Research Article

Comparative Analysis of Biogas Production Efficiency from Anaerobic Digestion of Cow Dung and Mixed Dung Substrates

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Abstract | This study evaluated the efficiency of biogas production from cow dung co-digested with wheat straw, goat manure, and poultry manure at 10%, 20%, and 30% proportions respectively. Laboratory-scale anaerobic digesters were used, and biogas yield was monitored daily. Biogas production was monitored using the water displacement method. The NPK (nitrogen, phosphorus, and potassium) values for all treatments and the control were measured both before and after anaerobic digestion following standard protocols. The collected data were analyzed using analysis of variance (ANOVA) techniques within a completely randomized design framework, and mean values were compared for significant differences using Duncan's Multiple Range Test (DMRT). The study found that co-digestion with wheat straw and poultry manure significantly enhanced biogas production compared to the control (cow dung alone), with the highest yield recorded for the 70% cow dung + 30% wheat straw mixture. Conversely, goat manure addition did not significantly ($p > 0.05$) enhance biogas production, indicating limited effectiveness. NPK analysis revealed that poultry manure addition resulted in the highest post-digestion nitrogen and potassium values, significantly exceeding the control ($p < 0.05$). These findings suggest that wheat straw and poultry manure are promising co-substrates for optimizing biogas yield and biofertilizer quality.

Received | December 11, 2024; **Accepted** | March 24, 2025; **Published** | June 17, 2025

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Citation | Khaliq, H., M. Younas, M. Sharif, S. Hussain, U. Younas, M. Sajid, Qudratullah, M.F. Khan, M. Usman and Q. Ullah. 2025. Comparative analysis of biogas production efficiency from anaerobic digestion of cow dung and mixed dung substrates. *Sarhad Journal of Agriculture,* 41(2): 881-893.

DOI | https://dx.doi.org/10.17582/journal.sja/2025/41.2.881.893

Keywords | Biogas production, Anaerobic digester, Comparative efficiency, NPK values

Introduction

Pakistan is predominantly an agricultural nation, with approximately 70% of its population residing in rural areas and relying extensively on agriculture and livestock farming (Ullah *et al*., 2021). Due to its significant reliance on livestock farming, Pakistan sustains a large livestock population. The substantial quantities of dung produced by these animals are predominantly utilized for land application or dried for energy purposes (Obaideen *et al*., 2022; Sammad *et al*., 2022). Energy is a fundamental requirement for every country. In many regions of Pakistan, natural gas is not accessible, making firewood a crucial energy source for daily use. The dependence on firewood for cooking has contributed to the degradation of forest areas (Sulaiman and Abdul-Rahim, 2022). The rapid population growth in the country has led to a shortage of firewood for cooking, significantly intensifying the pressure on forest resources to meet energy demands. Consequently, many rural inhabitants resort to burning livestock manure and other agricultural residues, which could otherwise enhance soil fertility. This practice has contributed to environmental degradation and diminished the soil's fertility potential (Maitima *et al*., 2009). This environmental issue can be mitigated by properly utilizing animal manure as a sustainable energy source, such as biogas (Akyürek, 2019).

Biogas, a renewable source of energy, can be generated via anaerobic digestion of various wastes, yielding differing methane content (Jingura and Kamusoko, 2017; Ijoma *et al*., 2021). The advantages of biogas technology are manifold, encompassing effective waste management, renewable energy recovery, job creation, and the enhancement of socio-economic conditions (Baloch *et al*., 2022). Additionally, it contributes to the reduction of CO_2 emissions (Manesh *et al*., 2020) and alleviates the burden on villagers who labor to collect firewood for their energy needs (Odejobi *et al*., 2022). Biogas also offers a proven alternative to household coal and kerosene consumption (Quinn *et al*., 2018) and mitigates environmental pollution caused by animal waste (Piwowar, 2020). The biosludge, a byproduct of biogas production, is rich in NPK and can significantly enhance soil's physiological and biochemical properties (Nur, 2020). Being free from pathogens and parasites, bioslurry is highly recommended for agricultural use to improve soil fertility (Basunia *et al*., 2020). This fermented manure promotes nutrient recycling, potentially reducing the need for chemical fertilizers in soils with low NPK levels (Mona *et al*., 2021).

