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Investigating the Influence of Organic Loading Rate, Temperature and Stirring Speed on Biogas Production Using Agricultural Waste in South Africa

Ephodia Sihlangu [1,*], Primrose Magama [2], Idan Chiyanzu [2], Thierry Regnier [3], Dibungi Luseba [4] and Khathutshelo Agree Nephawe [1]

[1] Department of Animal Sciences, Tshwane University of Technology, Pretoria 0183, South Africa; nephaweka@tut.ac.za
[2] Agricultural Research Council-Natural Resources & Engineering, Pretoria 0184, South Africa; magamap@arc.agric.za (P.M.); chiyanzui@arc.agric.za (I.C.)
[3] Biotechnology & Food Technology, Tshwane University of Technology, Pretoria 0183, South Africa; regniert@tut.ac.za
[4] Faculty of Veterinary Medicine, University of Kinshasa, Kinshasa P.O. Box 11850, Democratic Republic of the Congo; lusebad@gmail.com
* Correspondence: sihlangue@tut.ac.za

Abstract: Biogas production offers an alternate method for managing agricultural waste and contributes to sustainable renewable energy generation. Anaerobic digestion (AD) enables the transformation of organic waste, including agricultural substrates, into biogas, mostly consisting of methane, carbon dioxide, and trace gases such as ammonia and hydrogen sulphide. The objective of this study was to employ a 30 L semi-continuous stirred tank reactor to evaluate the effects of organic loading rate, temperature, and speed of stirring on biogas production. The reactor was inoculated with 8.6 L and filled with 11.4 L of a mixed substrate including cattle manure, potato waste, potato starch waste, fruit waste, and expired dry dog food. The reactor was evaluated with organic loading rates (OLRs) of 11.2, 12.2, and 13.2 g VS/L d, and stirring speeds of 25.5, 35.5, and 45.5 rpm. The results indicated that the maximum yield was 12.2 g VS/L d at 45.5 rpm, and in thermophilic conditions, the biogas yield surpassed that of mesophilic conditions, measuring 105,860 NmL/g VS compared to 69,800 NmL/g VS. This study emphasises the significance of optimising operational parameters to improve biogas output, thereby contributing to sustainable energy resources and advancing the Sustainable Development Goals (SDGs).

Keywords: substrates; Anaerobic digestion; methane; carbon dioxide; hydrogen sulphone; renewable energy sources; climate action; SDGs

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1. Introduction

The production of biogas by anaerobic digestion has emerged as a viable approach for managing agricultural waste while also generating renewable energy. Numerous studies have concentrated on cattle manure, poultry manure, agricultural residues, and food waste as suitable substrates for biogas production, owing to their elevated organic matter content and nutritional availability [1]. Agricultural waste is prevalent in KwaZulu-Natal, the Eastern Cape, and Free State. The scenario has led to multiple environmental issues, hence creating prospects for resource recovery [2]. Despite the availability of substrates, the adoption of biogas technology in South Africa is far lower than in places like Europe, where governmental policy frameworks promote biogas generation [3]. South Africa's biogas potential remains largely untapped, with only 300 of 700 plants operational as of 2016, primarily serving households. In contrast, Germany has over 8000 biogas plants, while China had 111,000 commercial plants by 2015, including 6737 large-scale facilities alongside numerous smaller installations [4]. This indicates a significant research gap that should

focus on optimising biogas generation, particularly in the context of South Africa, where agricultural waste is underutilised [5,6].

Agricultural waste, which includes agricultural leftovers, animal manure, and food industry by-products, represents a substantial and mostly underutilised resource for biogas production [7]. Nevertheless, despite its potential benefits, the efficient utilisation of agricultural waste for biogas production poses numerous challenges. An essential aspect that necessitates examination is the operational parameters and their impact on biogas quality [8]. Biogas is produced by anaerobic digestion (AD), a microbial process that decomposes organic waste in the absence of oxygen. The process occurs in several stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Hydrolysis entails the breakdown of complex organic compounds, such as carbohydrates, proteins, and lipids, into simpler soluble constituents [9]. These compounds experience fermentation during acidogenesis, producing volatile fatty acids, alcohols, hydrogen, and carbon dioxide. In acetogenesis, intermediate products are converted into acetate, hydrogen, and carbon dioxide, serving as substrates for methanogens in the final stage of methanogenesis [10]. Methanogens, a unique group of bacteria, convert their substrates into methane (CH_4) and carbon dioxide (CO_2), producing biogas. The methane concentration in biogas typically ranges from 50% to 75%, influenced by the feedstock and operational conditions [11]. Furthermore, biogas holds considerable promise for reducing greenhouse gas emissions, enhancing energy security, and promoting rural development [12].

Given the diverse properties of agricultural waste and its viability as a substrate for biogas generation, it is crucial to assess the influence of operational parameters, particularly organic loading rate (OLR), temperature, and stirring speed, on the quality of biogas derived from agricultural waste [12]. The OLR denotes the volume of organic material delivered into the digester, which can affect the microbial community's ability to process the material efficiently [9]. Temperature is a vital factor affecting microbial metabolic activity, with the mesophilic (30–40 °C) and thermophilic (50–60 °C) ranges being the most well studied [13]. The stirring speed is essential for maintaining uniform conditions inside the digester, promoting sufficient substrate interaction, and preventing the formation of dead zones. An exact balance of these elements is crucial for effective biogas production [14]. Evaluating these parameters is essential for a thorough understanding and enhancement of the biogas generation process, thereby boosting its efficiency and environmental sustainability [15].

