Journal of Energy, Environmental & Chemical Engineering
2022; 7(2): 36-47
http://www.sciencepublishinggroup.com/j/jeece
doi: 10.11648/j.jeece.20220702.13
ISSN: 2637-4331 (Print); ISSN: 2637-434X (Online)

Effect of Cow Dung to Maize Silage Mix Ratios and Temperature Variation on Biogas Production in Laboratory Batch Digester

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To cite this article:

Monyluak Makaj Yai Chol, Nicasio Maguu Muchuka, Daudi Nyaanga. Effect of Cow Dung to Maize Silage Mix Ratios and Temperature Variation on Biogas Production in Laboratory Batch Digester. *Journal of Energy, Environmental & Chemical Engineering*. Vol. 7, No. 2, 2022, pp. 36-47. doi: 10.11648/j.jeece.20220702.13

Received: March 28, 2022; **Accepted**: April 20, 2022; **Published**: May 10, 2022

Abstract: Optimization of biogas production from a given substrate and digester is an issue that needs to be addressed during the development of anaerobic digestion. To maximize the biogas production rate, the operating parameters that influence anaerobic digestion must be controlled and monitored. This research was carried out using a 0.15 m^3 laboratory digester. The study evaluated the effect of cow dung and maize silage mix ratios (1:1, 1:3, and 3:1) on biogas production which were compared to their pure substrates at a constant temperature of 20°C. The temperatures (20°C, 25°C, and 30°C) were then evaluated using the optimal mix ratio of 3:1 as feedstock. The Temperature of the digester was controlled and monitored using Programmable Temperature Controller (Multispan UTC 421) and the (PLC) running on SIEMENS LOGO. The mix ratios and temperatures showed a significant effect on biogas production ($P \leq 0.05$) with mix ratios of 3:1 and 1:1 improving biogas production by 31.24% and 15.52% respectively compared to cow dung. The temperatures of 25°C and 30°C increased biogas by 26.99% and 47.35% and methane increased by 3.92% and 11.76% respectively compared to the mesophilic temperature of 20°C. The study thus, recommends a mix ratio of 3:1 and the optimal temperature of 30°C for a 0.15 m^3 laboratory temperature-controlled fixed-dome anaerobic digester of cow dung and maize silage as a substrate when fed as a batch reactor.

Keywords: Anaerobic Digestion, Biogas Production, Optimization, Mix Ratios, Maize Silage, Cow Dung, Temperature

1. Introduction

Since the independence of South Sudan in 2011, there has been a great concern for energy demand, especially in rural households where people rely on firewood and charcoal for cooking, lighting, and heating houses. It has been estimated that around 86% of the population rely on firewood as their primary fuel to fulfill their energy needs on daily basis [1, 2]. Recently, the country had experienced random and illegal cutting down trees in the natural forest for firewood and charcoal production, and that has resulted in high deforestation leading to adverse environmental effects such as desertification, land degradation, drought, and famine in many parts of the country.

In the quest for an alternative source of energy, especially for rural households, there is a need to explore and exploit new sources that are renewables as well as friendly to the environment. In most rural areas including in South Sudan, various biomasses such as cow dung and energy crops could be potential sources to help meet household's energy demand. Anaerobic digestion of those biomasses to produce biogas could reduce the pressure on natural forests, improve environmental sustainability through emission reduction of Green House Gases (GHGs) to the atmosphere [2]. Also, it could reduce waste disposal and management problems. Biogas, a renewable and environmentally friendly energy could provide the communities with clean non-pollutant fuel for cooking, improve energy security, sanitation and health conditions of the rural households. Biogas is generated by anaerobic digestion of organic materials in which methanogenic bacteria break-down and convert the organic matter into biogas in an environment free of oxygen [3, 2]. It is mainly a mixture of components in which methane accounts for 50-70%; carbon dioxide accounted for 30-40% and low amount of other gases [4, 2]. Optimization of biogas production rate from a given feedstock and the type of digester used are critical issues that need to be addressed during the development of anaerobic digestion system. The factors such as temperature, total solid, substrate retention time, pH, carbon to nitrogen ratio, organic loading rate and the stirring of digester content among other factors influence and affect the digestion process, they also enhance the proper breakdown of organic materials being digested for biogas production [5].

Since the development of anaerobic digestion system, there are wide ranges of organic materials that have been used as feedstocks. These feedstocks include energy crops such as maize, sugar beets, sorghum and sugar cane [6-8], animal manures such as cow dung, poultry manure and pig manure [9, 10]. Biogas production depends on various elements such as nutrient content in the feedstock being fed into the digester and other factors affecting the digestion process. Anaerobic digestion of energy crops have gained attention in the renewable energy industry with the potential of environmental and economic benefits [11]. Mixing different feedstocks has been recommended to improve nutrient balance in the digester especially the C/N ratio, hence, increases biogas production rate [12, 13]. The optimum C/N ratio of the substrate recommended for anaerobic digestion lies between 20:30 [14, 15]. The high C/N ratio of the material being digested indicates rapid nitrogen consumption by methanogenic bacteria and consequently, a lower biogas production rate [16]. A lower C/N ratio in the substrate being digested in the other hand leads to the accumulation of ammonia inhibitors which are toxic to methanogenic bacteria, hence, degrade biogas production or even system failure [17, 16].

