Impact of Different Mixing Ratios on Biomethanation of Cattle Blood and Rumen Content

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Abstract:- **Biomethane produced by anaerobic digestion of organic waste is a renewable and sustainable energy that can supplement global energy needs. Existing literature shows that different mixing ratios of the same co-substrates have an impact on biomethane production. In this study, the impact of different mixing ratios of cattle blood and rumen contents on biomethane production was investigated. The physicochemical characteristics of seven samples with different blood and rumen contents were determined. Their biomethane yield was then assessed in laboratory-scale batch digesters at 37° C (mesophilic). The biomethane yields of the samples gave a mean value of 11.25±13.34 which indicates significant variability ($p < 0.05$). The sample containing 10 ml of bovine blood and 50 ml of rumen contents (S_6) gave the highest biomethane yield and can be considered for optimization of biomethane production from these feedstocks.**

Keywords:- **Co-digestion, biomethane, mixing ratios, cattle blood, rumen content**

I. INTRODUCTION

Energy is an inevitable resource in socio-economic development. However, the supply of fossil fuels, which are currently the main global source of energy, is running out, while their regular use generates greenhouse gasses that contribute to climate change. It is therefore a global concern to search for alternative energy sources that are renewable and sustainable. Nevertheless, biomethane produced by anaerobic digestion (AD) of organic waste is a proven renewable and sustainable energy. Biomethane has a calorific value of $36MJ/m^3$ and therefore can be combusted to produce thermal energy and electricity [1, 2].

The anaerobic digestion process involves four steps which are interdependent. These are hydrolysis, acidogenesis, acetogenesis and methanogenesis [3, 4]. In this process, microorganisms digest organic material for their growth and produce biogas which contains about 50 – 70% biomethane as a by-product [1, 5]. Different organic wastes have different biomethane potentials. However, the suitability and biomethane potential of substrates depend on their physicochemical characteristics and operating conditions such as pH, temperature, loading rate, pre-treatment and complete absence of molecular oxygen [6].

The activities of microorganisms involved in anaerobic digestion (AD) depend on the above factors. The biomethane producing bacteria (methanogenic bacteria) are sensitive to temperature, oxygen and pH [6, 7]. Temperature instability has a negative effect on biomethane production process. Fluctuations that do not exceed the following limits can be considered safe when operating AD processes: Psychrophilic range (±2 °C/h), Mesophilic range (±1 °C/h), Thermophilic range (±0.5 °C/h) [6]. The pH of a substrate is a critical parameter that determines the success of the AD process. This is because outside the optimum pH range of 6.5 and 8.5 [8], methanogens are strongly inhibited. Therefore, the proper slurry pH must be maintained in the digester for stable biomethane production. [5, 3].

The pH depends on the Carbon - Nitrogen ratio (C/N ratio) of the substrate fed to the digester. C/N is the ratio of the number of organic carbon atoms to the number of nitrogen atoms in the substrate. [3]. It determines the stability of the digestion process. Microorganisms feed on carbon as a source of energy and nitrogen to build cell structures. If the C/N ratio is too high, the pH of the process drops rapidly due to the formation of fatty acids, which inhibits the methane-producing bacteria. If the ratio is too low, the process suffers from ammonia inhibition [5, 3]. It is difficult to state the exact optimal C/N ratio because it varies with different substrates and with process conditions. However, for optimized biomethane production, the C/N ratio of the feedstock mixture should be between 20 and 30. Microorganisms consume carbon about 30 times faster than they use nitrogen, so they need a C/N ratio closer to 30. Table 1 shows the C/N ratio of some organic materials [9, 5].

Table 1: C/N of some organic materials

Substrate	Nitrogen (%)	C/N ratio
Chicken dung	8	6-15
Turkey litter	2.6	16
Human use	5-7	5-10
Pig manure	3.1	14
Cow manure	2.4	19

Sheep manure	2.7	16
Slaughterhouse waste	7-10	2-4
Horse manure	1.6	25-30
Whole carrot	1.6	27
Potato top	1.5	25
Raw sawdust	0.11	511
Rotted sawdust	0.25	200-500
Rice hull	0.3	121
Soybean meal	7.2-7.6	4-6
Tomato	3.3	12
Corn hubs	0.6	56-123
Corn husk	0.6-0.8	60-73
Grass clipping	2.1	12-19
Water lily	2.9	11.4
Algae	1.9	100
Fallen leaves	1	50

Biomethane yield also depends on the total solids (TS) and volatile solids (VS) of the substrates. TS is the dry solids portion of the wet substrate after heating the substrate to a temperature of about 105o C. VS is a portion of TS, which is lost when the dry matter is combusted at 550o C or 600o C. The total solids portion of the substrate is the volatile solids portion. It is the digestible portion of the substrate. It determines the concentration of the slurry introduced into a digester and the amount of biomethane produced from it per unit weight of slurry introduced into the digester [5, 3]. The total solids and volatile solids of some substrates are shown in Table 2 [10, 9, 11].

