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Research Article

Production and Characterization of Biogas from Cow Dung, Poultry Manure and Their Co-digestion

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Abstract

The global demand for energy grows rapidly, and therefore, it is a time to look alternative and renewable resources of energy to replace fossil fuels that harm the environment. On other hand, improper waste management creates environmental pollution and makes it unpleasant and unattractive for residences. Cow dung and poultry manures are the wastes produced from livestock and chicken, and they are important feedstock for biogas production. The main objectives of the present study was therefore, production of biogas from cow dung, poultry manure and their co-digestion, and evaluate the effect of biogas production parameter on the performance of anaerobic digestion process. In this study, batch mode of experimental digesters operated for 60 days at 37±0.5°C using five different ratios of cow dung to poultry manure mixtures as a feedstock. The feedstock were 100% of cow dung (T_1), 100% of poultry manure (T_2), 50% cow dung and 50% poultry manure mixture (T_3), 75% poultry manure and 25% cow dung mixture (T_4) and 75% cow dung and 25% poultry manure mixture (T_5). The feedstock was characterized in terms of moisture contents (MC), total solids (TS), volatile solids (VS), pH, organic carbon (OC), total Kjeldahl nitrogen (TKN), carbon to nitrogen ratio (C: N) and ash contents (AC). Each digester was operated in triplicate and one way ANOVA was used to compare the characteristics of feedstock, amount and chemical composition of biogas produced from the different mixtures of feedstock. The volatile solid (VS) content was more than 58% in all feedstock and, which indicates that, the feed-stocks were biodegradable and suitable for biogas production. The Carbon to Nitrogen (C: N) ratio was in the range of 6.26±0.25 to 28.75±1.23. Lower C: N ratio (T_2) indicates the biogas produced from this feedstock will be low and hence T_2 is less preferred for biogas production. The pH value of all the feedstock were feasible for biogas production, except T_1 (6.3), and significant difference was observed in all parameters among the feedstock. The total amount of biogas produced from T_1, T_2, T_3, T_4 and T_5 was 2820ml, 1509ml, 3994ml, 15796ml and 6709ml, respectively. The highest biogas yield was recorded in T4. The quality of biogas in term of methane content was 56.3±0.91, 56.9±10, 57.8±0.95, 60.7±0.1 and 63.6±0.7 for T_1, T_2, T_3, T_4 and T_5, respectively. In general, the present investigation revealed that, co-digestion encourages the feasibility of biogas from these feedstock. Even though, the methane concentration is higher in T_5, T_4 (75% poultry manure and 25% cow dung mixture) can be considered as the best feedstock for biogas production as it gives significantly high biogas yield as compared to the others. However, optimization of production parameters and analyzing other production factors need to be investigated in the future.

Keywords

Biogas, Co-digestion, Cow Dung, Feedstock, Poultry Manure, Treatment

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1. Introduction

Historical evidences indicate that the anaerobic digestion is one of the oldest technologies for different fermentation products. The industrialization of anaerobic digestion began in 1859 with the first digestion plant in Bombay, India [22]. Anaerobic digestion has been exploited to produce biogas and organic fertilizers for hundreds, perhaps thousands of years by the Chinese, Assyrians and Persians. In developed countries, significant potential for biogas use exists. By 1895, biogas was recovered from a sewage treatment facility and used to fuel street lambs in Exeter, England [18]. In the 1930's anaerobic bacteria and the conditions that promote methane production were identified [22]. In Sweden, a biogas powered train has been in service since 2005. In the United Kingdom, biogas is estimated to have the potential to replace about 17% of vehicle fuel [29].

Following to the oil crisis in the 1970's and the high oil prices in recent years, the interest in renewable energy such as biogas increased in most parts of the world. Biogas technology was introduced in Ethiopia as early as 1979, when the first batch type digester was constructed at the Ambo Agricultural College [19]. In the last three decades, around 1000 biogas plants, ranging in size from 2.5m^3 to 200m^3 were constructed in households, community and governmental institutions in various parts of the country [44]. The technology is believed to have a huge contribution to fulfill the household energy demand of the country has no access to use advanced fuels such as petroleum and natural gas. In Ethiopia, almost all fuel energy requirements in the rural area is fulfilled by traditional biomass [64].

Energy is one of the most important needy resources. It is generally classified in either renewable or non-renewable. Bio-gas comes in the category of renewable energy sources. Renewable energy is energy generated from natural resources and can be replenished within a short period of time [50]. Biogas technology provides a very attractive route to utilize certain categories of biomass for meeting partial energy needs. Anaerobic digestion (AD) is a technology widely used for treatment of organic and biological waste for biogas production and provides a sustainable source of energy while simultaneously resolving ecological and agrochemical issues. The anaerobic fermentation of poultry manure for biogas production does not reduce its value as a fertilizer supplement, as available nitrogen and other substances remain in the treated sludge [29].

Anaerobic digestion (AD) is a natural biological decomposition of organic material in a controlled environment in the absence of oxygen. In this deoxidized zone, different bacteria types are employed to decompose the proteinaceous and carbonaceous materials to producing biogas and sludge. Reports indicate that depending on the type of raw material, biogas contains on average 50-70% methane, 30-40% carbon dioxide, 1-2% nitrogen, 5-10% hydrogen, and trace amounts of hydrogen sulfide and water vapor [24, 59]. Other showed that a mixture of gases comprising 50 to 75% methane (CH_4), 25 to 45% carbon dioxide (CO_2) and 0 to 5% a combination of hydrogen sulfide (H_2S), N_2, H_2 and others [53]. The carbon dioxide that is released when biogas is combusted and mixed with the oxygen in the air does not contribute to the greenhouse effect as the carbon in the methane molecule originates from carbon dioxide in the air that growing plants have previously taken up by photosynthesis [19].

Biogas is a renewable source of energy. It is a mixture of colorless, flammable gases obtained by the anaerobic digestion of organic waste materials. It is a well-established fuel that can supplement or even replace wood as an energy source for cooking and lighting in developing countries. Currently, as the fossil-based fuels become scarce and more expensive and the economics of biogas production is turning out to be more favorable. Biogas is a readily available energy resource that significantly reduces greenhouse gas emission compared to the emission of landfill gas to the atmosphere [6].

