

Article

Investigating Methane, Carbon Dioxide, Ammonia, and Hydrogen Sulphide Content in Agricultural Waste during Biogas Production

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Abstract: The agricultural industry produces a substantial quantity of organic waste, and finding a suitable method for disposing of this highly biodegradable solid waste is a difficult task. The utilisation of anaerobic digestion for agricultural waste is a viable technological solution for both renewable energy production (biogas) and waste treatment. The primary objective of the study was to assess the composition of biogas, namely the percentages of methane, carbon dioxide, ammonia, and hydrogen sulphide. Additionally, the study aimed to quantify the amount of biogas produced and determine the methane yield (measured in NmL/g VS) from different agricultural substrates. The biochemical methane potential (BMP) measurements were conducted in triplicate using the BPC Instruments AMPTS II instrument. The substrates utilised in the investigation were chosen based on their accessibility. The substrates used in this study comprise cattle manure, chicken manure, pig manure, tomato plants, tomatoes, cabbage, mixed fruits, mixed vegetables, dog food, and a co-digestion of mixed vegetables, fruits, and dog food (MVMFDF). Prior to the cleaning process, the makeup of the biogas was assessed using the BIOGAS 5000, a Geotech Analyser. The AMPTS II flow cell automatically monitored and recorded the volume of bio-methane produced after the cleaning stage. The data were examined using the Minitab-17 software. The co-digestion of mixed vegetables, mixed fruits, and dog food (MVMFDF) resulted in the highest methane level of 77.4%, followed by mixed fruits at 76.6%, pig manure at 72.57%, and mixed vegetables at 70.1%. The chicken manure exhibited the greatest levels of ammonia (98.0 ppm) and hydrogen sulphide (589 ppm). Chicken manure had the highest hydrogen sulphide level, followed by pig manure (540 ppm), tomato plants (485 ppm), mixed fruits (250 ppm), and MVMFDF (208 ppm). Ultimately, the makeup of biogas is greatly affected by the unique qualities of each substrate. Substrates containing elevated quantities of hydrogen sulphide, such as chicken manure, require the process of biogas scrubbing. This is because they contain substantial amounts of ammonia and hydrogen sulphide, which can cause corrosion to the equipment in biogas plants. This emphasises the crucial need to meticulously choose substrates, with a specific focus on their organic composition and their capacity to generate elevated methane levels while minimising contaminants. Substrates with a high organic content, such as agricultural waste, are optimal for maximising the production of methane. Furthermore, the implementation of biogas scrubbing procedures is essential for efficiently decreasing carbon dioxide and hydrogen sulphide levels in biogas. By considering and tackling these problems, the effectiveness of biogas generation can be enhanced and its ecological consequences alleviated. This strategy facilitates the advancement of biogas as a sustainable energy source, hence contributing to the attainment of sustainable development goals (SDGs).



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Keywords: biogas; agricultural substrates; methane production; methane production rate; carbon dioxide; hydrogen sulphide; renewable energy sources; climate action; SDGs

1. Introduction

Agricultural waste consists of residual materials generated as a result of farming operations, including but not limited to livestock manure, waste from abattoirs, and excess fruits and vegetables from agro-processing enterprises [1]. Due to the projected increase in agricultural production driven by population expansion and rising affluence, there will undoubtedly be a considerable rise in the amount of agricultural waste generated [2].

Research projects that worldwide food production must increase by approximately 50–60% in order to fulfil the anticipated global food demand by the year 2050 [3,4]. An increase in agricultural production would inevitably result in a rise in agricultural waste generation, intensifying the adverse effects of inadequate agricultural waste management methods such as landfilling, open fire burning, open dumping, and incineration [5].

Several studies [6–8] have recorded the negative consequences resulting from insufficient waste management procedures, including noxious smells, the rapid increase in disease-causing microorganisms, the spread of illnesses, the release of greenhouse gases, and the pollution of land and water resources. An effective strategy to tackle these problems, especially regarding agricultural waste, is the use of anaerobic digestion as a more environmentally friendly waste management method. Utilising agricultural organic waste as a substrate in anaerobic digesters can be a valuable resource. This process generates renewable energy in the form of biogas, providing a sustainable way to fulfil a portion of the global energy requirements [5]. Moreover, this waste management approach simultaneously produces excellent fertilisers, thus improving agricultural methods and promoting environmental sustainability [9]. Moreover, the use of non-renewable resources, particularly fossil fuels, leads to the exhaustion of natural resources. This highlights the necessity of redirecting our focus towards other, sustainable sources of energy [10]. Anaerobic digestion (AD) is a naturally occurring biological process where microorganisms break down biodegradable materials in an oxygen-free environment, leading to the creation of biogas. The unprocessed biogas primarily contains methane (60–65%) and carbon dioxide (35–55%), along with trace amounts of hydrogen sulphide (0.1–3%) [11]. Furthermore, the biogas composition can differ based on the kind of substrate and pre-treatment process, containing small quantities of hydrogen sulphide, ammonia, oxygen, hydrogen, carbon monoxide, siloxanes, and different volatile organic compounds [9,12].

According to the study conducted by Rollo et al. [13], the methane levels in cow, chicken, and pig manure vary in their ability to produce biogas. Pig manure exhibited the highest overall biogas yield in comparison to chicken and cow manure. The pig manure produced a gas volume of 13.0561 ft³, suggesting a greater concentration of methane. The chicken manure had the largest concentrations of contaminants, including hydrogen sulphide and carbon dioxide, which likely affected its methane levels. In summary, the study indicates that pig manure is the optimal substrate for biogas production due to its high methane content. Various factors can influence the methane levels in different types of animal manure used for biogas production. These factors include the composition of the manure, the presence of anaerobic microorganisms, and the type of feedstock employed [14]. Based on the study conducted by Sebola, Tesfagiorgis, and Muzenda [15], the methane levels of chicken manure, pig manure, and cow manure varied depending on the ratios and conditions of anaerobic co-digestion. The optimal methane yield was obtained by using equal proportions of cow dung, chicken manure, pig manure, and sewage waste, with a methane yield of 58% CH₄/d. Furthermore, it was shown that the ideal temperature for anaerobic co-digestion is 40 °C, resulting in a methane production of 62% CH₄/d. It should be emphasised that the methane readings can fluctuate depending on the varied ratios and conditions of the digestive process [16]. The composition of different types of

animal manure differs, which can affect their capacity for methane production. Anaerobic microorganisms are necessary for the decomposition of organic matter into biogas, and their effectiveness can vary depending on the specific type of manure [15]. The type of substrate used in the anaerobic digestion process can also impact methane production, since certain materials may exhibit more susceptibility to methane conversion compared to others. The effectiveness of methane synthesis from animal manure can be influenced by various elements, such as temperature, pH levels, and retention time, which determine the completeness of the anaerobic digestion process [17].

Methane is the main constituent of biogas and acts as its primary source of energy. Organic matter undergoes microbial decomposition during anaerobic digestion, resulting in the generation of methane and carbon dioxide. The methane-to-carbon dioxide ratio in biogas is crucial as it directly affects the energy content and combustion characteristics of the gas produced. Biogas produced from agricultural waste displays different levels of methane and carbon dioxide, which depend on the composition, moisture content, and processing conditions of the waste [18].

In addition, methane (CH_4) is accountable for its energy content. With a calorific value of around 35.8 MJ/m^3 , it is a highly efficient fuel for producing heat and power. Methane, when burned, emits energy that can be used for different purposes, including generating power, heating systems, and as a fuel for vehicles in the form of compressed natural gas (CNG) [19]. Carbon dioxide (CO_2) is a substantial constituent of unprocessed biogas, typically comprising 30–50% of the blend. Although carbon dioxide (CO_2) does not burn, it reduces the concentration of methane in the biogas, resulting in a decrease in the overall energy density. Elevated concentrations of carbon dioxide (CO_2) diminish the calorific value of the biogas, necessitating a larger volume to attain an equivalent energy output to that of pure methane. This impacts the efficiency and efficacy of biogas as a fuel [20]. Ammonia (NH_3) is another significant component in agricultural waste, particularly in livestock manure. Ammonia (NH_3) is produced during the anaerobic digestion of nitrogen-rich substrates like chicken manure. While ammonia can enhance biogas production by providing essential nitrogen for microbial activity, excessive levels can inhibit methanogenesis and lead to process instability. At high concentrations, ammonia can be toxic to the microorganisms responsible for breaking down organic matter. This leads to a slowdown or even a halt in the anaerobic digestion process, reducing biogas yield [21]. Therefore, monitoring and controlling ammonia levels are crucial for optimising biogas production efficiency. The breakdown of sulphur-containing molecules that are found in organic matter results in the production of hydrogen sulphide (H_2S), which is a byproduct of anaerobic digestion. The presence of high concentrations of hydrogen sulphide presents several issues, such as the deterioration of equipment, the transmission of toxicity to microorganisms, and the occurrence of environmental risks. In order to guarantee the quality and safety of biogas for a variety of uses, it is vital to make sure that hydrogen sulphide is removed effectively [22,23]. Hydrogen sulphide (H_2S) is highly corrosive, particularly to metals and infrastructure components such as pipes, engines, and storage tanks. The presence of H_2S can lead to significant maintenance issues and increased costs for biogas plants [23]. H_2S is also highly toxic, posing health risks to humans and animals. Even at low concentrations, it can cause respiratory problems and other health issues. Due to its corrosive and toxic nature, the effective removal of H_2S from biogas is essential. Techniques such as iron sponge filtration, activated carbon adsorption, and biological desulphurisation are commonly used to scrub H_2S from the biogas [24].

Methane is the key contributor to the energy content of biogas, while carbon dioxide reduces its purity and energy value. High concentrations of ammonia can inhibit the anaerobic digestion process, and hydrogen sulphide is both corrosive and toxic, necessitating effective removal techniques [22]. By addressing these challenges, the efficiency and environmental benefits of biogas production can be significantly enhanced, contributing to the development of renewable energy sources and the achievement of sustainable development goals (SDGs).

The choice of substrate is crucial in biogas generation since it directly impacts the efficiency and results of the anaerobic digestion process. The composition of the substrates influences crucial aspects such as the organic content, moisture level, and nutritional balance, which are necessary for achieving optimal microbial activity and methane production [25]. The organic content of a substrate, quantified as volatile solids (VS), is a crucial factor in determining the amount of biogas produced. Substrates rich in organic matter, such as food waste, agricultural wastes, and animal manure, offer a greater amount of material for microorganisms to decompose, leading to an increased production of biogas. Substrates exhibit different levels of biochemical methane potential (BMP) [26]. Food waste and specific crop residues exhibit a higher biochemical methane potential (BMP) in comparison to lignocellulosic materials such as straw, which are more resistant to degradation [27].

Achieving optimal microbial activity in anaerobic digestion necessitates maintaining a well-balanced moisture level. An optimal moisture level usually falls within the range of 50–80%. Insufficiently moist substrates might impede microbial operations, but excessively moist substrates can dilute the concentration of organic material, resulting in a decrease in biogas production [26]. Ensuring the appropriate moisture level is crucial for maintaining process stability. Optimal moisture levels promote the blending of substances, ensuring uniform microbial exposure to the organic material and limiting the development of arid pockets or excessively compressed regions inside the digester [28].

An ideal C/N ratio for anaerobic digestion is recommended to fall within the range of 25 to 30. The ratio is crucial because very low or high values may hinder or disrupt the process of digestion. High levels of nitrogen can be quickly depleted by methanogens, leading to reduced gas generation. Conversely, insufficient nitrogen levels can result in ammonia inhibition. Hence, for optimal anaerobic digestion, it is advisable to maintain a C/N ratio between 25 and 30, as this will facilitate the efficient degradation of the highest proportion of carbon. Combining substrates with C/N ratios below 40 with dairy wastes prevents reactor instability and maintains a suitable nutritional balance. Combining feed materials with varying levels of carbon and nitrogen content can be beneficial to attaining the ideal carbon-to-nitrogen ratio for maximising the efficiency of anaerobic digestion [29]. Nutrients such as sulphur, phosphorus, and trace elements are essential for microbial health and activity. A balanced nutrient profile supports the growth and function of various microbial communities involved in different stages of the anaerobic digestion process [30].

