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Biogas Production from Mixture of Fruit Peels Co-Digestion with Cattle Manure Under Anaerobic Condition

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Abstract: Biogas is one of an alternative source of energy that is produced by methanogenic bacteria through the bio-degradation of organic material under anaerobic conditions. The aim of this study was to measure daily biogas production from mixture of fruit peels with cattle manure in solo and five mix ratios under anaerobic condition at 25°C using batch digestion under room temperature conditions (25ºC) using batch fermentation. In all treatments, parameters such as total solids (TS), volatile solids (VS), pH, organic carbon (%), total nitrogen and C:N ratio were measured before and after digestion. The daily biogas production was subsequently measured by water displacement method for 30 days. All measured of physico-chemical parameters of each substrate were significantly different among digesters before and after anaerobic digestion. Gas production was clearly observed in all of the substrates types starting from the fifth day of digestion and increase gradually and also decrease sequentially at the end in all substrates. Among all digesters, the one fed with 30% PM and 70% MFL was showed in the highest cumulative biogas and the lowest was recorded from 100% PM. The result suggested that mix ratio of the two substrates in 30% PM and 70% MFL was optimal for maximum biogas yield. Overall results indicate that the increment of biogas yield and VS, and TS reduction can be significantly enhanced when GM and WMP are co-digested. However, the quality of biogas produced was not subjected to be tested chromatographically in terms of the methane content.

Keywords: Biogas, Methanogenic Bacteria, Co-digestion, Cattle Manure, Fermentation

1. Introduction

1.1. Background of the Study

Globally, the demands of energy consumption are continuously increasing from time to time with increasing of human populations. To overcome these problems, an alternative energy sources have recently become more and more attractive due to the environmental concerns and the strategy to survive post-fossil fuel economy era [18].

Energy production and environmental protection are the burning issues of the world. Development and civilization of a society have largely been achieved through extensive harnessing of various forms of energy [10].

About 80-90% of African households rely on biomass fuel such as fire wood, and charcoals for cooking their daily meals [5]. However, due to rapid deforestation in many parts of Africa, fire wood and charcoal are becoming increasingly scarce. Because of scarcity of fuel wood, rural people have switched to burning animal dung and crop residues for fuel which in turn has resulted in progressive land degradation [1]. If rapid deforestation continues in the future, many people in Sub-Saharan Africa may be unable to afford fuels to cook their food [8].

As experts predict, mankind is quickly approaching an age where fossil fuels, particularly petroleum and natural gas, will reach a point where they will no longer be viable energy resources for meeting societal needs. Therefore, stakeholders must strive to develop an "energy portfolio" to contribute to alternative energy sources [15]. Problems of growing energy consumption and diminishing supplies of fossil fuels have also led researchers to develop new technological processes of energy production [10].

One of the renewable energy resources is biogas. Biogas is a good fuel resource similar to natural gas containing methane. Anaerobic digestion is the biological technology used to produce renewable and clean energy from biomass. Anaerobic digestion involves microbiological processes of decomposing organic matter in the absence of oxygen.

Besides, it conserves the fertilizer value presented originally in the waste [4]. Ethiopia has proved potential for renewable energy such as wind, solar and biogas resources. However, it has the lowest rate of access to biogas energy services although sources of energy supply are available [7]. This study is, therefore, designed to produce biogas from mixture of fruit peels and cattle manure in sole and mixture in the laboratory under anaerobic conditions.

1.2. Objectives of the Study

1. The general objective of the study was to measure the biogas production from mixtures fruit peels and cattle manure in different mix ratios.
2. Specific objectives were to:
   1) Optimize biogas production from mixture of fruit peels and cattle manure in different mixing ratio;
   2) Compare the average and cumulative daily biogas production from solo and mixture of samples combined in different proportions.

2. Materials and Methods

2.1. Description of the Study Area

This study was conducted at Wachemo University, Biotechnology department, Molecular Biology Laboratory which is found at Hossana Town. [11].

2.2. Sample Collection and Preparation

Fresh mixtures of fruit peels (MFP) and cattle manure (CM) were collected from restaurants, fast food making launches…etc. and cattle farms of selected areas respectively. Both MFP and CM were dried and crushed to fine powder by using simple machine and characterized for physicochemical properties such as: TS, VS, % organic carbon, total nitrogen, pH and C to N ratio following the standard methods.

