Black Sea Journal of Engineering and Science
doi: 10.34248/bsengineering.1594171

Open Access Journal
e-ISSN: 2619 – 8991

Research Article
Volume 8 - Issue 2: 398-404 / March 2025

EFFECT OF DIFFERENT SUBSTRATES ON H_2S AND N_2 PRODUCTION IN BIOGAS PRODUCTION

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Abstract: The aim of this study is to determine the hydrogen sulfide (H_2S) and nitrogen (N_2) ratio of biogas produced from various combinations of cattle waste (CW), three different Switchgrass (SG) (*Panicum virgatum* L.) plant varieties (Kanlow (SG1), Shawne (SG2), Alamo (SG3) and sugar beet (*Beta Vulgaris* L.) leaves (BL). In the study, a laboratory-scale biomethane application setup was established to determine the biogas potential. Within the scope of the research, a trial design was created as 1st application group, 2nd application group and 3rd application group. In the established application setup, biogas measurements were recorded in a computer environment for 16 days, 30 days and 43 days, considering the end of biogas production of the materials. During the measurements, temperature and pH values were checked at certain periods and mixing was done by shaking by hand every day. Applications were carried out considering 10% dry matter ratio. Methane (CH_4) in biogas is a valuable energy source, other components constitute a major obstacle to the commercial use of biogas. The variable composition is due to the various materials that can be used for the production of biogas. In the study, the effect of H_2S and N_2 ratios of biogas obtained from mixtures of different materials at different ratios on biogas production was investigated. As a result of the measurements made, the biogas components of each mixture were measured using a gas analyzer. H_2S and N_2 ratios obtained from different substrates were determined. The highest H_2S ratio was measured from cattle waste with 12 ppm, and the highest N_2 ratio was measured in the CW-SG3 mixture with 32.1%.

Keywords: Hydrogen sulfide, Nitrogen, Renewable energy, Biogas, Biomass

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Received: November 30, 2024
Accepted: January 6, 2025
Published: March 15, 2025

Cite as: Filikci C, Marakoğlu T. 2025. Effect of different substrates on H_2S and N_2 production in biogas production. BSJ Eng Sci, 8(2): 398-404.

1. Introduction

Energy can be defined as the ability of an object or system to do work. The main types of energy are chemical energy, heat energy, electrical energy and mechanical energy. These energies can be converted into each other with energy conversion systems. However, they become capable of doing work. The current source of energy in the world is solar energy. Most renewable energy sources get their energy directly or indirectly from the sun. For this reason, these resources are not exhausted because they are constantly renewed.

Biogas, whose production has increased rapidly in the world and in our country in recent years, has been the most remarkable among these energy sources, especially the fact that its production is easy and cheap and that it can provide the energy needed in many areas such as fuel, heating, lighting, etc. has caused the studies on biogas to gain momentum. In addition, the fact that the raw material left over from biogas production can be used in an area such as greenhouse cultivation is another advantage (Khalil et al., 2019).

Anaerobic bacteria produce biogas, a high-calorie gas produced by the decomposition of plant waste, animal feces and agricultural wastewater in special organic-loaded hermetic containers. In other words, biogas is a colorless and flammable gas mixture produced by the decomposition of organic materials such as plant and animal wastes in the absence of oxygen, containing 40-75% methane, 20-50% CO_2 and a small amount of H_2S and very little hydrogen, water vapor, ammonia, carbon monoxide and nitrogen (Filikci, 2024).

In the acid-forming step, acetogenic bacteria convert soluble organic matter into polycarbonate volatile fatty acids (acetic acid, butyric acid, propionic acid, valenic acid); carbohydrates into ethanol, H_2 and CO_2 and; amino acids, succinic acid and H_2; fatty acids into acetate and H_2. In the third step, biogas is produced with organic acids, H_2 and acetate with the help of methane-forming microorganisms. In this step, 70% of the methane produced comes from decarboxylation of acetate and the rest from CO_2 reduction reactions of methane bacteria using hydrogen. The stages of biogas formation are given in Figure 1 (Anonymous, 2023a).