Table 2: The TS and VS content organic materials

Substrate	TS (%)	VS (%TS)
Rumen content (untreated)	12 – 16	85 – 88
Cattle slurry	5 – 12	80
Chicken manure	10 – 29	67 – 77
Brewery spent grain	20 – 26	80 – 95
Corn Silage	20 – 40	94 – 97
Municipal organic waste	15 – 30	80 – 95
Sheep manure	18 – 25	80 – 85
Human Faeces	14 – 22	79 – 84; 93
Sewage sludge	3 – 5	75 – 85
Cattle excreta	25 – 30	75 – 85
Pig stomach content	12 – 15	80 – 84
Pig manure	20 – 25	75 – 80
Pig slurry	3 – 8	70 – 80

Studies have shown that biomethane production and process stability can be improved by co-digestion of high- carbon materials with low C/N ratio substrates. Moreover, different mixing ratios of the same co-substrates lead to different biomethane yields [12, 13, 14]. The addition of nutrient medium to the substrate increases the adaptability of the microorganisms involved in the anaerobic digestion process [15]. [12], investigated the biogas potential of anaerobic co-digestion of poultry slaughterhouse waste with sewage sludge. [16], evaluated the co-digestion process of different waste mixtures from agro-industrial activities. [17], studied the methane production, long-chain fatty acids (LCFAs) profile and predominant microorganisms in anaerobic digestion (AD) of lipid-rich swine slaughterhouse (SSW) waste. [18] investigated the biomethane production potential of slaughterhouse waste in the United States. [19] investigated the effects of varying the inoculum to waste ratio on the anaerobic treatment of slaughterhouse waste and proposes a cost-effective anaerobic treatment system.

Although cattle blood and rumen contents (which often constitute the majority of organic slaughterhouse waste) have been identified as suitable feedstock for biomethane production, their biomethane potential has not been investigated in relation to different mixing ratios [20, 21, 22]. This work was carried out to fill this gap by evaluating the biomethane potential of cattle blood and rumen contents from Kumasi Abattoir Company Limited. The study helps in identifying a mixing ratio that increases biomethane production from these organic effluents from the abattoir, as well as highlighting the effects of different mixing ratios of the same co-substrates on biomethane.

II. METHODS AND MATERIALS

2.1 Collection of Raw Material

Cattle blood (fluid) and rumen contents (slurry) were collected at 9:00 am from the evisceration unit of Kumasi Abattoir Company Limited at Ahinsan in Kaase Industrial Area, Kumasi, Ghana. The blood of the cattle was collected in a clean, dry plastic bottle while the rumen contents were collected in a clean, dry plastic container. They were stored in a temperature-controlled ice chest and transported to Biotechnology Laboratory of Chemical Engineering Department at Kwame Nkrumah University of Science and Technology, Kumasi, Ghana for analysis.

2.2 Experimental Set-up

Seven samples were prepared for the study with different mixing ratios of cattle blood, rumen contents and nutrient medium. Each sample was analysed for its physicochemical characteristics and then digested in a 1- litre laboratory digester at a constant temperature of 37o C (mesophilic) under anaerobic conditions to determine the biomethane yield. The digester system consisted of digesters, gas counter, infrared sensor (BlueSensBACCom 12) and BACVis software (see Figure 1).

Figure 1:The digester system and some of its components.

2.3 Sample Preparation

Samples were prepared by mixing various ratios of bovine blood and rumen contents. To each sample, 100 ml of nutrient medium was added and stirred at 200 rpm for 30 minutes to obtain a homogeneous mixture. The mixing ratios of the seven samples are shown in Table 3. The composition of the nutrient medium is shown in Table 4.

2.4 Determination of Physicochemical Characteristics of Samples

The following physicochemical characteristics of the samples were determined. Samples were prepared from cattle blood and rumen contents on three consecutive Wednesdays and the average was determined.

2.4.1.pH and Temperature

The pH and temperature of the samples were determined using the multiparameter from Hanna Instrument (HI9829). Each measurement was taken three times and averaged. All standard laboratory protocols were followed and the manufacturer's instruction manual was strictly followed. Each measurement was repeated three times and the average was determined.