Rapid biogas production is facilitated by easily degradable substrates such as food waste and fresh manure, as they serve as a readily available energy source for bacteria. On the other hand, substrates containing complex chemicals, like lignin found in plant biomass, necessitate a longer duration and specific microorganisms for decomposition [27]. The microbial activity can be influenced by the pH level of the substrate. Anaerobic microbes generally flourish within a pH range of 6.5 to 7.5, which is considered neutral. Microbial communities and biogas generation can be disrupted by substrates that are excessively acidic or alkaline [31].

The composition of the substrate has an impact on the necessary hydraulic retention time (HRT) for achieving complete digestion. Substrates that degrade easily may necessitate a shorter hydraulic retention time (HRT), whereas fibrous or complicated substrates may demand longer retention durations to obtain the highest possible biogas production. The choice of substrates has a direct impact on the temperature at which the digester operates [32]. Mesophilic settings, typically ranging from 30 to 40 °C, are commonly suitable for most substrates. However, certain substrates may necessitate thermophilic conditions, with temperatures between 50 and 60 °C, to achieve optimal breakdown. This requirement can impact energy inputs and system design [33].

The choice of substrate is frequently influenced by the accessibility and reliability of local resources. The uninterrupted functioning of the biogas plant is guaranteed by the dependable availability of substrates. The expense associated with substrates can significantly affect the overall financial feasibility of the biogas facility [34]. The utilisation

of inexpensive or discarded resources can improve the cost-efficiency of biogas generation. Selecting substrates that promote waste reduction and recycling, such as agricultural wastes and food wastes, is in line with environmental sustainability objectives and helps decrease the use of landfills and the emission of greenhouse gases [25]. A prudent choice of substrates is crucial for optimising the anaerobic digestion process and maximising the production of biogas. To guarantee effective microbial activity and steady digestive performance, it is important to consider factors such as organic content, moisture level, and nutritional balance. By considering and addressing these issues, the production of biogas can be improved, thus making a significant contribution to the generation of renewable energy and the sustainable management of waste [34].

Unaddressed contaminants in biogas present significant hazards to both the environment and public health. The presence of impurities such as hydrogen sulphide and ammonia in biogas can lead to health problems such as pulmonary paralysis, asthma, and other respiratory disorders. Additionally, these impurities contribute to global warming and climate change [24]. Additionally, these contaminants might result in corrosion and malfunctions in processing equipment.

To enhance the quality of biogas for valuable uses, it is essential to eliminate carbon dioxide and other impurities. Impurities reduce the calorific value of biogas when it is burned, resulting in decreased fuel efficiency. Eliminating these contaminants not only increases the energy density of biogas but also mitigates its environmental impact and safeguards human health [35]. By enhancing and refining biogas through the elimination of pollutants, its quality can be enhanced to match that of natural gas, rendering it appropriate for a wide range of valuable uses. The process of purifying enhances the energy content of biogas and reduces the adverse impact of contaminants on the environment and human health. Hence, the implementation of efficient biogas purification techniques is crucial for the advancement of cleaner energy solutions and the promotion of sustainable energy production [19,24].

There is a scarcity of research that establishes a connection between the type of agricultural substrate and the content of biogas. Although there have been notable improvements in biogas production, there is still a limited understanding of how different agricultural wastes affect the levels of methane (CH_4), carbon dioxide (CO_2), ammonia (NH_3), and hydrogen sulphide (H_2S) produced during anaerobic digestion. Current research frequently concentrates on specific categories of waste or lacks comprehensive comparative study across various agricultural wastes. Furthermore, there is a scarcity of data regarding the impact of different compositions of these wastes on the equilibrium between advantageous (methane) and harmful (CO_2 , NH_3 , H_2S) by-products. This gap impedes the optimisation of substrate selection and process parameters to maximise methane yield and minimise detrimental emissions.

The objective of this study is to examine the levels of methane, carbon dioxide, ammonia, and hydrogen sulphide in different agricultural waste materials during the process of biogas production. Comprehending the composition and qualities of agricultural waste is essential for optimising biogas generation. Through the analysis of methane, carbon dioxide, ammonia, and hydrogen sulphide levels in various substrates, customised strategies can be formulated to optimise methane production while minimising potential environmental hazards. This study contributes to the progress of sustainable energy solutions and highlights the potential of agricultural waste as a useful resource for producing biogas. Biogas is a highly promising renewable energy source that may make significant contributions to achieving the Sustainable Development Goals (SDGs), particularly SDG 7, which focuses on Affordable and Clean Energy, and SDG 13, which addresses Climate Action [36].

2. Materials and Methods

2.1. Ethical Approval

The research strategy, which encompasses the collection and management of various forms of waste, underwent review and received approval from the Animal Re-

search Ethics Committee of the Faculty of Science, Tshwane University of Technology (AREC2021/02/001), to ensure adherence to ethical standards.

2.1.1. Collection and Handling of Animal Manure

Measures were taken to ensure that the process of collecting animal manure did not result in any harm or distress to the animals. The manure was gathered from animals in their undisturbed natural habitats without any kind of compulsion or disruption. The manure was obtained from Agricultural Research Council (ARC) farms that comply with established animal care standards. The collection techniques were specifically developed to minimise any negative effects on the environment by utilising sterile airtight containers, ensuring that water sources and soil were not contaminated throughout the process of gathering manure.

2.1.2. Collection and Handling of Vegetable and Fruit Waste

Compliance with local and national waste management regulations is necessary to ensure that the collection of vegetable and fruit waste aligns with environmental protection legislation. The authorisation to collect the waste was acquired from firms producing waste and ensuring informed agreement and openness on the research objectives. The collection and transportation of vegetable and fruit waste were carried out with strict adherence to hygienic conditions to prevent the transmission of pathogens and ensure the safety of researchers. Personal protective equipment (PPE) was used and strict adherence to safety regulations was implemented while collecting and processing waste.

2.1.3. Collection and Handling of Expired Dog Food

Expired dog food was collected from suppliers with clear and explicit permission, providing transparency on its intended use in research. Prioritising the reuse of expired dog food as a means of reducing waste is in line with ethical and environmental sustainability objectives. Sterile airtight containers were used when handling expired dog food to avert potential health risks.

2.2. Study Area

The study was conducted at the Agricultural Research Council (Agricultural Engineering) in Silverton (Pretoria).

2.3. Substrate and Inoculum Collection and Analysis

The secondary collection of cattle, pig, and poultry manure took place at the Agricultural Research Council's research farm located in Irene, Pretoria. The expired dry dog food, shredded vegetable waste (consisting of spinach, carrots, beetroots, onions, lettuce, potatoes, and butternuts), and fruit material (containing banana peels, pineapples, oranges, apples, and watermelons) were collected from Marabastad (Tshwane market) in Pretoria. The collection technique utilised sterile nitrile hand gloves and airtight containers from Lasec, a company based in South Africa. Prior to use, all samples received mechanical pre-treatment using a blender. The inoculum was sourced from a fully operational biogas facility situated in Bronkhorstspuit, Pretoria. Before starting the testing, the inoculum was subjected to pre-treatment by being kept at a temperature of 37 °C for a period of 5 days. This action was taken to mitigate the production of biogas from internal sources.

The substrate and inoculum were stored at a temperature of 4 degrees Celsius prior to use. The collected substrates and inoculum were analysed for total solids (TS), volatile solids (VS), fixed solids (FS), moisture content (MC), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), nitrogen (N), carbon (C), sulphur (S), carbohydrates, protein, and fat using the AOAC method (2000). The pH was measured using the HI8424 General Purpose pH/mV Metre.

2.4. Biochemical Methane Potential (BMP) Set-Up and Operation

The biochemical methane potential (BMP) tests were conducted in triplicate using the AMPTS II system from Bioprocess Control (BPC) in Sweden. The instrument consists of three primary components: (i) twelve 500 mL biodigester glass reactor bottles, (ii) a CO₂ scrubbing unit, and (iii) a gas monitoring unit (Figure 1). The system is an automated batch system that was configured and left to operate until the process of digestion finished. Sodium hydroxide was employed for the purpose of eliminating carbon dioxide. A solution of 3M NaOH was made for use as the scrubbing solution to eliminate contaminants. A pH indicator solution was added to ascertain the saturation point of the cleaning solution for replacement.

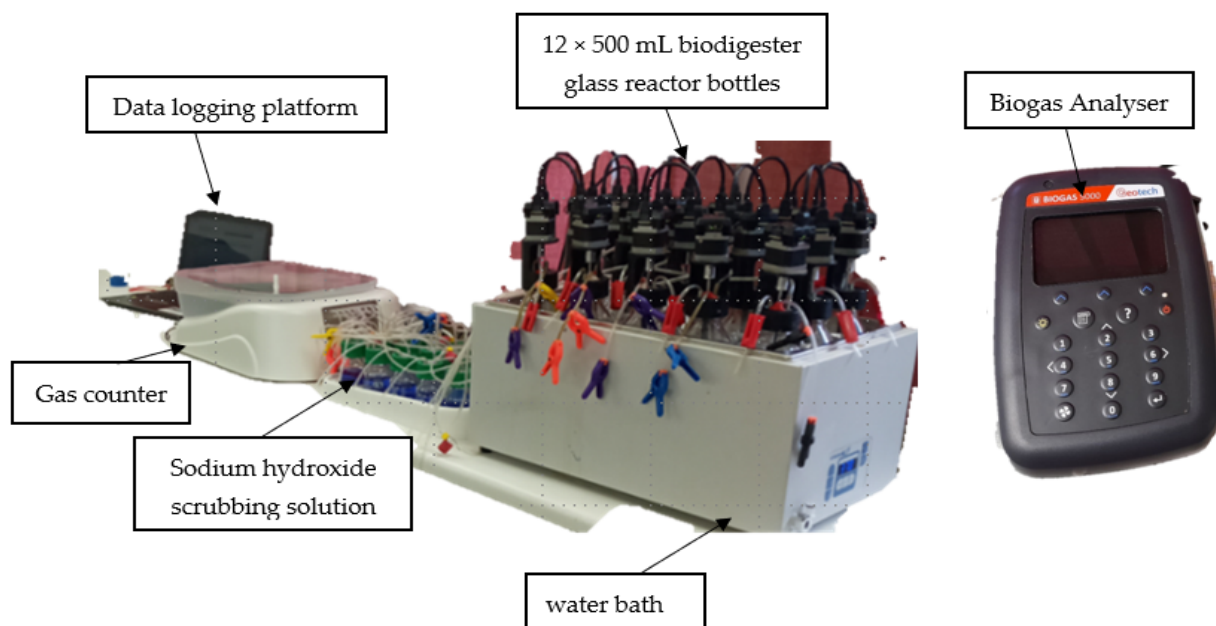


Figure 1. Biochemical methane potential (BMP) set-up including the Biogas 5000 analyser.

A potential constraint of the study is that only three replicates per treatment were achievable, which could lead to inadequate data for reliably detecting subtle yet significant treatment effects. To overcome this challenge, preliminary experiments were undertaken before the actual experiment. This initial stage facilitated the improvement in experimental protocols, the optimisation of circumstances, and the identification of any possible problems or factors contributing to inconsistency. Through the implementation of trial runs, the primary experiment was carefully organised and carried out, hence increasing the probability of identifying significant treatment effects, even with a restricted number of duplicates per treatment.

Substrates such as animal waste (specifically cattle and pig manure), dog food, and fruit and vegetable waste were used. A domestic blender thoroughly mixed the substrate for a duration of one minute and then transferred it into the digester reactors. Each reactor was filled to a capacity of 400 mL, with a headspace of 200 mL. The reactors were filled with a mixture of inoculum and substrate in a ratio of 2:1, based on the amount of volatile solids (VS).