Maize silage is an agricultural feedstock rich in cellulosic materials and easily degradable by bacteria in anaerobic digestion. The total solid contents of maize silage for anaerobic digestion was reported to be 30% [18], and it's considered an ideal crop for silage because it fulfils most of the biochemical requirements needed for silage preparation. Maize has a relatively low moisture content, low buffering capacity and has adequate water-soluble carbohydrate [19, 20]. The ensiling is a dynamic process that goes through several stages with a competitive environment and microorganisms, the process goes through four different stages based on biochemical and microbial transformation occurring during the ensiling. These four stages are namely; initial stage, fermentation stage, stabilisation stage and feed out stage [20]. In the initial stage, biomass respiration occurs immediately after filling and sealing the silage bag. The respiration process continues taking place for hours consuming sugars and producing carbon dioxide (CO_2) and water until the oxygen trapped inside the silage bag is all removed. In the fermentation stage, different bacteria's such as Lactic Acid Bacteria (LAB), Clostridia and Yeast competes for the available organic matter. Lactic Acid Bacteria produces lactic acid decreasing the pH of the silage to around 4.0 for efficient preservation [21]. In the stabilisation stage, the fermentation activities of microorganisms and bacteria are maintained low to stabilise anaerobic digestion. In this stage, the pH of the ensiled product remains stable, while the microbial and enzymatic activities continue until the feed out stage. The feed-out stage is the last in the ensiling process and it is critical because, once the silo or silage bag is opened, the remaining silage is exposed to oxygen and the microorganism is reactivated, which may spoil the remaining silage and lead to 15% energy losses [22, 20]. The feedstock's particle size is one of the parameters that enhance biogas production in anaerobic digestion, it speeds up the lactic acid bacteria (LAB) fermentation in the ensiling process and therefore, less organic matter losses [20]. Reducing the chopping lengths of the silage plant before ensiling has improved the fermentation process through the additional release of the easily fermentable substrate, leading to more extensive lactic acid fermentation [23]. Chopping the lengths of silage crop at the harvest stage to particle sizes of 7-8 mm could reduce anaerobic deterioration risk at the feed out stage by enabling higher silage densities and minimising air escaping into the silage [24].

The anaerobic digestion process is carried out by several bacteria and microorganisms that operate in a specific environment in the digester. These bacteria operate at three different temperature ranges: psychrophilic temperature range (below 25°C), the mesophilic temperature range between (25°C-45°C) and the thermophilic temperature range between (45°C-60°C) [25, 26]. Sudden change in the environment of bacteria and microorganisms inside the digester due to temperature fluctuation could minimise and slow down the bacterial activities or lead to death, subsequently leading to a decrease in biogas and methane production. The optimum temperature of anaerobic digestion may vary depending on the feedstock composition and the type of digester used for biogas production. Still, in most cases of anaerobic digestion processes. The operating temperature is maintained relatively constant to sustain the biogas production rate [27].

Limited studies have investigated the effect of mix ratios of cow dung and maize silage on biogas production. The effect of cow dung and maize silage was investigated at different mix ratios of 1:1; 3:1 and 1:3 at 8% dry matter content for 60 days retention time at a mesophilic temperature of 37°C [28]. The findings showed that mix ratio 3:1 of cow dung and maize silage had given the highest biogas production compared to other two mix ratios. The effect of nutrient balance on biogas production was evaluated from cow dung and maize silage mixtures under the mesophilic temperature of 36°C for 20 days substrate retention time [29]. The study indicated that specific gas yield had increased with the increasing dose of cow dung in the mix ratio.

The operating temperature of anaerobic digester has a direct effect on the physiochemical properties of the digester content. It affects the solubility of organic matter in the digester making it more accessible to microorganisms [30]. It also increases the chemical and biological reaction rate in the digester, thus, speeds up the conversion process resulting in a shorter retention time. The operating temperature of the digester has a strong influence over the quality and quantity of biogas production [31]. Several studies have reported that increasing the temperature of the digester enhance the biogas production rate [32, 33]. The optimum temperature of anaerobic digestion may vary depending on the feedstock composition and type of digester used for biogas production. Still, it should be maintained relatively constant in most anaerobic digestion processes to sustain the biogas production rate [26, 34, 35].

The effect of mesophilic temperature on biogas production was investigated using a blend of agro-based materials [26]. Cassava waste (CW), Brewery spent grain (BS), Powdered rice (PR), Cow dung (CD) and swine dung (SD) were blended and mixed at different mix ratios under mesophilic temperatures of 25°C, 30°C, 35°C and 40°C. The steady increase in biogas production was observed at temperatures of 35°C and 40°C and the highest yield was recorded under the optimum mesophilic temperature of 40°C [26]. The effect of temperature was investigated using Bamboo and Sawdust as substrates [34]. Bamboo and Sawdust were mixed with cow dung at a ratio of 1:3 in a batch feeding regime at different temperatures of 35°C, 45°C and 55°C. The study had shown that biogas production increases with the increase in temperature. In the mesophilic condition, the highest biogas production rate was recorded at 35°C compared to 45°C [34]. The effect of mesophilic temperature on biogas production was investigated using waste-water [35]. The study varied three temperatures of 34°C, 38°C and 42°C. The results show that the temperature of 38°C proved to be the most favourable for the anaerobic digestion process treating waste-water plants [35].

This study was aimed at evaluating the effect of different cow dung and maize silage mix ratios of 1:1; 1:3 and 3:1 which were compared to pure substrates at a constant temperature of 20°C digested in a 0.15 m^3 laboratory anaerobic digester for 20 days of retention time each mix ratio. The temperatures (20°C, 25°C and 30°C) were then evaluated using the optimal mix ratio of 3:1 (CD:MS) as feedstock for 15 days of retention time each treatment. The temperature of the digester was controlled and monitored by Programmable Temperature Controller (Multispan UTC 421).