Table 3: Composition of Samples.

Sampls	Nutrients (ml)	Blood (ml)	Rumen Content (ml)
S_1	100	60	0
S_2	100	50	10
S_3	100	40	20
S_4	100	30	30
S_5	100	20	40
S_6	100	10	50
S_7	100	0	60

Table 4: Composition of nutrient medium

Chemical	Mass (g)
NH_4Cl	0.53
KH_2PO_4	0.27
$Na_2HPO_4.2H_2O$	0.56
$CaCl_2.2H_2O$	0.075
$MgCl_2.6H_2O$	0.1
$FeCl_2.4H_2O$	0.02

2.4.2. Total Solids, Moisture Content, Ash Content and Volatile Solids

To determine TS and moisture content, 50 ml of each wet sample was weighed for its mass (MWS) and dried at a temperature of 105° C for twelve hours and the mass of dried sample (MDS) was weighed. The VS and ash content (AC) were also determined by burning the dried samples to ash at a temperature of 550° C for four hours and weighing the mass of ash (MA). They were then calculated using equations (1), (2), (3) and (4) as used by [23, 24] in their study. Each measurement was repeated three times and the average was taken.

$$TS(\%) = \frac{M_{DS}}{M_{WS}} \times 100 \quad (1)$$

$$MC(\%) = \frac{M_{WS} - M_{DS}}{M_{WS}} \times 100 \quad (2)$$

$$AC(\%) = \frac{M_A}{M_{DS}} \times 100 \quad (3)$$

$$VS(\%) = \frac{M_{DS} - M_A}{M_{DS}} \times 100 \quad (4)$$

2.4.3. Determination of Carbon – Nitrogen ratio (C/N)

To determine the C/N ratio, 50 ml of each sample was used. The organic carbon content of the samples was determined by dry combustion while the nitrogen content of the samples was determined by Kjeldhal Method [25, 24, 4]. Each measurement was repeated three times and the average was determined.

2.5. Determination of Bio-methane Yield

Each of the prepared samples was digested in a digester system to assess its biomethane yield. For each sample, the gas counter and infrared sensors (BlueSensBACCom 12) of the digester system recorded the cumulative volume of biogas (ml) and the corresponding cumulative methane concentration (%), respectively. Measurements were taken in each 1. The BlueSensBACCom 12 was connected to the BACVis software, which displayed the measured data on a computer

screen. The biomethane yield of each sample was determined by multiplying the cumulative biogas volume and the cumulative methane concentration.

III. RESULTS AND DISCUSSION

3.1. Physicochemical Characteristics

The physicochemical characteristics of the samples are shown in Table 5. Itis noticed that there is variability in the characteristics of the samples suggesting that different mixing ratios of the same co-substrates produce substrates with different physicochemical characteristics. This agrees with the findings of [1, 4, 13] in their studies that different mixing ratios of the same co-substrates produce different physicochemical characteristics. The TS and VS of the samples show that they all had biomethane potentials. In [26], it was recommended that the TS and VS in substrates for biomethane production should be 3 – 10% and 75 - 95 %, respectively. In the study of [29] moisture content of substrates increases the biomethanation production therefore higher range of moisture content (93.5$\pm$0.01 to 97.0 $\pm$0.00) suggest that the samples have high biomethane potential. This could also account for the highest biomethane potential of sample S_5.

The C/N ratio of the samples ranged from 2.6 - 11.3, which is close to the findings of [26] in their study that cattle blood and rumen contents have C/N of 3.41 and 19.2, respectively. It was found that the C/N ratio of the samples was inversely proportional to the composition of the blood. This indicates that C/N ratio depends on the type of substrate. It is known that blood contains more protein and therefore the addition of blood to the rumen contents reduces its C/N ratio. Therefore, co-digestion of rumen contents and cattle blood may improve the C/N of the substrate. The pH of the samples was within the optimal pH values for methanogens (6.5 - 8.5) and their temperatures were within the mesophilic temperature range [5, 9,]. This indicates that the methanogens in the substrates had suitable conditions for biomethane production.

3.2. Bio-methane Potential of the Samples

The trend of cumulative biomethane production of the samples is shown in Figure 2. Although the trend of cumulative methane production varied among the samples, they all followed the bacterial growth curve. It took some time for each of the samples to begin producing biomethane gas. During this elapsed time, the microorganisms adapted to their new environment before actively engaging in the process. However, S_1, S_2, S_3, and S_4 began production on the first day, while S_6 and S_7 began production on the second day and S_5 on the third day.

