Investigation into the Biogas Production Potential of Dairy Cattle Manure

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***Abstract*—We investigated the biogas production potential of dairy cattle manure obtained from the Fort Hare Dairy Farm, Eastern Cape Province of South Africa. A balloon type digester was charged with slurry of manure and operated under anaerobic digestion mode for six months. Fifty milliliters of slurry was withdrawn at different time intervals to analyze the microbial counts and physicochemical parameters by viable plate count method and standard methods, respectively. Data demonstrated that the pH and temperature ranged from 5.68-7.63 and 17.0 -25.04 ^{o}C, respectively. The total aerobic and anaerobic bacteria counts ranged from1.0 $\times10^{4}$-7.5$\times10^{6}$ and 4 $\times10^{2}$-1.8$\times10^{6}$ cfu/g, respectively, as well as the total yeast counts ranged from 2x10^{2}- 1.0$\times10^{6}$cfu/g. A linear regression model was developed that predicted the relationship between log total bacteria count and average slurry temperature, pH and days of digestion. The 11.2% TS, 61.5% VS and 32.5 % ash content of the manure indicated that the dairy manure constituted of biodegradable portion. Biogas (4600cm^{3}) was produced during digestion and was combustible after 120 days. Thus, we concluded that dairy manure harbors a considerable level of anaerobic bacteria and methanogens that participated effectively to degrade the organic portion of manure generating renewable energy.**

***Index Terms*—Anaerobic digestion, balloon digester, biogas, dairy manure, microbial count.**

I. Introduction

Livestock practices generate copious quantities of animal manure that warrants proper management. Consequently, there is a need for prompt intervention for proper disposal and management of these wastes in a bid to evade the adverse environmental and public health consequences (e.g. pathogen contamination, odor, air borne ammonia, green house gases etc) [1]. Interestingly, anaerobic digestion of animal manure in biogas digester has shown promise as a technology that will generate biogas, a renewable energy source that could be used for heating and other purposes and in addition aids in the proper management of these wastes by reducing the microbial load [2].

Furthermore, digestion of dairy manure through the anaerobic process entails the breakdown of organic matter contained therein by the concerted interplay of four sets of metabolically linked microbes via the hydrolytic, acidogenic, acetogenic and methanogenic stages to yield methane, carbon dioxide and other trace gases [3]. Overall, organic waste materials constitute of sufficient quantities of nutrients vital for the growth and metabolism of the anaerobic bacteria involved in biogas production [4]. Apart, from these bacteria, protozoa and fungi are the other groups of microorganisms that are present in animal manure [5].

Traditionally, at the Fort Hare Dairy Farm, the animal manure is being flushed with water into a lagoon located some distances from the farm for storage and treatment. However, this creates a nuisance to the environment due to the fact that the lagoon is uncovered and the stored manure generates and releases methane and carbon dioxide into the atmosphere [6]. These green house gases contribute in global warming [7]. In addition, the air around and close to the vicinity is polluted with malodorous compounds resulting from the incomplete breakdown of the organic fraction in the manure by the indigenous rumen microorganisms under uncontrolled environment. Therefore, the installation and implementation of a biodigester on the farm and utilization of the dairy waste as a feedstock to recover biogas will be a cheaper source of energy as well as a good waste management option.

Regardless of the fact that animal manure has been employed in biogas production through mono or co-digestion studies elsewhere [8]-[11], however, the biogas potential varies with the chemical composition, microbial and biological availability of the nutrients present in animal wastes. Concisely, the weather and soil characteristics might influence the physicochemical characteristics of these wastes as well as the species of animal, dietary sources, health status of the animals and factors affecting growth and age of the animals [5], [12].

In this paper, we investigated the biogas production potential of dairy manure obtained from the Fort Hare Dairy Farm, Eastern Cape Province of South Africa by monitoring the physicochemical parameters and microbial level of the digesting substrate throughout the anaerobic digestion process in a balloon type digester operating in a batch mode under mesophilic temperature condition. In addition, a model was developed to elucidate the relationship between the bacterial activity, pH and temperature and also to predict biogas yield in future studies.