2.3. Experimental Design and Substrate Loading

The two substrates were divided into five treatments in sole or combinations on their TS basis and placed in a 500 ml plastic bottle digester. The five treatments were 100% CM; 70%: 30% mix of CM and MFP; 50%:50% mix of CM and MFP; 30%:70% mix of CM and MFP and 100% MFP. In this study, rumen fluid was used as inoculum. For this, fresh rumen fluid was collected from the slaughter house and filtered through a cloth of 0.5 mm sieve diameter to separate solid content from slurry. Prior to using it as a microbial seed, the inoculum was stored for a week by incubating at 25ºC to remove the easily degradable volatile solid (VS) present in inoculums [12]. Then, 100 ml of inoculum (rumen fluid) was mixed with the substrate and the total solids of the substrate was adjusted to the recommended level (8%) [17] using appropriate amount of distilled water. After that the pH of the slurry was maintained within the pH range for optimal biogas production (6.8-7), by adding sodium hydroxide and hydrochloric acid to the organic substrate [19] to the initial pH of all substrates to about neutral. The slurry was mixed thoroughly by shaking and AD incubated for 30 days at 25°C for anaerobic digestion.

Table 1. *The proportion of different substrates added in the five digesters in three replicates.*

Tx	TS of CM (g)	TS of MFP (g)	Water (ml)	Inoculum (ml)	CM (g)	MFP (g)	Working vol (ml)
A	40	0	160.00	100ml	40	0	300ml
B	32	8	160.00	100ml	32	8	300ml
C	20	20	160.00	100ml	20	20	300ml
D	8	32	160.00	100ml	8	32	300ml
E	0	40	160.00	100ml	0	40	300ml

A=100%CM B=70%CM: 30%MFP C=50%CM: 50%MFP D=30%CM: 70%MFP E=100%MFP

2.4. Digester Arrangement and Measurement of Biogas

The experiment was arranged randomly in three replicates with three plastic bottles containing the slurry, acidified brine solution and empty bottle (to collect brine solution). The acidified brine solution was prepared by dissolving NaCl in water until a supersaturated solution is formed to prevent the dissolution of biogas in the water. Thus, the biogas produced by fermentation of the slurry was driven from the digester (first bottle) to the second bottle that contained a brine solution. As it enters into the second bottle, a volume of the solution estimated to be the amount of biogas was displaced to the third empty bottle in line [2, 3]. After AD, the slurry was also be analyzed for physicochemical properties indicated above. TS and VS were determined gravimetrically [9], pH and electrical conductivity was determined using Glass-electrode method, Organic carbon was determined using Titrimetric method [18].

2.5. Measurement of Biogas

The Amount of gas produced was measured by water displacement method. The daily gas production was recorded for different treatments for 30 days until the gas production ended and eventually the cumulative yield was expressed as the total sum of the 30 days production.

In this study, co-digestion of mixture fruit peels and cattle manure had been considered for biogas production under anaerobic condition in sole and mixed in different ratio.

2.6. Analysis of the Physicochemical Characteristics of the Substrates

2.6.1. Total Solids

Total solids are the amount of solids present in the sample after the water present in it is evaporated at about 105°C in oven. For the determination of TS, a clean evaporating dish was first dried at 105°C for 1 hour, cooled in desiccators and weighed immediately before use. Then, 10 g of freshly collected samples of each substrate was weighed using a digital balance, and placed onto a pre-dried and weighed evaporating dish. Then, the dish was put inside an oven at 105°C using a crucible. The crucible was allowed to stay in the oven for 24 hours, and then was taken out, cooled in desiccators and weighed [3]. Then, the percentage of the TS was calculated as follows:

$$\%\ \text{Total solids}=\frac{wDS}{wFS}\times 100\%$$

Where:
wDS=weight of dry sample (in grams)
wFS=weight of fresh sample (in grams)

2.6.2. Volatile Solids (VS) and Fixed Solids

The volatile solid contents of the samples were determined by transferring the dried samples into muffle furnace, heating at 550°C for 3 hours and weighing after cooling at room temperature. The percentage of volatile solids and fixed solids for all samples was calculated by using the following equation [3].

$$\%\text{VS}=\frac{MDS-M(ash)}{MDS}\times 100\%$$

Where
%VS=percentage volatile solid
MDS=mass of dried sample
M (ash)=mass of ash remaining mass after ignition=Fixed solids in grams
i.e., TS=VS + fixed solids

Then percentage of VS removal were calculated using the equation below

$$\text{VS removal}=\frac{VSi-VSf}{VSi}\times 100$$

Where,
VS i=initial volatile solids before AD
VS f=final volatile solids after AD

2.6.3. Determination of pH

The pH values were determined using digital pH meter before and after anaerobic digestion. The pH of the samples before anaerobic digestion was measured by diluting the samples using distilled water before inoculation with rumen fluid. Then, an electrode was inserted into samples of substrate and measured in triplicates. However, pH measurements after anaerobic digestion were done using pH electrode, which was inserted into samples of substrate that is digested at the end of the experiment.