As a result, the use of biogas is thus an important step in climate change mitigation. The development of biogas represents a strategically important step away from oil dependency that will contribute to a sustainable energy supply in the long term. Biogas is also produced locally, meaning that it is not dependent on trade relationships. This also contributes to improved energy security [36, 25]. Even though, anaerobic digestion technology has more than a century historical background scientific interest and efforts in researching biogas production technology are still relevant because of the very high costs of energy supply worldwide. Another rationale for their relevance is the fact that the rampant use of firewood for domestic cooking in low income countries invariably results in the destruction of forests which is harmful to the environment [29].

Almost all Ethiopians around rural area depend on low quality biomass fuels like wood fuel, dung, charcoal and crop-residue for their household energy sources. This high dependence on biomass fuel leads to negative impact on socially, economically and environmentally [22]. Burning of wood, charcoal and animal dung causes indoor air pollution. The soot and dust which is produced during the burning can go deep in to the lungs and causes respiratory infections, results eye illness and may causes blindness. Wastes of animals and plants are also highly responsible for the cause of water borne diseases, sanitation problem and make an environment unpleasant and unattractive [2, 17]. Women and children walk a long distance and spend their time to gather firewood for cooking and lighting purpose. To overcome those problems; alternative renewable energy source is needed. On the other hand, the use of fossil fuels as primary energy source has led to climate change, global warming, greenhouse gas emission, environmental degradation and human health problems.

Biogas generation is simple and economical which can plant in large and small scale and generated renewable energy.

It reduces the use of traditional biomass as the source of energy and that contributes for GHG emission reduction [3, 25]. Taking the situation in to consideration, Ethiopia has launched the implementation of successive domestic biogas programs in the selected districts of four regional states: Amhara, Oromia, the former SNNPR and Tigray from 2009-2017 [19]. More than 2,800 biogas digesters were installed in two phases under the program. However, [16] showed that 47.12% of installed biogas digesters were non-functional due to feedstock limitation among other factors. Dung and poultry manures are the wastes produced from the animal and important feedstock for biogas production. Recently, poultry farms in Ethiopia have been rapidly expanding to meet the growing demand for meat and eggs [26]. There are approximately 60 million chickens found in Ethiopia and it is estimated that approximately 800,000.00 metric tons of poultry manure is produced in each year [12]. Using poultry manure alone as a feedstock for biogas production is not suitable. Because, it has high nitrogen content and low C: N ratio. Cow dung is also one of potential feedstock for biogas production. But, most of the rural area people in Ethiopia use the dung as a fertilizer for their garden and they do not use it as feedstock. As a result, many digesters are failed. However, production of biogas gives bio slurry as a byproduct with better plant nutrients. Therefore, the present work was proposed with the objective of production and characterization of biogas from cow dung, poultry manure and their codigestion for household consumption.

2. Materials and Methods

2.1. Description of the Study Area

The study was conducted at Bioenergy Laboratory in College of Agriculture, Hawassa University, Ethiopia. Hawassa University is found at Hawassa city, the capital of Sidama region located about 278 km south of Addis Ababa. The feedstock for the present investigation was collected from Hawassa University main campus dairy and poultry farms.

2.2. Materials and Chemicals

The following materials and chemicals were used in this research. Brine solution (mixture of NaCl and drop of lemon slaves), Distilled water, Tap water, Boric acid, NaOH (0.1 N), HCl (0.1 N), H_2SO_4 and H_2O_2 were chemicals used during anaerobic digestion experimental analysis. Oven, Furnace, PH meter, Asmaco epoxy steel, Digital balance, Distillation kit, Nitrogen digester, Geo tech gas analyzer, Plastic bottles, Water bath, IV set, graduated cylinder, Pestle and Mortal, Crucibles, Desiccator and Conical flask were the materials used for this investigation.

2.3. Feedstock and Inoculums

Two types of feedstock; namely cow dung and poultry manure were used as feedstock for this anaerobic digestion. A plastic container with cover was used for collection purpose. To start up the digestion process microbial inoculum was used from actively running anaerobic digester found at private house behind Central Hotel, Hawassa.

2.4. Sample Collection and Preparation

Fresh cow dung and poultry manures (10 kg of each) were collected from Hawassa university main campus small and medium enterprise dairy and poultry farms randomly. The feed-stocks were allowed to dry under open sun for four consecutive days and then pulverized using a pestle and mortar. Pulverizing is done to reduce the particle size and increase surface area of the feedstock particles for anaerobic digestion.

2.5. Slurry Preparation

Five hundred grams of finely powdered cow dung and poultry manure was taken according to proposed proportion and mixed well with one liter of distilled water in 10 liters plastic drum to transfer slurified feedstock in to digester. The contents were thoroughly mixed manually to achieve homogeneity as shown Figure 1. The contents were maintained at feedstock to water ratio of 1:2 for maximum biogas production as described by [1, 2]. Then slurified mixture was added in the digester and actual biogas production was takes place.

Figure 1. *Slurry preparation.*

2.6. Experimental Design

A total of 500 g feed stocks was added in an anaerobic digester setup for generation of biogas in five different combination treatments as indicated in Figure 1. The experimental treatments include 100% of cow dung and 0% of poultry manure (T_1), 100% of poultry manure and 0% of cow dung (T_2), 50% of cow dung and 50% of poultry manure mixture

(T_3), 75% of poultry manure and 25% of cow dung mixture (T_4) and 75% of cow dung and 25% of poultry manure mixture (T_5) at 37 ℃ ±0.5 temperature as indicated Figure 2. The feedstock was prepared from total 500 g of cow dung and poultry manure wastes according to the proposed proportion. Then, feedstock was mixed with 1 L of distilled water and transferred in to two liter capacity digester [34]. After the appropriate amount of feedstock transfer in to the digester, 75ml of inoculum was added to each digester to start the fermentation process [62]. After adding 75ml of inoculum, the digester was reached around 1600ml by volume. The experiments were conducted in triplicate and the design was completely randomized design (CRD). The digestion was continued until biogas generation was stopped [45].

Table 1. Experimental Design Summary.