2. Materials and Methods

2.1. Material Preparations

The materials for the study were cow dung and maize silage. Maize was harvested from Njoro maize farms, and the silage was prepared at Egerton University Agricultural Engineering biogas lab in Kenya. The preparation included chopping the whole plant into smaller lengths of 7-8 mm [24, 2] by using a chopping machine. The chopped maize was stored in well-sealed plastic bags (Silage bags) for two months to allow proper fermentation of the silage [2]. Fresh cow dung was collected from Egerton University Farm (Tatton Farm). The stones and other unwanted materials were first removed from cow dung before mixing them with maize silage at different mix ratios. Maize silage and cow dung different mix ratios were then diluted with water at TS of 8% to make a slurry that was fed into a fixed-dome lab digester for biogas production [2].

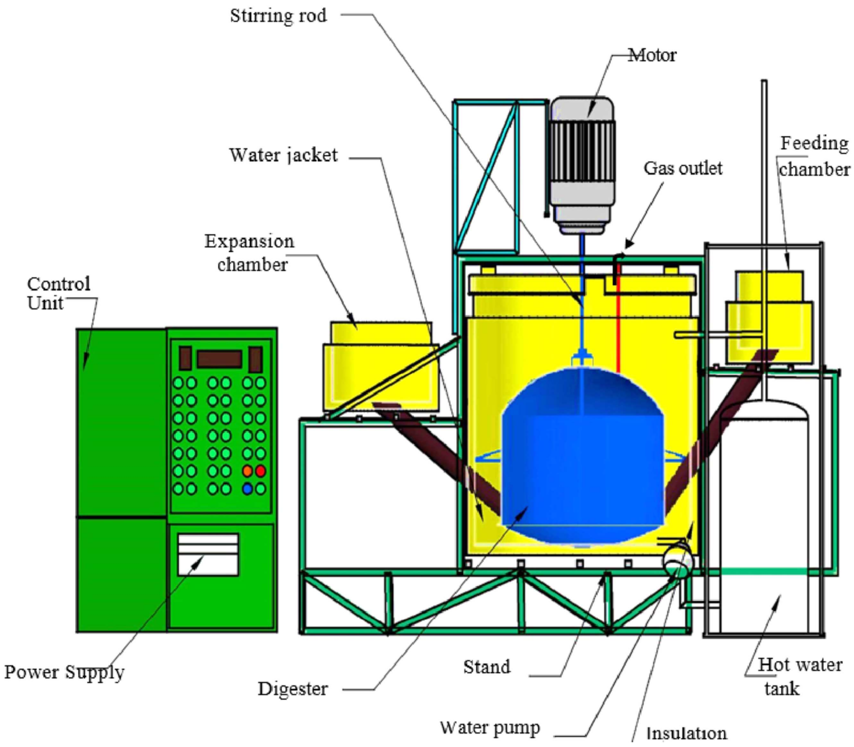

Figure 1. *Laboratory anaerobic digestion system [2].*

2.2. Experimental Setup

The laboratory anaerobic digestion system used for the study consists of four units namely; digester unit, heating unit, stirring system and control panel. The digester has a maximum volume of 0.15 m^3 with a net capacity of 0.12 m^3 and is enclosed by a water jacket, as shown in Figure 1. The digestion unit consists of a feeding chamber connected by a pipe to the digester used for feeding the system with the substrate. The expansion chamber connected to the main digester is used as an extra vessel to expand and remove the effluent from the digester. A water jacket is a metal sheet that enclosed the main digester and is used for thermoregulation of the digester's temperature by allowing hot water to be pumped through from an external water heating tank and circulated around the digester. The heating units consist of an external water tank with immersed heaters connected by a centrifugal pump to circulate hot water around the digester. The circulation of water between the water jacket and the external heater allows precise temperature control of the digester. A three-phase induction motor was mounted to the digester for stirring the substrate in the digester. The stirring speed and intervals were adjusted by the PLC programme running on LOGO SOFT SIEMENS. The control panel is an electrical box consisting of a power supply, Programmable Logic Controllers (PLC), Schneider Electric variable frequency drive (ATV12HU15M2), Programmable Temperature Controller (Multispan UTC 421) and setting buttons that are used to control different parameters in the digester such as temperature and stirring units. Biogas generated from the digester is collected through a biogas collection chamber using the water displacement method [2].

2.3. Substrate Pertinent Characteristics

Before the digestion process, feedstocks (cow dung and maize silage) were analyzed for total solids (TS, %), volatile solids (VS, %), Total Carbon (TC) and Total Nitrogen (TN) using the protocols given in Table 1 at the laboratories of Egerton University and Kenya Agricultural and Livestock Research Organization (KALRO).

Table 1. *Physical and chemical analysis methods used for the substrate samples.*

Parameter	Description of the method	Reference
TS (%)	Drying the sample in the laboratory oven at a temperature of 105°C for an hour.	[36, 2]
VS (%)	The residue obtained from total solids was ignited at a temperature of 550°C to a constant weight using a muffle furnace.	[36, 2]
TC (%)	Calorimetric: Organic carbon in the sample is oxidized by acidified dichromate at 150°C for 30 minutes. Barium chloride is added to the cool digests. The carbon concentration is read on the spectrophotometer at 600 nm.	[37, 2]
TN (%)	Digestion of feedstock in a tube with H_2SO_4 – Salicylic Acid- H_2O_2 and Selenium. The digestion completed by concentrated H_2SO_4 at elevated (330°C) under the influence of Se as a catalyst. Nitrogen was measured by distillation followed by titration with standardized 0.3 *N* HCL.	[38, 2]

TS= Total Solid, VS= Volatile Solid, HCL= Hydrochloric acid, S_e= Selenium, H_2O_2 = Hydrogen peroxide, H_2SO_4= Sulfuric acid, TC= Total Carbon, TN= Total Nitrogen.

