

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY



Laboratory Scale Biogas Production and Evaluation Using Different Substrates

Research Project Final Report

Investigators: Mr. Haftu Abraha (PI) &
Mr. Mekuanint Lemlem (co)

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Acknowledgement

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Abstract

The purpose of this study was to assess the potential of different readily available agricultural wastes for the production of biogas. Cattle manure, sugar cane bagasse, chicken manure, liquid slaughter house wastes and a 1:1 (w/w) combination of cattle and chicken manure were tested for biogas generation at retention time of 15,30,45 and 65 days. By taking one month predigested fresh cattle slurry as a starter culture, 10% dry matter of the substrates were digested in newly constructed 5 liters plastic bio digester at ambient temperature. The result showed that a maximum methane production of 54.7% for cattle manure, 50% for sugar cane bagasse, 30.7% for chicken manure and 65% for the 1:1 combination of cattle and chicken manure. However, the 1:1 cattle and chicken manure appeared as the best potential raw materials for biogas production (65%, n=3) with in retention time of 30 days. In conclusion, the tested agricultural wastes showed variable biogas production potential on different retention times. Therefore, anaerobic digestion of agricultural wastes in proper incubation period enables to get maximum concentration of methane, which is considered as green energy and eco-friendly that fosters sustainable development in energy utilization.

Key words: Biogas, Cattle manure, Chicken manure, Methane, Sugar cane bagasse

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List of Acronyms AD: Anaerobic digestion
ASTU: Adama Science and Technology University
CH₄: Methane
CO₂: Carbon dioxide
FAO: Food and Agriculture Organization
GTZ: German Agency for Technical Cooperation
H₂S: Hydrogen sulfide
HCl: Hydrochloric Acid
NaOH: Sodium hydroxide
NH₃: Ammonia
pH: Hydrogen ion concentration

PPM: Parts per million

SD: Standard deviation

1. Introduction

1.1 Background

Biogas is derived from organic materials on decomposition by a group of microorganisms in the absence of oxygen which is called anaerobic digestion (Weiland, 2010). The components of the biogas vary depending on the raw material being decomposed. It mainly contains mixtures of combustible gases methane (CH₄) and carbon dioxide (CO₂) and other trace amounts such as hydrogen sulfide (H₂S), hydrogen (H₂) and ammonia (NH₃) etc [CITATION Jør091 \l 1033]. Biogas plants can use different origin of starting materials. Agricultural manure and plant biomass, municipal food waste and industrial waste can be converted in to biogas[CITATION Luo11 \l 1033] .

Biogas can be utilized in various forms. For instance, heat and electricity can be generated from it. When the biogas is purified, it can substitute vehicle fuels with reduced nitrogen oxide, hydrocarbon and carbon mono oxide emissions, which minimizes the release of green house gases [CITATION Zha101 \l 1033]. Therefore, biogas plants provide an alternative energy source and play pivotal role in protecting environmental resources by minimizing deforestation and desertification [CITATION Mor081 \l 1033].

Biogas production process encompasses four basic biological steps. The first step is the hydrolysis process where complex organic compounds are degraded in to simpler organic compounds. Hydrolytic bacteria in a community are responsible for this process. In the second phase, the end products of hydrolysis (sugars, amino acids and fats) are degraded in to organic acids and alcohols by bacterial consortium called acidogenic bacteria and hence the process named after as acidogenesis. The third step is the acetogenesis, where hydrogen and carbon dioxide and (or) acetic acid (acetate) are generated from the second phase final products of biogas. This step is conducted by group of acetogenic bacteria. The last step is methanogenesis, which results in methane and carbon dioxide from the output products of the third step. Now it is called biogas which is obtained by consort of methanogenic bacteria [CITATION Raj171 \l 1033]. However, in a natural process of

AD, all of the microbial communities responsible for the above biodegradation steps dwell in harmony together.

Biogas was introduced to Ethiopia not more than a half century of age (1958). The biogas production centers were monitored by two directorates namely: Alternative energy technical dissemination and promotion directorate and the alternative energy design and development directorate. Two pilot biogas plants were attempted by FAO project in 1970. About 25 years later, nearly 1000 biogas plants were installed by governmental and nongovernmental organizations. It was in 2006 that national biogas program for Ethiopia formulated from previous responsible agencies. Because of this, in 2009 to 2013, about 14,000 plants were planned to be installed [CITATION Sm1111 \l 1033].

This experience tells us that the focus and awareness is increasing from time to time. However, getting quality service of the digesters remained a challenge that requires further improvement.

1.2 Statement of the problem

Currently, although the biogas plants constructed have been increasing in Ethiopia, they are not fully operational; users are not getting proper support from professional trainers. A clear observed communication gap exists between stake holders and variability in reactor design intensifies complexity in understanding biogas production process. Therefore, this study was motivated to do a research to harvest biogas for energy production, which might contribute in increasing our understanding and take part in addressing the technical problems that could happen in local biogas production services. This source of energy particularly benefits poor people with readily available by products from their farming activity. Therefore, by choosing easily available biomass ensuring bio-digester functionality for biogas usage is a continuous challenge and need to be studied intensely.

1.3. Objectives

1.3.1 General Objective

- To produce biogas from different agricultural wastes in a laboratory scale

1.3.2 Specific Objectives

- ◆ To validate biogas production at economically feasible temperature conditions
- ◆ To identify the best raw material for maximum biogas production
- ◆ To determine optimum retention time of substrates for improved biogas production

1.4 Significance of the study

The utilization of biogas as an alternative source of energy in Ethiopia is at its infant stage. Therefore, biogas plant construction and biogas production validation will improve users understanding and cater the energy demands in rural areas that do not have access to electricity. Such research pipeline is in line with the efforts that foster sustainable, green energy. This study has a particular benefit for:

- ✓ Farmers, rural and semi rural communities around Adama science and technology university either for cooking, lighting, provide bio-slurry which establish a basis for organic farming activities from house hold pollutants and biomass.
- ✓ Adama science and technology university (ASTU) would be a training center for biogas production technology and become an active participator of community service.
- ✓ The neighbor industries can be benefited from their by-products generating energy for their consumption and avoid environmental contamination by converting their toxic wastes in to beneficial products which are eco-friendly.