Recent studies have demonstrated that the organic loading rate (OLR) significantly affects microbial community dynamics and biogas production [16]. At an organic loading rate (OLR) under 10 g VS/L, methane is the dominant component in biogas generation, achieving an ideal yield of 184.4 mL/g VS at an OLR of 4 g VS/L. Conversely, with an OLR of 10 g VS/L, hydrogen synthesis intensifies, attaining its peak output of 61.3 mL/g VS at an OLR of 20 g VS/L, whilst methane production markedly declines. Moreover, the microbial population undergoes substantial alterations with differing OLR. At low organic loading rates, the proliferation of hydrolytic bacteria and methanogens enhances methane production. As OLR increases, hydrolytic bacteria and methanogens diminish, whereas hydrogen-producing and chain-elongating bacteria proliferate, leading to enhanced hydrogen generation and diminished methane emissions [12]. The study by Jurgutis et al. [17] revealed analogous results, with an initial organic loading rate (OLR) established at 2.24 kg/VS/m^3/day, which remained steady for the initial 98 days. Following the escalation to 3.14 kg/VS/m^3/day after day 110, fluctuations in biogas quality were noted. The methane concentration in generated biogas varied with alterations in the OLR. The initial level was 60%, which then decreased to 51.1% at specific intervals, suggesting that elevated OLR levels may induce stress in the microbial community, notably due to heightened total ammonium nitrogen (TAN) concentrations. The research conducted by Sudiartha et al. [18] demonstrates the critical role of temperature in the anaerobic digestion (AD) process, significantly influencing the metabolic activity of methanogenic microorganisms, which in turn impacts the efficiency and stability of the system. Temperature fluctuations can disrupt the balance between acidogenic and methanogenic microbial communities,

leading to reduced biogas production and overall process instability. Murillo-Roos et al. [19] discovered that thermophilic digestion increases biogas production and modifies microbial populations, hence enhancing methanogenic activity during the anaerobic co-digestion of animal manures and food waste.

The efficiency of biogas production is greatly affected by several operational parameters, including organic loading rate (OLR), temperature, and stirring speed, which influence microbial community dynamics and the quality of the produced biogas [20]. This study optimises biogas production from agricultural waste in South Africa by examining key operational parameters such as organic loading rate, temperature, and stirring speed. South African agriculture generates substantial organic waste, necessitating the development of efficient valorisation systems for waste-to-energy conversion. This study aimed to enhance methane production by minimising harmful contaminants through optimisation of operational parameters such temperature, organic loading rate, and stirring speed to ensure effective waste management and renewable energy provision. This project introduces novelty using an atypical feedstock, expired dry dog food, which has been rarely investigated in biogas production research. Conventional dog food typically possesses elevated concentrations of protein, lipids, and carbohydrates, thereby significantly influencing microbial activity and methane production, in contrast to typical agricultural leftovers. This offers novel insights on biogas production, contrasting with the predominant literature that primarily emphasises conventional agricultural waste. It also tackles the waste management challenges pertinent to South Africa and responds to the global demand for sustainability articulated in the United Nations Sustainable Development Goals and the African Union's Agenda 2063.

2. Materials and Methods

2.1. Ethical Approval

The study's research received approval from the Animal Research Ethics Committee of the Faculty of Science at Tshwane University of Technology (AREC2021/02/001). Ethical considerations were diligently addressed by adhering to the relevant regulations and practices governing local agricultural waste products.

2.2. Study Area

The research was carried out at the Agricultural Research Council of South Africa: Agricultural Engineering located in Silverton, Pretoria.

2.3. Substrates Collection and Handling

Manure was collected from animals undisturbed in their natural environment on ARC farms in line with accepted animal welfare rules to avoid injuries and distress to the animals. Collection methods were undertaken to minimize impact on the environment using sterilized, sealed containers (SteriSeal Products, Johannesburg, South Africa) to avoid the contamination of water bodies and soil in the process. The inoculum was taken directly from an operational biogas plant at Bronkhorstspruit, Pretoria, and kept at 4 °C up to the time of application.

Expired dry dog food, mostly consisting of chicken meal, fish meal, maize, and fats, was obtained from a local pet food supplier. The product was collected before being disposed of as landfill waste. In addition to mixed fruit waste, which included banana, pineapple, orange, apple, and melanin peels, non-consumable potatoes and potato starch waste by-products were collected from the Marabastad food market.

Sanitation was well adhered to during the collections; sanitised gloves (Hygienic Touch Ltd., Durban, South Africa) and sealed containers were used. The samples were pre-treated mechanically for a period of five minutes using a blender (Oster Blender Pro 1200, Sunbeam Products, Boca Raton, FL, USA). The pre-treated samples were stored at 4 °C in a laboratory-grade refrigerator (Thermo Scientific TSX Series, Thermo Fisher Scientific, Waltham, MA, USA) until processing. Waste management followed all the local

and national rules such that there would be no violation of environmental protection laws. Collection and storage of vegetable and fruit waste were performed with consent from the producers, hence assuring transparency and informed consent of the research objectives.

2.4. Substrates Analysis

All substrates collected and the inoculum were analyzed for total solids (TS), volatile solids (VS), fixed solids (FS), and moisture content (MC) using the Official Methods of Analysis of AOAC international [21]. Analytical-grade reagents (supplied by Sigma-Aldrich, St. Louis, MO, USA) were used where necessary. The pH was determined using the HI8424 General Purpose pH/mV Meter (Hanna Instruments, Woonsocket, Rhode Island, United States). Mixed substrates, i.e., the combination of cattle manure, potatoes, potato starch, mixed fruits, and dog food, were loaded into the reactor as presented in Table 1.

Table 1. The physical properties of substrates.