Table 5: Physicochemical characteristics of samples

Samples	Moisture Content (%)	Total Solids (%)	Ash Content (%)	Volatile Solids (%)	pH	Organic Carbon (%)	Nitrogen (%)	Carbon Nitrogen Ratio	Temperature (%)
S1	95.4$\pm$0.00	4.6$\pm$0.03	6.3$\pm$0.01	93.7$\pm$0.01	8.0$\pm$0.2	47$\pm$0.1	16.8$\pm$0.01	2.8$\pm$0.01	28$\pm$0.01
S2	93.5$\pm$0.01	6.5$\pm$0.01	6.4$\pm$0.01	93.6$\pm$0.00	8.1$\pm$0.00	46.2$\pm$0.01	15.7$\pm$0.01	2.9$\pm$0.01	25.4$\pm$0.01
S3	95.0$\pm$0.01	5.0$\pm$0.01	8.8$\pm$0.00	91.2$\pm$0.01	7.9$\pm$0.01	44.7$\pm$0.01	13.6$\pm$0.01	3.3$\pm$0.01	27.7$\pm$0.01
S4	96.4$\pm$0.01	3.6$\pm$0.04	13.3$\pm$0.01	86.7$\pm$0.00	7.8$\pm$0.01	41.3$\pm$0.01	16.0$\pm$0.00	2.6$\pm$0.01	27.8$\pm$0.01
S5	95.7$\pm$0.01	4.3$\pm$0.05	15.1$\pm$0.01	84.9$\pm$0.00	7.8$\pm$0.00	35.2$\pm$0.01	6.4$\pm$0.00	5.5$\pm$0.01	27.6$\pm$0.01
S6	96.4$\pm$0.01	3.6$\pm$0.03	18.6$\pm$0.00	81.4$\pm$0.00	7.8$\pm$0.01	34.5$\pm$0.01	5.5$\pm$0.01	6.2$\pm$0.00	27.7$\pm$0.02
S7	97.0$\pm$0.00	3.0$\pm$0.01	24.8$\pm$0.00	75.2$\pm$0.00	7.7$\pm$0.00	33.1$\pm$0.01	2.9$\pm$0.01	11.3$\pm$0.01	28.0$\pm$0.01

S_1 did not produce measurable amounts of biomethane gas after the first day until digestion was complete. This may be due to the high digestibility of the blood, allowing the bacteria to digest it quickly [27]. It may also be due to the pH of the digestion process dropping below the required level (6.5 to 8.5) and then inhibiting the process due to the high nitrogen content of the sample. Also, it may be due to lack of balanced nutrients and sufficient microorganisms in the sample. S_3 and S_5 finished production on the seventh day, while S_4 finished production on the eighth day and S_1, S_2, S_6 and S_7 finished production on the fourteenth day. The longer digestion times of S_7 and S6 may be due to the fact that they contained a high percentage of cellulose and hemicellulose, which require more time to digest.

However, the longer time of S_2 may be due to the higher proportion of volatile solids [14, 4].

S_3 increased production gently until the second day, after which it increased steeply until the third day, from which production remained constant. S_2 and S_6 gently increased production from the first and second days, respectively, until the fourteenth day, after which production ceased. Day after which production stopped. This could be due to the fact that there was a balanced supply of nutrients and microorganisms that kept the pH in the appropriate range, which caused the microorganisms to remain active to digest the sample [4, 9]. Meanwhile, the production in S_4 increased slightly from the second day until it remained constant on the third day. The variation in the trend of biomethane production of the samples indicated that the different mixing ratios affected the production of biomethane gas.
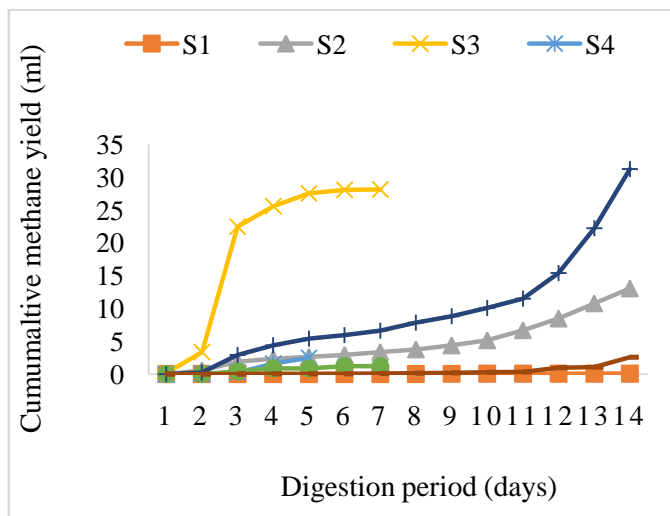

Figure 2: Trend of bio-methane production of samples.