2.6.4. C:N Ratio

In order to determine the C:N ratio, the amount of organic carbon was first determined by Walkley-Black method [18] while the N was determined using macro-kjedahl method. Thereafter, C:N ratio of each substrate was determined One gram dried organic substrate was weighed and transferred to a 500-mL Erlenmeyer flask. About 10ml of 0.167 M $K_2Cr_2O_7$ was added by means of a pipette and 20mL of concentrated H_2SO_4 was added by means of a dispenser and was swirled gently to mix thoroughly, (avoiding excessive swirling that was result in organic particles adhering to the sides of the flask out of the solution). This mixture was allowed to stand for 30 minutes. The flasks were placed on an insulation pad during this time to avoid rapid heat loss. The suspension was diluted with 200mL of water to provide a clearer suspension for viewing the end point. Then 10mL of 85% H_3PO_4 and 0.2g of NaF were added using a suitable dispenser, (The H_3PO_4 and NaF were added to complex Fe^{3+} which was interfere with the titration end point).

Finally, 10 drops of ferroin indicator was added. (The indicator was added prior to titration to avoid deactivation by adsorption). The mixture was then titrated with 0.5 M $FeSO_4$ to a burgundy end point. The color of the solution at the beginning was yellow-orange but turned to dark green at the endpoint (the change in color depends on the amount of un-reacted $Cr_2O_7^{2}$ remaining, which shifts to a turbid grey before the endpoint and then changes sharply to a wine red at the end point). Use of a magnetic stirrer with an incandescent light made the end point easier to see in the turbid system (fluorescent lighting gives a different end point color).

Calculation of %OC was done as follows.

$$\%C=\frac{(\text{FB}-\text{FS})\times\text{N}\times 0.39\times\text{mcf}}{\text{Wo}}$$

Where:
FB=ml of $FeSO_4$ solution used to titrate blank
FS=ml of $FeSO_4$ solution used to titrate sample
N=Normality of $FeSO_4$ (0.5N)
0.39=mill equivalent weight of C in g
mcf=moisture correction factor
Wo=dray sample weight in g

The total nitrogen in the sample was determined using the Kjeldahl method. One gram of sample and 6 ml of concentrated H_2SO_4 were added into a test tube and mixed carefully. Then 3.5 ml of H_2O_2 was added step by step. Violet color due to reaction was observed. As soon as the violent reaction was ceased the tube was shaken by hand. After adding 3g catalyst mixture the sample was allowed to stand for 5 to 15 minutes in the test tube rack before digestion. Then the digester was allowed to wait until its temperature reached 370°C. As the digester reached the temperature 370°C and the digestion continued for about 4 hours until a clear solution was observed.

After the digestion process, tube was transferred to the fume hood for cooling. About 50 ml of distilled water was added and shaken by hand to avoid sulphate precipitation in the solution. At this time 25 ml of 40% NaOH solution was added into the digested and diluted solution. Then 250 ml of conical flask containing 25 ml of boric acid, 25 ml of distilled water and an indicator solution was placed under the condenser of the distiller with its tip immersed into the solution and the distillation continued for about 8 minutes until the total volume became between 200 ml to 250 ml. Finally the solution was titrated using 0.1N H_2SO_4 to a reddish color and %Nitrogen was calculated using the following formula:

$$\%N = \frac{V \times No \times 0.014 \times 100 \times mcf}{Wo}$$

Where,
V=Volume H_2SO_4 in ml consumed during titration
No=Normality H_2SO_4 (0.1N).
0.014=mill equivalent weight of nitrogen in g
mcf=Moisture correction factor
Wo=Sample weight on dry matter in g
Finally C/N ratio was calculated by,

$$\%\frac{C}{N} = C:N$$

2.7. Data Analysis

All statistical analyses were performed using SPSS (IBM©SPSS Statistics v. 16, New York, USA). One-way ANOVA was performed to investigate statistical significance between the different treatments. For all statistical analysis, differences were considered to be significant at $p < 0.05$.