Treatments (T)	Feedstock proportion	
	Cow dung (%)	Poultry manure (%)
T_1	100	0
T_2	0	100
T_3	50	50
T_4	25	75
T_5	75	25

2.7. Experimental Set-up

In this study, batch mode of experimental digesters was operated for biogas production from cow dung, poultry manure and the co-digestions. Anaerobic digestions processes of substrates were carried out in plastic bottles and that had plastic stoppers and each with a capacity of two litters. Three plastic bottles were arranged for an experimental processes and in a way that the first bottle was contained feedstock substrate and it is commonly known as a digester; the middle one was contained acidified brine solution and the last one was for collecting the acidified brine solution that was expelled out (displaced) from the second plastic bottle [18, 20]. As control group, one independent digester was prepared to the inoculum mixing with one litter of distilled water and this digester was important to give hint how much gas was produced from the mixed inoculum [35].

All those plastic bottles were interconnected with plastic glucose drip (tubes) having a diameter of 1 cm and length 1 m. The tube connecting the first plastic bottle to the second was fitted just above the slurry in the first bottle to the top of the second plastic bottle to help gas collection in to second plastic bottle. Valve (tubes) was used to control gas flow from digester to second plastic bottle during digestion process. During digestion time, the valve was opened to allow biogas collection in the second plastic bottle. The volume of biogas produced was measured using water displacement method and displaced water was measured by using graduate cylinder. Thus, the biogas produced by fermentation of the feedstock slurry was driven from the first bottle to the second bottle that contains a brine solution and so as to displace a volume of the brine solution equivalent to the volume of biogas produced. The lids of all digesters were sealed tightly using asmaco epoxy steel glue in order to control the entry of oxygen and loss of biogas. One water bath was used at a temperature adjusted for 37 ℃ ± 0.5 and the digesters effective volume was fully immersed in this bath. Automatic shaker were added in the digester to insure contact between the substrate molecules, nutrients and microbial cells. Then gas stored plastic bottle was connected to gas analyzer to determine CH_4, CO_2, O_2 and H_2S compositions in biogas [31, 48].

Figure 2. Laboratory experimental set up.

2.8. Feedstock Sample Analysis

2.8.1. Moisture Content

To determine the percentage of moisture content (%MC) in the samples, 5 g of fresh sample was taken and dried in an oven (Memmert, Germany; 100-800) at 105 ℃ for 24 hours and then reweighed. Finally, the moisture content was calculated as follow according to APHA [4].

$$\%MC = \frac{W-D}{W} \times 100 \quad (1)$$

Where:
%MC = Percent of moisture content
W = Initial weight of sample in grams
D = weight of sample after drying at 105 ℃ in grams

2.8.2. pH

One of the most determinant factors for anaerobic digestion process is the pH [42]. In this experiment, pH of the feedstock samples was measured by using laboratory pH meter. This pH meter was first calibrated at neutral pH. The sample mixed with distilled water and converted in to aqueous. Then the pH meter was inserted in to the aqueous sample solution and the reading was taken.

2.8.3. Total Solids

Total dry solids (TS) can be defined as the solid substance present in the sample which contains both organic and inorganic matter. To analyze it, first the crucibles was cleaned, dried in the oven and weighted. Then the sample was added to the crucible and weighted again. According to the Standard Methods for the Examination of Water and Wastewater 2540 B [54] oven was switched on and allowed to reach 105 ℃. Crucible with each sample type was placed in the oven and allow to dry overnight to ensure constant weight of the sample [11]. The dried sample was weighted immediately to avoid absorption of moisture due to its nature. Finally, the following calculation was computed to determine percentage of total solid in the sample.

$$\%TS = \frac{WDS}{WWS} \times 100 \tag{2}$$

Where:
%TS = Percent of total solids
WWS = Weight of wet sample in gram
WDS = Weight of dry sample in gram
WDC = Weight of dry crucible in gram
WWS = (WDC + WWS) - WDC
WDS = (WDC + WDS) - WDC

2.8.4. Volatile Solids

Volatile solids (VS) are the amount of solids that are lost under ignition of dried material at about temperature of 550 ℃. It explains the organic content of the substrate. In the present work, samples dried at 105 ℃ were further heated in the muffle furnace at 550 ℃ for 20 minutes [4]. Then the feedstock was removed from muffle furnace and the crucibles were cooled in the desiccator and after then the samples were weighted. Then the percentage of volatile solid was determined according to Equation (3).

$$\%VS = \frac{WVS}{WDS} \times 100 \tag{3}$$

Where:
%VS = Percent of volatile solids
WVS = Weight of volatile solids
WDS = Weight of dry sample at 105 ℃
WVS = WDS - Ash

2.8.5. Organic Carbon (OC) Determination

The OC was determined using the volatile solid data and calculated according to following equation.

$$\%OC = \frac{\%VS}{1.72} \times 100 \tag{4}$$

Where 1.72: the factor parameter [8].

2.8.6. Total Nitrogen

The total nitrogen content in the sample was determined by using the Kjeldahl method. This method has three main steps. These steps were digestion step, distillation step and titration step. Two gram sampled and 15ml of concentrated H_2SO_4 was added in tecator tube and mixed carefully. Then 5ml of H_2O_2 was added step by step. Violent color due to reaction was observed. As soon as the violent reaction has ceased, the tube was shacked by hand. Then the digester switched on and waits until it reaches 370 ℃. As the digester reached at this temperature, the rack was placed in it and the digestion continued for about 4 hours until clear solution was observed. Then the tube in the rack was transferred to the fume hood for cooling. About 50ml distill water was added and shacked by hand to avoid sulphate precipitation in the solution. At this time 25ml 40% NaOH solution was added into digested and diluted solution. Then 250ml conical flask containing 25ml of boric acid and 25ml of distilled water was placed under the condenser of the distiller for about 10 minutes until a total volume become between 200ml to 250ml. Finally the solution was titrated using 0.1N HCl to a reddish color and the following calculation computed to get $\%N_2$.

$$\%\text{Nitrogen } (N_2) = \frac{V \times 0.1 \times 14 \times 100}{Wo} \tag{5}$$

Where:
V = Volume of HCl in Liter consumed to end point of titration
Wo = Sample weight on dry matter basis
14= The molecular weight of nitrogen
0.1 = Normality of HCl