The common methods for biogas production include batch digesters, continuous flow digesters, and fixed-dome digesters. Batch digesters operate in cycles, requiring periodic reloading and unloading, which limits their efficiency. Continuous flow digesters, while more effective, demand constant monitoring to maintain optimal conditions for microbial activity (Jegede *et al*., 2019; Mapantsela *et al*., 2024). Due to their long lifespan, fixed dome fermenters are most commonly used in rural areas, but require high technical expertise regarding gas tightness and fluctuating pressure (Jegede *et al*., 2019). Additionally, challenges such as long retention times, low biogas yield from certain feedstocks, and the need for co-digestion to optimize nutrient balance hinder widespread adoption (Kelif *et al*., 2024). Another limitation is the high initial investment cost for biogas plants, which poses a significant barrier for small-scale farmers (Mwirigi *et al*., 2014). The use of pure cow dung as a feedstock also has limitations due to its lower carbon-to-nitrogen ratio (C/N), which can result in suboptimal biogas yields (Nkuna *et al*., 2022).

Therefore, improving biogas production efficiency through co-digestion with different organic substrates is a promising approach that needs further exploration. This study therefore planned to evaluates the potential of co-digesting cow dung with wheat straw, goat manure, and poultry manure to check biogas yield and biofertilizer quality. The efficiency of different substrate combinations at varying proportions (10%, 20%, and 30%) is assessed through laboratory-scale anaerobic digestion. The study aims to identify optimal combinations for maximizing energy production and organic fertilizer quality to promote sustainable waste management in agricultural settings.

Materials and Methods

Collection of sample and substrate preparation

The research was carried out at the Livestock Department using laboratory-scale anaerobic digesters to assess the efficacy of cattle dung both alone and in conjunction with three distinct manure mixtures. Fresh dung and organic matter were sourced from the Livestock Experiment Station at the University of Agriculture Faisalabad. These materials experienced with an equal volume of water prior to introduction into the anaerobic digesters. The mixtures underwent thorough agitation to ensure uniformity. Temperature changes within the biodigester were monitored using a laboratory thermometer before and after the fermentation process.

Treatment plan

The experiment comprised ten treatment plans (T1-T10) conducted as follows: T1 involved pure dung (100% cow dung); T2, T3, and T4 utilized dung mixed with wheat straw in ratios of 90% dung + 10% wheat straw, 80% dung + 20% wheat straw, and 70% dung + 30% wheat straw, respectively. T5, T6, and T7 used dung mixed with goat manure in ratios of 90% dung + 10% goat manure, 80% dung + 20% goat manure, and 70% dung + 30% goat manure, respectively. T8, T9, and T10 employed dung mixed with poultry droppings in same above-mentioned ratios. Each treatment was replicated four times. Samples of dung and dung mixtures were collected before anaerobic digestion to analyze their NPK composition.

Determination of biogas

Biogas production was quantified using the method of water displacement, as illustrated in Figure 1 (Obuya *et al*., 2020).

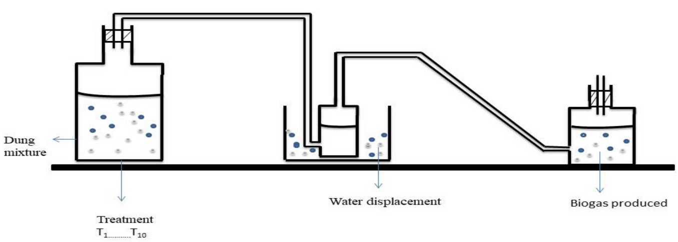

Figure 1: *Schematic diagram of Biogas production by downward water displacement method.*