Table 1. Composition Ratios of biogas

Biogas Component	Chemical Formula	Ratio (%)
Methane	CH_4	40-75
Carbon dioxide	CO_2	20-50
Water Vapor	H_2O	0.10
Nitrogen	N_2	0-2
Oxygen	O_2	0-0.5
Hydrogen	H_2	0-1
Ammonia	NH_3	0-0.5

Figure 1. Biogas ProductionStages(Anonymous, 2023a).

Metogenic and anaerobic bacteria use carbon (C) atoms for energy and nitrogen (N) atoms are essential components for bacterial growth and reproduction. The C/N ratio cannot be more than 23 and less than 10. If it is less than 10, ammonia formation occurs in the reactor and this formation negatively affects biogas production. If it is more than 23%, volatile fatty acids are formed and slow down biogas production by showing an inhibitory effect(Anonymous, 2023b).

If C/N is not in equilibrium (suitable), this ratio can be reduced or increased by using gypsum or urea in certain amount(Buğutekin, 2007). If substrates with very low C:N ratio are used, ammonia production increases and methane production is prevented. If substrates with too high C:N ratio are used, protein formation is negatively de due to nitrogen deficiency and the growth and reproduction rate of microorganisms slows down (Deublein andSteinhauser, 2008). Co-fermentation method is mostly used to ensure nutrient balance in anaerobic process. In the co-fermentation method, anaerobic fermentation is realized by using different waste types together. Thus, problems that may arise due to the use of a single type of substrate are prevented (Karlsson et al., 2014).

Methane-producing bacteria constitute the most sensitive group to toxicity in anaerobic digestion, but they are to some extent resistant to many toxic substances and have the ability to acclimatize against them. Many waste contents are toxic to the digestive system. Ammonia has a positive effect on the system in the concentration range 200-1000 $mg.L^{-1}$, while exceeding a concentration of 3000 $mg.L^{-1}$has an inhibitory effect. Similarly, many cations (sodium, potassium, calcium, magnesium, etc.) have a positive effect on the system at low concentrations, while inhibition can occur if concentrations increase (Allegue et al., 2012).

Carbon dioxide (CO_2) is a gas that reduces the consumption of biogas and reduces its calorific value, but it is not as harmful and destructive as H_2S and N_2. Hydrogen sulfur and nitrogen causes environmental pollution and causes deterioration of metallic engine parts, siphons, blowers, gas capacity tanks, valves and shortens the life of the energy (Huertas et al., 2011). The presence of these gases is the limit of the heat energy produced by the combustion of methane. For a better quality methane, biogas should be purified from these gases using biogas purification techniques. (Allegue et al., 2012).

In this study, different mixtures were determined and biogas was produced. As a result of the measurements, the biogas components of each mixture were measured using the optima gas analyzer device. H_2S and N_2 ratios obtained from different substrates were determined. The effect of these ratios on biogas production was analyzed.

2. Materials and Methods

Two different agricultural wastes (beet leaves and switchgrass) and cattle waste were used in the study. The reason for this is that switchgrass is selected as a sample plant among 37 plants in the United States, where its production is the highest, due to its value as a feed source and its strong bio-energy capacity. It is a plant that is encouraged for production for energy and animal feed purposes due to its high net energy production per unit area, low cultivation costs, low ash content, high water utilization rate, developed adaptation ability, easy application of seed production in all lands and sufficient carbon storage in the soil (Samson and Omielan, 1992; Sanderson et al., 1996). However, the reason why sugar beet leaves are preferred is that the production area in our region (Konya, Aksaray) is large and to determine the ways to convert this green biomass into energy. The study was carried out in three different application groups. In the 1st experimental group, biogas production was realized separately from all materials. In the 2nd experimental group, cattle waste was kept constant at a rate of 50% and mixtures were formed as 1:2 / 1:2 with other materials and biogas production was realized. The 3rd experimental group was prepared by selecting the switchgrass plant variety with the highest biogas yield in the 2nd experimental group. In the 3rd experimental group, the amount of cattle waste was kept constant at 50% and the other materials (switchgrass and beet leaves) were mixed at different ratios and biogas production was realized. The experimental design is given in Table 2.