2. Literature Review

2.1 Factors affecting biogas production process

Various parameters can be considered for maximal biogas production. Among the well studied key parameters: Temperature, length of incubation period, nutrient composition, and pH are very few to mention. The effects of these parameters are elaborated in the following sections.

2.1.1 Operating temperature

Temperature is one of the key parameters for maximum biogas production. In similar way to other chemical reactions, increasing the working temperature of a reactor increases biogas yield [CITATION Cha081 \l 1033]. A comparative study of anaerobic digestion of fruit and vegetable waste depicted that a maximum biogas yield attained under thermophilic temperature condition than in low and moderate temperature conditions [CITATION Bou041 \l 1033]. The substrate conversion efficiency is robust in this state causing improved biogas production [CITATION Vin091 \l 1033].

2.1.2 Feeding load

Anaerobic digesters also prefer optimum feed load for better production of biogas. Under load and over load can result in low biogas yield [CITATION Cha081 \l 1033]. When excess amount substrate added in each cycle, it heavily affects the biogas production by over production of intermediate products, which can have process inhibition effect. On the other hand, substrates become rate limiting to the inoculums size. Anaerobic digestion process operating on mesophilic condition is vulnerable to high organic feeding load than system based on thermophilic condition [CITATION Gou141 \l 1033]. Therefore, it is crucial to tailored the optimal biomass loading rate to the type digesters running with differing set ups.

2.1.3 Inoculum to substrate ratio and source

Methane yield of biogas reactor can be affected by the amount of inoculums employed. A variable methane output was observed by in one study [CITATION Gun951 \l 1033].

Increasing the substrate to inoculums ratio negatively affects the biogas yield [CITATION Liu092 \l 1033]. In species specific study of anaerobic digestion for biogas yield, a 75%

decrease in inoculum ended up in biogas yield reduction of 33% [CITATION Zen101 \ 1033]. Moreover, the source of the inoculums also matter. For instance, keeping other parameters the same, digested manure as starter culture brought a high biogas yield than taking sludge for the same purpose [CITATION GuY141 \ 1033].

2.1.4 Raw material pretreatment

For efficient biogas production, subjecting the substrates to biological agents (yeast and bacterial) can be prepared in a dried powder form to maximize biodegradability [CITATION Zho111 \ 1033]. Similarly, mechanical size reduction of starting material of sisal fiber enhanced biogas yield by a factor of more than two fold [CITATION Msh061 \ 1033]. However, substrate over reduction in size can have adverse effect on biogas production because of bulk production of volatile fatty acid in early phase, which inhibits the digester process [CITATION Izu101 \ 1033].

2.1.5 Carbon to Nitrogen ratio

Although there is no perfect, single optimum combination of carbon and nitrogen, about 25-30:1 combination of the two nutrients portrayed maximal methane production in anaerobic digester [CITATION Wan121 \ 1033]. This holds true when other conditions such as temperature, pH are controlled and under low level of free ammonia. For this reason, anaerobic digestion of algal sludge that was previously reported as poor substrate, improved on addition waste paper, which balanced the elemental composition of the substrate close to the ratio stated above [CITATION Yen071 \ 1033]. Differences in nutrient composition carbon and nitrogen ratio can affect not only the biogas yield but also can influence the time elapsed for the maximal biogas production [CITATION Sin031 \ 1033]. If the optimal combination is not attained, in addition to the effect on biogas yield at specific time point, it can inhibit the whole anaerobic digestion process. Therefore, negligible amount of biogas was obtained from substrates such as leather fleshing waste with high nitrogen content, which of course, can be utilized by co-digesting with biodegradable municipal wastes that normalizes the carbon to nitrogen ratio [CITATION Sha091 \ 1033].

2.1.6 Hydrogen-ion (pH) concentration

Operating pH condition of anaerobic bio-digester is among the various parameters that can directly affect biogas productivity. This is because microorganisms prefer specific pH range for their maximum growth. A study using rice husk as energy source depicted a peak production of biogas at pH 7.00 [CITATION Oke141 \l 1033]. Fermentation at neutral pH condition of waste activated sludge recorded high methane production potential compared to both acidic and basic pH regimes [CITATION Zha091 \l 1033]. In general, pH regimes 6.5 to 8.5 support anaerobic digestion process efficiently [CITATION Msh061 \l 1033].

3. Materials and methods

3.1 Raw material collection

Fresh, clean chicken manure was collected from local, small poultry farms in Adama city, Ethiopia. Slaughter house waste was also obtained from Adama governmental slaughter house which is found on main high way crossing Adama , exit road to Wonji town. The third substrate, sugar cane bagasse, was obtained from Wonji sugar factory. Fresh cattle slurry and manure was also obtained from house reared cattle for milk production, Bole, Adama city.

3.2 Raw materials pre-treatment

After collection, all the raw materials were dried under sun light for about two weeks. There was a particle size differences that matters the bio-degradation of the biomass. To compensate for this initial biomass size discrepancy, each substrate was shredded in to small size by mortar and pestle. To increase surface area for the degradation process, the raw materials were sieved by local tea strainer whose mesh size is two millimeter (2mm). The dry weight of each substrate passing the mesh was collected separately and then subjected for further process and the bulk biomass was excluded from the study. Only the waste collected from slaughter house facility was directly incubated after sieving.

3.3 Total solid (TS) preparation

After sieving, each of the collected substrates were subjected to further dry at 105°C in drying oven for 24 hours using clean aluminum foil. The drying process was continued for each substrate until a constant dry weight recorded in an electronic weighing balance.

3.4 Substrate preparation

All substrates except slaughter house waste were mixed with the working tape water after dry matter preparation. In brief, 350g dry matter (TS) of each substrate was mixed with 3.5L of tape water. 2.5L of liquid slaughter house waste was mixed with 1L of tape water. The same inoculums and size (10% V/V) was utilized for all treatments.