2.8.7. C: N Ratio

The relationship between the carbon and the nitrogen amount of the organic material is expressed in term of C: N ratio. The performance of the anaerobic digestion process mainly depends upon C: N ratio. The optimal growth and activity of bacteria require some essential nutrients in the correct concentration. The carbon (carbohydrates) supplies energy and the nitrogen (proteins) used for building up the cell structure [28, 54]. To compute the C: N ratio, firstly the content of organic carbon found in sample was determined according to Equation (4) and then, the amount of total nitrogen found in the sample was calculated using Kjeldahl method according to following equation. At the end, the ratio of carbon to nitrogen was calculated according to the Equation (6).

$$\text{C: N ratio} = \frac{\%\,C}{\%\,N} \tag{6}$$

Where:
C = Amount of organic carbon
N = Total Kjeldahl nitrogen

2.8.8. Ash Content

The ash content refers to the weight of the mineral content of biomass remaining after dry oxidation of the total solids of a given sample at 550 ℃. To determine the ash content, firstly the total solid was determined according to Equation (2). The obtained total solid (TS) was ignited at 550 ℃ in a muffle furnace for 5 hours to determine the fixed solid (ash) content of the sample [30]. These samples were cooled in desiccators to constant weight. The difference in weights in each case gave the volatile solids and the residue after ignition was the fixed solids.

$$\%\text{Ash content} = \frac{C-B}{A-B} \; X \;\; 100$$

Where:
A= Weight of dried residue + crucible in g
B= Weight of only crucible in g
C= Weight of residue after ignition + weight of crucible in g

2.9. Measuring the Amount of Produced Biogas

The amount of biogas produced was measured by water displacement method. The amount of gas produced is equivalent to the amount of water displaced in the water chambers according to Archimedes principle of floatation. In order to prevent the dissolution of biogas in the water, brine solution was prepared as suggested by [22]. An acidified brine solution was prepared by adding sodium chloride (NaCl) to water until a supersaturated solution was formed. Then, drops of citric acid from two slaves of lemon were added to acidify the brine solution. Since the biogas is insoluble in the solution, a pressure build up provides the driving force for displacement of the solution. The displaced solution was measured by using graduate cylinder to represent the amount of biogas produced [22]. Biogas volumes were measured starting from the third day after digestion set up and stayed for 60 days until biogas production was zero for consecutive three days.

2.10. Biogas Quality Determination and Statistical Analysis

The quality of biogas for each treatment was analyzed using Geo Tech gas analyzer from Addis Ababa University, School of Chemical and Bio-Engineering laboratory. Biogas produced was collected from five different digesters using plastic bottles and it was directly connected to the gas analyzer for its composition analysis. This gas analyzer predicts the percent composition of CH_4, CO_2, O_2 and H_2S in the biogas. The data obtained from the present investigation was analyzed using analysis of variance (one-way ANOVA) using SAS version 9.1. Fishers Least Significant Difference (LSD) was used to investigate statistical significance between the different treatments. The statistical significance level was selected at p-value < 0.05. It was used to compare mean at 95% confidence level. Excel sheet was used to generate graphs and charts to illustrate the results.

3. Results and Discussions

3.1. Feedstock Characterization

The cow dung and poultry manure were used as feed stock and their mixtures in proportions were characterized through various standard parameters and procedures. The parameters in each treatment were determined with three replications and their average values are presented under Table 2.

Table 2. *Physicochemical characteristic of the feedstock.*

Parameters	Treatments				
	T_1	T_2	T_3	T_4	T_5
%MC (db)	22.00±1.00	20.00±1.00	19.00±1.00	16.00±0.00	13.00±1.00
%TS (g/VS)	17.43±0.30	16.83±0.11	16.03±0.05	16.23±0.11	15.60±0.02
VS (as %TS)	80.45±0.62	58.33±1.67	68.80±0.69	75.30±0.01	67.94±0.04
pH	6.30±0.10	7.50±0.10	6.60±0.01	6.90±0.08	6.50±0.15
%OC	46.77±0.32	33.91±0.04	39.97±0.01	43.77±0.34	39.53±0.05
TKN (g/l)	1.80±0.05	5.41±0.21	1.85±0.10	1.52±0.05	1.61±0.03
C: N	25.99±0.82	6.26±0.25	21.64±1.22	28.75±1.23	24.50±0.34
%AC	22.98±0.61	45.23±0.54	36.25±0.85	28.39±1.11	33.33±0.42

Where: T_1, T_2, T_3, T_4 and T_5 are treatments, TS=Percent of total solid, VS=Volatile solid, OC=Organic carbon, TKN=Total Kjeldahl Nitrogen, C: N=Carbon to Nitrogen ratio, SD=Standard deviation, MC=Moisture content at dry base, pH=Power of hydrogen, AC=Ash content.

3.1.1. Moisture Content (MC)

Moisture content (MC) of the feedstock has a major influence on anaerobic fermentation in the course of biogas production process. In this study, the moisture contents of cow dung, poultry manure and their co-digestion in different proportions were in the range of 13.00±1.0 - 22.00±1.0% at dry base (Table 2). The moisture content of the feedstock was not in line with the proportion of one component of the mixture. The variation in moisture content may be due to the level of dryness of the feedstock or the type of feed of the chickens or cows. T_1, T_2, T_3, T_4 and T_5 were 13.0±1.0, 16.0±1.0, 20.0±1.0, 19.0±1.0 and 22.0±1.0 percent respectively at a dry base. The moisture content of the samples was found as low. The moisture content of feedstock highly influences the rate of degradation before fermentation as anaerobic bacteria can easily access liquid substrate for relevant reactions.

3.1.2. Total Solid (TS)

Total solid concentration of the feedstock is one the most important parameter that affects performance of biogas production. Result of the present investigation showed that, the total solid content of the feedstock were in the range of 15.6±0.02 to 17.43±0.3%. The maximum TS was 17.43±0.3% in T_1 (100% CD and 0% of PM) and the minimum TS value was 15.6±0.02% from T_5 (75% of CD and 25% PM). The finding was in agreement with [22], who reported 15 to 20% of TS. According to [7, 32] animal manure feedstock has the %TS in the range of 2-20 in which this finding also falls.