Substrate	TS %	VS %	FS %	MC %
Inoculum	2.202 ± 0.02^{e}	53.05 ± 0.06^{d}	46.95 ± 0.06^{a}	97.80 ± 0.02^{a}
Cattle manure	19.44 ± 0.12^{c}	84.90 ± 1.73^{b}	14.74 ± 0.33^{cd}	80.46 ± 0.07^{c}
Potato waste	25.89 ± 0.19^{b}	93.91 ± 0.07^{a}	6.09 ± 0.07^{e}	74.11 ± 0.19^{d}
Potato starch waste	78.90 ± 0.25^{a}	69.75 ± 3.38^{c}	30.25 ± 3.38^{b}	73.70 ± 0.62^{d}
Mixed fruits waste	11.49 ± 0.54^{d}	92.22 ± 0.32^{a}	7.78 ± 0.32^{e}	88.51 ± 0.54^{b}
Expired dog food	87.33 ± 0.31^{a}	82.81 ± 0.74^{b}	17.53 ± 0.32^{c}	12.67 ± 0.31^{f}
Mixed substrate	35.09 ± 0.71^{b}	86.92 ± 0.30^{b}	13.08 ± 0.30^{d}	64.91 ± 0.72^{e}

$^{a, b, c, d, e, f}$ Column means with different superscripts differ significantly ($p < 0.05$).

2.5. Semi-Continuous Stirred Tank Reactor (CSTR) Set-Up

A semi-continuous stirred tank reactor (SCSTR) (Supplied by Jezreel Eduscience, Pretoria, Gauteng, South Africa) was used as a lab-scale anaerobic digester with a total capacity of 30 L and an operating volume of 20 L, as presented in Figure 1 below. The anaerobic digestion processes were carried out under mesophilic conditions at 37 °C and thermophilic conditions at 55 °C. The start-up phase begun with 8.6 L of the inoculum mixed with 11.4 L of substrate, which consisted of a mixture of cattle manure, potatoes, potato starch, all kinds of fruits, and dog food. The substrate mixture was diluted with water to achieve an OLR of 11.2 g VS/L d. The reactor was firstly acclimatized for 7 days before the beginning of the routine of daily feeding. Considering the acclimatization, daily addition of 800 mL substrates was accomplished, and the same volume was removed, to maintain the same volume within the reactor. Biogas was directly extracted from the headspace of the anaerobic digester using a gas-tight pipe system. The biogas was thereafter stored in flexible gas bags constructed from double-layered rubber to prevent air infiltration. The relief valves were installed in the system to prevent over-pressurization. Consequently, biogas production was quantified volumetrically by the water displacement biogas flow meter, while its composition was analysed using the Biogas 5000 Geotech analyser. The pH, biogas production, and its composition were measured every week throughout the experimental period. The detailed steps of the experimental methodology are presented in Figure 2.

2.6. Biogas 5000 Geotech Calibration

The Geotech BIOGAS 5000 analyser was first calibrated according to the prescription of the manufacturer included in the BIOGAS 5000 Analyzer Operating Manual, QED Environmental Systems, Dexter, MI, USA. It was first activated to turn it on and reach its operating level. During preparation, it was connected to a standard calibration gas mixture using appropriate tubing and connectors. The calibration procedure begun with zeroing the analyser by introducing a gas mixture composition of 0% CH_4 and 0% CO_2 with the balance being nitrogen (N_2). A span gas calibration was undertaken for the mixture composition of 50% CH_4, 35% CO_2, and 0% O_2. Once the zero and span calibration had

been completed, the accuracy of the analyser was checked by exposing it to a known concentration of calibration gas. The measurements made were then compared with the known ones to ascertain their compliance with acceptable parameters of normally ±2% of the known value. Duplicate samples of biogas taken were sent for analysis with the certified laboratory using standard techniques of gas chromatography to cross-check the results obtained.

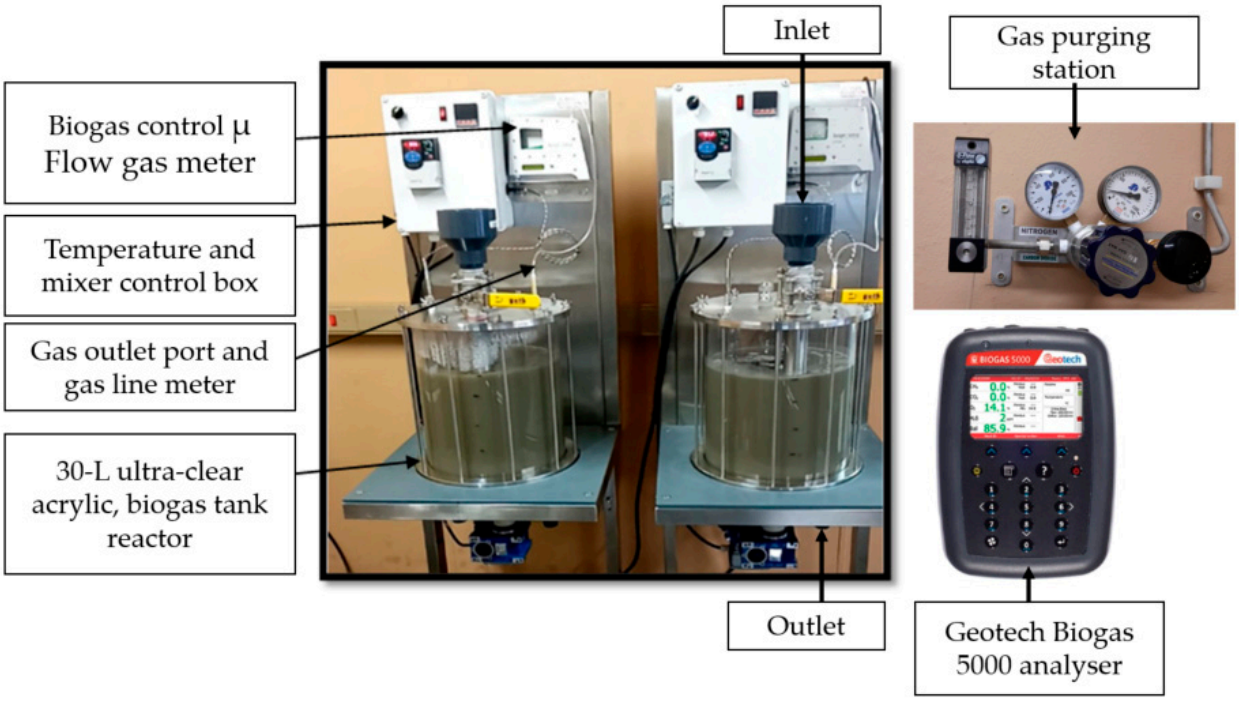

Figure 1. Continuous stirred tank reactor (CSTR) set-up.