3.5 Experimental setup and Reactor design

In this study, the reference working volume was taken as described elsewhere [CITATION Anu04 \l 1033]. In brief, the anaerobic reactor working volume was set to be at a laboratory scale which is five liters. Five liter's plastic bottles were adapted in to anaerobic digester. Initially, it was thoroughly washed with detergent and cleaned. Then, each plastic bottle was painted with black ink to block light penetration, creating dark condition and avoid free oxygen release by algal photosynthesis. Perforations for gas outlet and pH sampling were properly sealed by rubber tubes and adhesives, creating perfectly airtight anaerobic bio-digester. The components of bio-digester were assembled and seed up as shown in Figure 1. All experiments including controls were conducted in triplicates, operating under batch conditions at ambient temperature (14.2-29.4°C) and the pH was adjusted to 7.00. Each anaerobic reactor contents were mixed manually every day by gentle agitation [CITATION Kap081 \l 1033]. In order to correct for the back ground biogas production from the inoculums, the same inoculums size was mixed with equal volume of boiled tape water for anaerobic digestion. The initial substrate characteristics and operating conditions are shown in Table 2.

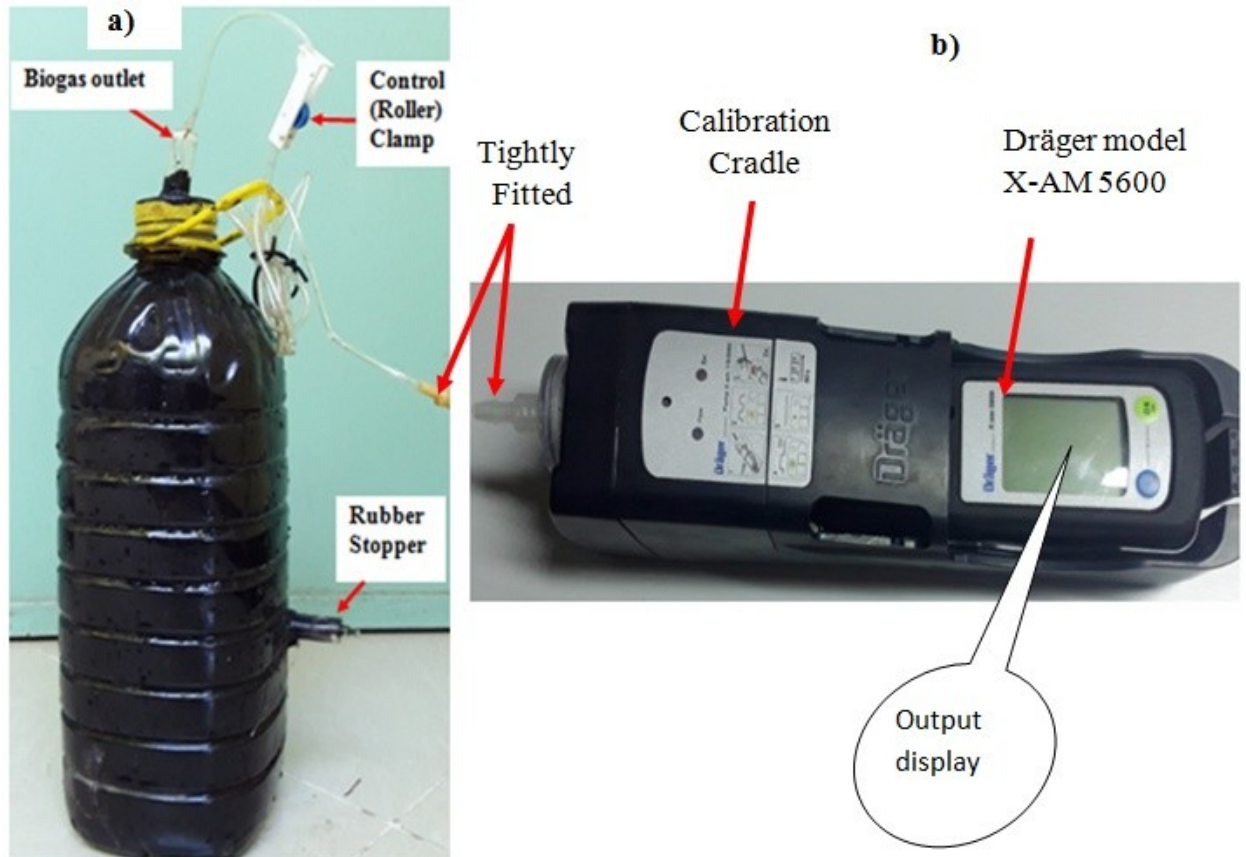


Figure 1: Newly constructed laboratory scale plastic anaerobic bio-digester design and set up. a) Customized anaerobic reactor (left side). b) Dräger X-am 5600, multi gas detector device inside calibration cradle (right side). The digester gas outlet via I.V Infusion set and signal receiving end of the measuring device are connected by protruding tube of calibration cradle.

3.6 Feeding inoculum preparation and size

The inoculum was prepared according to Pound, B., *et al.*, (1981), with slight modification. Mixed fresh cattle slurry and pre-boiled, cooled tape water (1:1 W/V respectively) were digested for 30 days, in an air tight sealed container. This methanogenically active inoculum and each substrate were mixed according to Manikadan & Arumugan, (2010), in a 1: 10 ratio V/V respectively. In brief, the inoculums size was taken in 10% of the total working volume.

3.7 Initial pH measurements and adjustment

Tape water was initially boiled to kill oxygen producing algae and cooled for few hours. Then, the raw materials were mixed with the cooled tape water; the initial pH was

recorded for each substrate (Table 1). All the pH adjustment measurements (initial, during digestion) were done by using digital pH meter (Pillow bread, Inc). Then, the initial pH of each substrate was uniformly adjusted to pH 7.00 by adding 2N NaOH and 2N HCl. During the digestion process, a sample volume of 40 ml was taken using syringe from each reactor in each data measuring points for pH determination within few hours.