Table 2. Experimental design

1st experimental					2nd experimental				3rd Experimental		
CW	SG1 (Kanlow)	SG2 (Shawne)	SG3 (Alamo)	BL	CW-SG1	CW-SG2	CW-SG3	CW-BL	CW(%50)- SG(%25) -BL(%25)	CW(%50)- SG(%30) -BL(%20)	CW(%50)- SG(%20) -BL(%30)

2.1. Determination of Biomethane Potential (BMP)

In the applications, 1000 ml volume glass jar bottles were used as reactors in determining the biomethane potential (Figure 2). The application setup was set up in an isolated area to protect it from sunlight. The reactors were operated in mesophilic (37±° C) conditions. JSR - JSIB-22T Series / circulating water bath device and BW-10H heating bath (11.5l) device were used to keep the temperature constant. In order to measure the amount of biogas produced in the reactors, 2 glass jars operating according to the water displacement principle were connected to each other with pneumatic sealing elements. The first glass jar connected to the reactor was filled to the brim with water acidified with sulfuric acid (H_2SO_4) (pH<2) and closed (Durgut, 2020). The volume determination was made by drawing on the glass jars from the Solidworks program on the PC and the volumes corresponding to each mm length were determined and added to the glass jars in order to make the readings. In order to measure the gas content of the gas between the reactor and the glass jar filled with acidified water, a valve was added to the connection line between the two glass jars. At the end of the applications, the gas storage balloon collected by means of the valve was taken and the gas content was determined. As a result of the applications, measurements were made with the help of scales added to the glass jars filled with water in order to determine the amount of biogas released and recorded in the computer environment. With the termination of the gas output, the gases collected in the balloons by means of the valve were stored and the biogas contents were measured with the help of a measuring device.

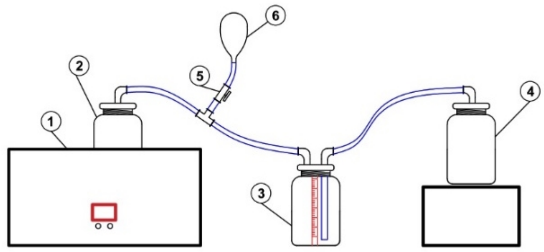

Figure 2. Experimental setup (1. Water Bath Device, 2. Reactor (Glass Jar), 3. Acidified Water (Gas Outlet), 4. Water Inlet, 5. Gas Sampling Valve, 6. Gas Storage Flask).

2.2. Determination of Mixing Ratios Used in Experiments

One of the desired reactor conditions for the best use of bacterial groups performing fermentation in an anaerobic environment in terms of producing biogas and methane content is the dry matter level of the feed materials used (Nagamani andRamasamy, 1999; Von Mitzlaff, 1988). Biogas production is best when the total dry matter ratio of the feed materials is between 6-13% (Šarapatka, 1993). Different mixing ratios were determined by optimizing the three different switchgrass varieties and beet leaf samples with the cattle waste kept constant.

2.3. Basic Characterization Analysis Values

Dry Matter, Raw Ash and Organic Matter analyses were carried out in the laboratory of the Soil Science and Plant Nutrition Department within the Faculty of Agriculture of Konya Selçuk University.

3. Results and Discussion

3.1. Basic Characterization Analysis Values

Basic characterization analyses were carried out to determine the amounts of energy plant switchgrass, sugar beet leaves and cattle waste to be added to the application mechanism established for the Determination of Biomethane Potential and to provide the desired amount of solid matter in the reactor (Table 3).

3.2. Amounts of Biogas Obtained From the Experiments

In the experimental setup, biogas measurements were carried out after 30 days in Experiment 1, 16 days in Experiment 2 and 43 days in Experiment 3, after the biogas production of the materials ended. Biogas production values are given in Table 4.