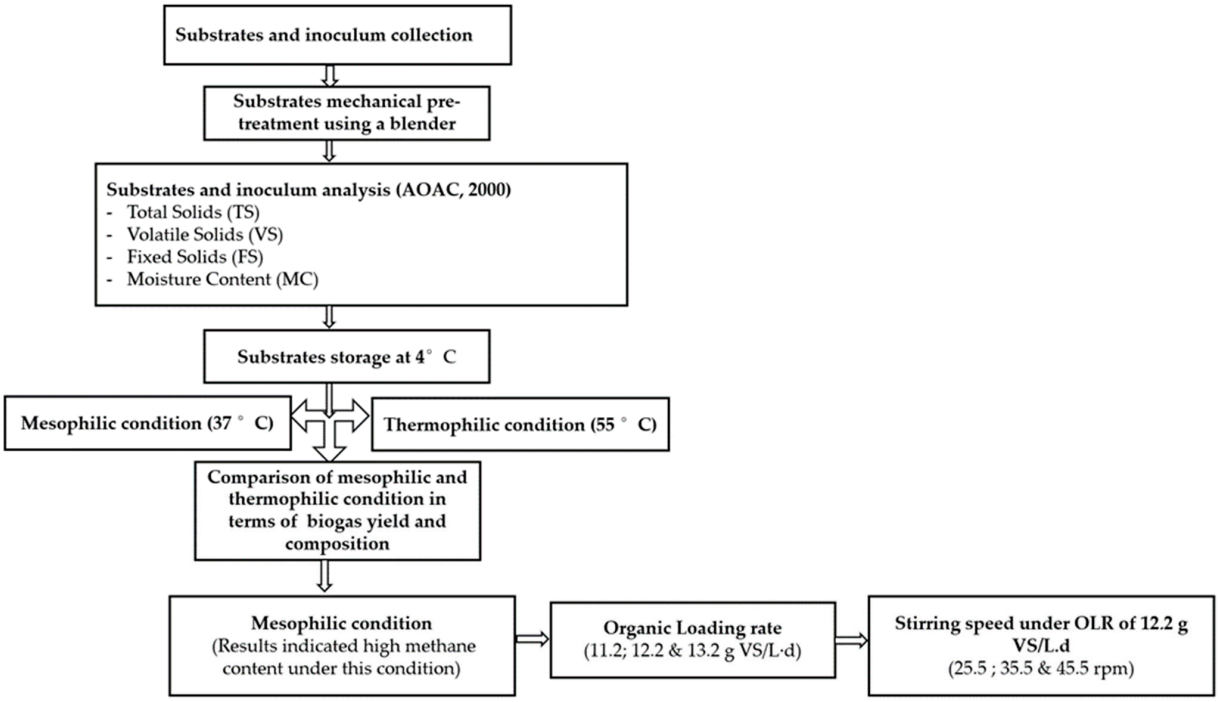

Figure 2. Flowchart of the methodology.

2.7. Statistical Analysis

The General Linear Model (GLM) technique in Minitab-17 was used to assess substrate properties, biogas output, and biogas composition. Fisher's Protected LSD test was employed to compare treatment means ($p < 0.05$). A statistical model was used for each dependent variable:

$$y_{ij} = \mu + \tau_i + e_{ij} \quad (1)$$

y_{ij} (dependent variable) is the effect of the j-th observation on the i-th treatment
μ is the overall mean
τ_i (independent variable) is the effect of the i-th treatment
e_{ij} is the residual component (error part)

3. Results and Discussion

3.1. The Effect of Temperature on Biogas Production

Biogas production at thermophilic conditions (55 °C) consistently exceeded that at mesophilic conditions (37 °C) over the 45-day experimental duration, as illustrated in Figure 3. During the initial lag phase (days 0 to 9), biogas production was minimal for both conditions, remaining below 5000 NmL/g VS. On day 15, biogas production under thermophilic conditions was approximately 10,000 NmL/g VS, while mesophilic conditions yielded about 5000 NmL/g VS. This indicates that thermophilic digestion enhances the rate of biogas production at an earlier stage [22]. From day 21, a significant increase in biogas production was observed, with thermophilic conditions achieving approximately 40,000 NmL/g VS by day 30, nearly doubling the mesophilic production of about 20,000 NmL/g VS. At day 45, thermophilic digestion reached approximately 90,000 NmL, in contrast to about 60,000 NmL/g VS under mesophilic conditions, indicating a 50% yield increase under thermophilic conditions at the end of the experiment. The standard deviation bars reflected variability in biogas production, under thermophilic conditions, presumably resulting from variations in microbial activity. The findings indicate that thermophilic digestion markedly improves biogas yield, though it necessitates monitoring to address potential variability [23].