The 2:1 ratio, based on volatile solids (VS), guarantees the presence of sufficient inoculum to efficiently degrade the substrate. The inoculum comprises the essential microbial populations that facilitate the process of anaerobic digestion. Ensuring a sufficient volume of inoculum in proportion to the substrate is crucial for sustaining microbial activity and stability during the digestive process [37]. The control setup involved loading the reactor with 15.71 mL of inoculum and 384.29 mL of water. The treatment involving mixed fruits and inoculum required loading the reactor with 8.81 mL of inoculum, 3.67 mL of mixed fruits, and 387.52 mL of water. The treatment involving cattle waste and inoculum consisted of loading the reactor with 9.43 mL of inoculum, 3.54 mL of animal manure, and

387.03 mL of water. The reactor was filled with 9.77 mL of inoculum, 3.52 mL of dog food, and 386.71 mL of water for the treatment process. The treatment using mixed vegetables and inoculum required the reactor to be filled with 8.78 mL of inoculum, 4.33 mL of mixed vegetables, and 386.89 mL of water. The MVMFDF treatment involved loading the reactor with 9.59 mL of inoculum, 3.41 mL of MVMFDF, and 387 mL of water. The treatment involving tomato plants and inoculum consisted of adding 10.06 mL of inoculum, 4.71 mL of tomato plants, and 385.23 mL of water to the reactor. The treatment involving tomatoes and inoculum consisted of loading the reactor with 9.24 mL of inoculum, 3.96 mL of tomatoes, and 386.8 mL of water. The treatment involving cabbage and inoculum consisted of loading the reactor with 9.15 mL of inoculum, 3.81 mL of cabbage, and 387.04 mL of water. The treatment using chicken manure and inoculum consisted of loading the reactor with 10.47 mL of inoculum, 4.63 mL of chicken manure, and 384.9 mL of water. The treatment using pig manure and inoculum consisted of adding 10.52 mL of inoculum, 4.33 mL of pig manure, and 385.15 mL of water to the reactor.

The headspace of each reactor was purged with a gas combination of carbon dioxide and nitrogen (20/80) for one minute at a flow rate of 1 m³/min and a pressure of 0.1 bar. This process was performed to remove oxygen and establish an anaerobic condition within the reactors. The reactor was run at a temperature of 37 °C for a duration of 21 days.

Opting for mesophilic settings in biogas production research is crucial since these conditions provide optimal microbial activity, process stability, energy efficiency, and economic viability. Mesophilic digestion creates a strong and durable environment for anaerobic microbes, guaranteeing reliable and effective methane production. In addition, the lower energy needs and operational ease of mesophilic digestion make it a realistic and scalable alternative for a variety of biogas applications. These elements collectively promote the wider acceptance and long-term viability of biogas technology, hence enhancing renewable energy generation and environmental advantages.

The entire test was performed as instructed by the AMPTS II standard operation manual and in triplicate [38].

2.5. Gas Measurement and Analysis

Biogas 5000 Geotech, QED Environmental systems, Inc., Dexter, MI, USA was used to determine the biogas composition before passing through sodium hydroxide scrubbing solution for all treatments. Methane production (NmL) was retrieved from the data logging platform of the AMPTS II and used for the calculation of daily cumulative methane production and methane yield (NmL/g VS).

Biogas 5000 Geotech Calibration

The BIOGAS 5000 calibration was performed following the manufacturer's instructions, as summarised (BIOGAS 5000 Analyser operating manual. QED Environmental systems, Inc., Dexter, MI. www.qedenv.com, accessed on 14 February 2022).

The BIOGAS 5000 analyser was turned on and allowed to warm up, then the analyser was attached to a known standard calibration gas mixture using appropriate tubing and connectors. The calibration was zeroed using a gas mixture containing 0% CH₄, 0% CO₂, and a balance of nitrogen (N₂). Span gas calibration with known concentrations of 50% CH₄, 35% CO₂, and 0% O₂ was performed. After performing zero and span calibrations, the accuracy was verified by measuring another calibration gas with known concentrations. The measured values were compared with the known values to ensure they were within acceptable limits (usually within ±2% of the known value). For the validity of the results, the parallel biogas samples were sent to a certified laboratory for analysis using gas chromatography standard methods.

2.6. Statistical Analysis

The collected data were used for statistical analysis. The GLM approach in Minitab 17 enables the investigation of treatment effects while considering probable causes of variation. Once significant treatment effects were identified using ANOVA, Fisher's protected LSD

test was utilised, assuming that the conditions of homogeneity of variances, normality, and independence were satisfied. Thorough examination of these assumptions is crucial for accurate and dependable statistical inference in the evaluation of treatment effects.

Means were separated using Fisher's protected LSD test ($p < 0.05$). The statistical equation can be represented as follows:

$$Y_{ij} = \mu + T_i + \epsilon_{ij} \quad (1)$$

where

Y_{ij} = substrate characteristics

μ = mean

T_i = treatments (substrates)

ϵ_{ij} = random residual error

To examine the effect of substrate type on biochemical methane potential, methane production rate, and biogas composition, the repeated measure technique was applied. Means were separated using Fisher's protected LSD test ($p < 0.05$). The statistical equation is expressed as follows:

$$ijk = \mu + T_i + \epsilon_{ij} + D_k + (TD)_{ik} + \epsilon_{ijk} \quad (2)$$

where

Y_{ijk} = response variable (biochemical methane potential or methane production rate or biogas composition)

μ = mean

T_i = treatments (substrates)

ϵ_{ij} = random effect associated with the j th sample on the i th treatment

D_k = the effect of the k th time on the measurements ($k = 1, 2, 3, \dots, 20$)

$(TD)_{ik}$ = interaction between the i th substrate and the k^{th} time

ϵ_{ijk} = random residual error

The Pearson correlation coefficient test was computed to determine the relationship between the nutrients and biogas composition ($p < 0.05$).

3. Results and Discussion

3.1. Substrate Characteristics

The effectiveness of anaerobic digestion is dependent upon several elements, such as the pH level and the nature of the substrate [39]. The findings of the present investigation, as depicted in Table 1, reveal that the pH of the inoculum (8.7) and cattle manure (8.8) provide a more alkaline environment. The high alkalinity of the pH helps to create and maintain the optimal environment for methanogenic bacteria, which play a crucial role in the production of methane during anaerobic digestion. Excessive alkalinity can hinder the activities of other microbial groups participating in anaerobic digestion. Therefore, it is essential to simultaneously digest substrates [40,41]. The pig manure exhibited a pH of 7.1, indicating its proximity to a neutral substrate. This pH level is suitable for the activities of several microbial groups that are engaged in the breakdown of organic materials. The cabbage had a pH of 6.8, whereas MVMFDF and mixed vegetables had a pH of 6.7 and chicken manure had a pH of 6.6. These pH values indicate that the cabbage and vegetables are slightly acidic to neutral. The pH range is generally conducive to anaerobic digestion, facilitating the presence of various microbial populations engaged in the process [42]. A pH of 5.8 was recorded from mixed fruits, 5.6 from tomato plants and dog food, and 4.7 from tomatoes, which provide acidic conditions. While acidic substrates can slow down methanogenesis, they can still contribute to biogas production when properly balanced with other materials [43].

Table 1. Substrates characteristics.

Substrates	pH	% TS	% VS	% VS (ww)	%FS	% MC	% N	% S	% C	% NDF	% ADF	% ADL	% Carbohydrate	% Protein	% Fat
Inoculum	8.7± 0.03 ^a	3.87 ±0.08 ^g	63.64 ±1.04 ^c	2.49 ±0.07 ^f	35.49 ±0.39 ^b	96.14 ±0.08 ^a	1.9 ±0.84 ^e	0.22 ±1.89 ^e	20.1 ±2.54 ^e	25.79 ±0.07 ^e	18.3 ±0.1 ^b	3.74 ±0.33 ^e	27.17 ±0.17 ^c	12.53 ±0.03 ^e	2.7 ±0.12 ^d
Cattle manure	8.8± 0.03 ^a	19.41 ± 0.16 ^d	84.77 ±1.73 ^{ab}	16.68 ±0.06 ^c	14.07 ±0.96 ^{ef}	80.59 ±0.16 ^d	6.5 ±1.25 ^a	0.5 ±0.11 ^d	25.06 ±0.23 ^d	60.13 ±0.01 ^a	28.5 ±0.23 ^a	6.67 ±0.07 ^b	30.6 ±3.64 ^b	10.25 ±1.25 ^f	1.23 ±0.55 ^f
Chicken manure	6.6± 0.09 ^d	23.23 ±2.84 ^c	72.0 ±5.07 ^{bc}	16.11 ±0.53 ^c	34.71 ±5.46 ^b	76.77 ±2.84 ^e	4.5 ±3.22 ^b	0.82 ±0.56 ^a	28.60 ±0.87 ^b	48.52 ±0.13 ^f	17.27 ±0.33 ^d	4.73 ±0.03 ^d	25.66 ±2.58 ^d	20.22 ±2.15 ^c	3.6 ±1.62 ^c
Pig manure	7.1± 0.05 ^a	20.45 ±0.03 ^{cd}	78.50 ±0.86 ^{abc}	17.64 ±0.69 ^c	21.50 ±1.26 ^{cd}	79.55 ±0.03 ^{de}	1.5 ±2.36 ^f	0.59 ±1.32 ^c	26.83 ±3.23 ^c	36.77 ±0.03 ^c	19.27 ±0.67 ^c	5.13 ±4.26 ^c	23.23 ±0.41 ^f	15.6 ±3.65 ^d	2.5 ±2.58 ^e
Mix fruits	5.8± 0.06 ^e	8.02 ±0.23 ^f	76.64 ±4.63 ^{abc}	6.59 ±0.05 ^e	17.53 ±2.48 ^{de}	92.65 ±0.93 ^b	1.6 ±0.21 ^f	0.73 ±1.44 ^b	35.6 ±1.65 ^a	30.63 ±0.04 ^h	7.87 ±0.03 ^f	1.77 ±2.13 ⁱ	2.5 ±1.58 ^j	0.5 ±0.89 ^k	0.1 ±0.23 ^g
Mix veg- etables	6.7± 0.04 ^{cd}	12.33 ±0.29 ^e	64.4 ±3.02 ^c	6.35 ±0.18 ^e	48.53 ±0.64 ^a	87.67 ±0.29 ^c	0.44 ±0.88 ^g	0.05 ±0.99 ^f	8.2 ±1.25 ⁱ	35.31 ±0.02 ^d	18.5 ±1.25 ^b	3.23 ±3.03 ^f	2.1 ±0.11 ^k	1.2 ±0.71 ⁱ	0.1 ±2.63 ^g
Tomato plants	5.6± 0.07 ^{ef}	17.94 ±0.89 ^d	67.87 ±4.18 ^c	11.32 ±0.69 ^d	36.96 ±0.72 ^b	82.06 ±0.89 ^d	4.5 ±0.67 ^b	0.6 ±0.49 ^c	6.8 ±3.18 ^k	45.48 ±0.21 ^b	25.6 ±1.22 ^a	7.47 ±3.12 ^a	6.5 ±0.52 ^g	3.6 ±2.33 ^g	0.1 ±0.04 ^g
Tomatoes	4.7± 0.09 ^g	4.86 ±0.09 ^g	74.20 ±1.13 ^{abc}	3.61 ±0.12 ^f	25.80 ±1.13 ^c	95.14 ±0.09 ^{ab}	2.67 ±1.96 ^d	0.52 ±0.11 ^d	10.2 ±0.65 ^h	16.56 ±0.01 ⁱ	7.2 0.82 ^f	2.17 ±0.07 ^h	3.5 ±0.69 ⁱ	1.3 ±3.08 ^h	1.3 ±0.26 ^f
Cabbage	6.8± 0.06 ^a	14.46± 0.64 ^e	77.08 ±0.44 ^{abc}	11.11 ±0.44 ^d	23.17 ±0.53 ^{cd}	85.54 ±0.64 ^c	3.45 ±0.18 ^c	0.22 ±0.42 ^e	7.2 ±0.17 ^j	17.68 ±0.21 ^g	15.2 ±0.85 ^e	2.77 ±0.33 ^g	5.2 ±2.21 ^h	1.02 ±0.16 ^j	0.1 ±0.24 ^g
Dog food	5.6± 0.01 ^f	85.96 ± 0.50 ^a	88.15 ±2.15 ^{ab}	77.34 ±1.77 ^a	10.05 ±1.66 ^f	14.04 ±0.50 ^g	2.46 ±0.08 ^d	0.26 ±0.26 ^e	12.6 ±0.71 ^g	25.67 ±0.05 ^g	15.2 ±0.63 ^e	3.57 ±0.67 ^e	38.5 ±0.22 ^a	30.20 ±0.09 ^a	19.5 ±0.42 ^a
MVMFDF	6.7± 0.57 ^{cd}	29.42 ±0.78 ^b	90.02 ±0.38 ^a	26.49 ±0.82 ^b	9.98 ±0.38 ^f	70.58 ±0.78 ^f	0.34 ±0.38 ^g	0.02 ±0.56 ^g	15.7 ±1.01 ^f	32.83 ±0.01 ^d	18.53 ±0.03 ^b	3.63 ±0.63 ^e	25.01 ±2.55 ^e	24.52 ±2.31 ^b	14.2 ±1.28 ^b

^{a, b, c, d, e, f, g, h, i, j, k}: column means with different superscripts differ significantly ($p < 0.05$).