Physiochemical analysis of samples

The physicochemical parameters were evaluated using established methodologies (Gupta *et al*., 2022). For N analysis, the samples were initially dried to ascertain their dry matter (DM) content, followed by N quantification via the Kjeldahl method (Liao *et al*., 2021). The Percentage of crude protein (CP) was calculated by the formula CP% = Nitrogen% × 6.25. DM content determination adhered to AOAC (2000) guidelines, wherein samples were placed in Petri-dishes and desiccated in a hot-air-oven for 36 h. Moisture content was then measured by subtracting final weight from initial value, dividing by the initial weight, and multiplying by 100. For P analysis, 0.5g samples were processed with 10ml of nitric acid and 5ml of perchloric acid on a digestion stand for 10 min until only 2ml of solution remained. After cooling, the digested samples were diluted to 100ml with distilled-water in volumetric flasks, filtered, and the P content was measured using a UV spectrophotometer. K content was determined using the Jenway-PFP-7 flame-photometer, following the procedure outlined by the U.S. Salinity Laboratory Staff (1954).

Statistical analysis

The collected data underwent analysis using standard procedures of analysis of variance (ANOVA) within a Completely-Randomized-Design. The significant of differences among the means value were assessed using Duncan's new multiple-range test (DMR).

Table1: *Table of means for the Biogas production in all the treatments.*

Treatments	Biogas production (cm^3)	
T1	200.00	± 2.041^{G}
T2	273.75	± 2.839^{F}
T3	403.75	± 2.394^{B}
T4	440.00	± 2.041^{A}
T5	190.00	± 2.550^{H}
T6	167.50	± 3.227^{I}
T7	140.00	± 4.082^{J}
T8	291.25	± 3.881$_{E}$
T9	351.25	± 3.119^{D}
T10	383.75	± 2.394^{C}

Means sharing similar letter are statistically non-significant (P>0.05).

Results and Discussion

Biogas production in all the treatments

Biogas production from various treatments, as presented in Table 1, indicates that the differences in biogas production across treatments are highly significant. Biogas production from the control treatment (T1) was measured at 200 cm^3. The highest biogas yield was observed in the 70% cow dung + 30% wheat straw mixture (T4), which produced 440.00 cm^3, significantly higher than control ($p < 0.01$). Similarly, the 70% cow dung + 30% poultry manure mixture (T10) also yielded more biogas than the T1 ($p < 0.01$). On the other hand, biogas production was significantly lower in all cow dung-goat manure mixtures, with T6 (80% cow dung + 20% goat manure) showing

Table 2: *Table of means for Nitrogen percentage in all the treatments before and after anaerobic digestion.*

Treatments	Anaerobic digestion						Change of N % in bioslurry		
	N % before digestion			N % after digestion					
T1	0.350	±	0.007[h]	1.400	±	0.021[d]	0.875	±	0.199[H]
T2	0.700	±	0.008[g]	1.400	±	0.029[d]	1.050	±	0.133[F]
T3	0.870	±	0.022[f]	1.410	±	0.042[d]	1.140	±	0.104[E]
T4	0.870	±	0.010[f]	1.410	±	0.038[d]	1.140	±	0.104[E]
T5	0.870	±	0.014[f]	1.050	±	0.022[e]	0.960	±	0.036[G]
T6	1.050	±	0.018[e]	1.400	±	0.025[d]	1.225	±	0.068[D]
T7	1.430	±	0.037[d]	1.920	±	0.047[b]	1.675	±	0.097[C]
T8	1.430	±	0.026[d]	1.925	±	0.047[b]	1.678	±	0.097[C]
T9	1.570	±	0.028[c]	1.960	±	0.079[b]	1.765	±	0.083[B]
T10	1.610	±	0.046[c]	2.100	±	0.043[a]	1.855	±	0.097[A]

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

the lowest yield (167.00 cm^3, $p < 0.05$) compared to the control. These findings highlight the potential of wheat straw and poultry manure as effective co-digestion substrates for improving biogas production.