2.4. Experimental Design

2.4.1. Effect of Cow Dung and Maize Silage Mix Ratios on Biogas Production

The effect of cow dung and maize silage different mix ratios were evaluated at the psychrophilic temperature of 20°C for 20 days of retention time. The selected three mix ratios of cow dung and maize silage were set at 1:1; 1:3 and 3:1 compared to pure substrates of cow dung and maize silage. The influents were prepared at 8% TS by diluting with tap water to make 120 kg of slurry and was fed into the digester using the formula (1) and (2) according to the sample's total solid contents as shown in Table 2.

The TS of the digester below 10% helps proper reactor stirring for biogas production. The formulas (1) and (2) were derived using the active volume of the digester which is 120 L or 0.12 m^3 and the digester's total solid 8% TS to quantify the masses of substrate and the water needed for proper dilution process [2].

$$\text{Mass of substrate} = \left\{\frac{8}{y} * 120\right\} \text{kg} \tag{1}$$

The required water to prepare 120 kg of the substrate is given by;

$$\text{Mass of water} = \left\{1 - \frac{8}{y}\right\} * 120\text{kg} \tag{2}$$

Where Y is the total solids (TS) of the sample being digested.

Table 2. *Water to substrate ratios for different mix ratios and their pure substrates at 20°C.*

Treatments	Mix ratios	Influent at 8% TS			
		TS (%)	Feedstock $\left\{\frac{8}{y} * 120\right\}$ kg	Water $\left\{1 - \frac{8}{y}\right\} * 120\text{kg}$	Feedstock: water ratio
Cow dung	1:0	16.1	59.627	60.373	1: 1.012
50%CD+50%MS	1:1	19.9	48.241	71.759	1: 1.487
25%CD+75%MS	1:3	24.3	39.506	80.494	1: 2.037
75%CD+25%MS	3:1	18.7	51.336	68.664	1: 1.337
Maize silage	0:1	31.6	30.379	89.621	1: 2.950

*TS= Total Solid, CD= Cow Dung, MS= Maize Silage, Y= TS of the sample.

The different mix ratios of 50%CD+50%MS, 25%CD+75%MS and 75%CD+25%MS with their pure substrates were weighed and diluted by adding water according to the sample's total solids content, then stirred for 10 minutes to attain a slurry of a regular consistency [2]. The mixed slurry was then poured into the digester, and the biogas was recorded daily until the decline in the daily biogas production was realised. The single substrates of cow dung and maize silage were used as the data controller. All the mix ratios were digested at a constant temperature of 20°C.

2.4.2. Effect of Temperature Variation on Biogas Production

The effect of temperature variation on biogas production was evaluated by running a 0.15 m^3 laboratory batch digester at different psychrophilic and mesophilic temperatures of 20°C, 25°C and 30°C for 15 days retention time each treatment as shown in Table 3. The digester was placed in a hot water bath (water jacket) to regulate the temperature. The water was heated by electric immersion heaters placed in a water tank and circulated by a centrifugal pump to the water jacket to ensure that the heated water releases heat to the digester until the digester's pre-set temperature is achieved [2]. The temperature controller (Multispan UTC 421) measures the temperature of the water jacket using a temperature sensor (thermocouples) and by comparing a sensors signal with a pre-set temperature, it regulates the process in a way that; if the temperature is lower than the set point, the PLC turns on the heaters. If the temperature is higher than the set point, the PLC turns off the heaters to maintain a constant temperature of the digester [2]. The biogas was recorded daily using the water displacement method until a decline in the daily biogas production was realised.

The substrate was prepared at 8%TS by diluting the optimal mix ratio 3:1 of cow dung and maize silage with water to make a slurry of 120 kg and was fed into the digester using the formula 1 and 2. In the process, 51.336 kg of cow dung and maize silage mix ratio with 18.7% TS was measured and diluted with 68.664 kg of water at ratio (1: 1.337) as shown in Table 3. The diluted substrate was then stirred for 10 minutes to attain a slurry with uniform consistency [2]. Then, the mixed slurry was poured into the digester. The biogas was recorded daily using the water displacement method until the decline in the daily biogas production was realised. The quality of biogas produced was analysed by a portable pump composite gas analyser (HFP-0401, portable 4IN1 gas detector) to determine the methane percentage [2].

Table 3. *Water to substrate ratios for optimal 3:1 CD: MS digested at different temperature.*

Treatment	Temperature (°C)	Influent at 8% TS				
		CD75:MS25	TS (%)	Feedstock $\left\{\frac{8}{y} * 120\right\}$ kg	Water $\left\{1 - \frac{8}{y}\right\} * 120$kg	Feedstock: water ratio
T1	20	3:1	18.7	51.336	68.664	1:1.337
T2	25	3:1	18.7	51.336	68.664	1: 1.337
T3	30	3:1	18.7	51.336	68.664	1: 1.337

*Y= TS of the sample= 18.7, CD= Cow Dung, MS=Maize Silage.

2.5. Biogas Production and Analysis

Biogas measurement was done using a water displacement method [39, 40, 2]. The daily biogas generated from the digester passes through the gas outlet. A graduated glass cylinder of 500 ml capacity was used as the gasholder. It's filled with water and kept in an inverted position in a water bucket as the biogas is released from a controlled valve through a plastic tube. It displaces water in the cylinder into a bucket of water due to the biogas pressure, and the biogas is recorded at a set point of the graduated glass cylinder. The process is repeated several times until the daily produced biogas is exhausted in the digester. The volume of biogas produced per day is calculated by computing the production rate in cubic metres per cubic metre of the digester volume per day (m^3/m^3day) [39, 40, 2].