Table 1: List of the starting raw materials for biogas production and their pH

Substrate type	Initial pH (Mean \pm SD)
Cattle manure	7.2 \pm 0.2
Chicken manure	6.2 \pm 0.4
Sugar cane bagasse	7.6 \pm 0.3
Slaughter house waste	7.3 \pm 0.2
Mixed cattle and chicken manure	7.1 \pm 0.5
Predigested (30 days) cattle manure (inoculums,1:1w/v cattle slurry: tape water	6.8 \pm 0.4

NB: SD: standard deviation, \pm : plus or minus

Table 2: Main characteristics of substrates and digester operating conditions

Item	Value	Units
Particle size	≤ 2	Millimeter
PH after adjustment	7.0	
Substrate total solid	100	Gram/Litter
Digester volume (Total)	5	Litter
Working volume	3.5	Litter
Head space	1.5	Litter

3.8 Biogas measurement

Biogas components generated (methane, hydrogen sulfide, carbon dioxide) were measured with a portable equipment, Dräger model X-AM 5600 (Figure 2 a), which contains two types of sensors (electrochemical and infrared). This device simultaneously detects up to 6 gases at a time [CITATION Mac171 \l 1033]. Moreover, the device possesses a measuring detection limit for each biogas components [CITATION Sch141 \l 1033]. In this

experiment, the device was used to gage the amount of biogas components produced in different time points. Data from the experiment was collected in 15 days interval, by starting at day 15 up to day 60. The device was first calibrated by atmospheric oxygen level by placing it in a calibration cradle (Figure 2b). After confirmation, the biogas outlet of each treated digester was fitted in to the cradle station inlet facing to the device signal receiving end. Data was recorded manually for each treatment.



Figure 2: Multi Gas Detection Device a) X-am-5600 b) Calibration cradle

3.9 Data analysis

Collected data was analyzed using Microsoft office excels, 2007. All values were presented as mean plus or minus standard deviation.

4. Results and Discussions

4.1 pH determination of running digesters

In each incubation period of bio-digesters in AD, their respective pH conditions were recorded (Table 3). Among many other parameters, pH is a well established variable that ascertains the future stability and performance of anaerobic digester [CITATION Pol07 \l 1033]

Table 3: pH of bio-digesters (n=3 for each treatment)

Substrate type digested	Mean pH during incubation periods				pH SD over all incubation period
	15 th Day	30 th day	45 th day	60 th day	
Cattle manure	6.2±0.4	6.8±0.3	7.1±0.3	5.0±0.3	0.93
Sugar cane bagasse	6.5± 0.3	6.7±0.2	7.4±0.3	9.1±0.6	1.18
Chicken manure	6.8±0.2	6.9±0.4	7.3±0.4	9.2±0.7	1.12
Slaughter house waste	7.4±0.3	7.0±0.2	6.5±0.3	6.0±0.2	0.61
Cattle +chicken manure (1:1w/w)	6.9±0.4	7.5±0.1	7.4±0.4	7.3±0.3	0.26

NB: SD: standard deviation, ±: plus or minus

Most digesters tested here showed relatively higher pH variation (see Table 3). Specifically, digesters with sugarcane bagasse and chicken manure depicted higher pH changes in relation to the other digesters tested. On the other hand digesters with cattle manure and slaughter house waste illustrated moderate pH changes in the test categories. Co-culturing cattle and chicken manure showed relatively stable pH conditions. This pH stability would have process stability and should help in attaining maximum methane yield.

The pH change to the alkaline region observed in sugarcane bagasse (Table 3) may be associated to cation mobilizations from the biomass, leading to pH instability [CITATION Iny10 \l 1033]. This pH change directly affects the survival methanogenic microorganisms and therefore forced to focus on their homeostasis balance in place of synthesizing activities, which diminishes the biogas product [CITATION Gut09 \l 1033] . This outcome in the long run adversely affects the smooth running of the reactor.

Chicken manure anaerobic digestion ended in pH rise. The high protein content and high level of acetate can result in elevated pH. Acetate, which is an intermediate product, can react with ammonium increasing the alkalinity by forming ammonium acetate or ammonium bicarbonate [CITATION Sha091 \l 1033]. This could be one possible justification for the observed variation and the increasing trend.

The digesters with slaughter house waste experienced a slight pH drop (Table 3) in the later stages of incubation time. The pH drop in these reactors might be associated with rapid degradation of lipid particles at late stage. This causes formation of excess long chain fatty acids and volatile fatty acids reaching toxic level to methanogenic microbials, leading to pH drop [CITATION Bro98 \l 1033]. The pH reduction directly affects the process efficiency. Relatively lower methane content has been observed, indicating the negative consequence on the yield. However pH could not be the main reason for the observed low methane content. Despite there was pH decline, the observed pH range is not far from the optimum condition.

Our investigation showed that mixing substrates (cattle and chicken manure) resulted in more stable pH condition. Our finding is in agreement with Li et al., (2013). Co-fermenting cattle manure and chicken manure have synergic effects on anaerobic process stability by keeping appropriate carbon to nitrogen balance and buffers the system by maintaining low total volatile fatty acid to total alkalinity ratio favoring many microbial cells to grow. Therefore, this could be one reason that accounts for the observed pH stability [CITATION LiY13 \l 1033].