Percentage of N in all the treatments before and after anaerobic digestion

The nitrogen (N) content varied significantly among treatments, with notable differences observed before and after anaerobic digestion (Table 2). The control treatment exhibited N content of 0.50% before anaerobic digestion, increasing to 1.4% after digestion (called slurry). The highest N content was recorded in the dung-poultry dropping mixtures, with post-digestion values of 1.92% (T8), 1.95% (T9), and 2.10% (T10), which were highly significantly than the control ($p < 0.01$). The dung-wheat straw mixtures (T2, T3, and T4) also exhibited higher nitrogen content compared to the control, with T4 achieving 1.41% N, significantly higher than T1 ($p < 0.05$). In contrast, the lowest N levels post-digestion were observed in the dung-goat manure mixtures: 1.05% (T5) and 1.4% (T6), both showing no significant improvement over the control ($p > 0.05$). These results suggest that incorporating poultry manure and wheat straw into cow dung effectively enhances nitrogen content, improving the quality of the resultant biofertilizer compared to the control.

Percentage of CP in all the treatments

The crude protein (CP) content increased after anaerobic digestion across all treatments. The highest post-digestion CP values were observed in the dung-poultry dropping mixtures (T10: 13.10%, T9: 12.25%, and T8: 12.03%), which were significantly higher ($p < 0.05$) compared to the control (8.75%). The dung-goat manure mixture also exhibited an increase in CP content, with T7 (8.93% before, 12.00 % after digestion) showing significant difference ($p < 0.05$) from the control, whereas T5 (1.05% to 8.75%) and T6 (5.43% to 6.43%) had the non-significant difference comparing with control treatment. Similarly, T2-T4 also revealed non-significant results as compared to T1 ($p > 0.05$). These results suggest that the dung-poultry dropping mixture is the most effective in enhancing CP content, while the dung-goat manure mixture exhibits moderate improvement (Table 3).

Percentage of P in all treatments before and after anaerobic digestion

The P percentage across all treatments showed significant increases after anaerobic digestion compared to T1 (Table 4). The highest P content was observed in the cow and goat manure mixtures, while the lowest was in cow dung only. Before anaerobic digestion, the P percentages ranged from 0.1% (T1) to 0.45% (T7). Post-digestion, the P percentages increased significantly, with values ranging from 0.2% (T1, $p < 0.05$) to 0.513% (T7, $p < 0.01$). Notably, the P content in T7 (0.513%, $p < 0.01$), T6 (0.44%, $p < 0.01$), and T10 (0.284%, $p < 0.05$) was significantly higher compared to T1. These findings highlight the effectiveness of anaerobic digestion in enhancing the P content of organic manure, improving its nutrient profile for agricultural applications.

Table 3: *Table of means for CP percentage before and after anaerobic digestion in all the treatments.*

Treatments	Anaerobic digestion		Change of CP % in bioslurry
	CP % before digestion	CP % after digestion	
T1	2.180 ± 0.026^{h}	8.750 ± 0.165^{d}	5.465 ± 1.244^{H}
T2	4.370 ± 0.129^{g}	8.750 ± 0.187^{d}	6.560 ± 0.834^{F}
T3	5.430 ± 0.149^{f}	8.810 ± 0.222^{d}	7.120 ± 0.651^{E}
T4	5.430 ± 0.192^{f}	8.810 ± 0.209^{d}	7.120 ± 0.652^{E}
T5	5.430 ± 0.108^{f}	6.560 ± 0.154^{e}	5.995 ± 0.231^{G}
T6	6.560 ± 0.122^{e}	8.750 ± 0.188^{d}	7.655 ± 0.427^{D}
T7	8.930 ± 0.231^{d}	12.000 ± 0.327^{b}	10.465 ± 0.609^{C}
T8	8.930 ± 0.241^{d}	12.030 ± 0.328^{b}	10.480 ± 0.615^{C}
T9	9.810 ± 0.200^{c}	12.250 ± 0.299^{b}	11.030 ± 0.490^{B}
T10	10.060 ± 0.262^{c}	13.100 ± 0.366^{a}	11.580 ± 0.611^{A}

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 4: *Table of means for the Phosphorus percentage in all the treatments before and after anaerobic digestion.*