The biogas composition was analysed using a portable pump composite gas analyser (HFP-0401, portable 4IN1 gas detector) to determine the methane concentration from the biogas sample being pumped into the system [2]. The portable gas analyser is a battery-powered device used to detect and analyze the concentration of combustible gases such as natural gas (Methane). The analyser is equipped with a sensor to detect the lower explosive limit (LEL) of methane gas at which it was calibrated. 4.9% LEL was reported as a low explosive level of methane, approximately 5% by volume [41, 2]. The analyser works by signifying a high methane gas level through a series of audible and visible indicators (light flashes, alarm sounds and vibration) at a response time ≤ the 20s. The sensor response serves as the reference point or scale, and when the sensor's response surpasses a certain pre-set level, an alarm is activated. The low explosive level (LEL) of the methane in the sample is recorded and calculated by dividing its concentration by the 100% LEL of methane (5%) [2].

2.6. Data Analysis

Data recorded from different mix ratios and temperatures were subjected to the analysis of variance (ANOVA) using SAS software for windows 8.2 (TS2M0) 1999-2001 by (SAS Institute Inc., Cary, NC, USA) to determine their effect on biogas production. The treatment values (means) of different mix ratios and temperatures were separated using Fisher's least significant difference (Fisher's LSD) at $p \leq 0.05$.

3. Results and Discussions

3.1. Physical and Chemical Characteristics of Substrates

Before the digestion process, different samples of the feedstock (cow dung and maize silage) were analysed for total solids (TS%), volatile solids (VS%), carbon content (C%), nitrogen content (N%) and carbon to nitrogen (C/N) ratio. The analyses were done at the laboratories of Egerton University and Kenya Agricultural and Livestock Research Organization (KALRO). Table 4 shows the detailed physicochemical characteristics of five different samples being analysed.

Table 4. *Physicochemical characteristics of the substrate mix ratios.*

Samples	Ratio	Parameters				
		TS (%)	VS (%)	C (%)	N (%)	C/N ratio
Pure CD	1:0	16.1	87.0	5.34	0.38	14.1
Pure MS	0:1	31.6	78.8	22.3	0.58	38.4
50%CD+50%MS	1:1	19.9	87.9	-	-	-
25%CD+75%MS	1:3	24.3	83.1	-	-	-
75%CD+25%MS	3:1	18.7	87.2	-	-	-

TS= Total solids, VS= Volatile Solids, C= Total Carbon, N= Total Nitrogen, C/N= carbon to nitrogen ratio, CD= Cow Dung, MS= Maize Silage.

Table 4 presents the detailed physicochemical characteristics of cow dung and maize silage different mix ratios. The results presented in the table showed that the C/N ratio of pure cow dung and that of maize silage were 14.1 and 38.4 respectively. The favourable C/N ratio for optimum biogas production lies between 20:30 [14, 15], and the pure cow dung here had a lower C/N ratio, which is below the required range to optimise biogas production. On the other hand, pure maize silage had a C/N ratio of 38.4, which was significantly higher than the optimal C/N ratio required for biogas production. Therefore, mixing these two substrates supplement and bring the two C/N ratios into the favourable range required to optimise biogas production. The amount of dry matter in the feedstock being digested determine and adjust the quantity of water being added to the feedstock in the dilution process. The total solid content of the feedstock affects biogas production [42]. If the total solid content of the feedstock is up to 30%, it reduces the conversion rate, while the total solid ranging from 30-50% inhibits anaerobic digestion as it builds up volatile fatty acids in the digester [42]. The TS% of pure maize silage presented in the table is not in line with the required TS% for biogas production, while TS% of cow dung is in the range [43-45].

3.2. Effect of Cow Dung and Maize Silage Different Mix Ratios on Biogas Production

The effect of cow dung and maize silage different mix ratios were evaluated at a constant operating temperature of 20°C for 20 days of substrate retention time each treatment. The selected mix ratios were set at 1:1; 1:3 and 3:1 which were compared to pure substrates of cow dung and maize silage. The volume of biogas produced per day was calculated by computing the production rate in cubic metres per cubic metre of the digester volume per day (m^3/m^3day) and the daily biogas produced was recorded throughout the substrate retention time. Data recorded from different mix ratios were subjected to the analysis of variance (ANOVA) using SAS software to determine the significant effect of each mix ratio on biogas production and their means were separated using Fisher's least significant difference (Fisher's LSD) at $p \leq 0.05$. The total and average biogas production from different mix ratios were separated and tabulated in Table 5. The daily data recorded from 1:1; 1:3 and 3:1 compared to pure substrates of cow dung and maize silage were plotted in Figure 2. The trend comparing the total biogas production for different mix ratios was presented in Figure 3.

Table 5. *Experimental results from different mix ratios of cow dung and maize silage.*

Substrates	Ratios	Factors			Biogas production	
		SRT* (days)	Temperature (°C)	TS (%)	Mean (m^3/m^3d)	Total (m^3)
Pure CD	1:0	20	20	16.1	0.2297^{b}	4.594
50%CD:50%MS	1:1	20	20	19.9	0.2654^{ab}	5.307
25%CD:75%MS	1:3	20	20	24.3	0.1019^{c}	2.038
75%CD:25%MS	3:1	20	20	18.7	0.3015^{a}	6.029
Pure MS	0:1	20	20	31.6	0.0000^{d}	0.000

The means with the same letters (superscript) are not significantly different at $P \leq 0.05$.
*Key: TS= Total solids, C= Total Carbon, N= Total Nitrogen, C/N= carbon to nitrogen ratio, CD= Cow Dung, MS= Maize Silage.

Table 5 presents the results of different mix ratios of cow dung and maize silage. The least significant difference (LSD) test on the effect of different mix ratios on biogas production showed that mix ratio 3:1 had higher biogas production and was significantly different from pure cow dung at $P \leq 0.05$. Similarly, mix ratio 1:1 had produced slightly higher biogas compared to pure cow dung but not significantly different from each other at $P \leq 0.05$. Contrarily, mix ratio 1:3 had produced lower biogas compared to pure cow dung, while pure maize silage did not produce biogas during the anaerobic digestion period. The daily data obtained from different mix ratios of 1:1; 1:3 and 3:1 which were compared to pure substrates of cow dung and maize silage were plotted in Figure 2 below.