In the application, the highest daily biogas yield values were obtained as of the 11th day. It was measured as 242 $mL.gDM^{-1}$ in CW, 180.2 $mL.gDM^{-1}$ in SG1 (Kanlow), 119.2 $mL.gDM^{-1}$ in SG2 (Shawne) and 99.4 mL. gDM^{-1} in SG3 (Alamo), respectively (Figure 3). When the biogas yield value was examined, the highest yield value was determined as 3504.07 mL in CW at the end of 30 days. Other total biogas yield values obtained at the end of 30 days were 2148.8 $mL.gDM^{-1}$, 1971.4 $mL.gDM^{-1}$, 1058.4 $mL.gDM^{-1}$ and 822.5 $mL.gDM^{-1}$ from BL, SG1(Kanlow), SG2(Shawne), SG3(Alamo) materials, respectively. Liew et al. (2012) investigated methane production from corn cob, wheat straw, garden waste and leaves by anaerobic fermentation. The highest methane yield (81.2 L .kg DM^{-1}) was obtained from corn cob. This was followed by wheat straw (66.9 L .kg DM^{-1}), leaves (55.4 L .kg DM^{-1}) and garden waste (40.8 L .kg DM^{-1}).

In the application group 2, the highest biogas yield value was determined as 707.82 mL .g DM^{-1}in total from CW-SG1 mixture (Figure 4). Other biogas yield values were determined as 462.7 mL .g $DM^{-1}$from CW-SG mixture, 119 mL .g $DM^{-1}$from CW-SG2 mixture and 198 mL .g $DM^{-1}$from CW-SG3 mixture, respectively. Ahn et al. (2010) investigated the biogas production potential of switchgrass - animal (cattle, poultry and pig) manure mixtures. The maximum methane yield was determined as 337 mL .g DM^{-1}in pig manure, 28 mL .g DM^{-1} in cattle manure and 2 mL .g DM^{-1} in poultry manure.

Table 3. Organic matter (%), dry matter (%) and ash ratio (%) values

Sample Name	Ash (%)	Organic Matter (%)	Sample Weight (gr)	Oven Dry Weight (gr)	Dry Matter (%)
SG-1 (Kanlow)	93.08	6.92	4.6506	4.3286	93.076
SG-2 (Aloma)	93.58	6.42	3.1603	2.9573	93.576
SG-3 (Shelter)	93.70	6.30	3.7667	3.5295	93.702
Beet Leaves	14.29	85.71	20.4167	2.9174	14.289
Cattle Waste	9.88	90.12	14.8338	1.4657	9.880

Table 4. Total biogas yield values of the experiments (mL.gDM-1)

Material	Biogas Yield Values
CW	3504.07
SG1(KANLOW)	1971.4
SG2(SHAWNE)	1058.4
SG3(ALAMO)	822.5
BL	2148.8
CW-SG1	707.82
CW-SG2	119
CW-SG3	198
CW-BL	462.7
CW(%50)- SG(%25)-BL(%25)	151
CW(%50)- SG(%30)-BL(%20)	1913
CW(%50)- SG(%20)-BL(%30)	1997.5