3.1.3. Volatile Solid (VS)

The volatile solid contents as part of the total solids of the feedstock for different treatments were in the range between 58.33±1.67 to 80.45±0.62%. The maximum value was found to be 80.45±0.62 in the digester that contains cow dung alone (T_1) while the minimum volatile solid content was 58.33±1.67% the digester that contains poultry manure alone (T_2). The higher percentage of volatile solid indicates that, the feedstock contains high amount of organic carbon that can be converted in to biogas yield [9]. Increase in volatile content increases biogas production and, hence the present investigation revealed that cow dung has the highest volatile solid content and it was the best feedstock for biogas production. On the other hand, poultry manure has the least solid volatile content and it is the least preferred feedstock for biogas production. However, it is shown that addition of cow dung in to the poultry manure increases its volatile content [21]. This may be due to modification of the physicochemical properties of the poultry manure that affects growth and development of fermentative bacteria. According to [61, 64], feedstock with volatile solid content between 75 and 85% was recommended as a potential feedstock for biogas production. On the other hand, feedstock with volatile solid component close to 77% was proposed [45]. Hence, from the volatile solid content point of view, cow dung alone or 75% of poultry manure mixed with 25% cow dung is proposed as a potential feedstock for biogas production.

3.1.4. pH

The pH is another important parameter that must be monitored in an anaerobic digestion process. Several studies on anaerobic digestion of waste have shown that the pH of substrates have strong influence on the rate of biogas production [25, 56]. The value of the pH for feedstock evaluated in this study was in the range of 6.3 to 7.5. The maximum and minimum pH of the feedstock observed from T_2 and T_1, respectively. Previous studies have reported that, methanogens activity is optimum within a pH range of 6.5-8.0 for mesophilic digestion [20, 46]. In line with this report, except T_1, all other reactors or treatments were having pH with in recommended range for anaerobic digestion process and they are between in optimum range for anaerobic digestions. T_1 (100% CD) more acidic (6.3±0.1) while T_2 (100% PM) was slightly basic (7.5±0.1). The two feed-stocks can be complementing each other in terms of pH when they are mixed. Each feedstock will have a buffering capacity and enhance the rate of anaerobic digestion for biogas production [45].

3.1.5. Carbon to Nitrogen Ratio (C: N)

The carbon to nitrogen ratio (C: N) of the feedstock is another factor that affects the anaerobic digestion processes. The feedstock's total organic carbon (TOC), total nitrogen (TN) and their ratio are critical process parameters in the anaerobic digestion process. Methane yield and its production rates are highly influenced by the balance of carbon and nitrogen in the feeding material. The C: N ratio for the feed-stocks determined in this study was in the range of 6.26±0.25 to 28.75±1.23 in T_2 and T_4 respectively. From all treatments or feed-stocks, T_2 (100% of PM) was having the lowest C: N ratio (6.26±0.25), which is quite low for optimum biogas generation. This is because, the poultry manure observed to have the highest total nitrogen content (Table 2) that reduces its C: N value. The C: N ratio of poultry manure showed to be increased by mixing it with cow dung in T_4. This is because of co-digestion process. When a substrate with a low C: N ratio is mixed with a substrate that has high C: N ratio, a better anaerobic digestion performance is achieved [64].

A high C: N ratio was an indication of rapid consumption of nitrogen by methanogens and results in lower gas production. On the other hand, a lower C: N ratio causes ammonia accumulation and pH values exceeding 8.5, which is toxic to methanogenic bacteria. The nitrogen found in the anaerobic reactor was mainly derived from proteins, and necessary for microbial growth, although a low C: N ratio in the system (high amount of nitrogen) can produce an ammonia accumulation in the digester, resulting in toxic levels for the process [25, 33] affecting biogas and methane yields and eventually causing process failure [13]. The co-digestion process was an important to balance one or the other components to achieve stable digestion [22] and to give optimal C: N ratios of 20:1 to 30:1 [10]. Because; The C: N ratio of the cow dung was found adequate (25.99±0.82), and that was co-digested with T_2 (100%PM and 0% CD) that have low (6.26±0.25) C: N ratio to adjust substrate in the range 20:1 to 30:1. According to this, all other treatments (T_2, T_3, T_4 and T_5) not affected by the C: N ratio. Because of by feedstock nature and co-digestion process, they are in the range of 20:1 to 30:1.

3.1.6. Ash Content (AC)

Other most determinant factors for anaerobic digestion process was the ash content. It is the residue that left in the vessel after a sample of feedstock has been ignited and heated at 550 ℃. Fixed solid (ash) contents are the amount of solid that does not volatilize at 550 ℃. In the present investigation, the ash (fixed solid) content was in the range of 22.98 ±0.61 - 45.23± 0.54% (Table 2). The lowest and highest ash content was recorded in T_1 and T_2 respectively. The ash content of T_1 was 22.98± 0.61%, which resulted in high VS as indicated in Table 2. This indicates that, T_1 is a suitable feedstock for anaerobic digestion. However, this treatment was negatively influenced by having lowest pH (6.3) as indicated in Table 2. This pH value was not in the optimum range for the methanogens. In contrary with T_1, T_2 showed the largest ash content. This is because of having lowest VS (as %TS) (58.33±1.67). So, having highest ash content and lowest VS (as %TS) makes T_2 less biodegradable. As a result, final biogas production is expected to be reduced.

3.2. Amount of Biogas Content

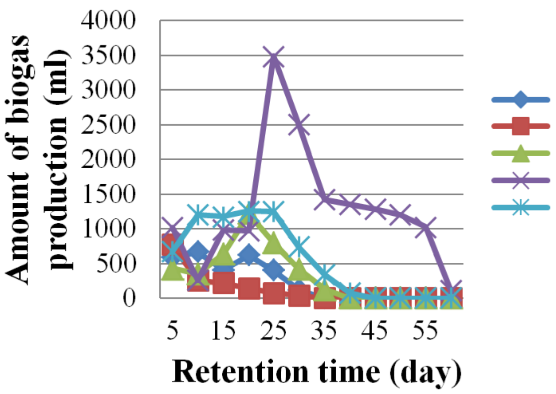

Figure 3. *Amount of biogas production in different retention time.*

The amount of biogas produced in a hydraulic retention time of 60 days of digesters was measured by using water displacement method in every day after brine solution fully displaced from gas stored bottle to brine solution stored bottle. The onset of gas production was seen starting from the second day for some digesters. The production of biogas was confirmed by observing the volume of displaced water from the gas storing plastic bottle to the displaced water storing plastic bottle. The amount of biogas yield was increased as the retention time increased in reactor with T_3, T_4 and T_5. But, in reactor one (T_1) the production was fluctuating and in reactor two (T_2) the rate of biogas production showed to decrease after 10 days. In all digesters after certain days of degradation time, production of gas was decreased and finally stopped. The amount of first 10 days biogas was the difference of digester feedstock and inoculum produced biogas. The rate of biogas production in 5 days interval under each reactor is given in Figure 3.