II. Materials and Methods

A. Raw Anaerobic Digestion Material (Dairy Cattle Manure)

A total of 1700L of fresh dairy cow manure was obtained

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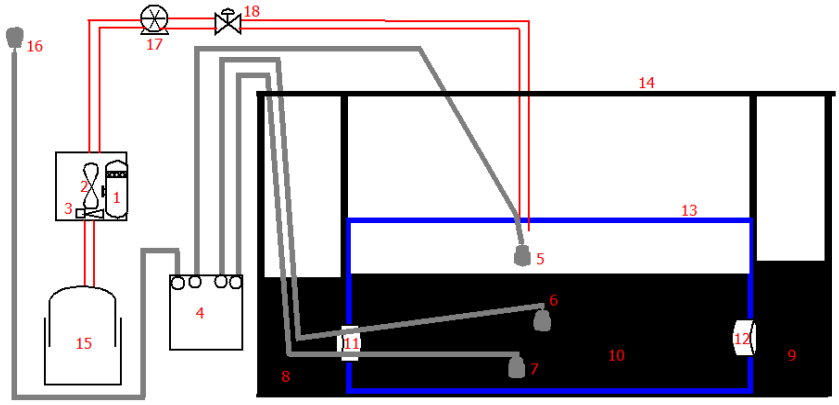
from the Fort Hare Dairy Farm, Alice on three consecutive days and were referred to as samples A, B and C. The characteristics of the wastes are presented in Table 1.The study was conducted from October 2013 to April 2014, at the Fort Hare Institute of Technology Research Centre, Alice campus.

TABLE 1: CHARACTERISTICS OF SAMPLES A, B AND C EMPLOYED IN FEEDING

Parameters	Sample A	Sample B	Sample C	Mean
%moisture content	86.6	89.3	90.4	88.8
%Total solids	13.4	10.7	9.6	11.2
%Volatile solids	72	47.8	64.6	61.5
%ash content	29.8	32.3	35.4	32.5
Ammonium level (mg/ml)	2.1	2.3	2.2	2.2
pH	6.3	6.95	6.46	6.57

B. Experimental Set up and Sampling

A balloon digester was housed within a concrete structure ($8m^3$) constructed originally into three compartments: feeding tank of height 83cm , width 89cm and length 95cm; bioreactor tank of height 3.25m and width 2m and lastly, the effluent tank (Fig. 1). The balloon digester was charged on three consecutive days with a homogenous mixture of dairy cow manure and water in the ratio 1:1. The mixing ratio of waste to water for the preparation of the slurry was determined by the moisture content of the waste [13]. The physicochemical parameters (pH, moisture, %TS, %VS, ammonium level and % ash content) of undigested wastes were determined before the slurry was prepared. The digester operated under batch mode over a six month period. Samples were withdrawn every three, seven and fourteen day interval for the analysis of microbial load while the pH and temperature were monitored daily throughout the study.



1, Campbell data logger; 2, Gas analyzer; 3, flow transducer; 4, hobo data logger; 5,produce gas temperature sensor; 6,intermediate slurry temperature sensor; 7, bottom slurry temperature sensor; 8, inlet chamber; 9,outlet chamber;10, slurry trap inside balloon; 11,slurry inlet;12,slurry outlet; 13,balloon digester; 14,insulation cover; 15,gas collection chamber; 16, ambient temperature sensor; 17, pump; 18, open and close valve

Fig. 1. Layout of the designed and constructed and balloon digester with the data acquisition system incorporated into the full schematic diagram.

C. Physicochemical Analysis of Slurry Samples

1) Determination of ammonium level of sample

Ammonium level was determined according to the method described by Ziganshin *et al*. [14]. Slurry samples were each centrifuged at 20,000xg for 20mins and the supernatant was decanted and colored with Nessler's reagent. The absorbance of the colored solutions was measured at 425nm wavelength by Hexios, Thermo Spectronics (Merck, Darmstadt, Germany) spectrophotometer and calculations were done considering that the standard (ammonia solution) had a concentration of 0.909g/mL. Also, distilled water was used as a blank to nullify the absorbance of water in both the samples and standard.