Observations revealed statistically significant variations ($p < 0.05$) in the total solids and volatile solids content among the substrates. The total solids content was highest in dog food at 85.96%, followed by MVMFDF (29.45%), chicken manure (23.23%), pig manure (20.45%), and cattle dung (19.41%). In addition, the study found that MVMFDF had the greatest concentration of volatile solids at 90.02%, followed by dog food at 88.15% and cattle manure at 84.77%. No major difference in total solids content was detected between cabbage (77.08%), tomatoes (74.20%), mixed fruits (76.64%), and pig manure (78.50%).

Li et al. [44] emphasised the substantial influence of substrate properties on biochemical methane potential. In the investigation conducted by Malolan et al. [45], the authors found that cattle manure had a volatile solids content of 82.23%, while poultry manure had a volatile solids content of 86.18%. These findings are consistent with the results of the current study. Nevertheless, discrepancies were seen in the overall amount of solids and moisture present, which can be attributed to differences in the conditions of the substrate during collection [41].

Furthermore, Wijaya et al. [46] found total solids of 20.07 for cattle manure and 23.30 for poultry manure, as well as volatile solids of 86.14 for cattle manure and 77.29 for poultry manure, which mirror the current study's findings despite variations in sample processing.

The findings of Li et al. [44] and Tang et al. [47] on the total solids and volatile solids of the inoculum, cabbage, and mixed vegetables were in agreement with the results obtained in this investigation. There is limited information on the use of tomatoes and tomato plants as substrates for anaerobic digestion. In a study conducted by Szilágyi et al. [48], it was found that tomato waste had a total solids content of 92.18% and a volatile solids content of 83.74%, which differs from the findings of this study. The variation seen in this case might be ascribed to disparities in substrate preparation. Specifically, Szilágyi et al. [48] employed heat-dried and crushed tomato fruits, stems, and leaves for the process of anaerobic digestion. In addition, Ambaye et al. [49] presented findings on fruit waste that were similar to those obtained in this study, but differences were observed in the composition of mixed fruit trash. There is a scarcity of information regarding the utilisation of dog food as substrates for anaerobic digestion.

The presence of nitrogen, carbon, and sulphur in various organic materials can have a significant impact on the process of producing biogas. Optimal biogas generation relies heavily on maintaining a proper equilibrium of essential nutrients, specifically nitrogen and carbon [50]. The nitrogen concentration in cattle manure was the greatest at 6.5%, followed by tomato plants and chicken manure, both containing 4.5% nitrogen. Inoculum had a nitrogen content of 1.7%, mixed fruits had 1.6%, pig manure had 1.4%, and lastly, cattle manure had 0.5% nitrogen. Nitrogen is important for microbial proliferation and metabolic processes. Organic materials that have a high nitrogen concentration tend to stimulate microbial activity, resulting in accelerated breakdown and increased production of biogas. Nevertheless, an excessive amount of nitrogen can lead to problems such as ammonia toxicity and instability in the process [51].

Carbon serves as the primary energy source for microbial metabolism during anaerobic digestion. High carbon content promotes methane production, while too low carbon content may result in slower digestion rates and reduced biogas [51]. The carbon content in mixed fruits was significantly higher ($p < 0.05$) at 35.5% compared to chicken manure (28.60%), pig manure (26.83%), and cattle manure (25.06%). The difference in carbon content identified in the cattle manure samples, in comparison to the findings published by Almonami [52], is significant. Almonami's [52] study reported a carbon content of 35.52%; however, our findings show a deviation from this value. The discrepancy could potentially be attributed to the variances in the diets of cattle.

The presence of sulphur can have an impact on both the quantity and quality of biogas produced. Although sulphur is necessary for microbial metabolism, an excess of sulphur can result in the creation of hydrogen sulphide (H_2S), which is undesirable because of its corrosive and poisonous characteristics. Materials that have a high amount of sulphur, such as some types of manure, may need to be treated beforehand or managed carefully

to avoid the creation of H_2S , for example, by biogas purification [50]. Chicken manure exhibited a significantly high sulphur content (0.82%), followed by mixed fruits (0.73%), tomato plants (0.6%), pig manure (0.59%), and cattle manure (0.5%) ($p < 0.01$).

Carbohydrates, which are organic substances composed of carbon, hydrogen, and oxygen, play an essential part in generating energy for microbes during anaerobic digestion. The substrates employed in the current investigation had a significant ($p < 0.05$) variance in their carbohydrate, protein, and fat composition. The dog food had the greatest carbohydrate content at 30.20%, followed by cattle manure at 30.6%, inoculum at 27.17%, chicken manure at 25.66%, MVMFDF at 25.01%, and pig manure at 23.23%.

Proteins are composed of amino acids and contain carbon, hydrogen, oxygen, nitrogen, and sometimes sulphur. During anaerobic digestion, proteins are broken down by hydrolytic bacteria into amino acids, peptides, and ammonia through a process called proteolysis. Excessive protein levels in the substrate can result in the accumulation of ammonia, which can inhibit methanogenic activity and cause process instability [33,44]. However, moderate protein content is essential for microbial growth and biogas production [9]. The dog food, MVMFDF, and chicken manure exhibited a significant ($p < 0.05$) protein content, with percentages of 30.20%, 24.52%, and 20.22%, respectively.

Substrates that include a significant amount of lipids, such as fats, have a greater capacity to produce methane because lipid molecules are highly concentrated sources of energy. Nevertheless, the breakdown of lipids results in the release of lengthy fatty acids, which might have harmful effects on the microbial community. These fatty acids can cause toxicity and result in a decrease in pH in the anaerobic digestion system [9]. In the current study, a noticeable high fat content was observed in dog food (19.5%) and MVMFDF (14.2%).

The research performed on neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) is a crucial evaluation of the fibre content in different substances. Although the direct influence of these parameters on biogas generation may not be as significant as carbon and nitrogen concentration, they do have an indirect effect on the anaerobic digestion process [53].

Out of the samples that were analysed, cattle manure had the greatest NDF concentration at 60.13%, followed by chicken manure at 48.52%, pig manure at 36.77%, and tomato plant material, respectively. In addition, cattle manure exhibited high levels of ADF at 28.8%, whereas tomato plants ranked second with 25.6%. However, there was no noticeable difference in ADF content between cabbage and dog food samples, as both had a recorded value of 15.2%. The acid detergent lignin (ADL) content was highest in tomato plants at 7.47%, followed by cattle manure (6.67%), pig manure (5.13%), and chicken manure (4.73%). It is worth mentioning that mixed fruits had the lowest amount of ADL, with a content of 1.77%. On the other hand, tomatoes had a slightly higher but still very low concentration of ADL at 2.17%.

3.2. Cumulative Methane Production of Various Substrates and Methane Production Flow Rate

Figures 2 and 3 depict the cumulative methane production and the methane production flow rate for different substrates. Figure 2 demonstrates that tomatoes had the highest cumulative methane production, followed by mixed fruits, MVMFDF (mixed vegetables and mixed fruits with dog food), cabbage, chicken manure, and dog food, in a descending sequence. The study conducted by Surendran and Shanmugam [54] found that the lowest biochemical methane potential (BMP) value among different vegetable waste samples was $0.08 \text{ Nm L CH}_4 \text{ g}^{-1} \text{ VSr}$ for okra waste, while the highest BMP value was $0.42 \text{ Nm L CH}_4 \text{ g}^{-1} \text{ VSr}$ for tomato waste. Furthermore, the average biochemical methane potential (BMP) value for all vegetable wastes was found to be $0.21 \text{ Nm L CH}_4 \text{ g}^{-1} \text{ VSr}$. Furthermore, the research conducted by Surendran and Shanmugam [54] aligns with the findings of the present study regarding the methane production capabilities of tomatoes, irrespective of the differences in substrate preparation.

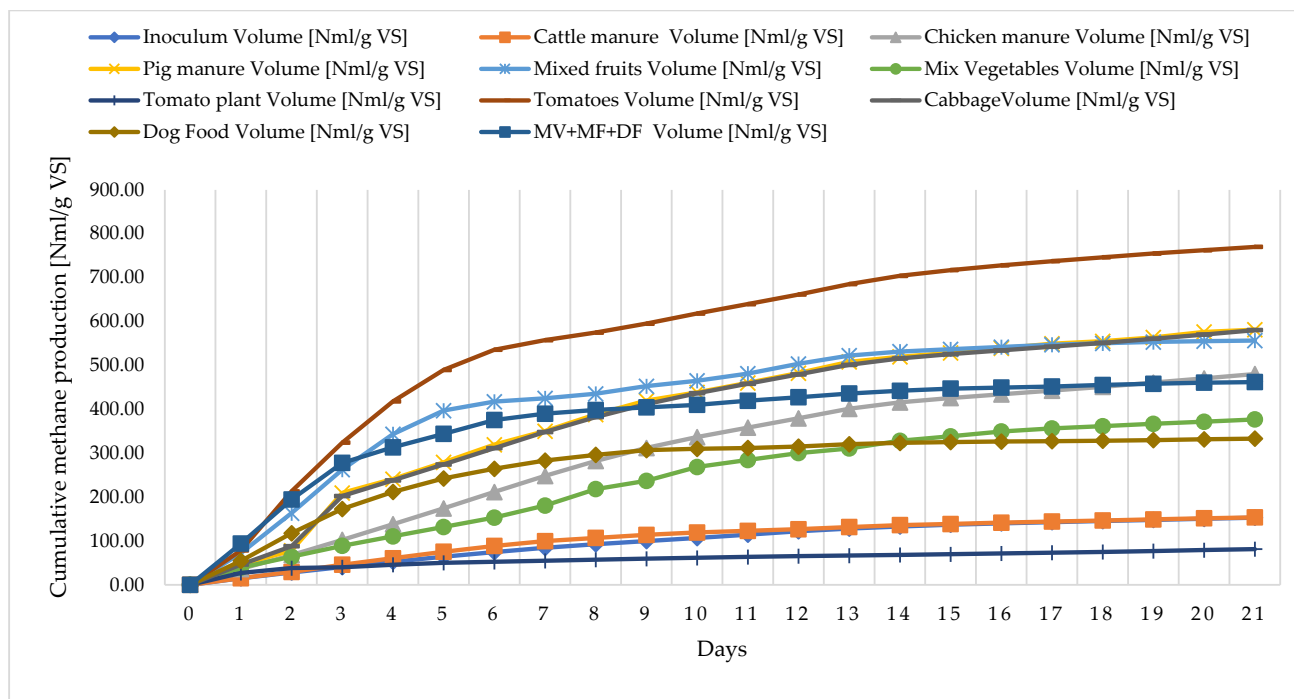


Figure 2. Cumulative methane production.