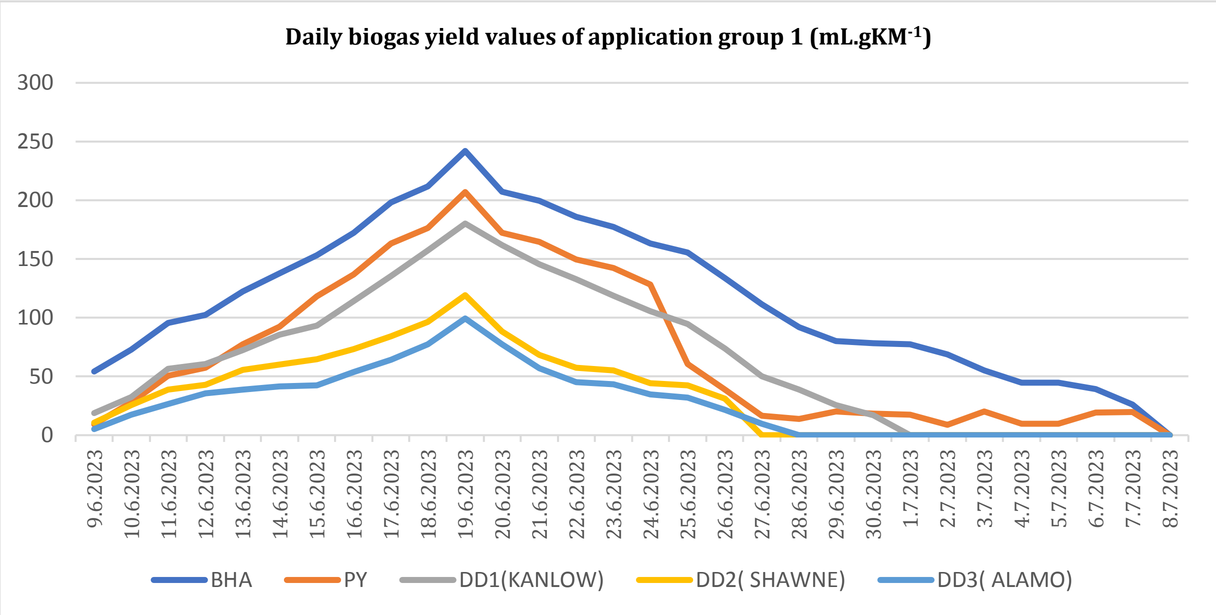

Figure 3. Daily biogas yield values of application group 1 (mL.gKM^{-1}). *BHA=CW, PY=BL, DD=SG.

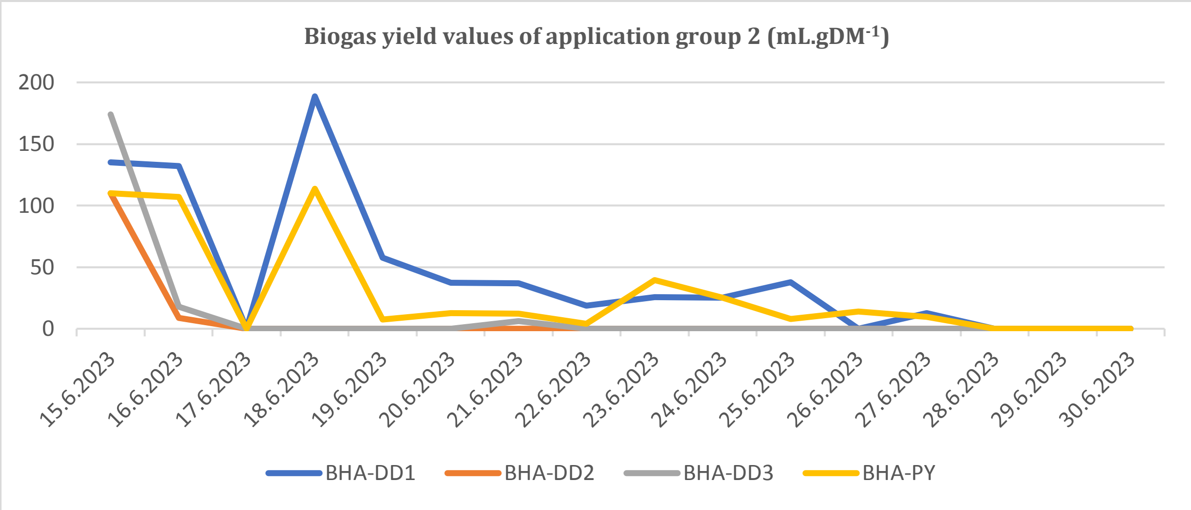

Figure 4. Daily biogas yield values of application group 2 (mL.gKM-1). *BHA=CW, PY=BL, DD=SG.

In the 3rd application group, the highest biogas yield value was determined as 1997.5 mL .g DM^{-1}in total from the mixture of CW(50%)- SG(20%)- BL(30%) (Figure 5). Other biogas yield values in the 3rd application group were determined as 1913 mL .g $DM^{-1}$from the mixture of CW(50%)- SG(30%)- BL(20%) and 119 mL .g $DM^{-1}$from the mixture of CW(50%) - SG(25%)- BL(25%), respectively. In the study conducted by Lehtomäki et al. (2007), anaerobic treatment of mixtures of energy crops and crop wastes with manure in a semi-batch complete mixed reactor was investigated. While the highest methane yield that could be achieved as a result of anaerobic fermentation of cow manure alone was 155 mL .g DM^{-1} , the highest methane yields were found as 268, 229, 213 mL .g DM^{-1}, respectively, with anaerobic fermentation of cow manure with grass, sugar beet and oat straw in certain proportions.