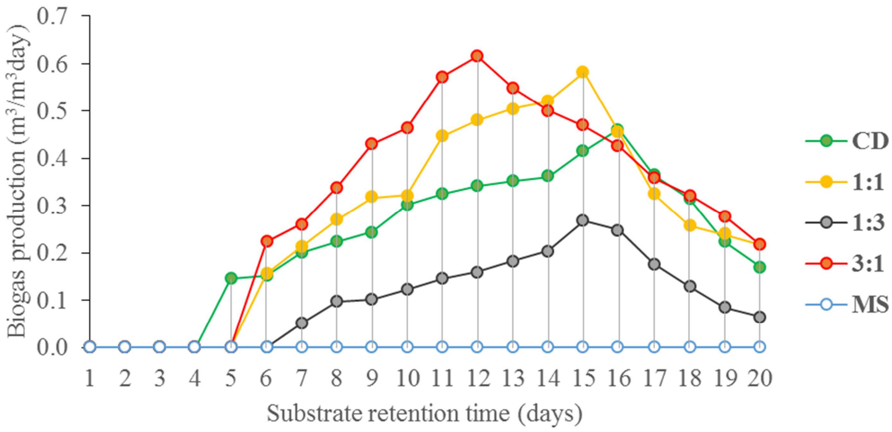

Figure 2. *Daily biogas production trend of different mix ratios.*

The results plotted in Figure 2 show that biogas production was observed on the fifth and the sixth day of retention time for cow dung, mix ratios 1:1 and 3:1, while it was observed on day seven for ratio 1:3. However, pure maize silage did not produce biogas during the digestion process. The delay in biogas production at the initial stage was probably due to slow microbial growth in the digester at the hydrolysis stage. During the hydrolysis stage, complex organic matter such as carbohydrates, proteins and fats are broken down to soluble organic molecules such as sugars, amino acids and long-chain fatty acids by different enzymes inside the anaerobic digester [46, 47]. A sample of biogas collected daily is burned to determine the anaerobic digestion stage. The unburnable biogas is an indication that CO_2 is higher than CH_4 in the biogas composition being produced, and that reveals the strong activity of hydrolysis. While the burnable biogas is an indication that CH_4 is higher than CO_2 in biogas composition being produced, this also reveals the strong activity of methanogenic bacteria at the final stage of the anaerobic digestion process [48, 2].

Cow dung to maize silage mix ratios 3:1 and 1:1 started producing biogas on day 6 of substrate retention time, while biogas was observed on day 5 and day 7 for pure cow dung and mix ratio 1:3 respectively. Thereafter, the biogas production increased gradually until the daily production reached the peak on day 12, 15 and 16 for mix ratios 3:1, 1:1 and 1:0 pure cow dung respectively, and that could be attributed to the increasing activities of methanogenic bacteria in the digester where the fermentation products such as acetate, hydrogen and carbon dioxide are converted to methane (CH_4) and carbon dioxide (CO_2) by methanogenic bacteria [49, 2]. The different peaks for 3:1, 1:1 and 1:0 pure cow dung could be attributed to different nutrients and biodegradable materials in each sample that the bacteria were acting on in the digester. After the peaks, biogas production started to decline gradually for the remaining days of the retention time due to the depletion of biodegradable organic materials in the digester, which resulted in slow microbial activities. The mix ratio 1:3 had produced very low biogas compared to pure cow dung while fermenting maize silage alone did not produce biogas during the anaerobic digestion process and that might be due to high lignin and non-degradable materials in maize silage that made it harder to be broken down by bacteria in the digester. Similarly, it could be attributed to the higher C/N ratio of maize silage as sample analysis shows that maize silage had a C/N ratio of 38.4 (Table 4) which was significantly higher than the optimal C/N ratio required for anaerobic digestion. The substrate's C/N ratio plays a vital role in the activity of microorganisms during the digestion process. The optimum C/N ratio required for anaerobic digesters lies between 20:30 [14, 15, 50]. The higher C/N ratio of the sample limits microbial growth in the digester due to the rapid consumption of nitrogen by methanogenic bacteria, leading to low biogas production or possibly process failure. On the other hand, a lower C/N ratio in the substrate reduces microbial growth as well due to carbon deficiency which leads to the accumulation of volatile fatty acids (VFAs) and ammonia (NH_3) and that reduces biogas production as well [13, 51].

Figure 3 compares the total biogas production for the digester loaded with cow dung and maize silage different mix ratios. The highest total volume of biogas produced was 6.029 m^3 recorded at mix ratio 3:1 then followed by 5.307 m^3, 4.594 m^3, 2.038 m^3 and 0.000 recorded at 1:1, CD, 1:3 and MS respectively. However, the statistical analysis results indicated that different mix ratios of cow dung and maize silage were significantly different at $P < 0.05$. Although mix ratio 3:1 produced higher biogas and is statistically different from pure cow dung, the trend of daily biogas production for ratios 1:1 and 3:1 was quite similar and were statistically not significantly different from each other at $P \leq 0.05$ (Table 5). Higher biogas production at mix ratios 3:1 and 1:1 could be probably due to the balance in the C/N ratio between maize silage and cow dung. Anaerobic codigestion of more than two different feedstocks improves the digester's nutrient balance by supplementing the missing nutrients to the digestion process, increasing and stabilizing the biogas production rate [12, 13]. Codigestion of materials with high carbon content such as maize silage with cow dung at ratios 3:1 and 1:1 had improved biogas production by 31.24% and 15.52% respectively compared to pure cow dung. The study concluded that anaerobic lab digester at mix ratio 3:1 of cow dung and maize silage had recorded biogas production higher than other mix ratios under the same temperature of 20°C. The results obtained had concurred with the results reported by other researchers at a mix ratio of 3:1 of cow dung and maize silage [28].