Treatments	Anaerobic digestion		Change in P % in bioslurry
	P % before digestion	P % after digestion	
T1	0.100 ± 0.001^{k}	0.200 ± 0.003^{j}	0.150 ± 0.019^{H}
T2	0.210 ± 0.004^{j}	0.250 ± 0.007^{i}	0.230 ± 0.008^{G}
T3	0.270 ± $0.005g^{hi}$	0.300 ± 0.003^{f}	0.285 ± 0.006^{E}
T4	0.270 ± $0.002g^{hi}$	0.300 ± 0.008^{f}	0.285 ± 0.007^{E}
T5	0.330 ± 0.006^{e}	0.360 ± 0.005^{d}	0.345 ± 0.004^{F}
T6	0.440 ± 0.009^{b}	0.512 ± 0.010^{a}	0.476 ± 0.008^{D}
T7	0.450 ± 0.007^{b}	0.513 ± 0.010^{a}	0.482 ± 0.024^{B}
T8	0.262 ± 0.006^{hi}	0.278 ± 0.003^{gh}	0.270 ± 0.007^{C}
T9	0.284 ± 0.003^{fg}	0.322 ± 0.007^{e}	0.303 ± 0.015^{A}
T10	0.300 ± 0.009^{f}	0.420 ± 0.012^{c}	0.360 ± 0.013^{A}

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Percentage of K in all the treatments before and after anaerobic digestion

The study also confirmed that the K percentage in most of the treatments was significant (Table 5). The highest K content was observed in the dung-poultry dropping mixture, followed by the dung-goat manure mixture, which had the second highest K value. The dung-wheat straw mixture also produced a higher K percentage compared to the control treatment. When comparing the K percentages before and after anaerobic digestion, it was evident that the bio-slurry produced post-digestion generally showed enhanced K values, except for the bio-slurry produced by the dung-goat manure mixture. Before anaerobic digestion, the lowest K content was observed in T1 (0.2%), while the highest was in T10 (1.24%). Post-digestion, the K percentage in T1 increased significantly to 0.6% ($p < 0.05$), while the highest recorded values were in T9 (1.28%, $p < 0.01$) and T10 (1.28%, $p < 0.01$). Notably, the K content decreased slightly in T7 (0.98%, $p < 0.05$) compared to its initial value (1.01%). These results highlight the effectiveness of anaerobic digestion in enhancing K content in most treatments, improving their agricultural potential.

Percentage of DM and moisture in all the treatments before and after anaerobic digestion

The DM and moisture content were analyzed across different anaerobic digestion treatments, revealing a significantly higher DM percentage in the dung-wheat straw mixture ($p < 0.01$). In contrast, T1, which used only dung, had the lowest DM percentage. The second highest DM percentage was found in the dung-goat manure mixture ($p < 0.05$). The differences

Table 5: *Table of means for Potassium percentage in all the treatments before and after anaerobic digestion.*

Treatments	Anaerobic digestion						Change of K % in bioslurry		
	K % before digestion			K % after digestion					
T1	0.200	±	0.005j	0.600	±	0.006hi	0.400	±	0.076G
T2	0.560	±	0.009i	0.900	±	0.015e	0.730	±	0.065F
T3	0.600	±	0.003hi	0.860	±	0.022ef	0.730	±	0.050F
T4	0.640	±	0.009h	0.900	±	0.022e	0.770	±	0.050E
T5	0.750	±	0.016g	0.750	±	0.010g	0.750	±	0.009EF
T6	0.900	±	0.023e	0.830	±	0.020f	0.865	±	0.019D
T7	1.010	±	0.013cd	0.980	±	0.016d	0.995	±	0.011C
T8	1.010	±	0.016cd	1.050	±	0.012c	1.030	±	0.012C
T9	1.160	±	0.017b	1.280	±	0.039a	1.220	±	0.030B
T10	1.240	±	0.010a	1.280	±	0.029a	1.260	±	0.016A

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 6: *Table of means for Dry matter percentage before and after anaerobic digestion in all the treatments.*

