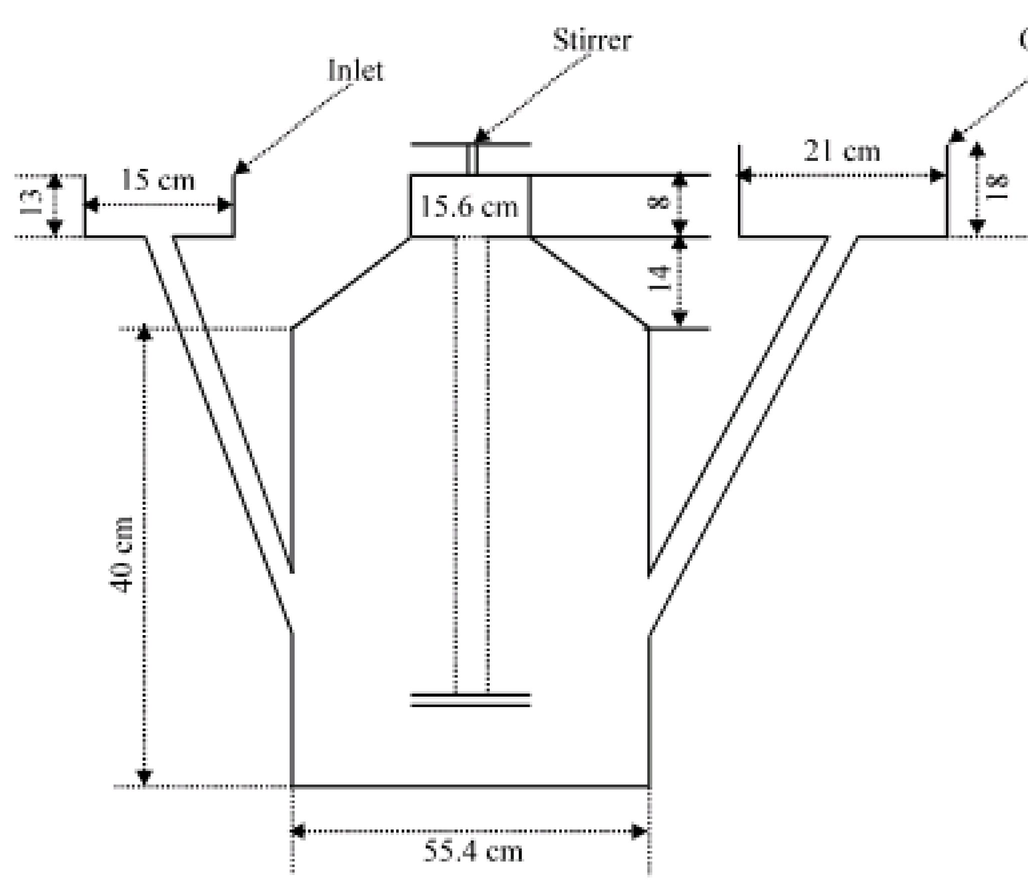

Fig. 1: Schematic diagram of the biodigester

operated for 98 days under atmospheric pressure and ambient temperature conditions. Gas production measured in dm^3/total mass of slurry was obtained by the downward displacement of water in the bucket by the gas.

Analysis of Wastes

Physico-Chemical Analysis

Ash, moisture and fiber contents of the undigested animal wastes were determined using AOAC (1990) method. Fat, crude protein and nitrogen contents were determined using soxhlet extraction and Micro-Kjedhal method described in Pearson (1976). Energy content analysis was carried out using the method of AOAC as described in Onwuka (2005). Total solids (TS) and volatile solids (VS) were determined using Meynell (1976) method.

Microbial Analysis

The Total Viable Count (TVC), isolation and identification of microorganisms in each digester during the anaerobic digestion process was carried out using the modified Miles and Misra method as described in Okore (2004).

Data Analysis

This was carried out using NLREG version 6.3 software; a specialized computer program designed for non-linear regression analysis.

RESULTS

The pathogens present in the raw and digested wastes were successfully isolated and identified. Table 1 shows that most of the organisms responsible for the digestion process were not found in the wastes after digestion. They were replaced by other organisms. Some of the pathogens observed before the digestion are also shown in the table. The various load/levels of the pathogens are shown in Table 2 with reduced levels after the digestion period. The physicochemical properties of the undigested wastes including moisture content, Ash, fibre, fat, nitrogen, protein, energy content, total and volatile solids content were characterized on the basis of which it was discussed that wastes with high fibre content such as swine and goat dung possess lower nitrogen and energy contents, respectively (Table 3). Higher ash content also corresponded with higher volatile solids content as can be seen from