3. Results and Discussion

3.1. Physicochemical Properties of the Substrates Used in Co-Digestion

The Physicochemical characteristics of Cattle Manure and Mixture of fruit peels in sole and mixed in different ratios were determined before and after anaerobic digestion and the results are shown in Tables.

Table 2. *Comparison pH before and after AD for the different combinations of feed (values are mean ± SE, n=3).*

Parameter		
Treatments	**Initial pH**	**Final pH**
A	7.00±0.12^{Aa}	6.92±0.12^{Aa}
B	6.87±0.08^{Bb}	6.77±0.03^{Bb}
C	7.00±0.00^{Aa}	6.64±0.03^{Ab}
D	7.00±0.005^{Aa}	6.67±0.04^{Ad}
E	6.96±0.04^{Aa}	6.29±0.18^{Cb}

A=100%CM, B=70%CM: 30% MFP, C=50%CM: 50% MFP, D=30%:70% MFP E=100% MFP

3.1.1. The pH Value of the Digesters

Originally, the pH of the slurry prepared for AD was adjusted to about neutral for all digesters. The initial pH values before anaerobic digestion were {6.8-7} ±0.12 for the 100% CM, 70% CM + 30% MFP, 50% CM + 50% MFP, 30% CM + 70% MFP and 100% MFP, respectively. But after AD, the pH in all digesters showed slight decrease, but most of them were not statistically significant from the original values. The pH values after anaerobic digestion were 6.93±0.01, 6.87±0.03, 6.67±0.03, 6.67±0.04 and 6.39±0.08 for the 100% CM, 70% CM + 30% MFP, 50% CM+50% MFP, 30% CM+70% MFP and 100% MFP were respectively. Hence, pH values remained within optimum values for biogas production. Marchaim indicated that the optimum biogas production is achieved when the pH value of the input mixture in the digester is between 6 and 7 [13]. A slight decrease after anaerobic digestion probably due to intermediate substances [16].

3.1.2. Total Solid Content and Volatile Solid Content

The sole and combine substrates were analyzed for their TS before and after AD. Results show that there was significant difference between treatments with maximum value (98.7) for 30%CM: 70% MFP combination and the minimum for 100% MFP. Similarly, significant difference was seen between treatments in TS after AD with maximum value for 100% CM and least for 70%CM: 30% MFP mix. (Table 3). Similar trend was observed in the case of VS, but the highest VS were that of 100%CM and the minimum was that of 30%PM: 70% MFP (Table 3). After AD, both TS and VS significantly decreased from the before AD. Reductions of TS and VS may due to conversion of the substrates into biogas through AD [14]. However, the maximum decrement of TS and VS was observed under the treatment that contained 30% CM + 70% MFP (49.49±0.03 to 20.60±0.03), suggesting more digestion of substrates by bacteria for either production of biogas or their own metabolic use. The maximum biogas yield was also observed in this treatment. For those treatments with lesser TS reduction, biogas yield was less relatively lower compared to the one that showed greater reduction.

Table 3. *Comparison of Total solids (% TS) and volatile solids (%VS) before and after AD for the different combinations of feed (values are mean ± SE, n=3).*

Treatment	Parameters			
	Initial %TS	**Final %TS**	**Initial %VS**	**Final %VS**
A	95.4±0.15^{Aa}	85.7±0.08^{Ab}	54.05±0.20^{Aa}	18.02±0.71^{Bb}
B	97.8±0.58^{Ba}	82.5±0.02^{Bb}	44.01±0.58^{Aa}	18.03±0.04^{Bb}
C	98.4±3.05^{Aa}	78.5±0.02^{Ab}	51.05±0.58^{Ba}	16.03±0.73^{Ab}
D	89.8±0.80^{Aa}	57.6±0.02^{Db}	50.09±0.55^{Ca}	14.05±0.43^{Bb}
E	99.5±1.51^{Aa}	67.6±1.06^{Cb}	45.07±0.50^{Ca}	15.06±0.33^{Ab}

Means followed by different small letters in row are significant at 0.05 probability level for paired samples T-test within treatment. Means followed by different capital letter in column are significantly different at 5% level of significance between treatments.
A. 100%CM B. 70%CM: 30% MFP C. 50%CM: 50% MFP D. 30%CM: 70% MFP E. 100% MFP.