4.2 Methane production from Cattle manure

As shown in Figure 3, anaerobic digestion of cattle manure gave 35.7 % of methane yield in retention time of fifteen days. After further digestion of this substrate for another 15 days, the methane concentration improved to 54.7%. The methane increment in the first two digestion time points can be supported by existence of optimum pH (Table 3). The 19% change in concentration appeared to be the highest among all incubation periods. This result is in agreement with Aremu, 2012. According to these authors, a maximum amount of methane was harvested in 30 days of mesophilic digestion time, which is similar to the current result. However, in our experiment, the increase in methane content from cow manure was not observed in the next retention time (45th day) of anaerobic digestion (49.7%), i.e the percentage of methane production reduced by 5 %. Methane content in the biogas in 60 days of retention time further declined in to 29.67%. The decrease in methane content in the 60th day may be partly explained by pH drop in the

reactor, but it does not hold true for 45th day incubation time (Table 3). The maximum methane content in this study (54.7%) however, is lower than to the previous finding obtained by Ukpai, 2012, which was 67.9%. Of course, the working volume of the digester in this literature was nine fold higher compared to the current experiment. This volume difference and variation in set up could be the reason for the observed variation.

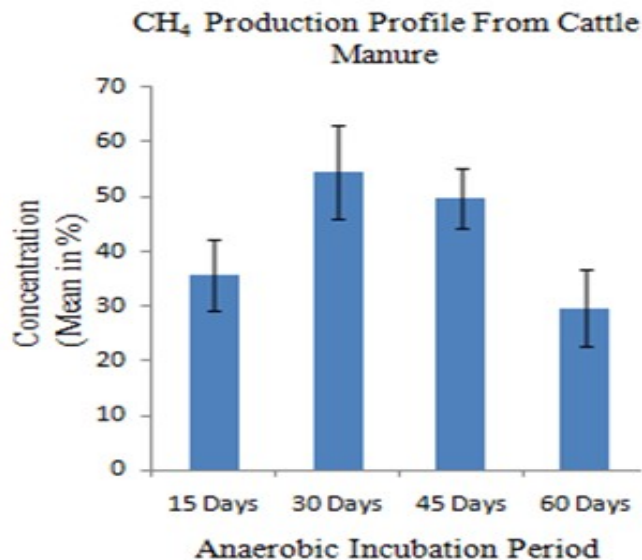


Figure 3: Cattle manure anaerobic digestion for Methane (CH₄) production. The Error bars shown on each bar graph were obtained from triplicate experiments. Average methane contents were corrected for background methane production.

4.3 Methane production from sugar cane bagasse

Sugar cane bagasse was anaerobically digested in similar parameters as that of cattle manure for biogas production (Figure 4). This substrate gave 31% concentration CH₄ in 15 days of retention time. This amount is comparable to cattle manure. Elongating the retention time to 30 days, resulted to 35%, which is a small change (4%). However, prolonging the retention time to 45 days highly improved the CH₄ concentration (50%), compared to the 30 days methane production profile. This result is consistent with the finding reported by Janke, 2015. Increasing the retention time gives more chance to degrade fiber rich raw materials like sugar cane bagasse and other straw. The increasing pattern was not observed in the last incubation time (60th day). Though the main reason is unknown, the digester showed a huge pH rise in 60th days (Table 3), which may be

difficult for the methane producing microbial consortium to run their normal growing condition and affecting their enzymatic activities. Therefore, this could be among the possible justification for the reduced methane content in the 60th day. The biogas in the form of methane generated in this experiment from sugar cane bagasse is very promising. But, the production from this substrate can be boosted by applying alkali pretreatments (Bolado-Rodríguez, 2016; Rabelo, 2011). This approach would have principal benefit for the initial incubation periods by making the biomass efficiently degraded. However, since alkaline treatment rises the pH, this is not the right option in last incubation period, where we noticed pH increment.

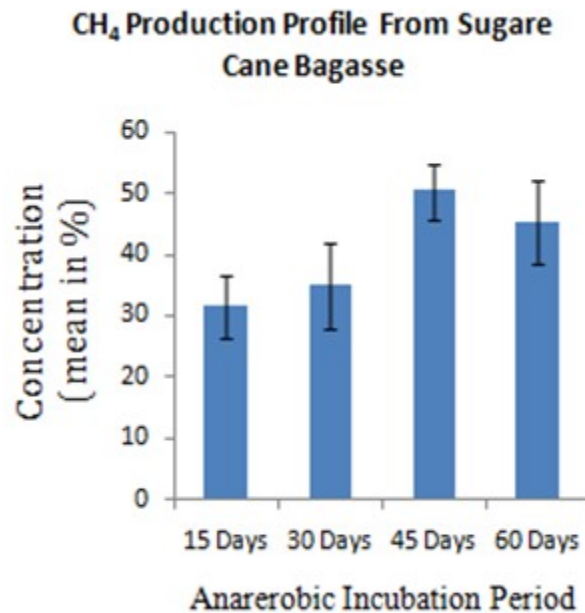


Figure 4: Sugar cane bagasse anaerobic digestion for Methane (CH₄) production. The Error bars shown on each bar graph were obtained from triplicate experiments. Average methane contents were corrected for background methane production.

4.4 Methane production from Chicken manure

Chicken manure was anaerobically digested for biogas production in the form of methane. Initial record from digestion of 15 days gave 5% of methane gas (Figure 5). Extending the anaerobic digestion time to 30 days enhanced the methane yield by more than two fold (11.7%). Further elongation of the retention time to 45 days maintained the increasing trend in methane yield (30.7%). However, drop in methane percentage was

noticed in 60 days retention time. The decrease in methane production is due to pH change to the alkaline region (Table 3), together with ammonia accumulation because of the natural richness in nitrogen (Callaghan, 1999). Therefore, methods that detoxify or reduce excess ammonia should be investigated.