Table 1: Isolation and identification of microbes before and at the end of digestion

Wastes	Microbes identified before digestion	Microbes identified after digestion
Cow Dung (CD)	*Proteus* sp., *Aerobacter cloacae*	*B. subtilis, E. coli, Clostridium, perfringes, S .typhimor*
Swine Dung (SD)	*Salmonella typhosa, Aerobacter cloacae*	*B. subtilis, E.coli*
Rabbit Dung (RD)	*E.coli, Salmonella typhosa, B. subtilis*	*B. subtilis, E.coli*
Poultry Dung (PD)	*Proteus* sp., *Salmonella typhosa*	*B. subtilis, E. coli*
Goat Dung (GD)	*Proteus* sp., *E.coli*	*B. subtilis*

Table 2: Total Viable Count (TVC) during the period of digestion fortnightly (cfu mL^{-1})

Days	Cow Dung (CD)	Swine Dung (SD)	Rabbit Dung (RD)	Poultry Dung (PD)	Goat Dung (GD)
0	1.33×10^8	6.17×10^8	1.50×10^9	3.33×10^8	3.92×10^8
14	1.45×10^9	1.03×10^{10}	2.07×10^9	1.99×10^9	1.08×10^8
28	4.98×10^9	4.30×10^9	2.02×10^9	7.38×10^8	5.13×10^9
42	1.42×10^8	2.50×10^7	4.52×10^9	3.25×10^8	1.08×10^8
56	1.25×10^8	1.97×10^7	9.07×10^8	6.83×10^7	4.33×10^7
70	3.95×10^7	1.08×10^7	8.38×10^8	2.87×10^6	2.01×10^6
84	2.25×10^6	1.58×10^6	4.09×10^7	1.88×10^6	6.25×10^5
98	2.40×10^5	1.80×10^5	1.68×10^5	1.05×10^5	1.50×10^4

Table 3: Physicochemical properties of the undigested animal wastes

Parameters	Cow Dung (CD)	Swine Dung (SD)	Goat Dung (GD)	Poultry Dung (PD)	Rabbit Dung (RD)
Moisture (%)	22.62	27.06	17.65	16.20	31.20
Ash (%)	41.00	40.15	37.65	37.90	31.01
Fibre (%)	21.25	51.05	55.20	28.70	23.20
Fat (%)	0.40	0.35	0.20	0.45	0.15
Nitrogen (%)	2.03	1.47	1.82	2.59	2.03
Protein (%)	12.68	9.19	11.37	16.18	12.68
Energy (Kcal g^{-1})	3.76	2.77	2.25	3.09	3.35
Total solids (%)	77.38	72.94	82.35	83.80	68.80
Volatile solids (%)	36.38	27.01	15.65	17.02	19.89