Temperature significantly influences anaerobic digestion, impacting both the rate and overall yield of biogas production from organic substrates [24]. In support of this study, Ji et al. [23] noted a reduction in biogas production rates at lower temperatures, with average production decreasing to 2.21 L/day at 15 °C, in contrast to the 5.17 L/day at 20 °C and 6.34 L/day at 25 °C that was observed by Rahman et al. [23]. Moreover, Alrowais et al. [25] found that thermophilic digestion at 55 °C enhanced biogas production by about 30% compared to mesophilic conditions at 35 °C in the co-digestion of sewage sludge and wheat straw, notwithstanding the increased energy requirements of thermophilic systems. Furthermore, Zahoor et al. [22] observed a significantly greater cumulative biogas yield of 3045 mL under thermophilic conditions during the co-digestion of banana peels and slurry, when compared to the yield of 2680 mL under mesophilic conditions. Wardani et al. [26] emphasised that thermophilic conditions accelerate the degradation of complex organic molecules, thus improving biogas production.

Thermophilic digestion provides increased biogas production due to enhanced microbial activity. However, it also presents significant disadvantages. For instance, a high operational temperature necessitates significant energy input, which raises operational costs and renders thermophilic digestion less economically viable in areas with elevated energy expenses or restricting access to external heating and insulation enhancements [27]. Temperature optimisation in anaerobic digestion is essential, as it directly affects microbial metabolism, organic matter degradation, and the efficiency of methane production [28].

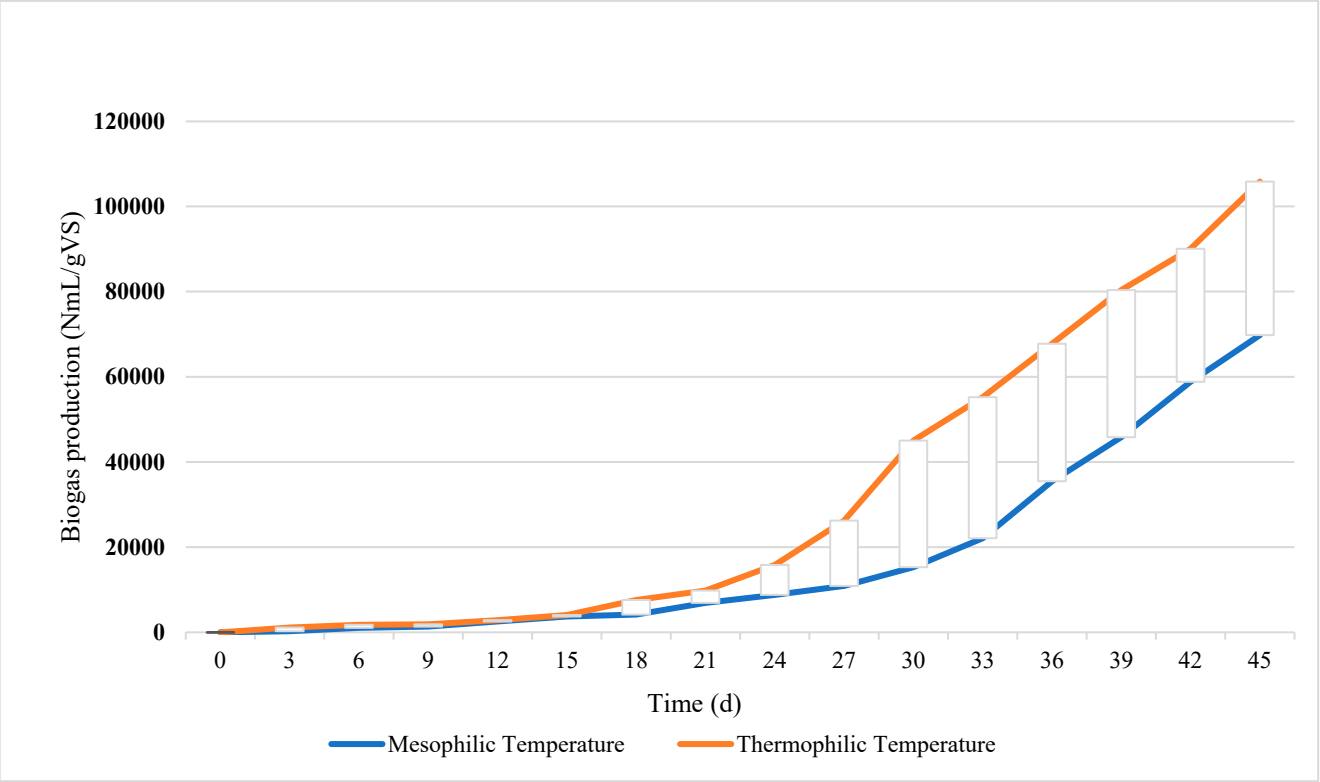

Figure 3. The effect of mesophilic and thermophilic condition on biogas production.

3.2. The Influence of Mesophilic and Thermophilic Temperature on pH

The pH levels under mesophilic (37 °C) and thermophilic (55 °C) conditions were monitored over the duration of the 45-day experiment as illustrated in Figure 4. The pH level for both conditions began at approximately 7.9 on day 0 and showed a slight decline over the course of the experiment. The pH levels in mesophilic conditions showed greater stability during the experiment. On day 15, the pH of the mesophilic condition stabilised at approximately 7.3, whereas in the thermophilic condition, the pH decreased slightly to around 7.2. During the period from day 20 to day 30, both variables exhibited minor fluctuations; however, the mesophilic maintained an average of approximately 7.2, whereas the thermophilic fluctuated around 7.1.

As the experiment progressed, from day 35 onwards, a more significant decrease in pH was observed under thermophilic conditions. On day 45, the pH under mesophilic conditions remained stable at approximately 7.1, whereas under thermophilic conditions, it decreased to around 6.9. The observed pH difference, indicative of increased acidification under thermophilic conditions, may also have resulted from the rapid degradation of organic matter into more acidic by-products [29]. Moreover, there was no statistical difference in pH levels between the mesophilic and thermophilic temperature.