3.1.3. Comparison of Organic Carbon (C) and Nitrogen (N) Before and After AD

Substrates prepared in sole and combinations were also analyzed for their organic carbon and nitrogen. From carbon and nitrogen values, C:N ratios of all treatments were also computed. Results show that there was significant difference between treatments both before and after AD in percent organic carbon, total nitrogen and C:N ratio. All of these parameters also significantly decreased after AD (Table 4). And this shows decomposition of organic matters into other compounds (composition of biogas) including methane and other intermediate compounds. The amount of reduction in organic carbon and total nitrogen was not equal k2between treatments. This might be the cause for variation in cumulative biogas yield measured at the end of incubation period [6]. Similar result has also been reported by [3] in his experiment of co-digestion of cow dung and poultry litter. Carbon to nitrogen ratio is a major factor affecting the anaerobic process which in turn affects methane yield and production rates. Therefore, the balance of carbon and nitrogen in a feed material is important. It is often suggested that an optimum C:N ratio is between 20:1 and 30:1 [13]. Therefore, the C:N ratios of all digesters were within optimal range though some variation.

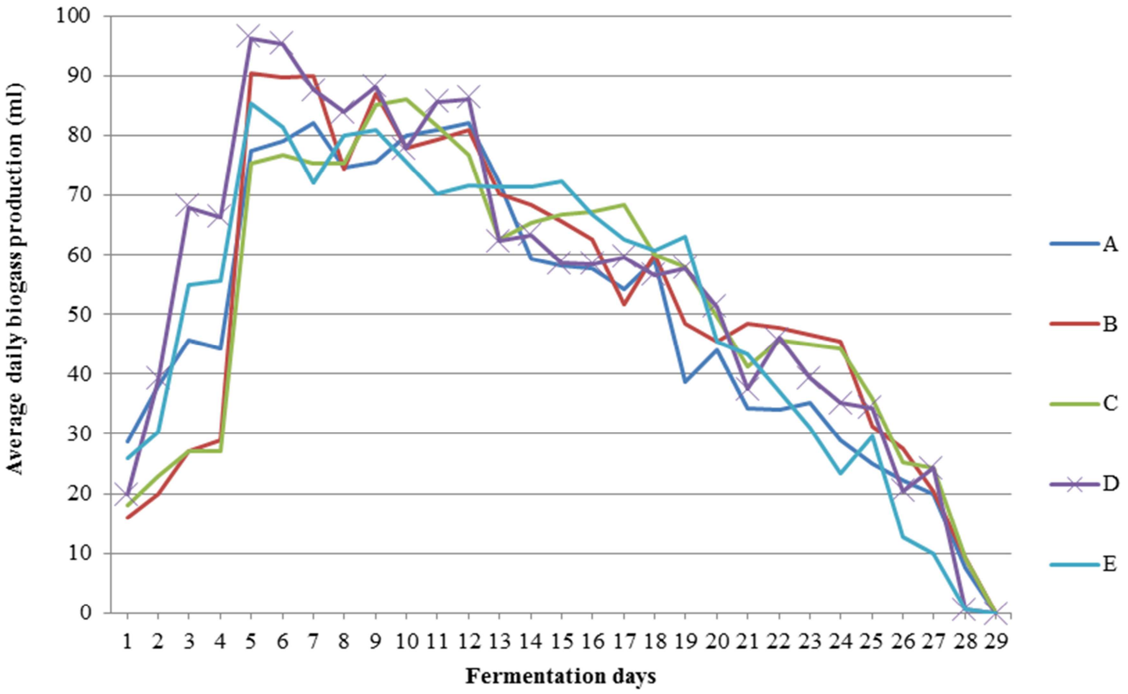
Table 4. *Comparison of Organic Carbon (C) and Nitrogen (N) before and After AD for the different combinations of feed (values are mean ± SE, n=3).*

Txt	% of Carbon		% of N_2		%C:N ratio	
	Initial	Final	Initial	Final	Initial	Final
A.	67.64±0.51 ^{Ba}	13.97±0.50 ^{Bb}	1.76±0.09 ^{Aa}	0.57±0.01 ^{Bb}	27.14±1.23 ^{Ba}	24.61±0.56 ^{Ab}
B.	75.27±1.22 ^{Aa}	36.50±1.11 ^{Ab}	2.86±0.04 ^{Aa}	0.64±0.03 ^{Bb}	49.67±0.03 ^{Aa}	45.17±0.01 ^{Ab}
C.	61.18±1.49 ^{Ca}	32.65±0.65 ^{Bb}	2.42±0.03 ^{Ba}	0.49±0.03 ^{Cb}	49.05±0.41 ^{Aa}	45.69±0.86 ^{Ab}
D.	54.48±2.38 ^{Da}	36.87±0.67 ^{Ab}	2.22±0.09 ^{Ca}	0.73±0.04 ^{Ab}	46.14±0.23 ^{Ba}	43.25±0.29 ^{Bb}
E.	66.5±1.22 ^{Ba}	37.17±0.83 ^{Ab}	2.7±0.06 ^{Aa}	0.72±0.02 ^{Ab}	47.28±0.12 ^{Ba}	43.92±1.78 ^{Bb}