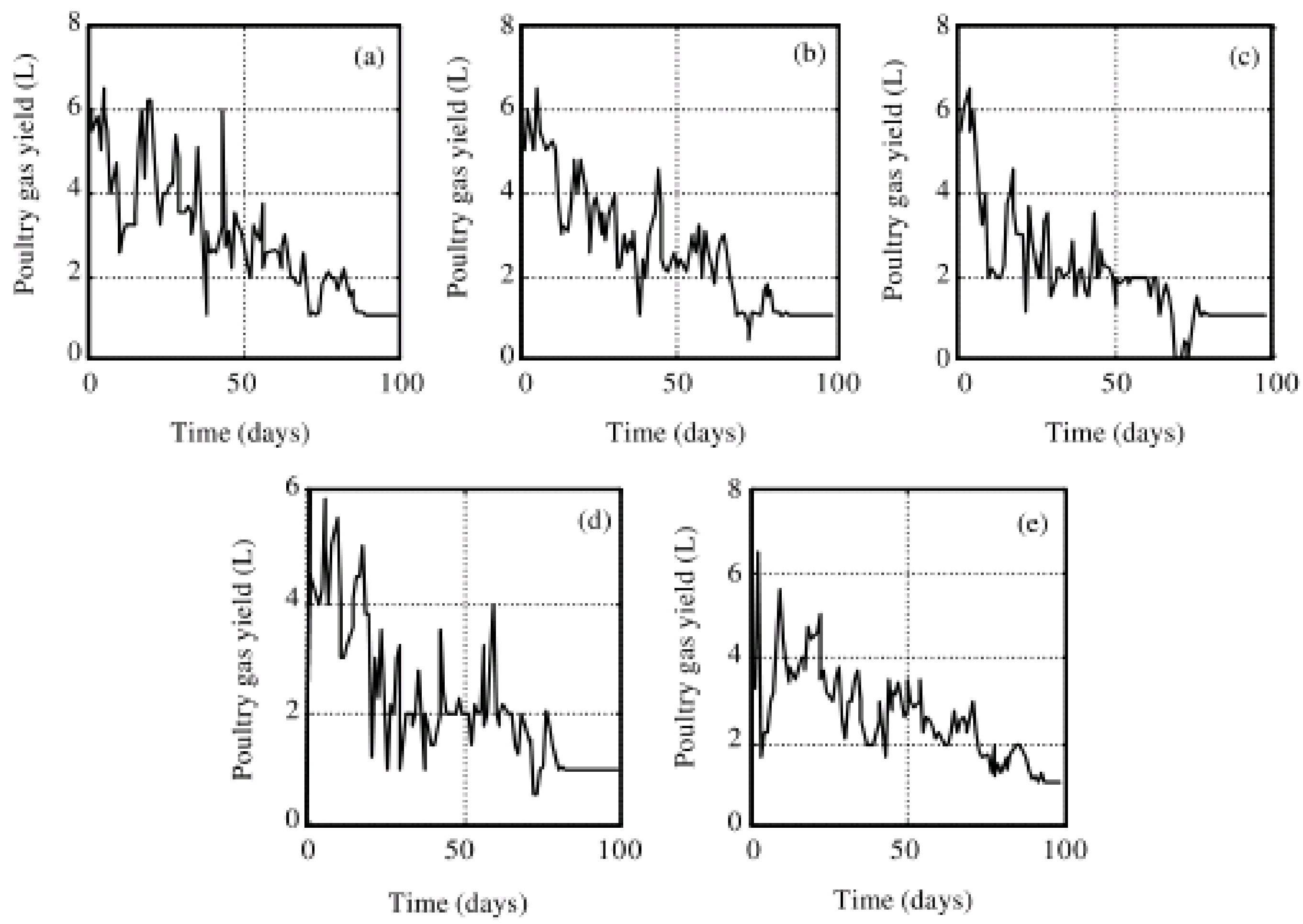

Fig. 2: (a-e) Plot of gas yield versus time for all wastes

the table. Adequate physicochemical properties are necessary for effective biogas production. The biogas yields of the five waste types were studied as a function of time (t) and Total Viable Count (TVC) for a total of 98 days. The plot presented in Fig. 2a-e and Table 4 shows that cow dung yielded the highest cumulative gas volume, whereas, goat dung produced the least, on the basis of which it was possible to determine the total biogas produced by each dung for the whole period as area under each curve.

Gas Yield (GY) was modelled as a function of time (t) using NLREG Version 6.3 Software, giving rise to the expression in Eq. 1, The regression parameters for the equation (Table 5) indicate that the equation can be reliably applied for the prediction of gas yield for most of the waste types except in the case of poultry dung ($Ra^2 = 0.89$). The coefficient of multiple determination Ra^2 is also shown for each of the five wastes studied.

$$GY = K_1 t^{a1} + K_2 t + K_3 \qquad (1)$$

Where:

GY = Gas yield (dm^3/total mass of slurry)

t = Time (days)

K_1, K_2, K_3, a1 = Constants (Table 5)

Table 4: Volume of biogas produced by the waste

Waste type	Cumulative gas volume (dm^3/total mass of slurry)	Average gas volume (dm^3/total mass of slurry)
Cow Dung (CD)	4,285	44
Swine Dung (SD)	3,900	40
Goat Dung (GD)	3,042	37
Rabbit Dung (RD)	3,633	33
Poultry Dung (PD)	3,199	31

Table 5: Regression parameters for GY vs. time

Waste type	K_1	a1	K_2	K_3	Ra^2 (%)
Cow Dung (CD)	59.2373	0.0859	-0.8075	2.9970	97.41
Swine Dung (SD)	67.8443	$3.35*10^{-13}$	-0.6088	2.5000	95.79
Goat Dung (GD)	51.9327	0.0288	-0.6166	4.2052	95.25
Rabbit Dung (RD)	31.9391	0.2396	-0.8516	1.8089	95.43
Poultry Dung (PD)	52.3850	0.0264	-0.5898	4.4140	88.96

Table 6: Regression parameters of different wastes

Parameter	Cow Dung (CD)	Swine Dung (SD)	Goat Dung (GD)	Poultry Dung (PD)	Rabbit Dung (RD)
K_1	73.225	57.548	44.282	53.723	48.099
K_2	-0.647	-0.507	-0.380	-0.517	-0.509
K_3	4.522	0.431	5.103	0.075	26.052
K_4	-125.468	-7.997	-145.639	-0.454	-795.423
K_5	1155.181	36.972	1382.849	-8.911	8079.865
K_6	-3526.351	2.767	-4364.822	63.080	-27303.720
a1	1.2×10^{-6}	1.74×10^{-7}	1.16×10^{-7}	1.02×10^{-8}	0.076
Ra^2 (%)	94.60	93.35	97.59	69.42	95.39