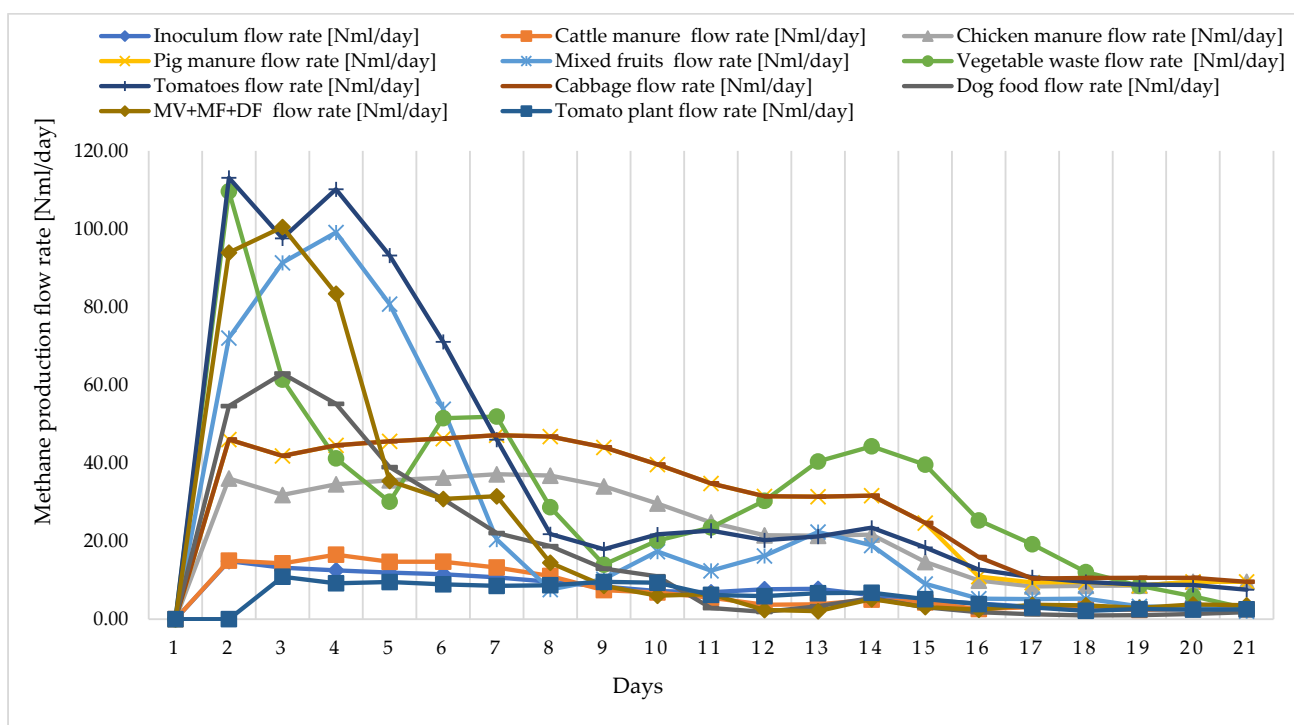


Figure 3. Daily methane production flow rate.

The high moisture level, glucose content, and fructose content in tomato waste make it more effective than other substrates. The fast breakdown of tomato waste through hydrolysis promotes the generation of a methane precursor known as volatile fatty acid, resulting in an enhanced output of biogas. In addition, the tomato waste has a significant amount of carbon, a high ratio of volatile solids, and a high carbon-to-nitrogen (C/N) ratio [55]. These factors contribute to its elevated biochemical methane potential (BMP) and calorific energy value (CEV). The lower toxicity of ammonia in tomato waste, which has a

low protein content and a high C/N ratio, also improves the efficiency of methane synthesis. As a result, the combination of these variables renders tomato waste a superior substrate for biogas production in comparison to other vegetable wastes. The elevated moisture content in tomato waste is essential for its effectiveness in biogas generation. The abundance of elevated concentrations of glucose and fructose in tomato waste speeds up hydrolysis, which involves the decomposition of organic substances into simpler chemicals. The rapid hydrolysis process results in the rapid generation of methane precursors, specifically volatile fatty acids (VFAs), which are crucial for the methanogenesis process responsible for biogas production [56].

Moreover, the abundant moisture in tomato waste provides an optimal setting for microbial activity, facilitating the effective fermentation of the organic matter. Additionally, the combination of the high levels of glucose and fructose in this context offers an abundance of material for the bacteria that are accountable for the generation of methane. Tomato waste, due to its high moisture content and the presence of glucose and fructose, creates an ideal environment for effective biogas production when compared to other substances. These characteristics enhance the efficiency of tomato waste in biogas generation procedures [57].

The observed pattern in methane production flow rate depicted in Figure 3 for tomato, mixed fruits, MV+MF+DF, dog food, and vegetable waste follows a typical trend seen in anaerobic digestion processes. From day 1 to day 8, there is a steady increase in methane production. This phase corresponds to the initial rapid degradation of easily biodegradable organic matter present in the feedstock. Similar trends were reported in the study conducted by Czubaszek, Wysocka-Czubaszek, and Tyborowski [58]. These substrates are readily available to the microbial community, promoting high microbial activity and resulting in a peak in methane production by day 8. However, after day 8, there is a significant decline in methane production. This decline indicates a depletion of the easily degradable substrates. As these substrates are consumed, microbial activity decreases because there are fewer readily available organic compounds to metabolise [59]. This leads to a drop in methane production, despite the presence of other organic materials. The slight increase observed after the significant decline and before the peak at day 21 may indicate a transition phase where microbial communities adapt to the remaining organic matter or less-easily-degradable components. This phase might involve slower degradation processes or the utilisation of more complex substrates, resulting in a gradual increase in methane production [60].

In the process of degradation, intermediate substances like volatile fatty acids (VFAs) may build up. High VFA concentrations can hinder methanogenic activity, causing a temporary drop in methane production. Changes in the composition of microbial communities can also affect methane production rates. Early stages of degradation may support the growth of rapidly multiplying acidogenic bacteria, while later stages may witness a shift towards slower-growing methanogens. This transition can lead to fluctuations in methane production rates [61].

The slight increase in methane production observed after the initial decline could be attributed to a secondary degradation phase, where more complex and resistant organic matter starts to break down. Typically, this phase generates methane at a slower pace compared to the initial phase. As time progresses, the availability of necessary nutrients for microbial activity may decrease, resulting in reduced metabolic rates and, consequently, lower methane production [62].

In the current study, cattle manure had the lowest methane potential, with mixed vegetables and tomato plants following in succession. The differences in methane production among various substrates can be attributed to their varied fibre compositions, which include neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL). Substrates containing a greater quantity of fibre, such as cattle manure, mixed vegetables, and tomato plants, often show a diminished capacity for methane production. The correlation suggests that increased levels of fibre components, particularly ADF to-

gether with its associated lignin content, can impede methane production by reducing the accessibility of the substrate and suppressing the microbial breakdown processes in the anaerobic digestion system [9,33].

Agrawal, Chaudhari, and Ghosh [62] reported that fruit waste generally has a higher methane potential compared to vegetable waste due to various factors. These results agree with the findings of the current study, regardless of variations in substrate preparation. One key factor is the higher sugar content present in fruits, which can be converted into methane more efficiently during anaerobic digestion [60]. Additionally, fruits often have a higher moisture content compared to vegetables, which can facilitate the microbial activity involved in the digestion process. Moreover, the presence of specific enzymes and organic compounds in fruits can also contribute to the higher methane potential. Overall, the composition and biochemical characteristics of fruits make them a more potent substrate for biogas production through anaerobic digestion compared to vegetable waste [59].

According to the study conducted by Arifan, Abdullah, and Sumardiono [63], cattle manure and chicken manure produced higher levels of methane compared to cabbage waste. These findings do not fully align with the results of the current study. In the current study, only chicken manure produced more methane than cabbage. Conversely, cabbage produced more methane than cattle manure. This discrepancy highlights the variability in methane production potential among different studies and suggests the influence of varying experimental conditions and feedstock compositions, which can be further influenced by factors such as the diet of the cattle and substrate pre-treatment [29].

Almeida et al. [56] documented the methane generation resulting from decomposing tomatoes, unripe tomatoes, and tomato plants. The study revealed that decomposed tomatoes emitted the highest levels of methane, whereas tomato plants emitted the lowest levels. The results align with the observations made in the current investigation. The branches and leaves of tomato plants had the least methane generation when compared to rotten tomatoes and green tomatoes.

The tomato plant has a high lignocellulosic content, which consists of a complex structure that is not easily broken down, resulting in it being a less effective substrate for anaerobic digestion. The presence of lignin in tomato plant remains interferes with the decomposition of organic matter and decreases the overall production of methane during anaerobic digestion [27]. Prior research has demonstrated that materials possessing a substantial amount of lignin exhibit a reduced ability to be broken down by biological processes and generate methane [27,32,64]. Hence, it is crucial to reduce the presence of tomato plants during the co-digestion of tomato wastes to maximise methane production or apply pre-treatment methods that will enhance tomato plants' degradability.

The study conducted by Belaid et al. [18] found that chicken manure had a higher methane potential than cattle manure. These results are consistent with the findings of the current investigation. Vegetable waste has been found to exhibit higher levels of methane production compared to the anaerobic digestion of cow manure [9]. According to the study conducted by Surendran and Shanmugam [54], tomatoes exhibited the maximum methane generation in comparison to mixed vegetables. These results are consistent with the findings of the current investigation. Additionally, it has been stated that this can be related to the elevated moisture content in tomatoes, which contains high levels of glucose and fructose that accelerate the process of hydrolysis and methanogenesis, resulting in a significant increase in the rate of methane synthesis [65].

Baek et al. [66] indicated that the process of digesting food waste (FW) alone resulted in the largest methane production, whereas the co-digestion of FW with pig manure (PM) created less methane. Conversely, the mono-digestion of cattle manure (CM) yielded the least amount of methane. The methane production rate exhibited a positive correlation with an increased proportion of food waste (FW) in the substrate combinations, while it showed a negative correlation with an increased proportion of cow manure (CM). Mixtures containing a high concentration of FW (food waste) exhibited a more rapid production of methane, most likely attributable to the presence of readily available organic components

in FW [67]. In addition, the methane production from the mono-digestion of pig manure was high compared to the mono-digestion of cattle manure; these findings agree with the findings of the current study. The results suggest that the organic matter content of the substrate mixture plays a crucial role in methane production efficiency. Mixtures with higher ratios of volatile solids (VS) to total solids (TS) had higher methane yields, indicating that the biodegradable organic content is an important factor in co-digestion efficiency [30].

The methane production of cattle manure is hindered by its high lignocellulosic content, particularly cellulose, hemicellulose, and lignin, which are resistant to anaerobic digestion. The significant lignocellulosic component present in cattle manure poses challenges for microorganisms to efficiently decompose and convert it into methane. Consequently, the production of methane from the anaerobic digestion of cattle manure alone is relatively modest in comparison to the co-digestion of cattle manure with other substances like pig manure and chicken manure [68]. This is because pig and chicken manure contain less lignin and have a greater potential for methane production. The co-digestion of cattle manure with other substrates fosters a harmonious habitat for the microbial population, enhancing the processes of hydrolysis, acidogenesis, and methanogenesis, ultimately leading to increased methane production. Hence, the utilisation of cattle manure alone in anaerobic digestion is less efficient in generating methane compared to the co-digestion of cattle manure with other substances such as fruit and vegetable waste [69].

These findings highlight the significance of comprehending the composition of substrate materials to optimise methane production rates in anaerobic digestion systems. This information can be used to make strategic decisions that improve the overall performance and efficiency of the biogas-producing process.

Biogas generation, achieved by collecting methane through anaerobic digestion, effectively avoids the release of methane, a highly potent greenhouse gas, into the atmosphere. Consequently, this process significantly reduces the overall emissions of greenhouse gases resulting from the breakdown of agricultural waste. Using agricultural waste for biogas production also aids in efficient waste management by decreasing the amount of waste that must be disposed of in landfills, consequently minimising the pollution of the land and water [67].