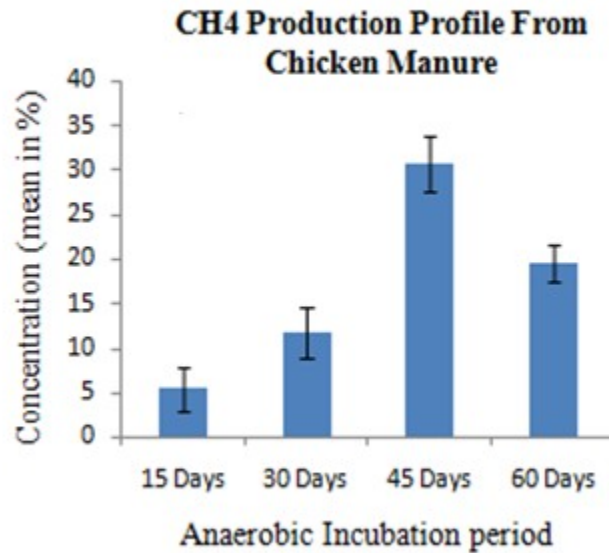


Figure 5: Chicken manure anaerobic digestion for Methane (CH₄) production. The Error bars shown on each bar graph were obtained from triplicate experiments. Average methane contents were corrected for background methane production.

4.5 Methane production from slaughter house waste

After physical separation of bulk materials from liquid slaughter house waste, it was subjected for an anaerobic digestion. Incubation of this substrate for 15 days resulted in methane content of 46% which is relatively higher than the cattle manure methane content at that time point. The methane concentration obtained from this substrate increased at 30 days incubation period (49.4%). However, a drop in methane content was depicted in 45 days and 60 days of retention times (Figure 6). Maximum methane content was gained in the first incubation periods (day 15 and day 30), which is in line with previous report by Palatsi, 2011. This can be explained due to the rumen content in the substrate which was used as seeding inoculums (Rismani-Yazdi, 2007).

The methane content reduction in the last two incubation periods (day 45 and day 60) may be due to the pH change to the acidic region (pH 6) (Table 3). Furthermore, the natural high fat content of the cattle slaughter house waste might have reached its inhibitory concentration (Harris, 2015).

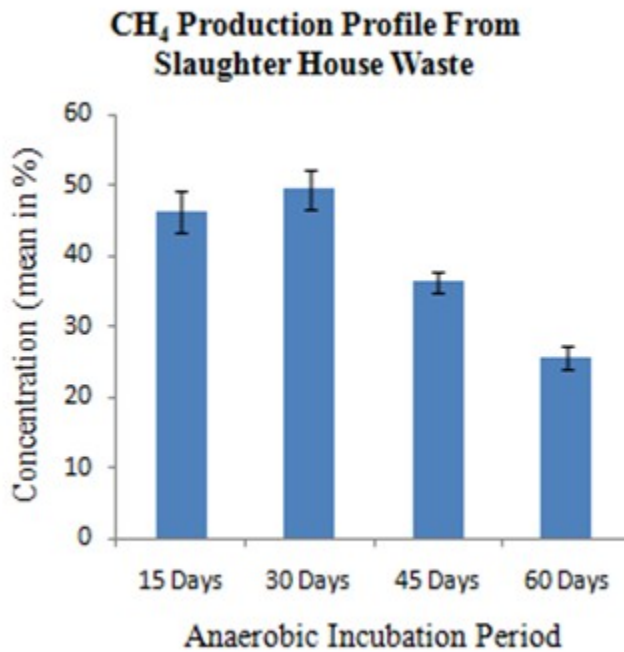


Figure 6: Slaughter house liquid waste anaerobic digestion for Methane (CH₄) production
The Error bars shown on each bar graph were obtained from triplicate experiments. Average methane contents were corrected for background methane production.

4.6 Methane production from mixed cattle and chicken manure

Although many combinations and different ratios are possible, in this experiment, we tested only one ratio and combination for resource reasons. Here, only the dry matter of the cattle manure and chicken manure in 1:1 ratio (W/W), then, the mixture was subjected to anaerobic digestion. In first measurement (day 15 retention time), the methane yield recorded as 40.7% (Figure 7). Elongating the incubation time to 30 days resulted in 65% methane content. Further extending of the digestion time to 45 days reduced methane content to 56.5%. According to Callaghan, 1999, chicken manure improves methane yield by balancing the carbon to nitrogen ratio, overfeeding of chicken manure for biodigesters inhibit the process due to the thigh nitrogen to carbon ratio. The

methane concentration further declined to 45% in 60 days of elapsed time in AD. The reduced methane content reported could be by ammonia inhibition (Callaghan, 2002), but the pH condition was relatively stable (Table 3). The inhibitory effect of chicken manure becomes more evident when extending the retention time of the bio-digester (Callaghan, 2002). However, the same authors showed that Co-fermenting cattle manure with vegetable, fruit wastes resulted in superior methane yield compared to digesting the substrate alone.

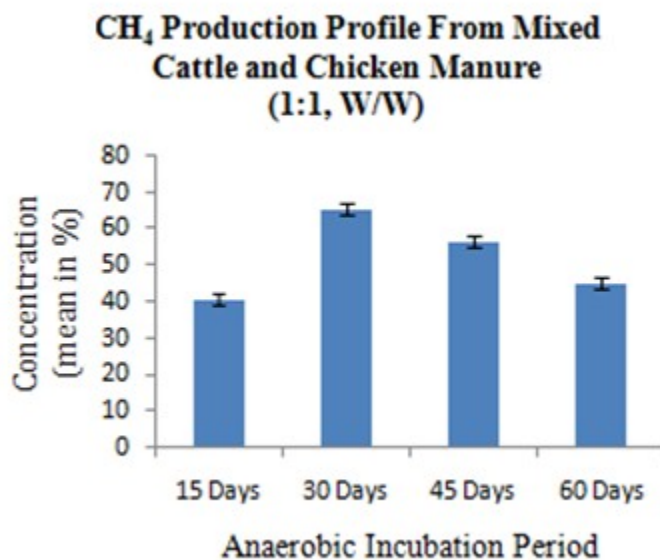


Figure 7 : Mixed Cattle and Chicken Manure anaerobic digestion for Methane (CH₄) production. The Error bars shown on each bar graph were obtained from triplicate experiments. Average methane contents were corrected for background methane production.