The total biogas content from each treatment was measured for about sixty days of retention time and indicated in Figure 4. From the study (T_4) 75% of PM and 25% of CD mixture gave a higher amount of gas (15,796ml) than all other treatments. The result was in agreement with the report by [41]. In T_1 (100% CD and 0% PM), the smallest amount of biogas production was observed (2,820ml) next to T_2 as compared to T_3, T_4 and T_5. This is because, the fermentation of dung as single substrate provides low methane yield due to a moderate anaerobic biodegradability [39, 49]. Moreover, the biogas production from cow dung is lower than that obtained from other farm animals, since cattle manure often contains an excess of lignin complexes from fodder and that are very resistant to anaerobic digestion [40, 43]. Low biodegradability of animal dung is often caused by large amounts of indigestible fractions such as lignocellulose, an element of the plant cell wall, residual from animal digestion, and composed by hemicellulose, cellulose and lignin. Lignin is a natural complex polymer and non-carbohydrate constituent of wood bond to cellulose fibers and it provides mechanical strength and structural support to the plants. Lignocellulose is very difficult to be degraded in anaerobic environments due to its rigid structure produced by lignin [23, 51].

Numerous studies have reported that lignin content and the efficiency of enzymatic hydrolysis have an inverse relationship [58]. Due to the different diets, cow dung generally shows the most abundant amount of lignin (i.e., around 18.0%) [58]. While in pig and poultry manure the content of lignin was 1.83% and 1.7%, respectively [37]. Having a less amount of lignin content makes poultry manure feedstock more degradable and suitable for anaerobic digestion process. Additionally, having high fraction of fiber makes cow dung an unsuitable substrate for the production of biogas by using single substrate according to explanation of [5].

T_2 (100% PM and 0% CD) gave the smallest amount of biogas production (1,509ml) than all other treatments. This is due to the presence of high amount nitrogen content in the poultry manure as compared to manure from other farm animals, and this creates a problem for the anaerobic digestion process [60]. According to [27, 57] ammonia inhibition is common problem for the anaerobic digestion process using feedstock such as poultry manure alone. The inhibitory effects of free ammonia on the metabolism of methanogens were observed by several authors while using poultry manure alone as feed stock for anaerobic digestion [63]. So, co-digestion of poultry manure with other type manure such as cattle manure has been evaluated and recommended by previous researchers [15, 60] and better result was observed.

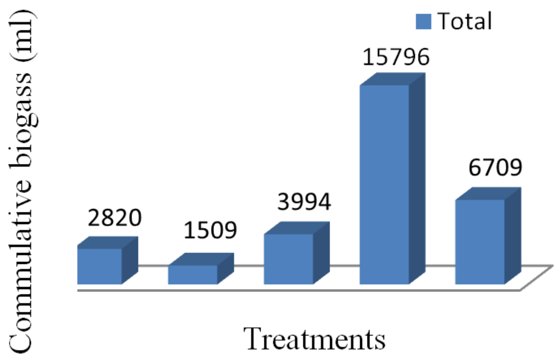

Figure 4*. Total biogas production in milliliter (ml) for different treatments.*

From all treatments, T_4 (75% PM and 25% CD) need more time for complete digestion. This is because, in a lag phase, there should be a sufficient period for acclimation for the organism to start up the digestion process. Reports showed that retention time for biogas production depends on type of substrate [38]. Accordingly, biogas production from T_1, T_2, T_3, T_4 and T_5 was ceased on 30th, 30th, 35th, 60th and 40th day of the batch fermentation commencement respectively as expressed Figure 3. This could also depend on the types of feed stock, amount and growth phase of the added inoculum (75ml) that might create prolonged lag phase of the methanogenic bacteria. The added inoculum itself is expected to have effect on the biogas production. To know the effect, the amount of biogas produced from the inoculum was recorded as a controlled group [41]. The cumulative biogas produced from the inoculum was 425ml within 10 successive days and after that the production was ceased.

3.3. Composition Analysis of Biogas

The composition of biogas largely depended on the type of feedstock used for its formation. In the present study, the collected gas was analyzed using Geo Tech gas analyzer to know the composition of biogas produced during the anaerobic digestion process. The analyzed biogas has different composition of gases like Methane (CH_4), Carbon dioxide (CO_2), Oxygen (O_2) and Hydrogen sulfide (H_2S). With the aid of this gas analyzer, the percentage of individual composition of gas was obtained and presented under Table 3. Analyzing the composition of biogas is needed to know the quality of produced biogas. According to [47, 55] report, the quality of biogas mainly depends on the percentage of CH_4 and, the better quality biogas contains 50-70% of CH_4. Accordingly, the result of the present investigation was in agreement with the report by [38].