Substrates characterised by low C/N ratios, such as animal manure, food waste, and specific industrial waste, possess an abundance of nitrogen compared to carbon [10]. An elevated nitrogen concentration results in an overabundance of ammonia synthesis during the decomposition of proteins and urea. An excessive amount of ammonia can elevate the pH level and generate a harmful setting for methanogens, limiting their functioning [21]. High levels of ammonia (ammonia toxicity) could affect the functioning of methanogens, the microorganisms that produce methane. Consequently, this leads to reduced methane production. Elevated nitrogen levels can promote the proliferation of ammonia-tolerant microorganisms at the expense of methanogens, disturbing the equilibrium necessary for optimal anaerobic digestion [70]. Combining substrates that have different carbon-to-nitrogen ratios can assist in attaining the most favourable ratio. One way to balance the ratio is by co-digesting animal manure, which has a low carbon-to-nitrogen ratio, with vegetable leftovers, which have a high carbon-to-nitrogen ratio [66]. Strategically choosing and combining substrates to obtain the required carbon-to-nitrogen ratio can improve the efficiency of digestion. This may entail the addition of carbonaceous elements to nitrogenous substrates, or vice versa. Occasionally, the introduction of exogenous carbon or nitrogen supplies can assist in modifying the C/N ratio. For instance, the act of incorporating straw or sawdust, which are carbon sources, into substrates that are high in nitrogen [21].

Moreover, many techniques can be utilised to enhance the efficiency of the anaerobic digestion process. To improve biogas quality, these strategies include altering substrate preparation methods, such as mechanical, thermal, and chemical pre-treatment, as well as mixing ratios and operational factors [31]. For ensuring the digester is kept at the most favourable temperatures for microbial activity, mesophilic settings, often ranging from

30 to 40 °C, are generally more stable. On the other hand, thermophilic conditions, which range from 50 to 60 °C, can enhance reaction rates and pathogen elimination. However, they demand more energy and precise monitoring [33]. To maintain a steady pH (often between 6.5 and 7.5) and promote optimal microbial activity, one can add chemicals such as bicarbonates or use substrates with natural buffering abilities to prevent acidification [42]. Modifying the hydraulic retention time (HRT) to guarantee sufficient duration for the microbial breakdown of substrates is important as an inadequate duration of hormone replacement therapy (HRT) might cause inadequate digestion, whilst an excessive duration of HRT can result in decreased system efficiency [27]. To prevent the formation of scum or dead zones, promote homogeneous microbial activity, and prevent stratification, it is important to ensure appropriate mixing. It is recommended to utilise intermittent mixing instead of continuous mixing to decrease energy usage while still ensuring efficient substrate contact and gas release [41].

Biogas is a sustainable energy source that has the potential to substitute fossil fuels in producing power and heat. This substitution aids in reducing reliance on non-renewable energy sources and mitigates carbon dioxide emissions linked to the combustion of fossil fuels. In addition, anaerobic digestion decreases the presence of odours and pathogens in agricultural waste, resulting in enhanced air quality and decreased health hazards for neighbouring communities [35].

Through the production of biogas, farmers and rural communities can achieve greater energy self-sufficiency, diminishing their dependence on external energy sources and strengthening energy resilience.

3.3. Biogas Composition of Various Substrates

The biogas composition changes based on the substrates used in the anaerobic digestion process. Biogas is primarily composed of methane (CH_4), which makes up 45% to 70% of its composition. It also contains carbon dioxide (CO_2) at levels of 30% to 45%, as well as small amounts of trace gases such as hydrogen sulphide (H_2S) and ammonia [71]. Carbon dioxide is a significant constituent of biogas, generated during the anaerobic digestion process. It can dilute the methane content of biogas, reducing its energy potential. Elevated concentrations of carbon dioxide can also impact the effectiveness of biogas utilisation in fuel cells and other technologies [20]. Ammonia is a relatively small constituent of biogas, found in very small quantities. It is generated from the breakdown of organic molecules that contain nitrogen during the process of anaerobic digestion. Although small amounts of ammonia may not have a significant impact on the process, excessive concentrations might hinder the activity of methane-producing bacteria and have a detrimental effect on biogas generation [70]. Hydrogen sulphide is a low-concentration sulphur chemical that is present in biogas. It is generated as a secondary product during the process of anaerobic digestion. It can lead to problems such as corrosion, an unpleasant smell, and toxicity. Elevated concentrations of hydrogen sulphide can also hinder the efficiency of fuel cells and other technologies that utilise biogas as a fuel source [24].

Carbon dioxide can be eliminated from biogas using techniques like pressure swing adsorption or membrane separation. These techniques aid in the separation and retention of carbon dioxide, hence diminishing its concentration in the biogas [19,20]. To mitigate the presence of ammonia in biogas, it is necessary to utilise appropriate desulphurisation processes. This process may entail the utilisation of iron compounds or other appropriate substances to decrease the concentrations of ammonia in the biogas. In addition, enhancing the digestive process and regulating the composition of the substrates may be beneficial in decreasing ammonia levels [21,70]. Implementing appropriate desulphurisation technologies is essential for mitigating the impacts of hydrogen sulphide in biogas. One method to eliminate hydrogen sulphide from the gas stream is by using iron chloride or air injection. Ensuring appropriate pH levels in the digester and monitoring the sulphur content in the substrate can aid in managing hydrogen sulphide levels [72]. To successfully reduce the presence of carbon dioxide, ammonia, and hydrogen sulphide in biogas, these methods

can be implemented. This will ensure the efficient operation of fuel cells and catalytic fuel upgrading units.

Table 2 illustrates the results showcasing the methane (CH₄), carbon dioxide (CO₂), ammonia (NH₃), and hydrogen sulphide (H₂S) levels. Upon analysing the raw biogas production, the highest methane content was observed in the MVMFDF substrate, which is the co-digestion of mixed vegetables, mixed fruits, and dog food, at 77.4%. Following closely was the methane yield from mixed fruits, registering at 76.6%. Conversely, the lowest methane yield was detected in the inoculum at 34.1%, tomato plants (62.9%), and cattle manure (63.5%), respectively. Notably, there was no statistically significant difference ($p > 0.05$) in methane content between dog food (72.5%) and pig manure (76.6%).

Table 2. Biogas composition of various substrates.

Substrates	CH ₄ (%)	CO ₂ (%)	NH ₃ (ppm)	H ₂ S (ppm)
Inoculum	34.1 ± 0.03 ⁱ	65.9 ± 0.53 ^a	24.33 ± 0.33 ^g	27.0 ± 0.46 ^h
Cattle manure	63.5 ± 1.62 ^g	36.5 ± 0.27 ^c	38.33 ± 0.88 ^f	108.0 ± 3.33 ^f
Chicken manure	69.4 ± 0.25 ^e	30.6 ± 0.69 ^e	98.0 ± 0.63 ^a	589.0 ± 1.12 ^a
Pig manure	72.57 ± 3.03 ^c	27.43 ± 0.29 ^g	65.0 ± 0.55 ^c	540.0 ± 2.29 ^b
Mix fruits	76.6 ± 1.43 ^b	23.4 ± 2.65 ^h	68.0 ± 1.27 ^c	250.33 ± 0.33 ^d
Mix vegetables	70.2 ± 0.13 ^d	29.8 ± 1.49 ^f	35.33 ± 0.88 ^f	105.0 ± 1.89 ^g
Tomato plants	62.9 ± 2.23 ^h	37.1 ± 4.01 ^b	78.0 ± 1.67 ^b	485.0 ± 0.73 ^c
Tomatoes	64.5 ± 0.07 ^f	35.5 ± 0.07 ^d	36.67 ± 2.72 ^f	6.0 ± 0.07 ^k
Cabbage	70.1 ± 0.15 ^d	29.9 ± 0.08 ^f	36.0 ± 0.58 ^f	18.0 ± 2.4 ⁱ
Dog food	72.5 ± 3.12 ^c	27.5 ± 0.97 ^g	43.33 ± 1.64 ^e	18.0 ± 1.32 ^j
MVMFDF	77.4 ± 2.25 ^a	22.6 ± 2.66 ⁱ	51.33 ± 3.38 ^d	208.0 ± 1.39 ^e

^{a, b, c, d, e, f, g, h, i, j, k}: column means with different superscripts differ significantly ($p < 0.05$).

According to the results reported by Rafiee et al. [73], 60% of methane content was recorded from cattle manure and poultry manure. Kafle and Chen, Ref. [74] reported a methane content of 69.1% from cattle manure and 70.1% from poultry manure. These results are comparable to the results obtained from this study. These similarities in methane and carbon dioxide content might have been instigated by similar initial and final pH values. According to the study conducted by Maile, Muzenda, and Mbohwa [2], the methane content of mixed fruits and mixed vegetables ranged between 50 and 60%. In the present study, the methane content recorded for mixed fruits was 76.6% and 70.2% for mixed vegetables. The variance in methane content could have been initiated by inoculum collected from a running biodigester, while the study conducted by Maile, Muzenda, and Mbohwa [2] used sodium hydroxide to adjust the initial pH of the substrates.

In terms of carbon dioxide content, the highest levels were noted in the inoculum (65.9%), tomato plants (47.90%), and cattle manure (36.5%), respectively. Notably, a significantly high level of ammonia was recorded in chicken manure (98.0 ppm) and tomato plants (78.0 ppm), with statistically significant differences ($p < 0.05$). Conversely, there was no significant disparity in the ammonia content between pig manure (65.0 ppm) and mixed fruits (68.0 ppm). The allowable hydrogen sulphide content in biogas ranges from 10 to 100 ppm; higher concentrations of hydrogen sulphide can be corrosive to downstream equipment [75,76]. A significantly ($p < 0.05$) high hydrogen sulphide content was observed from chicken manure (589.0 ppm), pig manure (540.0 ppm), and tomato plants (485.0 ppm), respectively. Only tomatoes, cabbage, and dog food had a hydrogen sulphide content that was well below the recommended H₂S range.

3.4. Oxygen Concentration during Anaerobic Digestion

Figure 4 depicts the changes in oxygen concentration throughout the anaerobic digestion process of different substrates, showing a gradual reduction over time. Tomatoes had the least amount of oxygen throughout the later stages of anaerobic digestion, specifically from day 16 to day 21. Conversely, cattle manure and pig manure had the most elevated

quantities of oxygen. There is a scarcity of research on the levels of oxygen present during anaerobic digestion. An oxygen-free environment is typically advised for the occurrence of anaerobic digestion. Nevertheless, despite attempts to remove gases using nitrogen, a certain amount of oxygen unavoidably persists within the biodigester. Studies suggest that anaerobic biodigesters may withstand a substantial level of oxygen without negatively impacting methane production [77,78]. Vilniškis [79] also observed similar results, with the oxygen concentration being below 0.5% from day 12 to day 20.