Treatments	Anaerobic digestion						Change in DM % in bioslurry		
	DM % before digestion			DM % after digestion					
T1	8.000	±	0.218ghij	7.500	±	0.107j	7.750	±	0.147f
T2	10.400	±	0.151b	8.200	±	0.157fgh	9.300	±	0.428c
T3	10.900	±	0.196b	8.700	±	0.149def	9.800	±	0.431b
T4	11.500	±	0.143a	8.900	±	0.200cd	10.200	±	0.504a
T5	8.500	±	0.207dg	8.000	±	0.161gj	8.250	±	0.154e
T6	8.800	±	0.187cde	8.100	±	0.135ghi	8.450	±	0.170de
T7	8.800	±	0.190cde	8.400	±	0.218dh	8.600	±	0.154de
T8	8.140	±	0.192fi	7.600	±	0.135ij	7.870	±	0.149f
T9	8.550	±	0.156dg	7.900	±	0.165hij	8.225	±	0.162e
T10	9.300	±	0.221c	8.300	±	0.190eh	8.800	±	0.232d

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

in DM percentages were significant across all treatments, both before and after anaerobic digestion, highlighting how the type of additive material affects the amount of solid matter in the mixture (Table 6). Regarding moisture content, Treatment with dung only had the highest moisture percentage because dung alone retains more water. This absence of dry, absorbent materials results in a more liquid mixture. Despite this, the overall moisture content across all treatments was statistically non-significant ($p > 0.05$). This means that while there were some differences in moisture levels, these differences were not substantial enough to be statistically significant compared with T1 (Table 7).

This study provides a comprehensive evaluation of biogas production efficiency from various dung substrates, including mixtures with wheat straw, goat manure, and poultry droppings. The findings align with previous studies highlighting the benefits of co-digesting organic materials to enhance biogas yields. For instance, Kaur and Kommalapati (2021) demonstrated that co-digestion of rice straw and cotton gin waste with livestock dung resulted in higher gas production in a shorter time, supporting the hypothesis that agricultural residues significantly improve anaerobic digestion efficiency. Similarly, Ali *et al.* (2022) found that co-digestion of cattle dung with water hyacinth optimized the carbon-to-nitrogen (C/N) ratio,

Table 7: *Table of means for Moisture % before and after anaerobic digestion in all the treatments.*

Treatments	Anaerobic digestion						Change of moisture % values in bi-oslurry		
	Moisture % before digestion			Moisture % after digestion					
T1	92.000	±	1.285	92.500	±	1.342	92.250	±	0.865A
T2	89.600	±	0.802	91.800	±	0.922	90.700	±	0.702A
T3	89.100	±	1.091	91.300	±	0.788	90.200	±	0.749A
T4	88.500	±	0.856	91.900	±	1.209	90.200	±	0.940A
T5	91.500	±	0.733	92.000	±	1.089	91.750	±	0.615A
T6	91.200	±	0.877	91.900	±	1.304	91.550	±	0.739A
T7	90.950	±	1.187	90.600	±	0.999	90.775	±	0.721A
T8	92.110	±	1.412	92.400	±	1.964	92.255	±	1.121A
T9	91.450	±	0.721	92.100	±	1.510	91.775	±	0.784A
T10	90.700	±	1.035	91.700	±	0.738	91.200	±	0.618A

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

enhancing biogas production, which supports the improvements seen in our study with mixed substrates.

The importance of substrate composition and ratio in optimizing biogas production is well documented. Haq *et al.* (2020); Ahmad *et al.* (2020) reported that co-digestion of jatropha fruit coat substrates at a 2:1 ratio yielded the highest biogas production. These findings reinforce our study's conclusion that substrate ratios significantly influence biogas yield. Tasnim *et al.* (2017) also demonstrated that co-digestion of cow dung with water hyacinth improved biogas yield, particularly when dilution was optimized. This observation supports our findings on the role of proper dilution levels in enhancing microbial activity during anaerobic digestion.