Methane (CH₄) production from different substrates

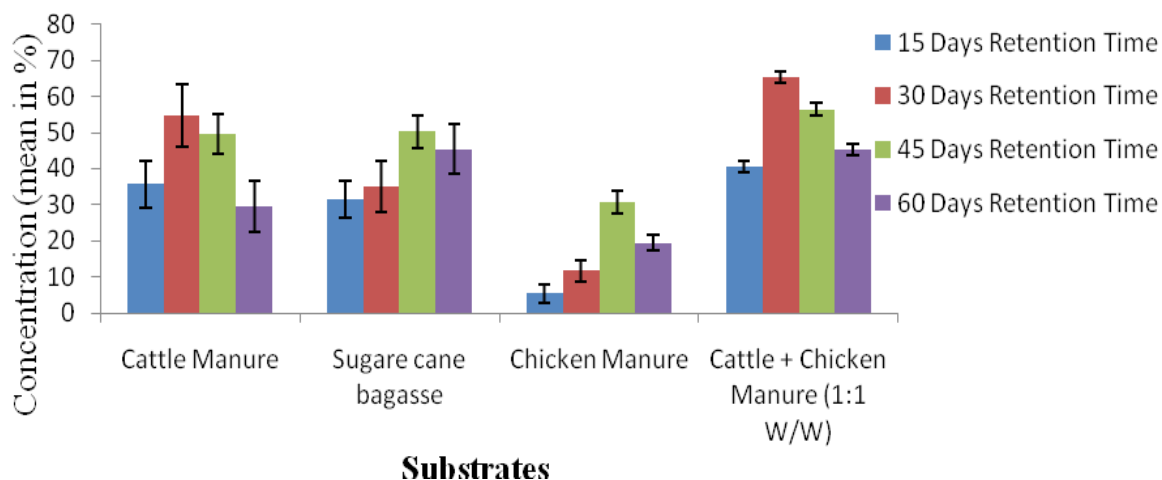


Figure 8: Methane production from different Substrates on different anaerobic incubation time points. The Error bars shown on each bar graph were obtained from triplicate experiments. Average methane contents were corrected for background biogas production.

5. Conclusion and Recommendations

5.1 Conclusion

In this study, we have generated methane using different substrates under ambient temperature (mesophilic condition). In controlled operating conditions, the raw materials gave a diverse methane concentration potential (Figure 8). We showed that charging biogas digesters with combination of cattle manure and chicken manure is superior in generating high methane content than other substrates fed with one substrate alone. Moreover, co-fermenting of substrates stabilized pH condition during the tested periods for anaerobic digestion process, which is one of the important factor for attaining maximum enzymatic activity by the microbial consortium and hence the methane yield. However, we were not able to directly compare the methane content generated from liquid slaughter house waste since its total solid was not prepared. For optimal biogas production, we have marked out that, substrate prefer a special retention time depending the biodegradability of its biomass nature.

5.2 Recommendations

In this current experiment, AD process was directly started up without flushing with the bio-digesters with nitrogen or other gases that can displace existing free oxygen level. Therefore, biogas production can be further enhanced by creating airtight anaerobic conditions by removing initial oxygen and hence speed up the start up of the digesters.

This experiment utilized fresh cattle manure as a starter culture. However, adding methane producing special natural strains or biotechnologically improved varieties in to bio-digesters can enhance the biogas production capability by degrading cellulose materials. We also recommend to incorporate methanogenic synthetic microbial consortium in to the current inoculums to completely digest the lingo-cellulosic materials under economically feasible working temperatures enhance bio-degradation.

For better understanding of the anaerobic process, instead of 15 days interval, the data measurement should be done online to track what is happening in between and there has to be a means to check the level of intermediate products (volatile fatty acids, acetic acid and others); thereby monitoring them to support a successful anaerobic digestion for maximum output.

In this report, we have controlled initial pH and utilized wider range, ambient temperature. For a comparative study, we recommend to conduct the research in buffered pH condition and narrow temperature range to account the biogas yield difference in to the substrate.

6. References

- Anunputtikul, W. &. (2004). Laboratory scale experiments for biogas production from cassava tubers. *Sustainable Energy and Environment (SEE). Hua Hin: Thailand* .
- Aremu, M. O. (2012). Comparison of biogas production from Cow dung and Pig dung under Mesophilic condition. *International Refereed Journal of Engineering and Science (IRJES)* , 1(4), 16-21.
- Bolado-Rodríguez, S. T.-J.-E. (2016). Effect of thermal, acid, alkaline and alkaline-peroxide pretreatments on the biochemical methane potential and kinetics of the anaerobic digestion of wheat straw and sugarcane bagasse. *Bioresource technology* , 201,18.
- Boleman T., F. J. (2011). *Building a laboratory scale biogas plant and verifying its functionality*. Bratislava : Slovak University of Technology.
- Bouallagui, H. H. (2004). Effect of temperature on the performance of an anaerobic tubular reactor treating fruit and vegetable waste . *Process Biochemistry* , 39(12), 2143-2148.
- Broughton, M. J. (1998). Anaerobic batch digestion of sheep tallow. . *Water Research* , 32(5), 1423-1428. .
- Callaghan, F. J. (2002). Continuous co-digestion of cattle slurry with fruit and vegetable wastes and chicken manure. *Biomass and bioenergy* , 22(1), 71-77.
- Callaghan, F. J. (1999). Co-digestion of waste organic solids: batch studies. . *Bioresource technology* , 67(2), 117-122.
- Chae, K. J. (2008). The effects of digestion temperature and temperature shock on the biogas yields from the mesophilic anaerobic digestion of swine manure. *Bioresource technology* , 99(1), 1-6.
- Gou, C. Y. (2014). Effects of temperature and organic loading rate on the performance and microbial community of anaerobic co-digestion of waste activated sludge and food waste. *Chemosphere* , 105, 146-151.
- Gu, Y. C. (2014). Effect of inoculum sources on the anaerobic digestion of rice straw. *Bioresource technology* , 158, 149-155.
- Gunaseelan, V. N. (1995). Effect of inoculum/substrate ratio and pretreatments on methane yield from *Parthenium*. *Biomass and Bioenergy* , 8(1), 39-44.