3.3. Biomethane Ratios of Biogas Produced from Experiments

The biomethane components of the biogas produced from the experimental groups were determined and the highest CH_4 (methane) yield was determined as 58.86% in BL (beet leaf) materials and 53.76% in CW-BL mixtures. The methane yields of other mixtures and materials in the experiments varied between 53.22% and 43.12 % (Table 5). In their study, Sheets et al. (2015) also investigated the effect of dry matter ratio in the mixtures with temperature on methane yield from switchgrass. In the reactor operating as solid fermentation in biomethane production, the highest biomethane yield was obtained at 13% and 22% under mesophilic conditions. Ciggin (2016) mixed switchgrass with process sludge in his study. According to the test results, the highest methane yield was found in the mixture with a ratio of 0.4-0.6. In their study, Liew et al. (2012) investigated methane production from corncob, wheat straw, garden waste and leaves by anaerobic fermentation. The highest methane yield was obtained from corncob (81.2 l/kgUKM). This was followed by wheat straw (66.9 L/kgUKM), leaves (55.4 L/kgUKM) and garden waste (40.8 L/kgUKM).

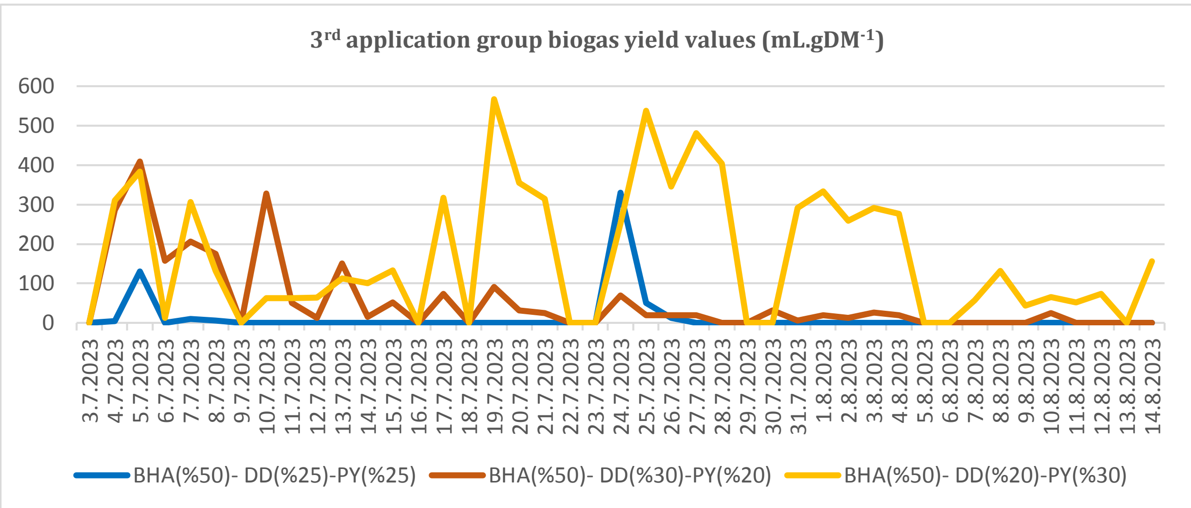

Figure 5. Daily biogas yield values of application group 3 (mL.gKM-1). *BHA=CW, PY=BL, DD=SG. * BHA=CW, PY=BL, DD=SG.

Table 5. Biomethane components of biogas produced from experiments

Material	CH_4 (%)
CW	53.42
SG1(KANLOW)	47.4
SG2(SHAWNE)	46.76
SG3(ALAMO)	43.12
BL	58.86
CW-SG1	49.78
CW-SG2	48.43
CW-SG3	44.86
CW-BL	53.76
CW(%50)- SG(%25)-BL(%25)	51.74
CW(%50)- SG(%30)-BL(%20)	50.86
CW(%50)- SG(%20)-BL(%30)	53.22

3.4. Hydrogen sulfide and Nitrogen Components of Biogas Produced from Experiments

The hydrogen sulfide and nitrogen ratios of the biogas produced from the experimental groups were determined and are given in Table 6. The highest values were reached as 26.18% and 27.79% in the CW-SG1 and CW-SG2 experiments, respectively.