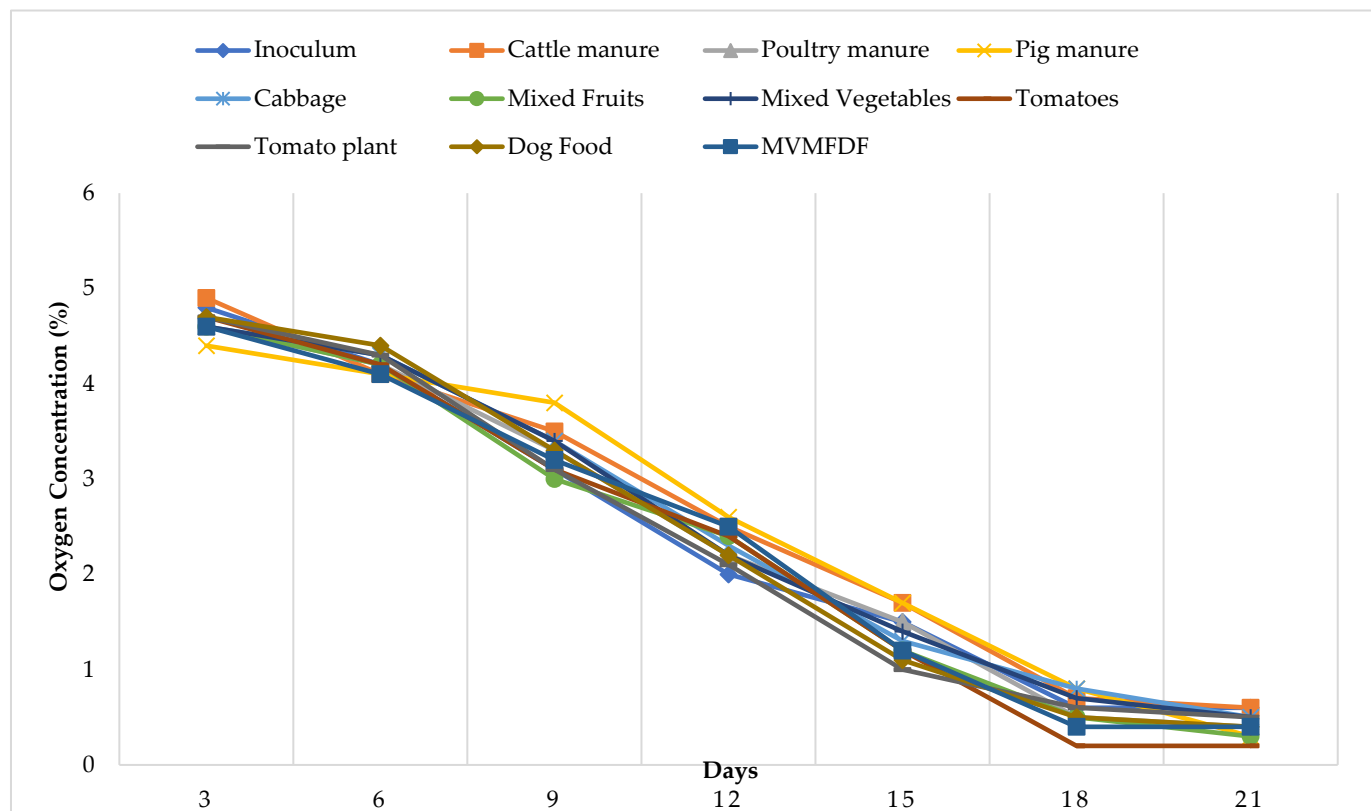


Figure 4. Oxygen concentration during biogas production.

Anaerobic digestion depends on the maintenance of exclusively anaerobic conditions. Oxygen can impede the activity of anaerobic bacteria, specifically methanogens, which play a crucial role in methane generation. Exposure to oxygen might result in an increase in aerobic microorganisms, which outperform anaerobic bacteria, hence reducing the efficiency of methane production [80]. The introduction of oxygen can transition conditions from anaerobic to aerobic, disturbing the intricate equilibrium of the microbial ecology that is essential for anaerobic digestion. This interruption can cease the production of methane and lead to the formation of substitute by-products, such as carbon dioxide and water, rather than methane [26].

Several studies [78,81] indicate that limited aeration can boost the activity of specific microorganisms, such as methanogens and sulphide-oxidising bacteria, hence improving the breakdown of organic matter and increasing biogas production. Nevertheless, an overly high quantity of oxygen might impede the growth of anaerobic organisms, resulting in a decrease in methane purity and the ability to degrade organic matter. Ensuring the optimal performance of anaerobic digestion systems relies on optimising the rate and quantity of oxygen. Wang et al. [82] reported that the addition of an oxygen supplement, specifically in the form of O₂-containing gas nanobubble water (NBW), to the anaerobic digestion (AD) of cellulose has several benefits. Firstly, it enhances cellulase activity, which is responsible for breaking down cellulose into simpler compounds, facilitating microbial digestion and methane production. This enhancement results in higher methane yields from cellulose during

the AD process. Furthermore, the oxygen supplement helps reduce cellulose crystallinity, further aiding its breakdown. This reduction in crystallinity improves methane production efficiency. Additionally, the oxygen supplement creates a micro-oxygen environment that enhances electron transport system (ETS) activity. This increased ETS activity provides more energy for the microbes involved in AD, ultimately leading to higher methane production.

The effect of oxygen concentration on anaerobic digestion bacteria can exhibit variability based on specific conditions and concentrations, highlighting the necessity for additional research in this area. Oxygen can reduce the methane levels in biogas since the presence of oxygen does not support the production of methane. In contrast, the process of aerobic digestion produces a higher amount of carbon dioxide. Decreasing the methane content reduces the energy potential of biogas, hence reducing its efficiency as a fuel source. Oxygen can also lead to the generation of unwanted by-products, like water, heat, and other gases, which can pollute biogas and reduce its quality [83].

Oxygen can undergo a chemical reaction with hydrogen sulphide (H_2S) present in biogas, resulting in the formation of sulphur compounds. These compounds have the potential to cause corrosion and other problems related to the functioning of the system [26]. A combination of aerobic and anaerobic conditions can be produced in the digester by elevated oxygen levels, which can lead to polluted biogas with lower methane levels and higher levels of carbon dioxide and other non-combustible gases. Furthermore, the high oxygen content of the upgraded biogas can limit its further applications as a renewable energy source [84]. The contaminated biogas may necessitate additional purifying measures to assure effective utilisation, hence increasing the overall expenses and complexity of the biogas-producing process [81].

3.5. Inoculated Initial pH and Final pH of Various Substrates

Figure 5 illustrates the results of the initial and final pH levels following inoculation. A significant decline in pH was detected between the early and end stages for substrates such as cattle manure, cabbage, mixed fruits, mixed vegetables, and dog food ($p < 0.05$). The pH level inside the anaerobic biodigester is vital in determining the effectiveness of the anaerobic digestion process. Previous research had indicated that anaerobic microorganisms flourish most effectively within a pH range of 6.7 to 7 [45,85]. Based on the findings shown in Figure 4, the initial pH values of the substrates that were inoculated ranged from 6.9 to 7.7, except for the inoculum. This suggests that the anaerobic digestion process was conducted within the ideal pH range. The elevated pH of the inoculum, exceeding 8, may be attributed to the specific feedstock employed in the biodigester plant where it was collected. Substrates that include a large amount of proteins or urea, such as animal manure or specific agricultural substrates, might result in elevated quantities of ammonia, thus leading to an increase in pH [70].

A pH 8.7 inoculum facilitated pH changes in the substrates. Previous studies have shown that pH values lower than 6.1 or higher than 8.3 are more likely to experience operational problems [10,42,86]. Santos et al. [65] observed an initial pH of 7.7 and a final pH of 6.8 for a combination of different fruits. These findings are consistent with the outcomes of the present investigation.

Methanogens, which are essential for methane generation, can be inhibited by acidic environments with a pH below 6.5. This might result in the buildup of volatile fatty acids (VFAs), which can further decrease the pH and create a harmful environment for microorganisms [86]. Alkaline environments with a pH greater than 8.0 might hinder the activity of hydrolytic and acidogenic bacteria. These bacteria play a crucial role in breaking down complex organic materials into simpler chemicals that can be utilised by methanogens. Deviation from the appropriate pH range can greatly decrease methane production [87]. Acidic conditions can impede methane formation by suppressing the activity of methanogens, whereas alkaline conditions can decelerate the initial decomposition of organic matter, limiting the availability of substances for methane production. Optimal pH levels are essential for maintaining a well-balanced microbial consortium,

which is crucial for promoting successful anaerobic digestion [88]. Deviation from the balance can disturb the equilibrium, resulting in the prevalence of undesirable microbial species that generate lower amounts of methane and higher amounts of by-products, such as hydrogen sulphide. Deviation from the optimal pH levels might lead to instability in the anaerobic digestion system. These factors can lead to varying rates of biogas production, the buildup of substances that hinder the process, and the risk of the system not functioning properly [89].

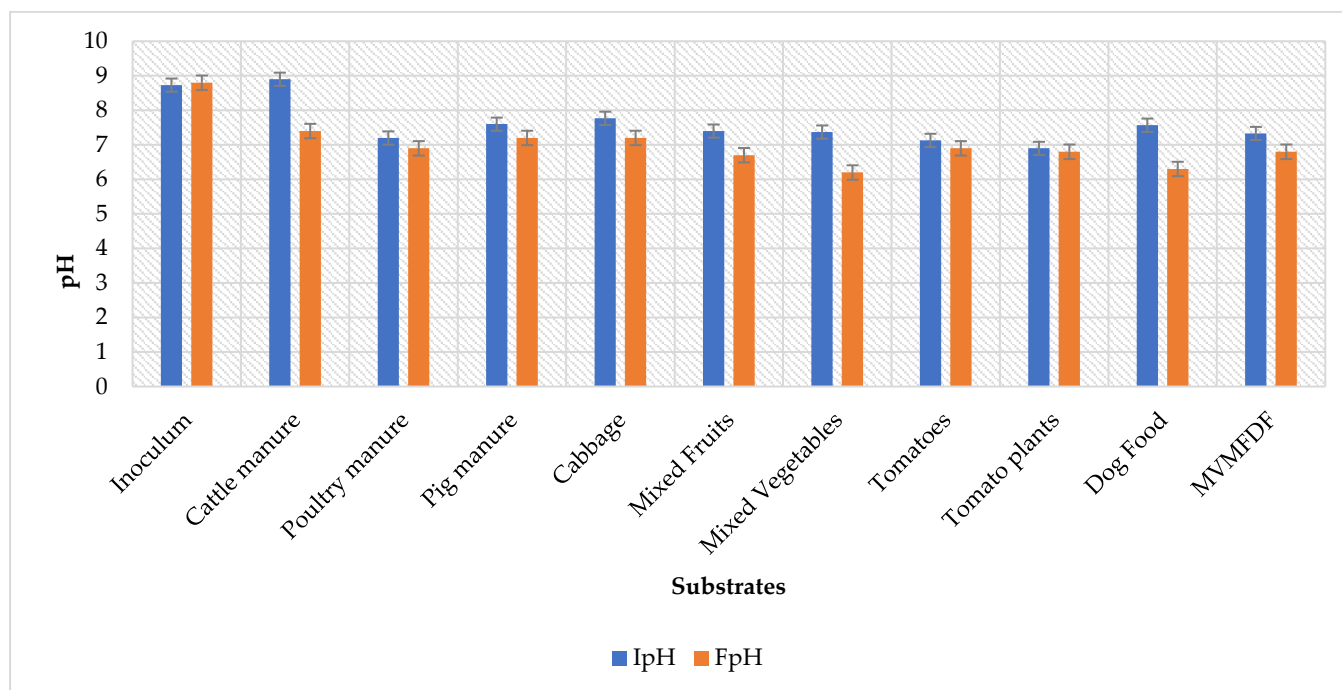


Figure 5. Inoculated initial pH and final pH of various substrates.

The ideal pH for anaerobic digestion often falls within the range of 6.8 to 7.4. These bacteria are highly active and effectively decompose complex organic materials into simpler molecules. Methanogens exhibit optimal activity and efficiency in the production of methane, hence guaranteeing superior quality and quantity of biogas [87]. Regular and ongoing monitoring of pH levels is crucial to promptly identify any deviations and implement necessary corrective measures. Ensuring an adequate buffering capacity in the digester can assist in reducing pH fluctuations [90]. The inclusion of buffering agents such as bicarbonates can help maintain stable pH levels. To minimise fast reductions in pH caused by the accumulation of volatile fatty acids (VFAs), it is important to modify the feeding regimen to avoid overloading the digester with substrates that are easily broken down [88] (Figure 5).

3.6. Correlation between Nutrients and Biogas Composition

The relationship between carbohydrates, protein, lipids, methane, carbon dioxide, ammonia, and hydrogen sulphide in anaerobic digestion processes is complex and interconnected. Carbohydrates, proteins, and lipids are organic compounds that act as substances for microbial metabolism in anaerobic digesters. The decomposition of these molecules by diverse microorganisms in microbial communities results in the generation of methane, carbon dioxide, ammonia, and hydrogen sulphide [91].