Besides, it was reported a significant increase in biogas production by amending bovine dung with mustard oil cake (Fajobi *et al*., 2022; Hafeez *et al*., 2020). This finding underscores the potential for using supplementary materials to enhance biogas production, a principle supported by our study's results. Phuttaro *et al.* (2023) found that mixing grass with various animal wastes yielded higher biogas production compared to grass alone. This supports our observation that the addition of other animal waste to dung can improve biogas yields. Pal *et al.* (2022) investigated the co-digestion of different crop residues with cow dung, finding that rice chaff and rice straw produced higher gas yields compared to rice husk. This is consistent with our study's results, which indicate that the type and composition of co-digested substrates significantly influence biogas production.

Among the tested treatments, the dung-wheat straw mixture proved to be the most effective for biogas production. This superior performance can be attributed to the high cellulose content in wheat straw, which serves as an excellent substrate for methanogenic bacteria. Previous studies (Atelge *et al*., 2020; Jin *et al*., 2022) have reported that lignocellulosic materials significantly boost methane production due to enhanced microbial activity, which aligns with our findings. Gaballah *et al.* (2020) also observed that co-digestion of cattle manure with crop residues resulted in higher biogas yields, supporting the effectiveness of the dung-wheat straw mixture in our study. Moreover, Rahmani *et al.* (2022); Haroon *et al.* (2022) found that co-digestion of dung with straw produced 20% more biogas compared to mono-digestion, further corroborating our results.

In comparison, the dung-goat manure mixture showed slightly lower biogas production efficiency. Previous studies have noted variability in biogas production potential based on the specific compositions of different manures (Fernandes *et al*., 2023). While goat manure is beneficial, it may not provide the same synergistic effects as wheat straw when mixed with dung. Wheat straw enhances the fermentation process due to its structural properties, leading to increased microbial activity and higher methane yields (Kaldis *et al*., 2020).

The dung-poultry dropping mixture also yielded significant biogas production, demonstrating its effectiveness compared to the control treatment. Poultry droppings are known for their high nitrogen content, which can significantly influence anaerobic digestion

(Jurgutis *et al*., 2020). Several studies have demonstrated that co-digestion of cow dung with poultry manure enhances biogas production due to the additional nitrogen supply (Miah *et al*., 2016; Manogaran *et al*., 2022). The high nitrogen content in poultry droppings stimulates microbial activity, accelerating the breakdown of organic material and improving methane production efficiency (Hammad *et al*., 2018). Our findings align with these studies, further highlighting the advantages of poultry manure as a co-substrate.

Regarding nutrient content, our study found that bio-slurry from anaerobic digestion had significantly higher NPK levels compared to fresh cow dung. This result underscores the effectiveness of bio-slurry as a fertilizer. It was demonstrated previously that digestate contains higher concentrations of essential plant nutrients compared to raw manure, providing improved soil enrichment (Czekała, 2022). Additionally, bio-slurry enhances the absorption of essential nutrients like magnesium, sulfur, boron, iron, and calcium. These nutrients are absorbed by plants and subsequently enter the bodies of humans and animals through the consumption of their fruits and leaves, supporting overall growth and development (Huang *et al*., 2015; Yang *et al*., 2015; Xiao *et al*., 2019). Furthermore, it was found that digestate application enhances soil organic matter, water holding capacity, and soil structure, contributing to better crop yields (Mayerova *et al*., 2023; Huang *et al*., 2023). Our results are further supported by a finding showing bio-slurry releases nutrients slowly, which is beneficial for maintaining steady plant growth and nutrient uptake (Yadav *et al*., 2023).

Our study's results on N and P levels indicate a significant increase in these contents in bio-slurry compared to cow dung. This enhancement is crucial for plant nutrition, as both are an essential nutrient for root development and energy transfer in plants (Fathi and Afra, 2023). The benefits of bio-slurry in providing a slow-release source of N and P align with findings by Jiang *et al*. (2023b), who noted that digested slurry contains abundant mineral elements and bioactive substances that enhance nutrient concentration and uptake in plants. The increased K content in bio-slurry in current findings also highlights its effectiveness as a fertilizer. K is vital for plant metabolism and stress resistance (Samadi *et al*., 2024). The results are consistent with the previous research, which reported that digestate contain improved nutrient content (Lee *et al*., 2021). Furthermore, it was observed a significant increase in biogas production by amending bovine dung with cabbage waste, as well as enhanced K levels of bio-slurry (Liu *et al*., 2022). This suggests that the addition of various amendments can further optimize nutrient content.