2) Determination of moisture content of samples

As per the method of Cioabla *et al*. [15], samples were weighed in a dish and dried in an oven at 105°C overnight. The weight of the dried sample plus dish was noted and the percentage moisture content was calculated by this equation;

$$\% \text{ moisture content} = (m_2-m_1)-(m_3-m_1) / (m_2-m_1); 100 \quad (1)$$

where m_1= mass in grams of the empty dish, m_2= mass in grams of sample plus the empty dish before drying, m_3= mass in grams of sample plus empty dish after drying.

3) Determination of dry matter (total solids)

Percentage of total solids was determined according to the method of Asam *et al*. [16]. A known weight of sample (W_S) in a dish was dried at 105°C in an oven for 24h. After drying, the weight of sample was measured and recorded as W_{DM}. Percentage of total solids was calculated as follows:

$$\% \text{ total solids} = 100 \times W_{DM} / W_S \quad (2)$$

4) Determination of volatile solid Content and ash content

Following the determination of total solids, the overnight dried sample was combusted in a muffle furnace at 550°C for 1h. The weight of the ash plus the dish was taken and percentage of volatile solid was then calculated from the formula:

$$\% \text{ volatile solid} = 100 \times (W_{DM} - W_{ash}) / W_{DM} \quad (3)$$

Furthermore, the percentage of ash content was determined from the expression proposed by Cioabla *et al*. [15] as follows:

$$\% \text{ ash content} = (m_3-m_1)/m_2-m_1; 100; 100/100-\% \text{ moisture} \quad (4)$$

where m_3= mass in grams of ash plus empty dish, m_2 and m_1 are as referred above.

5) Determination of pH, temperature and biogas production

Daily ambient and slurry temperature were measured by four temperature sensors connected externally to a data logger (U12, Hobo), configured to log every 30 minute interval. The pH was measured by a PHSCAN 30, pH meter. The cumulative volume of biogas generated was recorded by a gas analyzer. Triplicate determinations were carried out on each parameter.

D. Microbial Analysis of Samples

1) Bacterial counts

Total viable count was conducted on undigested and withdrawn samples during digestion according to the method described by Poudel *et al*. [17]. Each sample was aseptically collected, introduced into tryptic soy broth medium in sterile centrifuge tubes and transported on ice to the laboratory [18]. Samples were analyzed immediately upon arrival at the "Applied and Environmental Microbiology Research Group" laboratory. Evaluation of the total viable counts was conducted as follows: 1g of each sample was serially diluted tenfold in 9mL of sterile physiological saline. Dilutions from 10^{-1} to 10^{-5} were spread in triplicates on different microbiological media, including Nutrient agar (Merck, South Africa), Anaerobic agar (Conda, Spain), and Potato Dextrose agar (Conda, Spain) to obtain total aerobic bacteria counts, total anaerobic bacteria counts, and total yeast counts, respectively.

All inoculated plates were incubated at 37℃ for 24hrs, except that the plates for total yeast counts were incubated at 28^{o}C for 4days. After incubation, the number of emergent colonies on each plate was counted, recorded and each value represented the mean of triplicate plating [19].

E. Development and Building of a Mathematical Model

A linear regression model was developed to predict the relationship between slurry temperature, pH, days of digestion and log bacterial count (total number of aerobic and anaerobic bacteria count).

F. Statistical Analysis

The experimental data were processed and analyzed using Matlab software (R2013a).

III. Results and Discussion

Data on % total solids, total volatile solids, and ash content indicated that the cow manure constituted of biodegradable portion that could be digested for the release of biogas by the microorganisms contained therein [20].

At the point of charging, the total aerobic bacteria, anaerobic bacteria and yeast counts were high as shown in Table II. This affirms the fact that cow manure is a suitable substrate of biogas production since rumen microorganisms demonstrate significant roles in anaerobic digestion to degrade the organic portion of dairy cattle manure [1]. From the public health perspective, the total microbial load as depicted from the Table II, below revealed that dairy cow manure is a highly potential source of both water and soil pollution when the manure is not being treated effectively before it is released into the environment through soil application in agriculture for better crop yield. This poses threats to animals and humans as infection is possible since modes of transmission become feasible [21].