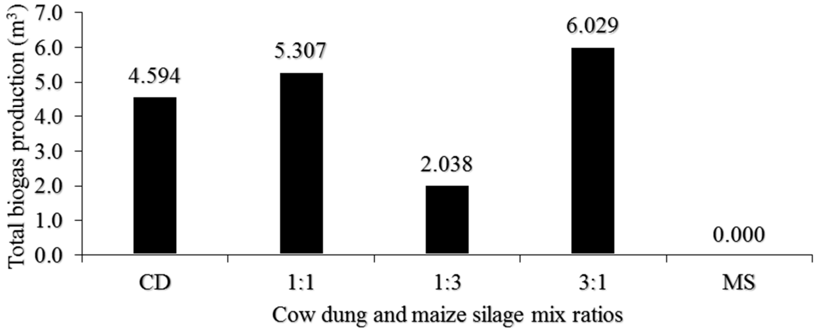

Figure 3. *Total biogas production from different mix ratios.*

3.3. Effect of Temperature Variation on Biogas Production

The effect of temperature variation on biogas production was investigated by running a laboratory batch digester at different temperatures of 20°C, 25°C and 30°C for 15 days of retention time each treatment. The substrate was prepared at 8%TS by diluting the optimum mix ratio of 3:1 of cow dung and maize silage with water to make a slurry of 120 kg that was fed into a fixed dome anaerobic digester. The volume of biogas produced per day was calculated by computing the daily production rate in cubic metres per cubic metre of the digester volume per day (m^3/m^3day). Data obtained from 20°C, 25°C and 30°C were subjected to the analysis of variance (ANOVA) using SAS software to determine the significant effect of different temperatures on biogas production. The means of data obtained from different temperatures were separated using Fisher's least significant difference (Fisher's LSD) at $p \leq 0.05$, then, the total and the average biogas production were calculated and tabulated in Table 6. The daily biogas recorded throughout the digestion period were presented graphically in Figure 4 and the trend of total biogas and methane percentages for the digester operated at different temperatures were presented in Figure 5.

Table 6. *Biogas production and methane percentage at different temperatures.*

Mix ratio	Factors			Biogas production		
	SRT* (days)	Temperature (°C)	TS (%)	Mean (m^3/m^3d)	Total (m^3)	Methane (%)
3:1	15	20	18.7	0.275^c	4.120	51
3:1	15	25	18.7	0.349^b	5.232	53
3:1	15	30	18.7	0.405^a	6.071	57

The means with the same letters (superscript) are not significantly different at $P \leq 0.05$.

Table 6 shows the results of both biogas and methane percentage obtained at different temperatures of 20°C, 25°C and 30°C. The least significant difference (LSD) test on the effect of temperature variation on biogas production had shown that the data recorded at 20°C, 25°C and 30°C were significantly different from each other at $P \leq 0.05$ with the digester operating at 30°C recording the highest biogas and methane percentage compared to the psychrophilic temperature of 20°C. The daily data obtained from 20°C, 25°C and 30°C were plotted in Figure 4 below.

Figure 4 presents the daily biogas production trend (m^3/m^3d) recorded at different temperatures of 20°C, 25°C and 30°C for 15 days retention time each. The biogas production was observed on days five and six of the retention time for the temperatures 20°C, 25°C and 30°C. The delay of biogas production at the initial stage was attributed to the slow microbial activity due to the strong activity of hydrolysis. During the hydrolysis stage, complex organic matters in the digester were first broken down into soluble organic molecules like sugars, amino acids and long-chain fatty acids by extracellular enzymes to be utilised by Acidogenic bacteria [46, 2]. A sample of biogas collected daily is burned to determine the anaerobic digestion stage. The unburnable biogas is an indication that CO_2 is higher than CH_4 in the biogas composition being produced, and that reveals the strong activity of hydrolysis. While the burnable biogas is an indication that CH_4 is higher than CO_2 in biogas composition being produced, this also reveals the strong activity of methanogenic bacteria at the final stage of the anaerobic digestion process [48, 2]. Biogas was observed on day 5 of the retention time for the temperatures of 2°C and 30°C, while it was observed on day 6 for the digester operating at temperature 20°C. The lag period between the digester operated at 2°C and that operated at 25°C, and 30°C shows that the digestion and decomposition process in the digester takes place quickly at the higher temperature. Thereafter, the production increased gradually until the digester reached a peak on day 11 for temperatures 25°C and 30°C. In contrast, at 20°C, biogas production reached the peak on day 12 of the retention time, which could be attributed to the increasing activities of methanogenic bacteria in the digester. After the peak, biogas production started to decline gradually for the remaining days of the retention time due to the depletion of biodegradable organic materials in the digester, which resulted in slowing down the activities of microorganisms inside the digester.