Bivariate regression analysis of the Gas Yield as a function of time (t) and total viable count (TVC) using NLREG v. 6.3 Software also led to good modells for virtually all the waste types according to Eq. 2, however a close observation of the regression parameters (Table 6) indicates that the gas yield is highly correlated to time and TVC for all the waste types except for poultry waste ($Ra^2 = 0.69$).

$$GY = K_1 t^{a1} + K_2 t + K_3 TVC^3 + K_4 TVC^2 + K_5 TVC + K_6 \quad (2)$$

Where:
GY = Gas yield (dm^3/total mass of slurry)
t = Time (days)
TVC = Total viable count (cfu mL^{-1})
K_1, K_2, K_3, K_4, K_5, K_6, a1 = Constants (Table 6)

DISCUSSION

The presence of the pathogens shown in Table 1, poses a lot of health problems for biogas researchers and workers who frequently handle these wastes (Ofoefule and Uzodinma, 2005). However, the same table shows that some of these microorganisms died during the anaerobic digestion giving rise to other species. This indicates that the microorganisms at the hydrolytic and acidogenic/acetogenic stages died giving rise to the methanogens which are different types of bacteria. Research findings have shown that anaerobic digestion does not actually destroy all the microorganisms but reduces them to a minimum where they no longer constitute health hazard (Anonymous, 2007). The present study seems to underscore this point because the result of the total viable count (TVC) in Table 2 indicated that the microbes were quite reduced. This confirms the report of

Elango *et al*., 2007 who maintained that after 35 days (stationary phase), the methanogenic bacteria are not capable to utilize the substrate because most of the bacteria changes to death phase and so the remaining waste material in the digester changes to acidic stage, ultimately reducing the generation of biogas.

Adequate physicochemical properties are necessary for effective biogas production (Energy Commission of Nigeria, 1998). The physicochemical properties shown in Table 3 indicates that the highest producing wastes which are cow, swine and rabbit dung had adequate nutrients (fat, protein and fibre), energy content and volatile solids (VS) which is the biodegradable portion of the waste. The Ash contents which contains the minerals in the wastes were high enough showing that they will be very good sources of fertilizer.

The biogas yields of the five waste types studied as a function of time and Total Viable Count (TVC) on the basis of which it was possible to determine the total volume of biogas produced by each dung for the whole period as area under each curve showed that Cow dung produced the highest volume of biogas, followed by Swine, Rabbit and Poultry while Goat dung produced the least volume of biogas. This underscores the superiority of Cow dung as a better biogas producer over other animal dung (Odeyemi, 1987; Ofoefule and Uzodinma, 2006). This also indicates that Cow dung, Swine dung and Rabbit dung are better starters or blending wastes for the low-producing wastes like plant wastes which are very difficult to biodegrade (Uzodinma and Ofoefule, 2009).

In view of the fact that anaerobic digestion of substrates is generally a function of time, whereas biogas production is highly dependent on microbial load, these relationships were quantified using regression analysis. From Eq. 2, the analysis indicated that Gas Yield can be predicted as a function of time and Total Viable Count (TVC).

The coefficient of multiple determination for this model as shown in Table 6 was quite high for all dung studied, an indication that the derived modells can be applied with a high level of accuracy, except in the case of Poultry waste. This observation collaborate the report of Ofoefule and Uzodinma (2006) on the abnormal behavior of poultry dung, which gives high volume of gas (that is not combustible) for the first 21 days after which it will produce flammable gas for one week and stop production. This necessitated the study carried out on the optimization of that particular waste which has the potentials to produce flammable biogas but only when it is blended with Cow dung. The results of the analysis indicate that it is possible to develop models for predicting biogas yield based on time and total viable count for animal wastes. Such approach would enable a prior selection of an appropriate waste type for application in a biogas plant without actually performing the experiments.

CONCLUSION

Results of the present study have shown that anaerobic digestion can be an effective means of waste management and pollution control as some of the pathogens and microorganisms were seriously reduced at the end of the digestion process. Consequently, the use of anaerobic digestion should be encouraged especially in the developing countries were waste disposal is a major problem. This will reduce the rate of sicknesses and diseases prevalent in those countries. Cow dung produced the highest amount of biogas while Goat dung produced the least volume of gas. Mathematical modells derived using regression analysis indicated that biogas production of animal wastes can be predicted based on digestion time and total viable microbial count. This is expected to help biogas users and researchers in the choice of animal waste for use and for blending (during optimization) with wastes that are low in biogas production.

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