Furthermore, the performance of an anaerobic digester is strongly influenced by the pH and temperature of digesting substrate [15]. Livestock wastes including dairy manure have been reported to have high buffering capacity producing alkalinity when degraded upon by the microorganisms [22]. Consequently, in this study the pH of the digesting medium was unregulated. However, in the first two months of digestion (Fig. 2), a decrease in pH of the medium (from 6.57 at the point of charging to 5.82) was observed which could be attributed to the high concentration of volatile fatty acids, bicarbonate alkalinity and carbon dioxide; end products of the early stages of anaerobic digestion process (hydrolysis & acidogenesis [3]. This result corroborates the findings of Li *et al*. [23] and Abubakar and Ismail [1]. As the process progresses, the volatile fatty acids were metabolized and the pH gradually increased to the sufficient buffering capacity (neutral pH) necessary for the production of biogas [23]. Moreover, both acidogenic and methanogenic microorganisms have their optimal pH for metabolism [11], but the methanogens are highly pH sensitive and thrive optimally within the pH range of 6.6-7.6 [24]. This explains the high flammability rate of the biogas in this study, at pH 7.45 on the 121st day of the digestion process owing to the increase in methanogenic activity of the digester system.

TABLE II: Total Microbial Counts before and during the Anaerobic Digestion Process

Days of digestion	TAC	TANC	TYC
0	2.7×10^6	2.3×10^6	7.9×10^5
3	3.6×10^6	2.0×10^6	2.6×10^5
6	7.0×10^5	3.0×10^5	2.0×10^5
9	5.0×105	3.0×10^5	1.1×10^5
14	4.4×10^4	6.9×10^3	1.7×10^4
19	1.8×10^4	2.0×10^3	2.5×10^4
24	5.5×10^4	1.0×10^3	9.3×103
29	9.0×10^4	2.6×10^3	2.0×10^4
34	4.0×10^4	3.3×10^3	1.6×10^4
41	8.5×10^4	7.0×10^2	2.2×10^4
48	2.1×10^5	4.0×10^2	2.0×10^4
55	4.8×10^5	4.1×10^3	3.0×10^3
62	4.5×10^5	1.1×10^4	2.5×10^3
76	3.0×10^4	1.0×10^4	8.0×10^2
83	6.6×10^5	8.0×10^3	3.3×10^3
121	5.0×10^4	5.0×10^3	5.0×10^2
133	2.6×10^4	1.3×10^3	2.1×10^3
143	4.5×10^4	2.8×10^3	1.3×10^4
150	2.3×10^4	1.6×10^3	1.5×10^4
161	1.5×10^4	4.5×10^4	2.5×10^3
171	2.3×10^3	3.5×10^5	2.3×10^3
178	2.3×10^3	9.8×10^3	2.4×10^3

TAC- Total aerobic bacterial counts; TANC-Total anaerobic bacterial count; TYC-Total yeast count.

In addition, the metabolic rate of microorganisms has been reported to be influenced by temperature thus it modifies the effectiveness of these anaerobic microbes relevant in the process of biogas production. Accordingly, the temperature

profile was monitored by four temperature sensors; two sensors were embedded at different levels in the slurry, one was floating in the biogas space and the last was located outside the digester (within the concrete housing detecting ambient temperature) but all were connected to a Hobo data logger for data recording and storage.

The variation of daily average temperature (slurry) over the duration of anaerobic digestion is presented on Fig. 3 and is unaffected by the ambient temperature. Generally, it was noted that there was combined regimes of psychrophilic process (< 20°C) for the first two months and mesophilic process (>20°C < 30°C) for the rest of the process (4months) [25]. This result is in accordance with the findings of Cioabla *et al*. [15], although they evaluated the factors affecting anaerobic digestion of agricultural vegetal residues.

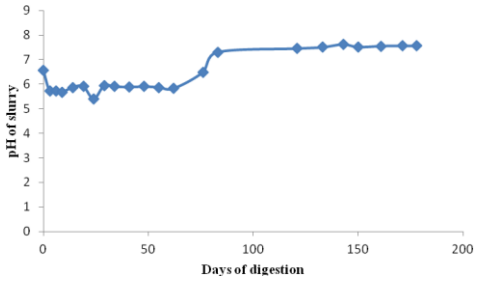


Fig. 2. Variation of pH of substrate during the anaerobic digestion process.