Tablo 6. Hydrogen sulfide and nitrogen components of biogas produced from experiments

Material	H_2S (ppm)	N_2 (%)
CW	12	19.61
SG1(KANLOW)	6	25.25
SG2(SHAWNE)	5	25.17
SG3(ALAMO)	5	26.8
BL	9	24.92
CW-SG1	9	26.18
CW-SG2	8	27.79
CW-SG3	7	32.1
CW-BL	10	20.68
CW(%50)- SG(%25)-BL(%25)	9	23.92
CW(%50)- SG(%30)-BL(%20)	9	24.92
CW(%50)- SG(%20)-BL(%30)	10	21.84

High percentage of CH_4 ratio is important in biogas production. In the study, it was observed that high hydrogen sulphide and nitrogen ratios naturally reduce methane production. Again, the low hydrogen sulphide and nitrogen ratios in our mixtures, especially in the mixtures containing switchgrass, showed that switchgrass has a higher methane production in terms of biogas production.

Ghatak and Mahanta (2016) conducted a Biogas Purification experiment using Chemical Absorption in their study. In this experiment, raw biogas was compressed and stored in air compressor. Compressed biogas is allowed to pass through scrubber by changing the inlet pressure from 1 bar to 5 bar in the range of 1 bar with the help of pressure regulator. The flow rate of inlet biogas is changed from 1 lpm to 5 lpm in 1 lpm steps with the help of rotameter. Raw biogas and purified gas exiting from scrubber are tested for composition analysis with the help of Gas Chromatography (GC). The percentage of raw biogas was found to be 41.5%. It was discovered that the process of using soda lime as absorbent is used where the percentage of carbon dioxide in the outlet gas decreases with the increase in inlet pressure and the percentage of carbon dioxide in the outlet gas increases with the increase in inlet gas flow rate in case of vertical scrubber. Here the minimum carbon dioxide content present in biogas can be reduced up to 1.34% in the off-gas at 5 bar and 1 lpm flow rate of biogas. The biogas was found to be enriched with 97.7% methane.

Song et al. (2020) conducted an experiment of Air Addition as a Stimulation Approach for the Enhancement of in Situ Desulfurization and Methanization of Anaerobic Digestion of Chicken Manure. Biogas produced by anaerobic digestion of chicken manure has a high H_2S level (5000-6000 ppm), which makes biogas plants economically unviable. In their study, they investigated in situ desulfurization by injecting restricted air into the headspace of a digester and the effects on process performance and injection techniques under various oxygen loads (1.4, 2.8, and 4.2 $mL.gVSin^{-1}$). The results showed that a constant oxygen load of 4.2 mL/gVSin removed 99.7% of H_2S (up to 1015 ppm) and simultaneously increased methane output by 6.4 percent.

4. Conclusions

This study evaluated various mixtures for their potential in biogas production. The biogas components of each mixture were analyzed using an Optima gas analyzer. The ratios of hydrogen sulfide (H_2S) and nitrogen (N_2) extracted from different substrates were determined, and their impacts on biogas production were examined. A laboratory-scale setup was created to conduct the biogas production experiments. In this context, three distinct applications were developed, along with a comprehensive experimental framework. Biogas measurements were recorded in a digital environment over periods of 16, 30, and 43 days, focusing on the endpoint of biogas production for the materials used.

Throughout the measurement period, temperature and pH values were periodically monitored, and the mixing process was performed daily through manual shaking. The applications were designed with an emphasis on maintaining a 10% dry matter content.

Biogas production has an important place among sustainable energy sources. However, the quality of biogas can vary depending on the amount of various gases in it. Among the most important of these gases are hydrogen sulfide (H_2S) and nitrogen (N_2). The effects of these two gases on biogas production are very important.

Author Contributions

The percentages of the authors' contributions are presented below. All author reviewed and approved the final version of the manuscript.

	C.F.	T.M.
C	60	40
D	60	40
M	60	40
DCP	60	40
DAI	60	40
LR	60	40
W	60	40
CR	60	40
SR	60	40
PM	60	40
FA	60	40

C= concept, D= design, M= management, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, LR= literature review, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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