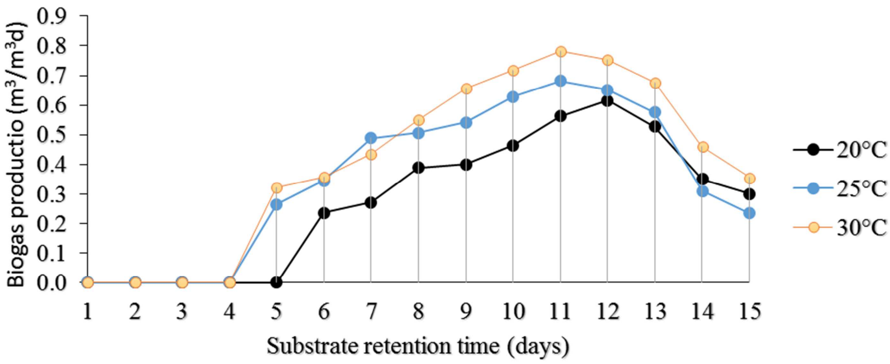

***Figure 4.** Daily biogas production trend for different temperatures.*

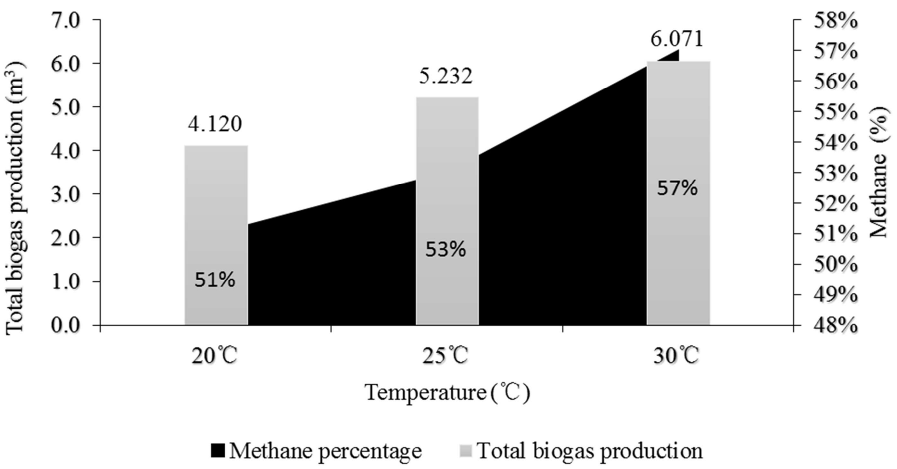

***Figure 5.** Total biogas and methane percentage for different temperatures.*

Figure 5 presents a trend of total biogas production and methane content percentage for three different temperatures. The highest biogas production was recorded at a temperature of 30°C with total biogas of 6.071 m^3 then followed by 5.232 m^3 and 4.120 m^3 recorded at 25°C and 20°C respectively. The highest biogas production recorded at 30°C was probably due to the high temperature in the digestion process. The digester temperature is the main factor affecting the performance and stability of anaerobic digestion [52]. Mesophilic temperature between (25-40°C) stabilises anaerobic digestion performance as it provides a favourable environment for intensive microbial activity in the digester and is less sensitive to inhibitors [47]. Different temperatures had a direct effect on biogas production as both the results of the evaluated temperatures and their means were statistically significantly different at $P \leq 0.05$ (Table 6). The biogas and methane content differed according to the digestion temperature, with the methane content accounting for 51%, 53% and 57% recorded at temperatures of 20°C, 25°C and 30°C respectively. The results show that, with the increase of the operating temperature, biogas and methane content continuously increased as the total biogas production increased by 26.99% and 47.35% at 25°C and 30°C, respectively compared to the psychrophilic temperature of 20°C. The quality of biogas obtained was dependent on the operating temperature as the methane content percentage increased by 3.92% and 11.76% with the temperature change from 20°C to 25°C and 30°C respectively.

The results obtained were in agreement with the results reported by many researchers stating that, increasing the temperature of the digester within the mesophilic range improves biogas and methane production [33, 26, 53]. In conclusion, it has been observed that the total cumulative biogas production and the methane percentage had improved with increasing the operating temperature of the digester. For the lab-scale anaerobic digestion temperature range 20°C, 25°C and 30°C, the higher the temperature, the better the biogas and methane production.

4. Conclusions

4.1. Effect of Different Cow Dung and Maize Silage Mix Ratios on Biogas Production

The results obtained from evaluating the effect of different cow dung and maize silage mix ratios on biogas production had shown that a mix ratio of 3:1 (75% CD: 25%MS) provided the highest cumulative biogas of 6.029 m^3 followed by 5.307 m^3, 4.594 m^3, 2.038 m^3 and 0.000 at 1:1, CD, 1:3 and MS, respectively. The mix ratio of 3:1 (75% CD: 25%MS) had improved biogas production by 31.24% compared to Cow dung alone. In addition, maize silage alone is not suitable for biogas production due to its high C/N ratio that limits the microbial activities in anaerobic digestion. Based on the results obtained from the study, the research work recommends cow dung and maize silage mix ratio of 3:1 (75% CD: 25%MS) for the 0.15 m^3 laboratory temperature-controlled fixed-dome anaerobic digester when fed as a batch system.

4.2. Effect of Temperature Variation on Biogas Production

The results obtained from evaluating the effect of temperature variation on biogas production from cow dung and maize silage mix ratio of 3:1 (75% CD: 25% MS) had shown that biogas and methane content increased according to the digestion temperature, with the methane content being 51%, 53% and 57% at 20°C, 25°C and 30°C, respectively. The results obtained revealed that the highest total cumulative biogas was achieved at 30°C with total biogas of 6.071 m^3 then followed by 5.232 m^3 and 4.120 m^3 recorded at 25°C and 20°C, respectively. The temperatures of 30°C and 25°C improved biogas by 47.35% & 26.99% respectively compared to 20°C. Methane percent increased by 11.76% and 3.92% at 30°C and 25°C compared to the psychrophilic temperature of 20°C. The mesophilic temperatures of 30°C and 25°C have shown a positive effect on biogas production from cow dung and maize silage feedstock at a mix ratio of 3:1. Therefore, the optimum temperature of 30°C is thus recommended for the 0.15 m^3 laboratory temperature-controlled fixed-dome anaerobic digester of cow dung and maize silage as a substrate when fed as a batch system.

Acknowledgements

The research project was supported by the Centre of Excellence in Sustainable Agriculture and Agribusiness Management (CESAAM), Egerton University, Kenya.

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