The stability of pH during the mesophilic phase reflects a balanced microbial environment, characterised by regulated acid production and consumption, which facilitates the process while mitigating the risk of acidification [25]. According to the study conducted by Jiang et al. [30], the pH from the thermophilic reactor increased from 7.52 to around 8.68, while in the mesophilic reactor, the pH increased from 7.83 to 8.66. These findings are inconsistent with those of the current study, which observed a decrease in pH for thermophilic conditions from 7.9 to 6.9 and for mesophilic conditions from 7.9 to 7.1. The variation in these results may be attributed to differences in substrates and hydraulic retention time [31]. Orhorhoro and Erameh [32] emphasized that the preferred pH range for optimal biogas yield is between 6.9 and 7.4 at a mesophilic temperature range of 36–37 °C, and that furthermore, thermophilic digestion operates effectively within a

broad pH range similar to mesophilic conditions, ideally around 6.5 to 8.0. Furthermore, the study by Orhorhoro and Erameh [32] highlighted that pH levels above 8 can be toxic to methane-forming bacteria due to free ammonia, while levels below 6 can be harmful due to the accumulation of volatile fatty acids.

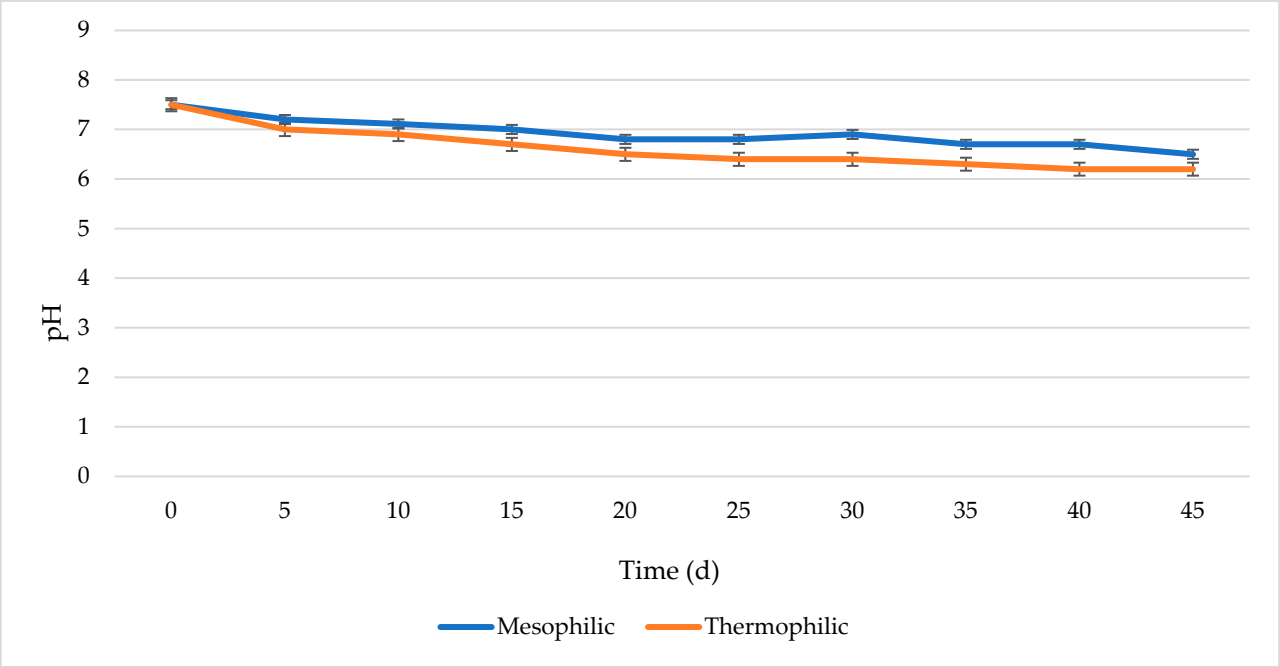

Figure 4. The influence of mesophilic and thermophilic temperature on pH.

3.3. Influence of Temperature on Biogas Composition

The findings in Table 2 indicate significant variations in some critical parameters under mesophilic (37 °C) and thermophilic (55 °C) conditions. In mesophilic digestion, the CH_4 concentration reached 65.77%, markedly above the 61.8% recorded in thermophilic digestion. Conversely, Alrowais et al. [25] reported that the average methane content in biogas generated under mesophilic settings in their study varied from 59.23% to 63.12%, but in thermophilic conditions it ranged from 60.34% to 64.25%. Furthermore, Begum et al. [31] noted that during specific intervals, the cumulative methane production was markedly greater in thermophilic reactors; however, they did not discount the stability and efficacy of mesophilic reactors, which can also yield considerable CH_4 concentrations, based upon the operational parameters. These discrepancies in results may be attributed to the substrates employed for biogas production under mesophilic and thermophilic conditions [33]. In the research conducted by Alrowais et al. [25], activated sludge and wheat straw served as substrates, but the present study utilised agricultural waste, including cattle manure, potato and potato starch waste, mixed fruit waste, and expired dry dog food as substrates. Moreover, wheat straw is regarded as a complex organic substrate and has superior performance under thermophilic conditions compared to mesophilic digestion [25]. Furthermore, thermophilic digestion results in increased solubilisation of substrates such as lignocellulosic materials (e.g., wheat straw), rendering them more amenable to microbial activity. This increased solubilisation frequently results in improved methane production during subsequent digestion phases [27].