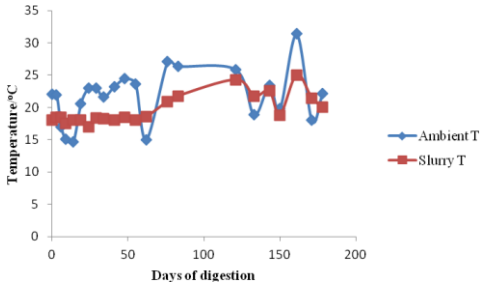


Fig. 3. Variation of temperature (slurry & ambient) during anaerobic digestion.

TABLE III: INPUT PARAMETERS AND THE OUTPUT THAT WERE UTILIZED IN DEVELOPING THE MULTIPLE LINEAR REGRESSION MODEL

No. of days	Average slurry temperature (°C)	pH value	Log of total Bacteria counts
0	18	6.57	6.12
3	18.49	5.72	5.80
6	18.46	5.73	5.85
9	17.53	5.68	5.70
4	18.02	5.87	4.64
19	8.02	5.91	4.26
24	17	5.4	4.74
29	18.38	5.94	5.34
34	18.24	5.9	5.24
41	18.02	5.88	5.11
48	18.49	5.9	5.09
55	18.02	5.87	4.90
62	18.54	5.82	4.80
76	20.92	6.48	5.037
83	21.77	7.3	5.39
121	24.27	7.45	4.99
133	21.7	7.5	4.42
143	22.55	7.62	4.40
150	18.83	7.51	3.90
161	25.04	7.55	4.18
171	21.43	7.56	3.78
178	20.09	7.56	3.39

A linear regression model was developed to predict the relationship between average slurry temperature, pH, and days of digestion and log bacterial counts. These are all the parameters that have been reported to influence the production of biogas [15]. From Table III, the predictors were number of days (n), average slurry temperature (T), and the pH while the response was the logarithm of the total bacterial counts (Y). The multiple linear regression model is as shown in the equation below:

$$Y = A0 + A1\ (n) + A2\ (T) + A3\ (\text{pH}) \quad (5)$$

where $A0$ = Forcing constant; $A1$ = Scaling constant of n; $A2$ = Scaling constant of T, $A3$ = Scaling constant of pH.

TABLE IV: SCALING VALUES OF THE RESPECTIVE SCALING CONSTANT

Predictor symbol	Scaling symbol	Scaling Value	Output symbol
constant	$A0$	1.671	Y
n	$A1$	-0.018	
T	$A2$	0.106	
pH	$A3$	0.383	

In addition, the measured log of total bacterial counts and the model of log of the total bacterial counts derived from the data set of Table I are as shown in Fig. 3. The measured and the modeled output were strongly correlated with a determination coefficient (r^2) and a P-value of 0.94 and 0.85, respectively. The large r^2 value showed that the measured Y fit the modeled equation and also the P-value depicted that the modeled equation has a statistical significance of over 80% in agreement to the calculated response. The modeled equation coefficients predicted that an increase in average slurry temperature and pH could likely result to an increase in the logarithm of total bacterial counts.

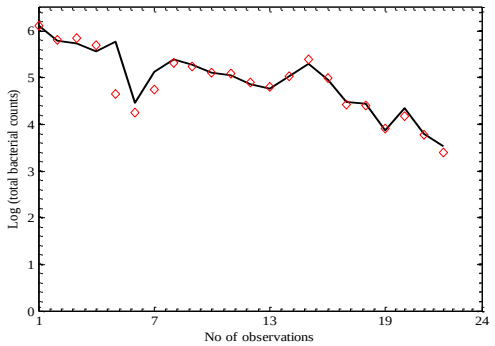


Fig. 3. Shows calculated and modeled log bacterial counts.