Means followed by different small letters in row are significant at 0.05 probability levels for paired samples T-test within treatment. Means followed by different capital letter in column are significantly different at 5% level of significance between treatments.
A. 100%CM B. 70%CM: 30%MFP C. 50CM%:50%MFP D. 30%CM: 70%MFP E. 100%MFP. CM=cattle manure and MFP=Mixture of Fruit Peels

3.2. Determination of Average and Cumulative Daily Biogas Production

Gas production was monitored for 30 days of hydraulic retention time and emission was noticed from the first day of incubation in all digesters. The amount of daily gas production went gradually rising within the first week of incubation with peak measurement for most of the digesters at about 6th day. After that production gradually dropped to zero at the end of incubation (Figure 1). The trend probably is explained by the population dynamics of microbes in the digesters and availability of substrates for biogas production [13].



A=100% CM, B=70% CM: 30% MFP, C=50% CM: 50% MFP, D=30% CM: 70% MFP and E=100% MFP

Figure 1. *Average Daily biogas production of different substrate combinations.*

The cumulative of biogas production of the substrates was 2438.25, 2510.90, 2495.28, 2591.91 and 2485.23 ml for 100% CM, 70% CM + 30% MFP, 50% CM + 50% MFP, 30% CM + 70% MFP and 100% MFP, respectively. The maximum biogas production was measured in 30% CM +70% MFP whereas the minimum biogas production was measured from 100% CM (Figure 2). Compared to CM alone, all substrate types resulted in higher cumulative biogas yield with the highest cumulative biogas production observed in 30% CM + 70% MFP mix substrate. Though its %VS was higher, the 100% MFP did not result more biogas than the three PM to MFP substrate mixtures. This might be due to the less favorable situation of 100% MFP to microorganisms as compared to the substrate mixtures. In general, studies on possible uses of Corn stalk have indicated its potential use in biogas production.

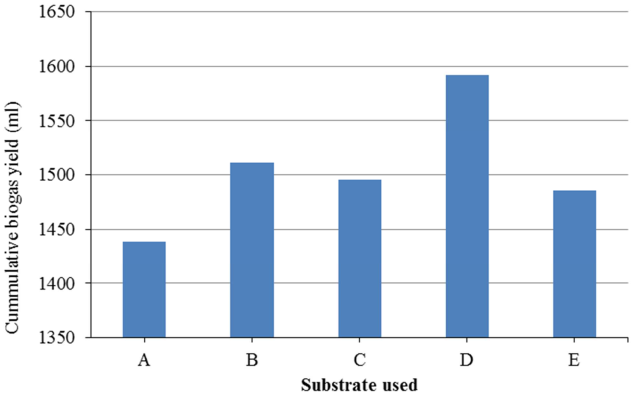


Figure 2. *Cumulative Biogas yield.*

A=100% CM, B=70% CM: 30% MFP, C=50% CM: 50% MFP, D=30% CM: 70% MFP and E=100% MFP

4. Summary, Conclusions and Recommendations

4.1. Summary and Conclusions

Anaerobic digestion was carried out to obtain suitable mix ratio for maximum biogas production from co-digestion of mixture of fruit peels with cattle manure at five mix ratios. The experiment was carried out in 500 ml batch digester at room temperature (25°C) for 30 days. The maximum biogas was produced in a combination of mixture of fruit peels with cattle manure in the ratio of 30% PM + 70% MFP. The highest cumulative biogas production was measured from 30% CM + 75% MFP ratio while the lowest was measured from 100% CM. Therefore, optimal co-digestion of mixture of fruit peels with cattle manure biomass in the ratio of 30% CM + 70% MFP has contributions for addressing and improving the level of feedstock for biogas production.

4.2. Recommendations

Based on the findings the following recommendations were forwarded:

Efforts should also be made to determine quality of biogas produced from mixture of fruit peels with cattle manure of the different combinations by Gas Chromatography.

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