Table 3*. The gas composition (mean value ± SD) of the biogas produced from the different feedstock (Treatments).*

Treatments	Composition of gas for each treatment (%)			
	CH_4	CO_2	O_2	H_2S
T_1	$56.3^{D}\pm0.91$	$33.6^{A}\pm1.25$	$0.9^{A}\pm0.06$	$0^{A}\pm0$
T_2	$56.9^{CD}\pm1.0$	$35.5^{A}\pm0.85$	$0.75^{A}\pm0.21$	$0^{A}\pm0$
T_3	$57.8^{C}\pm0.95$	$26.5^{B}\pm2.52$	$0.81^{A}\pm0.08$	$0^{A}\pm0$
T_4	$60.7^{B}\pm0.10$	$23.4^{C}\pm1.05$	$0.6^{B}\pm0.14$	$0^{A}\pm0$
T_5	$63.6^{A}\pm0.70$	$25.7^{BC}\pm1.95$	$0.8^{A}\pm0.01$	$0^{A}\pm0$
LSD (t) value	1.46	2.99	0.22	0
Pr.>F	0.024	0.0004	0.117	ND
ANOVA Decision	Significant	Significant	Non-significant	-
CV	1.36	5.69	15.91	0

As expressed in Table 3, the mean values followed by different capital letters in column are significantly different at 5% (0.05) level of significance between treatments. But, the mean values followed by same capital letter in column are not significantly different at 5% (0.05) level of significance between treatments. Additionally, the statistical significance level value that selected at p-value less than 5% (< 0.05) for biogas characterization was 0.024, 0.0004, and 0.117 for CH_4, CO_2

and O_2 respectively. From this, the values of probability > F less than 0.05 is for CH_4 and CO_2, while the value was greater than 0.05 for O_2. This indicates that, the gas composition (CH_4 and CO_2) was significantly different with the different treatments, while there was no significant variation in the percentage of O_2 and H_2S with the use of the different feed-stocks (treatments).

The concentration of the CH_4 in this study was in the range of 56.3±0.91 to 63.6±0.70% (Table 3). The lowest % CH_4 of methane (56.3±0.91) was recorded from T_1 when cow dung is used as a feedstock, while the highest % CH_4 of methane (63.6±0.70) was observed from T_5 when cow dung and poultry manure mixed at 75 and 25%, respectively. T_1 and T_2 were operated by the single subst rate alone; cow dung and poultry manure, respectively. The gas compositions from these two treatments were having the least CH_4 content. On the other hand, when one of the substrate supplemented with the other one at 75:25% (T_4 and T_5), the concentration (%) of CH_4 was high. This indicates that, the reactor environment better suits to the microorganism when the two substrates are mixed than used alone. According to [52, 53] explanation, biogas which comprises of 50 to 75% methane (CH_4), 25 to 45% carbon dioxide (CO_2) and 0 to 5% a combination of hydrogen sulfide (H_2S), N_2, H_2 and others. The gas compositions of the biogas produced in the present study was in agreement with this recommendation.

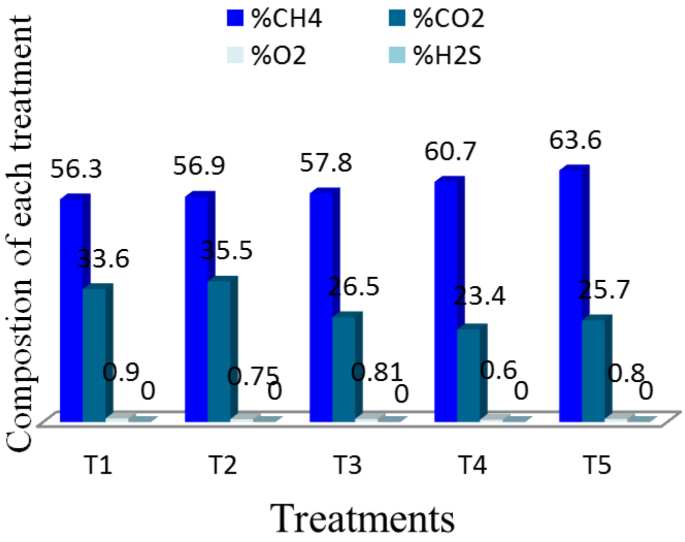

Figure 5. *The gas composition of biogases produced from the different feedstock.*

3.4. Statistical Significance of the Physicochemical Properties

The statistical significance of the physicochemical characteristics of the feedstock used in the present study is given under Table 4. The p-value for all parameters was less than 0.05 ($p < 0.05$). This showed that, all parameters are significant among the different treatments. This difference may be caused from the feed-stocks co-digestion proportions and the physicochemical composition of feed-stocks with in treatment.

Present investigation result resembles with the previous study reported by [41]. According to [41] explanation, feed-stocks parameters and its ANOVA decision may highly influenced by the composition of used feed-stocks and as a result influence biogas production. Accordingly, being a significant in case of ANOVA decision (Pr.>F less than 0.005), was caused significant difference for biogas production (Table 4).

Table 4. *ANOVA Summery table for feed stocks characterization.*

Parameters	Source of Variation	DF	SS	MS	F-Value	Pr.>F	CV	ANOVA decision
	Treatment	4	150	37.5	46.88	0.0016		
MC	Error	10	8	0.8				Significant
	Total	14	158					
VS	Treatment	4	837.35	209.33	286.38	< 0.0001	1.21	Significant

Parameters	Source of Variation	DF	SS	MS	F-Value	Pr.>F	CV	ANOVA decision
	Error	10	7.30	0.73				
	Total	14	844.66					
	Treatment	4	6.16	1.54	62.26	0.0002	0.95	
TS	Error	10	0.24	0.024				Significant
	Total	14	6.41					
	Treatment	4	2.56	0.64	64.38	0.001	1.47	
PH	Error	10	0.099	0.009				Significant
	Total	14	2.66					
	Treatment	4	282.72	70.68	1557.3	< 0.0001	0.52	
OC	Error	10	0.45	0.045				Significant
	Total	14	283.17					
	Treatment	4	33.42	8.35	679.38	< 0.0001	4.52	
TKN	Error	10	0.123	0.123				Significant
	Total	14	33.54					
	Treatment	4	942.28	235.57	301.65	< 0.0001	4.12	
C: N	Error	10	7.809	0.78				Significant
	Total	14	950.08					
	Treatment	4	844.85	211.21	375.99	<0.0001	2.25	
AC	Error	10	5.617	0.56				Significant
	Total	14	850.47					

3.5. Fishers Least Significance Difference (LSD) Comparisons (Mean ± SD) for Feedstock Characterization

As expressed in Table 5, the means value followed by different capital letter in row are significantly different at 5% level of significance between treatments. But, the mean values followed by same capital letter in row are not significantly different at 5% (0.05) level of significance between treatments. The variation in physicochemical characteristics of the feedstock will have effect on the fermentation process, amount and quality of the resulting biogas.