The CP content of bio-slurry in our findings was notably higher than in fresh dung, indicating improved nutrient quality. In terms of DM content, the dung-wheat straw mixture had the highest DM percentage, while T1, which used only dung, had the lowest. This result can be attributed to the high moisture content in fresh dung, which reduces the DM percentage (Nong *et al*., 2020). Organic amendments like bio-slurry improve soil structure, reduce evaporation, and stabilize soil temperature, which can also affect the DM content of substrates (Chaudhari *et al*., 2021; Jiang *et al*., 2023a). The low DM in T1, which used only dung, reflects the inherent high moisture content of raw dung, making it less effective in retaining DM compared to mixtures with straw or other materials. The moisture content was highest in T1, where only dung was used, compared to other treatments. This is consistent with the understanding that fresh dung has high moisture content, which affects its DM percentage. However, the overall moisture percentage across treatments was statistically non-significant. In short, dung-wheat straw and dung-dropping substrates are highly effective for biogas production, with results consistent with existing research that supports the benefits of co-digestion. The enhanced nutrient content associated with bio-slurry application further demonstrate its value as a superior fertilizer compared to fresh dung.

Conclusions and Recommendations

The optimal management practice for animal dung involves converting it into biogas, particularly through the co-digestion process with wheat straw and poultry droppings. This approach not only boosts biogas production but also enriches the fertility value of the resulting organic manure. The enhanced biogas output and improved nutrient content of the bio-slurry make it a valuable resource for sustainable energy and agriculture. However, additional research is necessary to identify other potential mixtures that could further optimize biogas production. This could involve exploring various organic materials and their combinations to enhance methane yield, improve the efficiency of the anaerobic digestion process, and increase the nutrient profile of the resulting bio-slurry. Such research would contribute to developing more effective and sustainable biogas production strategies, maximizing the benefits of animal waste management, and supporting agricultural productivity.

Acknowledgments

We sincerely extend our deepest appreciation to Dr. Anjum Munir and Dr. Riaz Virk for their expert guidance, insightful discussions, and unwavering support throughout the course of this research. Their valuable expertise and constructive feedback greatly enhanced the quality of our study. Additionally, we are profoundly grateful to the UAF High Tech Lab for providing essential resources, advanced research facilities, and technical assistance, all of which were instrumental in successfully conducting our experiments and analyses. Their contributions have played a pivotal role in the completion of this work.

Novelty Statement

This study presents a novel comparative analysis of biogas production efficiency from the anaerobic digestion of cow dung and mixed dung substrates while also evaluating the NPK value of the resulting bioslurry. Unlike previous studies that primarily focus on single-substrate digestion, our research systematically examines the synergistic effects of mixed substrates on biogas yield and nutrient composition. By comparing the biogas output and macronutrient content of bioslurry, we provide critical insights into optimizing substrate composition for enhanced bioenergy production and improved soil fertility. This integrated approach not only advances waste-to-energy technologies but also highlights the agricultural benefits of bioslurry, contributing to sustainable waste management.

Author's Contribution

Haseeb Khaliq and Muhmmad Younas: Designed the study.
Muhammad Sharif and Shujaat Hussain: Wrote the manuscript.
Muhammad Fahim Ullah Khan and Qudrat Ullah: Supervised the manuscript.
Umair Younas, Muhammad Sajid and Muhammad Usman: Collected and analyzed the sample.
Qudratullah and Qudrat Ullah: Edited and reviewed the final version of manuscript.

Funding Source

This research has not received financial assistance from any funding agency.

Ethical Approval

This study was approved by the Departmental Committee on Animal Ethics and Welfare, University of Agriculture Faisalabad, Pakistan.

Conflict of Interest

The authors declare no conflict of interest related to this research or its authorship.

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