The recorded CO_2 concentration was 38.2% under thermophilic conditions, exceeding the 34.23% seen under mesophilic conditions, indicating a more accelerated acidogenic process under thermophilic settings and leading to increased CO_2 production [26]. These observations accord with findings in other relevant studies [25,30]. The study by Alrowais et al. [25] demonstrated that thermophilic conditions result in a greater concentration of free ammonia and affect biogas composition, notably increasing CO_2 levels due to

intensified breakdown processes at elevated temperatures. Ammonia concentrations were significantly higher in thermophilic digestion, attaining 401 ppm, compared to 209 ppm in mesophilic digestion in the current study. Carbon monoxide in thermophilic conditions was notably higher at 137.33 ppm, but no detection was observed in mesophilic conditions. The presence of CO may arise from the incomplete breakdown of organic matter at high temperatures, suggesting that thermophilic conditions could produce other undesired by-products necessitating increased inspection [29].

Table 2. The influence of temperature condition on biogas composition.

Parameters	Mesophilic (37 °C)	Thermophilic (55 °C)
Methane (CH_4 %)	65.77 ± 0.43 ^{a}	61.8 ± 0.15 ^{b}
Carbon dioxide (CO_2 %)	34.23 ± 0.43 ^{b}	38.2 ± 0.15 ^{a}
Oxygen (O_2 %)	0.2 ± 0.01 ^{a}	0.2 ± 0.01 ^{a}
Ammonia (NH_3 ppm)	209.00 ± 4.58 ^{b}	401 ± 0.88 ^{a}
Carbon monoxide (CO ppm)	0.00 ± 0.00 ^{b}	137.33 ± 0.33 ^{a}
Hydrogen Sulphide (H_2S) ppm	2805.3 ± 0.67 ^{b}	9190 ± 0.88 ^{a}

$^{a, b}$ Means across the row with different superscripts differ significantly ($p < 0.05$).

The present study recorded high levels of H_2S at 9190 ppm in thermophilic digestion, in comparison with 2805.3 ppm in mesophilic digestion. Furthermore, Alrowais et al. [25] observed that thermophilic digestion markedly influences the composition of biogas, notably elevating H_2S concentrations. Consequently, it has been claimed that thermophilic digestion leads to elevated H_2S concentrations [31]. Increased concentrations of H_2S in biogas diminish its quality and pose corrosive threats to equipment, leading to health and environmental concerns [34]. The increased H_2S concentration during thermophilic digestion could result from enhanced metabolic activity of sulphur-reducing bacteria at elevated temperatures, which promotes the degradation of sulphur-containing compounds [35]. Multiple studies [19,28,30] have noted differences in biogas composition between mesophilic and thermophilic digestion conditions, rendering the choice of operational temperature essential for the specific objectives of the biogas production system. Nonetheless, thermophilic digestion accelerates decomposition and produces by-products more rapidly; yet, the increased concentrations of CO, NH_3, and H_2S suggest possible disadvantages impacting the quality of the generated biogas and operational costs [29].

3.4. Effect of Organic Loading Rate on Biogas Production Under Mesophilic Condition

Table 3 highlights the significance of modifying the organic loading rate (OLR) to improve biogas production and quality during anaerobic digestion. A notable discovery is that an OLR of 12.2 g VS/L d produced the best results, characterised by maximum biogas production rates and enhanced methane concentrations, while reducing undesired gases such as CO_2, NH_3, and H_2S.

Table 3. The effect of organic loading rate on biogas production (mesophilic condition).

OLR (g VS/L.d)	Biogas (NmL/g VS)	Flowrate (NmL/h)	CH_4 %	CO_2 %	NH_3 (ppm)	H_2S (ppm)
11.2	193,739 ^{c} ± 3.88	113.47 ^{b} ± 0.33	73.63 ^{b} ± 0.23	26.37 ^{b} ± 0.33	501.33 ^{b} ± 0.88	8109.7 ^{b} ± 5.33
12.2	235,481 ^{a} ± 0.98	314.00 ^{a} ± 1.79	85.10 ^{a} ± 0.55	14.9 ^{c} ± 0.43	352.00 ^{c} ± 1.86	652.67 ^{c} ± 2.04
13.2	201,214 ^{b} ± 4.48	89.07 ^{c} ± 0.35	64.53 ^{c} ± 0.35	35.47 ^{a} ± 0.44	561.33 ^{a} ± 1.53	9086 ^{a} ± 1.20

$^{a, b, c}$ Means within the column with different superscripts differ significantly ($p < 0.05$).

Several studies substantiate the findings of the present research within the framework of the existing literature [16,17,36]. Research on optimising anaerobic digestion of cattle dung indicated that modifying temperature and organic loading rates could enhance methane production; however, elevated organic loading rates occasionally led to reduced

degradation efficiency due to ammonia inhibition [33]. This corresponds with the concept that a critical threshold of OLR exists, above which the efficiency of biogas production begins to decline [17]. A study on municipal solid waste (MSW) revealed that methane generation can be maximised at organic loading rates (OLRs), highlighting the necessity for incremental modifications in organic loading to accommodate the microbial population and substrate utilised [33]. Moreover, sustaining OLR levels below a crucial threshold maximises methane recovery while reducing volatile fatty acid concentrations, which can be harmful at excessively increased organic loads.

Furthermore, a study on kinetic analysis of cow dung anaerobic digestion found that varied OLRs resulted in varying biogas production [37]. This study indicated that low to moderate organic loading rates (15–22 g VS/L d) facilitated rapid biogas production, whereas elevated loading rates (exceeding 22 g VS/L d) hindered the biogas generation process. This pattern was consistent with the findings of the current study, where an increase in organic loading rates correlated with a decrease in methane concentration and an increase in CO_2 emissions. These insights indicate agreement with the literature that modifying OLR is essential for efficient biogas production. The adverse impacts linked to both low and high OLRs, including reduced methane concentrations and elevated ammonia or hydrogen sulphide emissions, have been consistently observed [16,17,38]. Consequently, meticulous regulation of OLR enhances both biogas yield and quality while alleviating operational challenges [38]. Therefore, meticulous adjustment of OLR is essential for optimising the efficacy of anaerobic digestion processes across diverse substrates and conditions [36].