Carbohydrates are generally the main carbon source in the process of digestion. Fermentative bacteria convert them into organic acids, which are then metabolised to generate hydrogen, carbon dioxide, and volatile fatty acids. Methanogenic archaea utilise these chemicals to create methane. Protein-rich substances play a role in the production of am-

monia during the process of digestion. When ammonia levels are high, it might hinder the functioning of methanogens [27].

The interactions between these chemicals and microbial populations are crucial in influencing the effectiveness and output of methane generation. Ensuring a proper equilibrium of various nutrients, such as carbs, proteins, and lipids, is crucial for achieving optimal methane synthesis [92]. In addition, regulating variables such as pH, temperature, and retention time in digesters can impact the generation of methane and other by-products such as carbon dioxide, ammonia, and hydrogen sulphide.

The concentration of methane in biogas is influenced by the content of these organic compounds in the substrate. Additionally, the presence of carbon dioxide, ammonia, and hydrogen sulphide in biogas can affect its quality and usability [35]. Carbon dioxide, when present in high concentrations, lowers the heating value of biogas and reduces its efficiency as a fuel. Ammonia and hydrogen sulphide are impurities that can hinder the commercial use of biogas, leading to issues such as corrosion in equipment and the emission of harmful gases during combustion [27].

Analysing the connections between these substances and microbial populations using metagenomic and metabolomic investigations can offer valuable information about the metabolic pathways associated with anaerobic digestion and aid in improving the process for the sustainable production of methane. Through a thorough examination of the functions of various microorganisms and their mutually beneficial relationships, researchers can optimise the management of processes, increase effectiveness, and reduce the negative impact of substances such as ammonia and hydrogen sulphide on the synthesis of methane [93].

The data in Table 3 clearly indicate a negative association (-0.171) between carbohydrates and methane. This occurrence can be attributed to the fact that acidogenic and acetogenic bacteria tend to utilise carbohydrates as their primary substrate to produce volatile fatty acids (VFAs) rather than converting them directly into methane. This can hinder the availability of the essential intermediates required for methanogens to produce methane [91]. In addition, high carbohydrate content can lead to the accumulation of VFAs, which can lower the pH and inhibit methanogenic activity, thus reducing methane production [27].

Table 3. Correlation between nutrients and biogas composition.

Nurients	CH ₄ %	CO ₂ %	NH ₃ ppm	H ₂ S ppm
Carbohydrates	-0.171	0.171	-0.014	0.032
%	ns	ns	ns	ns
Protein %	0.110	-0.110	0.163	0.147
	ns	ns	ns	ns
Fat %	0.254	-0.254	-0.069	-0.186
	ns	ns	ns	ns
NDF %	0.057	-0.057	0.470^{**}	0.555^{**}
	ns	ns		
ADF %	-0.215	0.215	0.066	0.279
	ns	ns	ns	ns
ADL %	-0.170	0.170	0.346^{*}	0.515^{*}
	ns	ns		
C %	-0.086	0.086	0.396^{**}	0.438^{**}
	ns	ns		
S %	-0.130	0.130	0.776^{**}	0.795^{**}
	ns	ns	0.000^{*}	
N %	-0.183	0.183	0.804^{**}	0.798^{**}
	ns	ns		

$p < 0.01^{**}$, $p < 0.05^{*}$, ns: not significant, NDF (neutral detergent fibre), ADF (acid detergent fibre), ADL (acid detergent lignin), C (carbon), S (sulphur), N (nitrogen).

Furthermore, a negative correlation of -0.14 was detected between carbohydrates and ammonia. Carbohydrates lack nitrogen. Elevated quantities of carbohydrates can potentially decrease the concentration of nitrogen in the substrate, resulting in a decrease in the production of ammonia from nitrogen-containing molecules [94]. Conversely, there was a direct relationship observed between carbohydrates and carbon dioxide, with a correlation coefficient of 0.171 . Carbohydrates are highly fermentable and can enhance the activity of fermentative bacteria, resulting in increased carbon dioxide production because of fermentation [95]. Moreover, a direct relationship was seen between methane and hydrogen sulphide, with a correlation coefficient of 0.032 . Sulphate-reducing bacteria, which can produce hydrogen sulphide, can vie with methanogens for resources such as hydrogen and acetate. Nevertheless, the presence of a slight positive association implies an intricate interaction in which both gas-producing pathways may coexist under specific circumstances [96]. An association was found between fat and methane, as well as between protein and methane, both showing a positive correlation. Nevertheless, these findings lacked statistical significance.

A substantial connection ($p < 0.01$) was found between NDF and ammonia (0.470). A statistically significant positive connection ($p < 0.01$) was also found between NDF and hydrogen sulphide (0.555). The ADL showed a positive correlation with ammonia (0.346) ($p < 0.05$). The ADL showed a positive correlation with hydrogen sulphide (0.515) ($p < 0.05$). There was a strong positive correlation between carbon and ammonia ($r = 0.396$, $p < 0.01$). Carbon showed a significant positive correlation with hydrogen sulphide (0.438) ($p < 0.01$). The presence of sulphur showed a strong positive correlation with ammonia, with a correlation coefficient of 0.776 ($p < 0.01$). Sulphur exhibited a positive correlation with hydrogen sulphide (0.795) ($p < 0.01$). The correlation between nitrogen and ammonia was positive, with a correlation coefficient of 0.804 ($p < 0.01$). The correlation between nitrogen and hydrogen sulphide was positive (0.798) and statistically significant ($p < 0.01$).

The findings demonstrate many significant correlations among crucial factors, indicating complex interactions within the anaerobic digestion process. A strong positive connection ($p < 0.01$) was found between neutral detergent fibre (NDF) and ammonia (0.470). Furthermore, there was a strong and statistically significant connection ($p < 0.01$) between NDF and hydrogen sulphide (0.555), indicating that the amount of fibre in the sample had a notable impact on the formation of gas.

In addition, acid detergent lignin (ADL) showed strong positive associations ($p < 0.05$) with ammonia (0.346) and hydrogen sulphide (0.515), highlighting the impact of lignin levels on gas composition during anaerobic digestion. Furthermore, there were strong correlations ($p < 0.01$) seen between carbon and ammonia (0.396) as well as hydrogen sulphide (0.438), indicating a possible connection between carbon availability and the generation of these gases. Similarly, sulphur showed strong positive associations ($p < 0.01$) with both ammonia (0.776) and hydrogen sulphide (0.795), highlighting the influence of sulphur levels in determining the gas composition in the anaerobic digestion environment.

Furthermore, nitrogen displayed significant positive correlations ($p < 0.01$) with both ammonia (0.804) and hydrogen sulphide (0.798), highlighting the interconnectedness between nitrogen availability and the production of these gases. Similar results were recorded in the study conducted by Amaleviciute-Volunge, Slepetiene, and Butkute [53]. However, the study reported a significant ($p < 0.01$) negative correlation between ADF and methane, while in the current study, the negative correlation was not significant. The findings highlight the complex interactions between substrate components and gas production parameters, providing valuable insights into the underlying mechanisms governing anaerobic digestion processes. Such knowledge is crucial for optimising biogas production efficiency and mitigating potential environmental impacts.

In addition, nitrogen exhibited strong positive correlations ($p < 0.01$) with both ammonia (0.804) and hydrogen sulphide (0.798), indicating the close relationship between nitrogen availability and the generation of both gases. The study performed by Amaleviciute-Volunge, Slepetiene, and Butkute [53] yielded comparable findings. Nevertheless, the study

indicated a noteworthy ($p < 0.01$) negative relationship between ADF and methane. However, in the present investigation, the inverse association was not statistically significant. The results emphasise the complex connections between the components of the substrate and the parameters that affect gas production, offering a significant understanding of the fundamental mechanisms that control anaerobic digestion processes. Acquiring such knowledge is essential for maximising the efficiency of biogas generation and reducing any potential negative effects on the environment.

4. Conclusions and Recommendations

The study's findings emphasise the diverse biogas quality generated by distinct agricultural substrates. The co-digestion of mixed vegetables, mixed fruits, and dog food (MVMFDF) resulted in the greatest methane content, reaching 77.4%. Mixed fruits alone achieved a methane content of 76.6%. A high concentration of methane is essential for biogas as it increases its energy potential. In contrast, tomato plants (62.9%) and calf manure (63.5%) had lower methane yields, indicating that these substrates are less effective for producing high-quality biogas. The different concentrations of carbon dioxide, ammonia, and hydrogen sulphide also affect the overall quality and utility of the biogas. Elevated concentrations of hydrogen sulphide, found in chicken manure, pig manure, and tomato plants, possess corrosive properties and necessitate further measures to prevent harm to machinery.

This provides a substantial contribution to the objective of this study, which is to examine the levels of methane, carbon dioxide, ammonia, and hydrogen sulphide in different agricultural waste materials during the process of biogas production. The study offers a thorough assessment of the appropriateness of different agricultural wastes for biogas production by measuring the quantities of methane, carbon dioxide, ammonia, and hydrogen sulphide. This knowledge is crucial for optimising the processes of biogas generation and selecting suitable substrates to maximise the synthesis of methane while minimising the creation of dangerous by-products.

The study's results enhance the overall knowledge in the field of renewable energy by pinpointing agricultural residues that can be efficiently transformed into top-notch biogas. Using substrates such as mixed fruits and MVMFDF might improve the efficiency of biogas production, hence promoting the shift towards renewable energy sources. In addition, the promotion of sustainable waste management involves the valorisation of agricultural waste, which helps to reduce the environmental impact of waste disposal and provides an alternative energy source.

The study can provide valuable insights for policies aimed at boosting the use of agricultural residues for the generation of biogas, hence fostering the establishment of biogas facilities that employ highly productive substrates. Policymakers have the authority to establish criteria for the quality of biogas, which involves maintaining a high level of methane and minimising the presence of dangerous by-products. The study's findings can be used by biogas producers to optimise substrate combinations, maximising methane production and minimising hazardous emissions. The industry should consider investing in pre-treatment technology to improve the biodegradability of low-yield substrates. The study provides evidence to support the objective of enhancing the proportion of renewable energy by optimising the processes involved in biogas production. The study's promotion of biogas usage aids in the mitigation of greenhouse gas emissions resulting from waste.

The findings can be utilised in practical situations by providing guidance for the selection of substrates for biogas facilities. Operators can optimise methane output and raise the efficiency of biogas plants by prioritising high-yield substrates such as mixed fruits and MVMFDF. Furthermore, the study can provide valuable insights for optimising the design and functioning of biogas facilities to effectively manage substrates containing elevated quantities of ammonia and hydrogen sulphide by integrating essential treatment systems.

Using agricultural waste for biogas production helps to reduce the amount of waste that is disposed of in landfills, hence reducing environmental pollution. Biogas production involves the collection of methane, which would otherwise escape into the atmosphere during

the decomposition of organic waste. This process helps to decrease the amount of greenhouse gas emissions. The residue generated from the production of biogas can serve as a fertiliser that is abundant in nutrients, supporting the practice of sustainable agriculture.

The local production of biogas can decrease reliance on imported fossil fuels, resulting in reduced energy expenses. The biogas business has the potential to generate employment opportunities in trash collection, plant operation, and maintenance. Farmers and biogas plant operators can generate extra income by selling excess biogas and digestate as fertiliser.

The study's results may differ depending on the environmental conditions or the sources of the inoculum. The composition of agricultural wastes can fluctuate seasonally and geographically, which has an impact on the production of biogas. Performing trials in various settings and utilising diverse inoculum sources can yield more reliable data. Consistent monitoring and examination of the composition of the substrate can assist in modifying biogas production procedures to accommodate fluctuations.

Future investigations can conduct research on pre-treatment techniques to enhance the biodegradability of low-yield substrates; analyse the enduring ecological and financial consequences of employing various substrates in the manufacturing of biogas; create optimisation models for substrate mixes with the objective of maximising methane production while minimising the formation of harmful by-products; examine the potential of biogas production to expand and adapt using diverse agricultural waste materials in varying geographical locations and climates; And analyse the feasibility of combining biogas production with other forms of renewable energy to create a more holistic strategy for achieving sustainable energy.

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