The cumulative biomethane yields of the samples are shown in Table 6 and cattle blood and rumen contents produced the highest biomethane gas, followed by the sample with the ratio of 2:1 (S_3), while the sample with the ratio of 6:0 (S_1) produced the least. One-factor ANOVA showed that there was a significant difference in the biomethane yields of the samples ($p < 0.05$). As explained in [1, 4, 6, 13, 28], this difference can be attributed to the different mixing ratios of the co-substrates. This is because mixing co-substrates in different ratios results in substrates with different physicochemical characteristics, particularly TS, VS and C/N ratio, creating different environmental conditions for the microbes.

Although sample S_7 had a C/N of 11.3, which is within the recommended C/N ratio range of 20-30:1 [3], it could not produce higher biomethane compared to S_6 as it had the lowest TS and VS. However, S_6 had a C/N ratio of 6.2 but yielded more biomethane than S_7, which clearly highlights the influence of TS and VS. C/N ratio provides a balanced pH environment in which methanogens can operate, while VS is the digestible component of TS, so the higher the percentage of TS and VS, the higher the biomethane gas produced.

Moreover, the required C/N optimum depends on the operating conditions and substrate type [9, 11, 22]. S_1, S_2, S_3, S_4 and S_5 had both TS and VS higher than S_6 but lower biomethane yield than S7, which could be due to their lower C/N. When the C/N is low, the process suffers from ammonia inhibition, which increases the pH, which inhibits the activities of methanogens [3, 5]. The above discussion shows that mixing of co-substrates in different ratios produces substrates with different physicochemical properties, thus yielding different amounts of biomethane gas. This clearly shows that different mixing ratios have a significant effect on biomethane production.

Table 6: Cumulative biogas and methane yields of samples

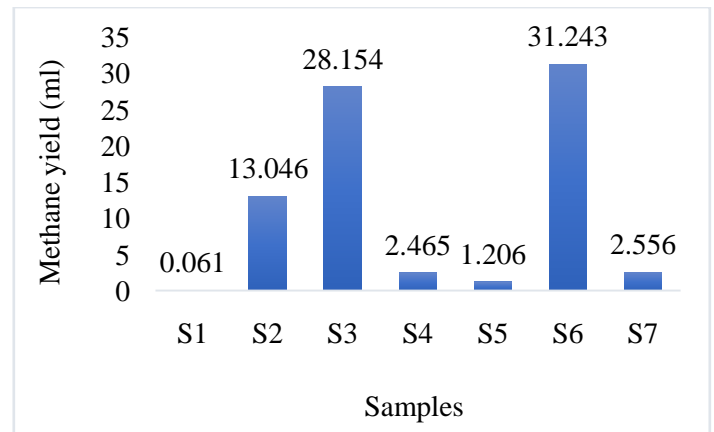| Sample	Biogas Volume (ml)	Methane Concentration (%)	Methane Yield (ml)	Digestion Period
S_1	4.2	1.46	0.061	14
S_2	90.6	14.4	13.046	14
S_3	265.6	10.6	28.154	7
S_4	39.7	6.21	2.465	5
S_5	8.2	14.71	1.206	7
S_6	118.3	26.41	31.243	14
S_7	9.6	26.63	2.556	14

Figure 3: Cumulative bio-methane yields of samples (ml).

IV. CONCLUSION

There was significant variability in the physicochemical characteristics of the samples, highlighting the fact that different mixing ratios of the same co-substrates have a significant effect on their physicochemical characteristics. The TS, VS and C/N ratio of cattle blood and rumen contents and their different blends ranged from 2.97-17.2%, 75.17 - 95.01% and 2.6 - 11.3, respectively. Their pH and temperature ranged from 7.58 - 8.14 and 25.43 - 28° C, respectively. The biomethane yields of the samples gave a mean value of 11.25 ± 13.34 which indicates significant variability ($p < 0.05$). The sample with mixing ratio 1:5 (S_6) produced the highest biomethane. The study concludes that different mixing ratios

of cattle blood and rumen contents produce substrates with different physicochemical characteristics and biomethane potentials. The maximum ratio of 1:5 of cattle blood and rumen contents should be considered for the production of biomethane from cattle blood and rumen contents.

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