3.5. Effect of Stirring Speed on Biogas Production and Composition

The effects of various stirring speeds, specifically 25.5, 35.5, and 45.5 rpm, on biogas production and composition are illustrated in Table 4. Increased stirring speeds enhanced yield and flow rate, with a peak overall production of 28,842 NmL/g VS observed at 45.5 rpm, corresponding to a flow rate of 640.10 NmL/h. Nevertheless, when accounting for methane concentration and purity, the best stirring speed was 35.5 rpm, as the methane concentration at this speed was the highest of the three, measuring 79.67%. Additionally, CO_2 levels at 35.5 rpm were lower (20.3%) compared to 25.5 rpm (31.67%) and 45.5 rpm (24.67%), indicating that at this intermediate speed, the reduction of CO_2 impurities was more effective.

Table 4. The effect of stirring speed on biogas composition.

Starring Speed (rpm)	Biogas (NmL/g VS)	Flowrate (NmL/h)	CH_4%	CO_2%	NH_3 (ppm)	H_2S (ppm)
25.5	15,725 ^{c} ± 5.17	89.27 ^{c} ± 0.28	68.33 ^{c} ± 0.24	31.67 ^{a} ± 0.33	521.67 ^{c} ± 1.45	8101.3 ^{a} ± 0.88
35.5	27,830 ^{b} ± 2.56	334.50 ^{b} ± 0.58	79.67 ^{a} ± 0.57	20.33 ^{c} ± 0.44	600.67 ^{b} ± 0.67	658.00 ^{c} ± 0.38
45.5	28,842 ^{a} ± 1.45	640.10 ^{a} ± 0.31	75.33 ^{b} ± 0.33	24.67 ^{b} ± 0.89	636.00 ^{a} ± 0.57	800.00 ^{b} ± 0.48

$^{a, b, c}$ Means within the column with different superscripts differs significantly ($p < 0.05$).

The stirring speed of 35.5 rpm was effective for both undesired impurities, such as NH_3 and H_2S. The average NH_3 concentration was 600.67 ppm, which was lower than the maximum concentration of 636.00 ppm at 45.5 rpm. At 35.5 rpm, the H_2S concentration was 658.00 ppm, significantly lower than the peak value of 800.00 ppm seen at 45.5 rpm, suggesting a reduced presence of sulphur-based contaminants. Conversely, the minimal stirring speed of 25.5 rpm resulted in the lowest methane concentration and biogas yield, while producing elevated levels of CO_2, NH_3, and H_2S, indicating less favourable conditions for the quality of the biogas produced. A stirring speed of 35.5 rpm minimised contaminants such as CO_2, NH_3, and H_2S, rendering it the most efficient speed for producing high quality biogas.

These results align with findings reported by Wahid and Horn [39], who observed a specific threshold beyond which excessive mixing disrupted biogas production. The

research conducted by Singh, Szamosi, and Simenfalvi [40] demonstrated that methane yields were greater at a stirring speed of 50 rpm in comparison to 80 rpm. Servati and Hajinezhad [41] also noted a similar observation when the stirring speed was increased. High stirring speeds can generate shear forces that compromise the stability of anaerobic granules. These granules are crucial for efficient methane production, as they create optimal conditions for methanogenic archaea. Negative impacts on these granules can result in reduced methane production [42]. Excessive stirring can result in the accumulation of intermediate products, such as volatile fatty acids (VFAs), due to increased substrate breakdown without sufficient consumption by methanogens. The accumulation may impede the methanogenesis process, thereby diminishing methane yields [40]. Increased mixing speeds can further modify the hydraulic conditions within the digester, including flow patterns and dead volumes, which may hinder the interaction between substrates and microorganisms, thereby reducing methane production [42]. Consequently, it is essential to adjust the stirring speed to the specified rate to increase methane concentration and reduce impurities such as carbon dioxide, ammonia, and hydrogen sulphide.

4. Conclusions and Recommendation

This study recommends an OLR of 12.2 g VS/L d to achieve maximum biogas yield and methane concentration. This OLR enhances microbial activity, thereby enhancing substrate degradation without system overload. Thermophilic temperatures promote biogas production, but mesophilic conditions increase methane concentration with minimal contaminants. Mesophilic conditions (37 °C) are advised when biogas quality is critical. The stirring speed of 35.5 rpm resulted in the highest quality of biogas produced. The study suggests that the productivity of biogas under optimal conditions, such as a stirring speed of 35.5 rpm, an OLR of 12.2 g based on biomass weight, and a VS of 86.92, is approximately 27,805.5 NmL/g VS. Increased stirring speeds, such as 45.5 rpm, may enhance biogas production; however, elevated levels of carbon dioxide, ammonia, and hydrogen sulphide subsequently reduce economic viability due to the necessity for additional purification processes. Additional research is necessary to examine the interactions among OLR, temperature, and stirring speed when these variables are altered concurrently. Additionally, the primary parameters influencing the optimisation of the biogas production process include pH, hydraulic retention time (HRT), and substrate composition. The study is limited by its consideration of only three levels of organic loading rate, stirring speed, and temperature conditions. Although significant insights are presented, more thorough results may be achieved by examining a broader spectrum of values. Subsequent research should also examine additional operating parameters. The duration of the experiment was limited, likely overlooking the long-term characteristics of biogas production. Additional research involving long-term continuous monitoring will be necessary to address potential variations in performance and stability over time. This experiment was conducted in a controlled laboratory setting, which may not accurately reflect conditions at an industrial-scale biogas plant. These are typically recommended to be succeeded by scale-up studies that will validate practical applications.

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Data Availability Statement: Tshwane University of Technology (TUT) and the Agricultural Research Council- Natural Resources & Engineering (ARC) remain the owners of any intellectual property because of this study. No information is allowed to be used without the prior consent of TUT and ARC.

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