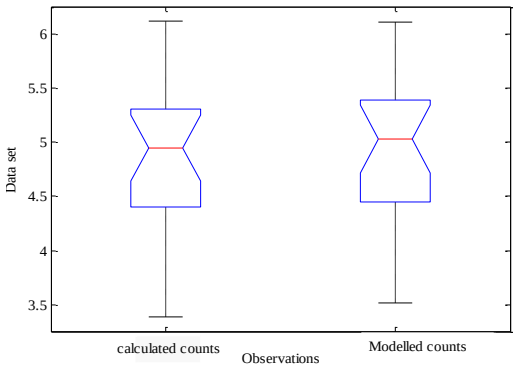


Fig. 4. ANOVA plot of calculated and modeled bacterial counts.

It is evident that temperature increases microbial metabolism thereby converting volatile fatty acids into the desired end products [6]. Consequently, the concerted interplay of these microbes culminated in the production of biogas whose cumulative value was 4600cm^3 at the end of the study. However, an increase in the number of days can often bring about a drop in the output. This may probably be owing to exhaustion of nutrients since anaerobic bacteria needs suitable nutrients to thrive in any environment that they are kept.

Furthermore, the one way ANOVA test was performed between the measured and modeled log of total bacterial counts as illustrated in Fig. 4. The test showed no mean significant difference. Moreover, the data set of both the measured and modeled output showed a normal distribution with no outlier. The mean of both dataset from the ANOVA plot was 4.945 and 5.030, respectively.

IV. Conclusion

From the results of this study, it is worth mentioning that dairy cattle manure is endowed with a considerable biogas production potential evaluated through anaerobic decomposition that offers numerous benefits of environmental, agricultural and socio-economic standards.

Acknowlegment

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Dr. Simon has published a number of research articles in peer-reviewed journals and conference proceedings. His current areas of research include solar energy technologies, energy efficiency as well as power engineering. He is also an energy consultant.

Anthony I. Okoh was born in Nigeria. He holds a Bsc (Hons), Msc and PhD degrees in microbiology at the Obafemi Awolowo University in Nigeria. He also holds 10 other diplomas and certificates in several aspects of biotechnology and molecular biology from reputable institutions on the continents of Africa, Asia, Europe and America. In the past, he was a senior lecturer at the Obafemi Awolowo University in Nigeria, and retired from the University to take up an appointment as associate professor of microbiology at the University of Fort Hare, South Africa in 2006. In January 2008, he was promoted to be a full professor of microbiology in the same university, and in January 2009, he was appointed to be the head of the Department of Biochemistry and Microbiology till now. He has therefore garnered extensive experience not only in teaching and research but also in administration, having also served in numerous committees in the University system including the appointment and promotion committee as well as elected for a two years term as president of Obafemi Awolowo University Staff Club in 2002.

His research expertise falls within the aegis of applied and environmental microbiology with particular emphasis on microbial water/ wastewater quality, and bioactive compounds of health and biotechnological importance. He has also been a recipient of such awards as Postgraduate Fellowship Award, Obafemi Awolowo University, Ile – Ife, Nigeria, 1990 – 1992; United Nations University Fellowship (1998); UNESCO Biotechnology Action Council Fellowship (2000), as well as several grants from the NRF, MRC, WRC, ESKOM, RS-DFID and ISRAR/APUA. Currently his publication stood at over 300 publications made of over 190 journal articles; several conference presentations and nucleotide sequences deposited in the GenBank in my academic career of over two decades.

Prof. Okoh established his research group called Applied and Environmental Microbiology Research Group in the Department of Biochemistry and Microbiology of the University of Fort Hare. In 2008, he won the University of Fort Hare Vice-Chancellor Emerging Researcher Award, and in 2011, the Vice-Chancellor Senior Researcher Award. In 2009, he was invited to represent South Africa in the international collaboration on the Surveillance of Reservoirs of Antibiotic Resistance (ISRAR) under the auspices of the Alliance for the prudent use of Antibiotics (APUA) with headquarters in the Boston, USA. In 2011, he was elected as president of the South Africa Society for Microbiology (2011- 2013), and in November 2013, he was appointed as leader of the Water Resources for Sustainable Development Niche Area at the University of Fort Hare for the next five years. He is also a member of the South Africa National Committee on IUMS, and also a C2 (Established Researcher) rated researcher.