Table 5. *Fischer LSD Comparisons (mean ± SD) for feedstock characterization.*

Parameter	Treatments T_1	T_2	T_3	T_4	T_5	LSD (t) value
VS	80.45^{A}±0.62	58.33^{D}±1.67	68.80^{C}±0.69	75.03^{B}±0.06	67.94^{C}±0.04	1.55
MC	13.00^{D}±1.00	16.00^{C}±0.00	20.00^{B}±1.00	19.00^{B}±1.00	22.00^{A}±1.00	1.63
TS	17.43^{A}±0.30	16.83^{B}±0.11	16.03^{C}±0.01	16.23^{C}±0.11	15.60^{D}±0.02	0.29
PH	6.30^{D}±0.10	7.50^{A}±0.10	6.60^{C}±0.01	6.90^{B}±0.08	6.50^{C}±0.15	0.18
OC	46.77^{A}±0.32	33.91^{E}±0.04	39.97^{C}±0.01	43.77^{B}±0.34	39.53^{D}±0.05	0.39
TKN	1.80^{BC}±0.05	5.41^{A}±0.21	1.85^{B}±0.10	1.52^{D}±0.05	1.61^{C}±0.03	0.20

Parameter	Treatments					LSD (t) value
	T_1	T_2	T_3	T_4	T_5	
C: N	$25.99^{B}\pm0.82$	$6.26^{D}\pm0.25$	$21.64^{C}\pm1.22$	$28.76^{A}\pm1.23$	$24.50^{B}\pm0.34$	1.61
AC	$22.98^{E}\pm0.61$	$45.23^{A}\pm0.54$	$36.25^{B}\pm0.85$	$28.39^{D}\pm1.11$	$33.33^{C}\pm0.42$	1.36

3.6. Fishers Least Significance Difference (LSD) Comparisons (Mean ± SD) for Cumulative Biogas Yield Between Treatments

Table 6 showed the least significant difference (LSD) comparison between treatments for cumulative biogas yield for different feedstock. The values of probability > F, less than 0.05 and that indicates the cumulative biogas yield was significant. This is because; the biogas yield was significantly influenced by the parameters shown in each treatment and codigestion of the two feedstock; namely cow dung and poultry manure in different digester at different ratio. This effect has been explained to be due to improvement of nutrient balance with mixture of feedstock which enhances digestion, sludge solublization, methanogens stability and biomethane production [25]. The present investigation result also concord with the finding of [14] that showed the effect of codigestion. This is confirmed by the observing the co-digested and single digested feedstock. The first two treatments; T_1 and T_2 were operated by cow dung and poultry manure alone, respectively, and their cumulative biogas was small as compared with T_3, T_4 and T_5. Treatment two (T_2) gave the smallest amount of biogas production than all treatments. This is because of having smallest VS (58.33±1.67) and C: N ratio (6.26±0.25) contents.

Table 6. Fischer LSD Comparisons (mean ± SD) for cumulative biogas yield (ml).

Treatments	Cumulative biogas (ml)	LSD (t) value	Pr. > F	CV Value	ANOVA decision
T_1	$2,820^{D}\pm285.11$				
T_2	$1,509^{E}\pm162.31$				
T_3	$3,994^{C}\pm121.11$	324.65	<0.0001	2.89	Significant
T_4	$15,796^{A}\pm162.31$				
T_5	$6,709^{B}\pm94.59$				

4. Conclusion

Biogas technology is growing as interests on biogas as a main approach for treating a variety of organic waste increases. Biogas production decreases environmental pollution through decomposing organic wastes and positively impacts the socio-economy of the society. Biogas is considered as a clean and a renewable form of energy that could replace the nonrenewable energy sources such as fossil fuels. It is produced through anaerobic degradation, engaging various microorganisms performing four major degradation steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

The main aim of this proposed research was production and characterization of biogas from cow dung, poultry manure and their codigestion at different ratio in mesophilic condition (37 ℃). The digester runs for 60 days using five different treatments (feedstock). The feedstock were co-digested according to the proposed proportion and they were characterized in terms of moisture contents, total solids, volatile solids, pH, organic carbon, total Kjeldahl nitrogen, C: N ratio and ash contents. The codigestion showed to influence most of the physiochemical characteristics of the feedstock significantly. The C: N ratio was in the range of 6.26±0.25 to 28.75±1.23. The lowest (6.26±0.25) and highest (28.75±1.23) value was recorded in T_2 and T_4 respectively. The C: N ratio is considered as one of the determinant factor for biomass to be considered as feedstock for biogas production.

From the present investigation revealed that T_4 (75% poultry manure and 25% cow dung) produced the highest (15,796±162.31) amount of biogas (ml). The results of the biogas quality analysis using Geotech gas analyzer was demonstrated as 56.3±0.91, 56.9±10, 57.8±0.95, 60.7±0.10 and 63.6±0.70 for CH_4 in T_1, T_2, T_3, T_4 and T_5, respectively. From the result we can concluded that, the values of biogas quality analysis was in the range of accepted value in the literature section. It also can be concluded that, the co-digestion improves the quality of biogas in term of methane contents. The present investigation showed that codigestion improves the quality of the feedstock for biogas production. However, further investigation and optimization of biogas production parameters is required before scaling up.

Abbreviations

AC	Ash Content
AD	Anaerobic Digestion
ANOVA	Analysis of Variance
C: N	Carbon to Nitrogen Ratio
CD	Cow Dung
CH_4	Methane
CO_2	Carbon Dioxide
CRD	Completely Randomized Design
H_2	Hydrogen
H_2O_2	Hydrogen Peroxide
H_2S	Hydrogen Sulfide
H_2SO_4	Sulfuric Acid
HCl	Hydrochloric Acid
HRT	Hydraulic Retention Time
KOH	Potassium Hydroxide
LSD	Least Significant Difference
MC	Moisture Content
N_2	Nitrogen
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
O_2	Oxygen
OC	Organic Carbon
OLR	Organic Loading Rate
pH	Power of Hydrogen
PM	Poultry Manure
R	Reactors
SD	Standard Deviation
T	Treatment
TKN	Total Kjeldahl Nitrogen
TS	Total Solids
VS	Volatile Solids

Author Contributions

Daniel Kebede: Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Writing – original draft, Writing – review & editing

Zerihun Demrew Yigezu: Conceptualization, Project administration, Supervision, Validation, Visualization

Conflicts of Interest

The authors declare no conflicts of interest.

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