- Gutierrez, O. P. (2009). Effects of long-term pH elevation on the sulfate-reducing and methanogenic activities of anaerobic sewer biofilms. *Water research* , 43(9), 2549-2557.
- Harris, P. W. (2015). Review of pre-treatments used in anaerobic digestion and their potential application in high-fat cattle slaughterhouse wastewater . *Applied Energy* , 155, 560-575.
- Inyang, M. G. (2010). Biochar from anaerobically digested sugarcane bagasse. . *Bioresource Technology* , 101(22), 8868-8872.
- Izumi, K. O. (2010). Effects of particle size on anaerobic digestion of food waste . *International biodeterioration & biodegradation* , 64(7), 601-608.
- Janke, L. L. (2015). Biogas production from sugarcane waste: assessment on kinetic challenges for process designing . *International journal of molecular sciences* , 16(9), 20685-20.
- Jørgensen, P. J. (2009). *Biogas-green energy*. Faculty of Agricultural Sciences, Aarhus University.
- Kaparaju, P. B. (2008). Effects of mixing on methane production during thermophilic anaerobic digestion of manure: lab-scale and pilot-scale studies . *Bioresource technology* , 99(11), 4919-4928.
- Li, Y. Z. (2013). Evaluating methane production from anaerobic mono-and co-digestion of kitchen waste, corn stover, and chicken manure. *Energy & Fuels* , 27(4), 2085-2091.
- Liu, G. Z.-M. (2009). Effect of feed to inoculum ratios on biogas yields of food and green wastes . *Bioresource technology* , 100(21), 5103-5108.
- Luostarinen, S. N. (2011). *Overview of biogas technology*. European Union-European Regional Development Fund, Denmark.
- Macuda, J. Ł. (2017). Monitoring of explosive gases. With a portable gas detector dräger X-AM 5600** . 34.2.543.
- Manikadan, M. &. (2010). Potentiality of calotropis procera on the yield of biocrudes and biogas production. *Journal of Phytology* , 2(4): 33–40.
- Moradi, K. Z. ((2008). Reducing natural resource degradation by introducing biogas technology to qezel village of kermanshah province: An action research approach. *Environmental sciences* , vol.5, no2.
- Mshandete, A. B. (2006). Effect of particle size on biogas yield from sisal fibre waste. *Renewable energy* , 31(14), 2385-2392.

- Okeh, O. C. (2014). Biogas production from rice husks generated from various rice mills in Ebonyi State, Nigeria. . *Renewable Energy* , 62, 204-208.
- Palatsi, J. V. (2011). Anaerobic digestion of slaughterhouse waste: main process limitations and microbial community interactions . *Bioresource Technology* , 102(3), 2219-2227.
- Poliafico, M. (2007). *Anaerobic digestion: decision support software*. An MSc Thesis Presented to the School of Graduate Studies of Cork Institute of Technology.
- Pound, B. D. (1981). Biogas production from mixtures of cattle slurry and pressed sugar cane stalk, with and without urea . *Trop Anim Prod* , , 6:1.
- Rabelo, S. C. (2011). Production of bioethanol, methane and heat from sugarcane bagasse in a biorefinery concept. *Bioresource technology* , 102(17), 7887-7895.
- Raja, I. A. (2017). *Biogas Production: The Fundamental Processes*.
- Rismani-Yazdi, H. C. (2007). Electricity generation from cellulose by rumen microorganisms in microbial fuel cells. *Biotechnology and bioengineering* , 97(6), 1398-1407.
- Schirmer, W. N. (2014). Methane production in anaerobic digestion of organic waste from Recife (Brazil) landfill: evaluation in refuse of diferent ages. . *Brazilian Journal of Chemical Engineering* , 31(2),373-384.
- Shanmugam, P. &. (2009). Optimising the biogas production from leather fleshing waste by co-digestion with MSW. *Bioresource Technology* , 100(18), 4117-4120.
- Singhal, V. a. (2003). Biogas production from water hyacinth and channel grass used for phytoremediation of industrial effluents. *Bioresource technology* , 86(3), 221-225.
- Smith, J. (2011). *The potential of small scale biogase digesters to alleviate poverty and improve long term sustanability of ecosystem services in sub-saharan Africa*.
- Ukpai, P. A. (2012). Comparative study of biogas production from cow dung, cow pea and cassava peeling using 45 litres biogas digester. *Advances in Applied Science Research* , 3(3), 1864-1869.
- Vindis, P. M. (2009). The impact of mesophilic and thermophilic anaerobic digestion on biogas production. *Journal of achievements in materials and manufacturing Engineering* , 36(2), 192-198.
- Wang, X. Y. (2012). Optimizing feeding composition and carbon–nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and

wheat straw . *Bioresource technology* , 120, 78-83.

Weiland, P. (2010). Biogas production: current state and perspectives. *Applied microbiology and biotechnology*, 85(4), 849-860.

Yen, H. W. (2007). Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresource technology* , 98(1), 130-134.

Zeng, S. Y. (2010). Effect of inoculum/substrate ratio on methane yield and orthophosphate release from anaerobic digestion of *Microcystis* spp. *Journal of Hazardous Materials* , 178(1-3), 89-93.

Zhang, P. C. (2009). Waste activated sludge hydrolysis and short-chain fatty acids accumulation under mesophilic and thermophilic conditions: effect of pH. *Water research* , 43(15), 3735-3742.

Zhao, Q. L. (2010). Purification technologies for biogas generated by anaerobic digestion. *CSANR Research report* .

Zhong, W. Z. (2011). Effect of biological pretreatments in enhancing corn straw biogas production. *Bioresource technology* , 102(24), 11177-11182.

Author (s):

1. Name: Mr. Haftu Abraha
(Corresponding Author)

Address: Adama, Ethiopia.

Tel. +251910253163

E-mail: ahmba2127@gmail.com

Fax:

Signature:

Date:

2. Name: Mekuanint Lemlem

E-mail: meki4emu@gmail.com

Signature:.....

Date: