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INTEGRATING ANAEROBIC CO-DIGESTION OF GOAT MANURE WITH BREWERY SLUDGE: PROMOTING RENEWABLE ENERGY AND IMPROVING ORGANIC WASTE SOLUTIONS

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Abstract

The growing accumulation of organic waste resulting from agricultural and industrial processes poses significant environmental and health challenges, especially in developing nations where sustainable waste management systems are limited. This research explored the feasibility of combining goat manure (GM) with brewery sludge (SB) through anaerobic co-digestion as a sustainable approach for producing renewable biogas and enhancing the value of organic waste. The amount of biogas was calculated using the water displacement method, while physico-chemical parameters, including volatile solids, total solids, total nitrogen, organic carbon, & pH, were analyzed before & after digestion. All indicators showed significant variations between the pre- and post-digestion stages ($p < 0.05$). Among substrates, 100% goat manure (GM) had the greatest initial pH (7.88), whereas 100% brewery sludge (SB) had the lowest pH (6.78). Maximum decrease in VS (100% SB) & TS (100% GM) were found, while the greatest carbon reduction occurred in the 25% SB + 75% GM mixture. After digestion, the amount of organic nitrogen rose, with the highest C/N ratio observed in 100% SB and the lowest in 100% GM. Cumulative biogas yield revealed that the 25% SB + 75% GM mixture produced the highest volume, identifying it as the optimal substrate ratio. The findings suggest that, especially for rural and agro-industrial communities, anaerobic co-digestion of goat manure and brewery sludge provides an affordable and sustainable method for producing clean energy, managing organic waste better, and protecting the environment.

Keywords: *Anaerobic digestion, biogas, co-digestion, goat manure, organic carbon, sludge of beer.*

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1. INTRODUCTION

The growing energy crisis, accelerating climate change, and the continuous accumulation of solid and liquid wastes have become pressing global concerns that threaten sustainable development worldwide [1]. Studies predict that global crude oil reserves could be depleted within the next 40-70 years, while natural gas resources may run out in about 50 years. Furthermore, by the year 2100, increase of 1.4 to 5.8 °C is anticipated in the average global temperature, with further warming expected thereafter [2, 3]. These climatic shifts are anticipated to intensify environmental disasters such as droughts, floods, hurricanes, and large-scale crop failures. According to [4-6], global warming primarily resulting from the combustion of fossil fuels and the subsequent accumulation of greenhouse gases (GHGs) in the atmosphere remains one of the most serious environmental challenges of the modern era. The excessive release of carbon dioxide, methane, and nitrous oxide has intensified the greenhouse effect, leading to rising global temperatures, unpredictable weather patterns, and severe ecological disruptions. These changes threaten food security, water availability, and human health on a global scale. Consequently, there is an increasing need to transition toward renewable and sustainable energy sources, such as biogas, solar, and wind power, to mitigate emissions, ensure environmental protection, and promote energy security for future generations.

The inputs of biogas production mainly consist of organic materials that act as substrates for anaerobic digestion, providing essential nutrients for microbial activity during methane generation. These include animal manure from cattle, pigs, poultry, and other livestock, which supplies nitrogen and beneficial microorganisms; plant-based biomass such as crop residues, straw, husks, and leaves that offer carbon-rich material; and food waste from households, restaurants, and markets, which contains easily degradable organic matter that enhances methane yield. In addition, sewage sludge from wastewater treatment and industrial organic waste from dairies, breweries, and food-processing industries are also valuable feedstocks [7]. The combination of these materials through co-digestion improves nutrient balance, microbial synergy, and process stability, resulting in higher methane productivity. In countries like Ethiopia, where dependence on traditional fuels has led to deforestation and pollution, the utilization of mixed substrates particularly livestock waste and plant biomass plays a crucial role in advancing sustainable energy production, waste recycling, and environmental protection while supporting rural livelihoods [8-10].

However, around 40% of these installations are currently non-functional due to technical failures, inadequate management, declining livestock populations, and water scarcity [11, 12]. These challenges underscore the need for optimized feedstock combinations, improved operational practices, and resilient management strategies to ensure long-term success and wider adoption of bio-methane technology in Ethiopia. Moreover, the growing scarcity and rising cost of fossil fuels have enhanced the economic feasibility of biogas production, which also helps lower greenhouse gas emissions [13, 14]. Considering global priorities on energy security, environmental sustainability, and rural development, biogas is increasingly recognized as a crucial and sustainable energy solution, particularly in livestock-rich countries like Ethiopia [15, 11]. By providing a clean and renewable alternative to wood-based fuels, biogas not only alleviates energy shortages but also mitigates environmental and health challenges associated with traditional biomass use.

Globally, energy demand continues to rise due to population growth and economic development [16, 17]. Currently, approximately 78% of the world's energy supply comes from fossil fuels, 18% from renewable sources, and 4% from nuclear energy. In Ethiopia, biomass remains the primary energy source, accounting for nearly 94.7% of total consumption, mainly in the form of firewood (Portugal-Pereira). Conventional fossil-based energy sources, including petroleum, liquefied gas, diesel, and coal, are being rapidly **exhausted**, highlighting the urgent need to expand renewable alternatives. Sources of renewable energy, including biogas, hydropower, wind, and solar are increasingly recognized as environmentally sustainable options to meet the growing global energy demand while minimizing environmental impacts [18-20].

In rural Ethiopia, heavy dependence on firewood, charcoal, and other biomass fuels has caused severe environmental, economic, and health problems. The use of these materials depletes forests, erodes soil, and reduces biodiversity while removing essential nutrients needed for soil fertility [21-23]. As a result, crop yields decline and food security is threatened. Moreover, indoor air pollution from traditional biomass fuels leads to respiratory illnesses, and reliance on imported fossil fuels strains the economy [24]. To address these challenges, biogas production from organic wastes such as goat dung and brewery sludge offers a sustainable alternative. Goat manure's high nitrogen content and stable fermentation properties make it an effective substrate for co-digestion, enhancing biogas yield and promoting eco-friendly waste management [25, 26, 27, 28].

Despite numerous studies on anaerobic digestion using common substrates like cow dung, pig manure, and food waste, limited research has focused on the **co-digestion of goat manure and brewery sludge**, which are abundant yet underutilized organic wastes [29]. Goat manure alone has high fiber content and low degradability, while brewery sludge is rich in readily available nutrients, suggesting that their combination could enhance microbial activity, balance the C/N ratio, and improve biogas yield. However, few studies have systematically **optimized the mixing ratios, retention time, and operational conditions** for this specific substrate combination. This research addresses this gap by evaluating and optimizing the anaerobic co-digestion of goat manure and brewery sludge, providing both **quantitative data on biogas production** and insights into **process stability and digestate quality**. Moreover, the study contributes to **effective environmental management** by reducing organic waste disposal issues and mitigating pollution. By converting these wastes into biogas, it offers a **sustainable solution for renewable energy production and resource recovery.**

2. MATERIALS AND METHODOLOGY

2.1 Sample Collection Process and Preparation

In this study, anaerobic digestion (AD) was utilised to manufacture biogas using goat manure (GM) and beer production sludge (SB) as feedstocks. To minimize contamination, 4.5 kg of fresh GM was collected from the Haramaya University goat farm in plastic bags, while 4 kg of SB was purchased from the Harar Brewery Industry in plastic bottles. To reduce moisture content, both substrates were sun-dried for 7 consecutive days. They were then cut into 10-20 mm pieces and mechanically processed in a high-speed crushing machine to 0.2-2 mm pieces [30, 31] to increase surface area and enhance biodegradability. The powdered materials were sieved through a 0.5 mm mesh to ensure uniform particle size, which is critical for consistent digestion performance. Prior to use, 800 g of each processed substrate was wrapped in aluminum foil and stored at 4 °C to maintain freshness and prevent microbial contamination. Physicochemical properties of the substrates, including moisture content, total solids, and volatile solids, were measured to characterize the feedstocks and ensure reproducibility in the AD experiments.

4 L of fresh **rumen fluid** was gathered from the Haramaya University main campus slaughter facility to serve as the inoculum for anaerobic digestion (AD). The fluid was first sieved by using a 0.5 mm cloth sieve to eliminate coarse solids, then pre-incubated at 38 °C for seven days and fully degassed to eliminate dissolved

methane and easily degradable organic matter. The presence of active anaerobic microorganisms in the inoculum was confirmed using the pour plate technique in an anaerobic jar [32-34]. To maintain microbial activity and prevent contamination, both the substrates and the inoculum were stored under refrigeration at 4 °C until the start of the digestion experiment. Prior to the experiment, the inoculum was thoroughly mixed to ensure uniform distribution of microbial populations, and its pH and temperature were monitored to maintain optimal conditions for anaerobic activity. This preparation step was essential to achieve consistent biogas production and reliable experimental results.

2.2 Fermentation and Design of Digester

The substrates were anaerobically digested in batch mode using 0.5 L plastic bottle digesters. Before incubation, the prepared substrates, as previously described, were placed into the digesters in three replications. Each digester received 100 mL of inoculum, and distilled water was added to adjust the total solids (TS) content to 8% [35, 36]. The pH of the slurry was maintained at approximately neutral optimal for biogas production by adding hydrochloric acid (HCl) or sodium hydroxide (NaOH) as necessary [37, 38]. Subsequently, the digesters were incubated in an oven at 38 °C for 30 days to promote anaerobic fermentation and biogas production.

The substrate composition and mixing ratios of goat manure (GM) and beer sludge (SB) were used in the experiment. Five treatments were formulated to assess the impact of varying substrate combinations on biogas generation efficiency. T1 consisted entirely of beer sludge (100% SB), serving as the control treatment with no goat manure. T2 combined 25% GM with 75% SB, while T3 had an equal ratio of both substrates (50% GM and 50% SB). T4 contained a higher proportion of goat manure (75% GM and 25% SB), and T5 was composed solely of goat manure (100% GM) [39, 40, 29]. This gradation of mixing ratios was designed to determine the optimal blend that maximizes biogas yield and enhances substrate biodegradability. The inclusion of goat manure aimed to improve microbial activity and provide sufficient nutrients for effective anaerobic digestion. Similarly, beer sludge served as a complementary substrate that balances moisture content and organic matter availability. The systematic variation of these mixtures provides valuable insight into the synergistic effects of co-digestion. Therefore, this design helps identify the most efficient substrate ratio for enhanced biogas generation and waste utilization [41-43].

To evaluate biogas production, a simple water displacement setup using three plastic bottles was utilized. The first bottle contained the digested slurry, the second held an acidified saline solution, and the third acted as a reservoir to collect the displaced brine. The bottles were connected in sequence with a plastic tube (1 cm internal diameter) [44]. To prevent biogas from dissolving in water, sodium chloride (NaCl) was dissolved in deionized water, and a few drops of sulfuric acid were added to achieve a highly concentrated solution. For efficient gas transfer, the tube connecting the first and second bottles was positioned in the first bottle just above the slurry surface. During fermentation, the biogas generated from the slurry displaced an equal volume of brine from the second to the third bottle, allowing for indirect measurement of gas production. All digesters were tightly sealed with super glue to prevent oxygen intrusion and biogas leakage [45, 7].

2.3 The Substrate Properties Both Prior and After AD

The substrates' physico-chemical characteristics were examined prior to and after anaerobic digestion using typical analytical procedures to analyze the persistence & digestion performance procedure [36, 46]. According to [47, 48], total solids and volatile solids were determined gravimetrically to assess the fraction of organic matter available for microbial degradation. The pH of the samples was measured using a calibrated pH meter to monitor the suitability of the environment for anaerobic microorganisms. Organic carbon (OC) was analyzed by the titrimetric method described by [49], providing an estimate of the degradable carbon content, while the Kjeldahl digestion method [50] was used to measure the amount of nitrogen available for microbial development, total nitrogen (TN). Comparing the samples' initial & final concentrations allowed for the determination of the decreases in TS & VS, indicating the extent of substrate degradation [51]. Lastly, to assess nutritional balance and digestion performance, the C-to-N ratio was computed using the measured OC and TN values [52].

2.3.1 Total Solids of the sample

To stop moisture from absorbing, a sanitised crucible was first dried in an oven set at 105°C for an hour before being cooled in a desiccator, and weighed immediately to obtain its initial mass [53, 54]. Subsequently, 10 g of goat manure (GM) and beer sludge (SB) were measured individually using mettler Teledo XPR56 micro-analytical balance and transferred into the dish that has been dried and is evaporating (Kutlar et al., 2024). After that, the filled plates were dried in an oven set at 105°C, following the procedure conducted by [54]. This technique ensured accurate determination of the sample's moisture content and prevented errors due to atmospheric moisture.

2.3.2 Volatile Solids of the sample

To ascertain the samples' volatile and fixed solids composition, the total solids were burned for five hours at 550°C in a muffle furnace, following the method described by [47, 55]. After being chilled in a desiccator to stop moisture from absorbing from the air, the samples were promptly weighed to determine their ash content. The calculation of the volatile solids (VS) were dodne by subtracting the mass of the residual ash (fixed solids, FS) from the initial total solids (TS). This procedure provides vital information about the organic matter content of the substrate, this is crucial for determining its capacity to produce biogas and biodegrade. All assessments were carefully performed in triplicate to ensure the correctness, reliability, and repeatability of the experimental findings [56, 57].

2.3.3 The ratio of carbon and nitrogen

To resolve the carbon-to-nitrogen (C:N) ratio, the amount of organic carbon (OC) in every substrate was first measured using the Walkley-Black method, while total nitrogen (N) was determined by the macro-Kjeldahl method [58]. The C:N ratio was then calculated for each substrate as described by [59]. After accurately weighing 1 g of the dried organic substrate using an analytical balance, the sample was carefully change into a clean 500 mL conical flask to facilitate the precise determination of its organic carbon content through subsequent chemical analysis. A clean pipette was carefully employed to add approximately 10 mL of 0.167 mM potassium dichromate ($K_2Cr_2O_7$) into the flask, after which 20 mL of concentrated sulfuric acid (H_2SO_4) was slowly and cautiously dispensed. This step was performed carefully to ensure thorough mixing, complete reaction, and to minimize any potential splashing or loss of reagents during the procedure. The mixture was swirled gently to avoid excessive adherence of organic particles to the flask walls and allowed to wait for half hour. During this period, the conical flask was placed on an insulating pad to reduce rapid heat loss, ensuring that the reaction temperature remained stable throughout the experiment [60, 61]. Following digestion, the liquid was mixed with 200 mL of distilled water to improve clarity for visual observation of the titration endpoint. Complex ferric ions (Fe^{3+}) were then treated with 10 mL of 85 percent phosphoric acid & 0.2 g of sodium fluoride (NaF), which might have affected the endpoint result [62, 63]. This procedure ensured accurate measurement of organic carbon content, which, together with total nitrogen, allowed precise calculation of the C-to-N ratio.

At the final step, 10 drops of ferroin indicator were incorporated to the thoroughly mixed solution prior to titration in order to prevent its deactivation, which can occur due to adsorption onto the substrate during the reaction process. After that, a 0.5 M ferrous sulphate ($FeSO_4$) mixed solution was added to the mixture until a clear burgundy endpoint was achieved. Initially, the solution appeared yellow-orange, gradually turning dark green as the reaction progressed. The color change depends on the amount of unreacted dichromate ions ($Cr_2O_7^{2-}$) in the solution, which towards the conclusion abruptly turns wine red after initially shifting to a muddy grey [64]. To improve the visibility of the endpoint in this turbid system, a magnetic stirrer combined with incandescent lighting was employed, as fluorescent lighting can alter the perceived color of the endpoint [65. 66]. This careful approach ensured accurate determination of organic carbon content in the samples.

The Kjeldahl method was employed to ascertain the sample's TN content, as explained by [67]. The three primary processes in this process are titration, distillation, and digesting. A test tube was filled with 1 g of the material for digestion, & Carefully mixing was done after adding 6 mL of strong sulphuric acid (H_2SO_4). Subsequently, a volume of 3.5 mL H_2O_2 solution was mixed gradually, resulting in the appearance of a violet color due to the oxidation reaction. Once the vigorous reaction subsided, the test tube was gently shaken by hand to ensure uniform mixing [68]. A catalyst mixture (3 g) was then added, and the material was left in a rack of test tubes for five to fifteen minutes prior to digestion. The tube was then placed in a digester, which was gradually heated to 370 °C. Digestion was continued at this temperature for approximately 4 hours until the answer became evident, showing absolute oxidation of organic material and conversion of nitrogen into ammonium sulfate. This clear digest was subsequently used for the distillation and titration steps to quantify total nitrogen [69]. Following the digesting procedure, the test tube was moved to a fume hood and let to cool. To stop the sulphates from precipitating, around 50 mL of deionized water was introduced, and the mixture was gently agitated by hand [70, 71]. Ammonia was then released from the digested and diluted sample by adding 25 mL of a 40% sodium hydroxide (NaOH) solution. The condenser of the distillation apparatus was set up underneath a 250 millilitre flask that contained 25 millilitres of boric acid, 25 millilitres of distilled water, and a suitable indicator solution, making sure the condenser tip was submerged in the solution. After around eight minutes of distillation, the volume of the distillate had increased to between 200 and 250 millilitres.The collected distillate was then titrated with 0.1 N sulfuric acid (H_2SO_4) until a reddish endpoint was observed, allowing the calculation of the total nitrogen content (%) in the sample [72, 73].

2.3.4 Measurement of pH

The samples pH value was assessed both prior to and after anaerobic digestion (AD) using a digital pH meter. To guarantee accurate results, prior to use, clean water was used to calibrate the pH meter to a neutral pH [74-75]. The mixture's pH was measured prior to inoculating the substrates with rumen fluid, as maintaining an optimal pH range of 6.8-7.4 is critical for efficient AD. For calibration and measurement, as advised by the 4500-H^+ B standard for single glass electrodes, the pH electrode was submerged in a standard buffer solution [76, 38]. Following the completion of the digestion procedure, the pH of every samples were carefully measured again by directly inserting the electrode into the thoroughly digested substrates, in order to accurately assess any variations or shifts resulting from the anaerobic digestion process [77]. This step ensured monitoring of the biochemical environment necessary for microbial activity and biogas production.

2.4 Anaerobic Digestion Substrate Treatment

One of the many benefits of using low-temperature pre-treatments (less than 100°C) is that they need less energy, ease of operation, and a reduced risk of forming Maillard reaction compounds due to slower reaction rates [78-79]. However, achieving the desired effect at low temperatures generally requires a longer contact time. [80, 81, 82], reported that thermal pre-treatment of sewage sludge at low temperatures enhanced both biogas yield and substrate biodegradability. In this study, two substrates were prepared in five treatments based on their total solids (TS) content, either individually or in combination, & placed in 500 millilitter flasks enclosed with plastic film to prevent contamination and moisture loss. The procedures were as follows in Table 1:

Table 01: Digester substrate mixing ratios [83, 84]

Digester setup	Goat manure (GM)	Brewery sludge (SB)	Mix ration (GM:SB)	Description
T1	0%	100%	0:1	Control brewery sludge only
T2	25%	75%	1:3	High GM proportion
T3	50%	50%	1:1	Equal mix of both substrate
T4	75%	25%	3:1	High SB proportion
T5	100%	0%	0:1	Control goat manure only

Each substrate combination was uniformly combined with 174 mL of distilled water & subjected to thermal pre-treatment for five hours at 80°C in a hot water bath. The flasks were periodically shaken to achieve homogeneous heat transfer patern and prevent settling of the substrates, promoting consistent exposure of all particles to the treatment temperature [85-86]. After thermal treatment, the substrates were permited to cool to room temperature before further handling. During the entire process, care was taken to monitor temperature and prevent spillage, ensuring that the integrity and homogeneity of each treatment were preserved.

2.5 Experimental Design and Procedure

The study's digesters had a combined working volume of 300 mL, which included the proper amounts of inoculum, distilled water, and substrate. Digesters made of 0.6 L plastic bottles were filled with the prepared substrates, and the total solids (TS) content was calibrated to 8% by adding the required amount of distilled water, taking into account the water already added during pretreatment and inoculation (100 mL) [87]. After measuring the slurry's pH, sodium hydroxide (NaOH) or hydrochloric acid (HCl) were used to bring it into the ideal range for producing biogas (6.8-7.2), ensuring near-neutral conditions before digestion [37, 88, 89]. The mixture, consists the inoculum, were thoroughly mixed by agitating to achieve homogeneity. Anaerobic digestion was then conducted for 30 days under mesophilic conditions at 38 °C in a hot air oven. This setup ensured controlled conditions for microbial activity and biogas production.

Three duplicates of the experiment, each comprising three connected plastic bottles, were set up in a fully randomised configuration (Fig 1).The slurry was in the first container, followed by an acidified brine solution in the second, and an empty bottle to catch the brine solution in the third. Sodium chloride (NaCl) was dissolved in distilled water to create the acidified brine solution [90-91]. After that, a few drops of strong sulphuric acid were added till the solution became supersaturated. Because of this configuration, biogas was unable to dissolve into the liquid phase. A 0.5-cm-diameter plastic tubing was used to link the three bottles. To enable effective gas collection, the tube that connected the slurry container to the brine bottle was placed slightly above the slurry surface [7]. Using ash and Amir Alpha (alpha-cyanoacrylate glue), the lids of every digester were firmly sealed to minimise methane leakage and stop oxygen intrusion. A constant-temperature oven was used to keep all digesters at 38°C, guaranteeing mesophilic conditions for the best anaerobic digestion.

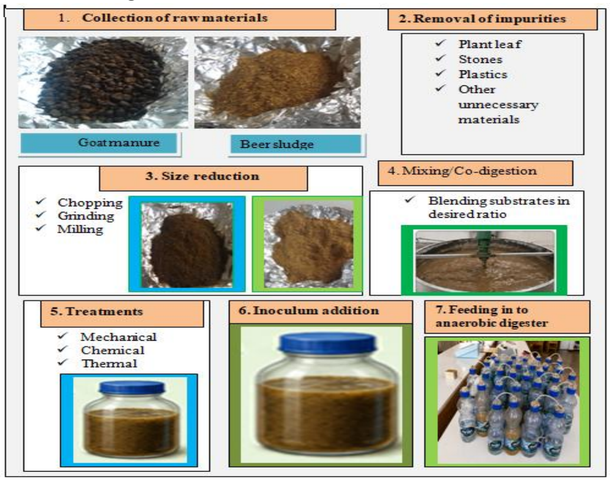

Figure 01: Highlights of the anaerobic digestion configuration and experimental process at the laboratory scale

2.6 Biogas Production Measurement

The technique outlined by [92] was used to measure the biogas production every day. When the fermentation chamber (digester) started producing biogas, the gas was provided through a connecting tube into a second chamber containing the acidic brine mixture. Because biogas is largely insoluble in the brine solution, the increasing pressure in the digester displaced the brine into a third collection bottle [93-95]. The displaced volume was measured daily, representing the total biogas produced by using the following formula:

$$Yb = \frac{Vb}{Mtv}$$

Yb= biogas yield (mL/g), Vb= total volume of biogas produced (mL), Mtv= mass of total volatile solid fed (g). This method not only allows continuous monitoring of biogas production but also minimizes gas loss and prevents oxygen ingress, which could inhibit the anaerobic process. Additionally, the acidified brine solution helps to maintain a stable pH in the collection system and prevents dissolution of carbon dioxide and other acidic gases, ensuring a more accurate estimation of total biogas yield. Daily measurements also allowed the construction of biogas production curves over the 30-day digestion period, providing insight into the kinetics of gas production and the effect of different substrate combinations and pretreatments on biogas yield [96, 97].

2.7 Analysis of Data

Analysis of variance in one direction (ANOVA) in SPSS for Windows, version 21, was used to examine the experimental data, to evaluate statistical differences between the various treatments. This analysis allowed for assessment of the effect of substrate composition, pretreatment, and other experimental variables on biogas production and substrate characteristics. To investigate changes within a treatment, such as differences prior and after anaerobic digestion, a paired t-test was determined [98]. At a probability level of $P < 0.05$, the differences between the treatments' mean values were determined to be statistically significant. To show variability, all findings are shown as mean ± standard deviation and reliability across replicates. When significant differences were discovered during post-hoc comparisons, Tukey's HSD test was employed. It analysis helped identify specific pairs of treatments that exhibited statistically meaningful variations. This comprehensive statistical approach ensured that the observed effects of pretreatment and substrate combinations on biogas yield and substrate properties were evaluated rigorously and objectively, confirming the validity and repeatability of the study's conclusions.

3 RESULTS

3.1. Physical and Chemical Properties of Substrate

3.1.2. Comparative analysis of organic carbon and pH

When assessing the stability and effectiveness of an anaerobic digester, pH is a main factor for this process. The initial pH of the 100% goat manure (GM) slurry before anaerobic digestion (AD) was approximately 7.88, while that of 100% beer sludge (SB) was 6.78 (Table 2). The pH of 100% GM was within the optimal range for biogas production, whereas the lower pH of 100% SB was less favorable (Perin et al., 2020). Mixing the two substrates resulted in an intermediate pH, which increased relative to SB alone but decreased compared to GM alone. Notably, the initial pH of the substrate mixtures was beneficially associated with the proportion of goat manure in the mix, indicating that goat manure contributes to buffering the system and maintaining conditions suitable for microbial activity and methane production ($p < 0.05$). These findings indicate that co-digestion of GM with SB can improve pH stability, enhancing the overall performance of the anaerobic decomposition procedure [99-101].

Mixing substrates is an effective strategy for adjusting the pH to the most favorable scope for anaerobic decopmposition [102]. A significant difference in pH values before and after digestion was observed using a paired-samples t-test ($p < 0.05$). Due to the creation of alkaline chemicals, especially ammonium ions, while organic material is decopmposing, the pH of all treatments rose after anaerobic digestion (AD) in comparison to the baseline values [103-105]. In the current investigation, the post-digestion pH values of treatments T1, T2, T3, T4, and T5 likely reflect the higher ammonia production associated with the greater organic matter content in goat manure (GM) compared to beer sludge (SB). pH dynamics during AD are influenced by the balance between acid and base production: protein-rich substrates generate ammonia, which increases pH and provides buffering capacity, whereas carbohydrate-rich substrates produce volatile fatty acids (VFAs), which lower pH. Consequently, maintaining an appropriate ratio of carbohydrate to protein in the substrate mixture can optimize biogas production by stabilizing pH throughout the digestion process [106].

Table 02: Analyzing the pH and organic carbon (OC) content

Substrate setup	PBAD	PAAD	CCBAD	CCAAD
T1	6.78±0.033[b]	7.44±0.021[b]	51.17±0.171[a]	44.68±0.226[b]
T2	7.08±0.020[a]	7.53±0.032[a]	49.61±0.241[b]	43.33±0.037[a]
T3	7.26±0.035[ab]	7.65±0.072[d]	47.30±0.173[c]	42.92±0.021[a]
T4	7.69±0.020[c]	7.79±0.024[a]	45.43±0.161[d]	37.30±0.029[c]
T5	7.88±0.013[b]	8.02±0.012[b]	42.38±0.147[e]	40.74±0.057[d]

Value represent mean ± standard deviation (n=3). Different subscript letters within same column indicate significant differences among treatment ($p<0.05$). PBAD= pH before anaerobic digestion, PAAD= pH after anaerobic digestion, CCBAD= content of carbon before anaerobic digestion (%),CCAAD = content of organic carbon after anaerobic digestion (%).

Table 2 presents the comparison of pH & OC content of the different substrate treatments before & after anaerobic digestion. The initial pH values interval derived from 6.78-7.88, suggesting that the environment is neutral to slightly acidic and conducive to microbial activity. After digestion, the pH increased in all treatments, ranging between 7.44 and 8.02, showing a shift toward slightly alkaline conditions. This increase in pH could be considered the findings from the consumption of volatile organic acids while methanogenesis & the release of ammonia from protein degradation, which neutralizes the acids formed during the early stages of digestion. Among the treatments, T5 (7.88 to 8.02) showed the highest pH after AD, suggesting stable methanogenic activity, while T1 (6.78 to 7.44) exhibited the lowest increase.

The percentage of OC different sustainabily ($p < 0.05$) across treatments prior to and after AD. In nearly all treatments, the % OC was significantly higher prior to AD. Specifically, the OC content of SB 100% (T1), SB 75% with GM 25% (T2), SB 50% with GM 50% (T3), SB 25% with GM 75% (T4), & GM 100% (T5) before AD were 51.17 ± 0.17, 49.61 ± 0.24, 47.30 ± 0.17, 45.43 ± 0.16, and 42.38 ± 0.15, respectively (Table 1). The OC content decreased in all treatments after digestion, indicating effective biodegradation of organic matter into biogas components. Before AD, OC values ranged between 42.38% and 51.17%, while after AD, they declined to 37.30 to 44.68%. The highest reduction in OC was observed in T4 (45.43% to 37.30%), suggesting more efficient microbial utilization of organic material. The overall reduction in organic carbon content confirms the conversion of biodegradable components into methane and carbon dioxide during digestion. A similar and comprehensive study was conducted by [107], which further demonstrated the effectiveness of anaerobic co-digestion in enhancing biogas generation efficiency and promoting the degradation of organic matter. In general, the results reveal that anaerobic digestion enhanced substrate stability, as evidenced by the increased pH and reduced OC content. The gradual rise in pH and significant decline in organic carbon across treatments indicate that co-digestion of goat manure and sugarcane bagasse improved biodegradability and promoted efficient methanogenic activity, particularly in treatments with higher proportions of goat manure.

3.1.3. Analysis comparing C/N and Nitrogen (N) ratio

Analysis was done on each treatment's C-to-N ratio and N results are presented in (Table 3). Across all treatments, nitrogen content was higher after anaerobic digestion (AD) compared to the values before digestion. This increase in nitrogen concentration can be ascribed to the mineralization of organic nitrogen into ammonium throughout the breakdown of proteins and other nitrogenous compounds in the digester. The observed changes in N content and C-to-N ratio highlight the effect of AD on substrate composition and indicate enhanced nutrient availability, which is critical for microbial activity and biogas production.

Table 03: Comparative analysis of carbon-nitrogen (C/N) and nitrogen (N)

Digester setup	nitrogen (%) BAD	nitrogen (%) AAD	carbon-to-nitrogen (%) BAD	carbon-to-nitrogen AAD
T1	1.85±0.011^{ab}	1.87±0.008^{ac}	27.88±0.214^{ab}	23.9±0.133^{aa}
T2	1.87±0.005^{ab}	1.89±0.008^{ac}	26.73±0.253^{ac}	23.34±0.074^{ab}
T3	1.93±0.008^{ac}	1.95±0.011^{ab}	24.98±0.324^{ab}	22.59±0.078^{abc}
T4	1.96±0.014^{ac}	1.98±0.012^{b}	23.03±0.111^{ab}	18.93±0.049^{bb}
T5	1.99±0.012^{ac}	2.05±0.017^{ab}	20.58±0.087^{ac}	20.96±0.012^{bb}

Values are presented as mean ± standard deviation (n=3). BAD= before anaerobic digestion, AAD= after anaerobic digestion. Different subscript letters within the same column indicate significant differences ($p < 0.05$).

Table 3 presents the N content (%) & C-to-N ratio of the different substrate treatments before and after anaerobic digestion (AD). The nitrogen content slightly increased in all treatments following AD, ranging from 1.87% to 2.05%, compared to 1.85% to 1.99% before digestion. This improve is likely due to the **mineralization of organic nitrogen** and the **loss of carbon as CO_2 and CH_4** during biogas production, which results in a relative enrichment of nitrogen in the residual digestate. Among the treatments, T5 (100% goat manure) exhibited the highest nitrogen content after digestion (2.05%), whereas T1 (100% sugarcane bagasse) showed the lowest (1.87%), indicating that substrates with higher manure content tend to retain more nitrogen. The differences in nitrogen content between treatments were statistically significant, as shown by the capital letter superscripts, while small letter superscripts indicate significant changes within each treatment before and after AD ($p < 0.05$). Microbial activity, methane yield, and production rates are all strongly impacted by the C:N ratio, making it a significant element in the anaerobic process. Optimal C:N ratio ensures proper proliferation of bacteria during substrate degradation [108].

The C/N ratio decreased in most treatments after AD, reflecting the **effective breakdown of organic carbon by microbial metabolism**. The largest reduction in C/N ratio was observed in T4 (from 23.03 to 18.93), suggesting a higher rate of carbon consumption in co-digested substrates with more goat manure. In contrast, T5 showed a slight increase in C/N ratio (from 20.58 to 20.96), possibly due to slower carbon degradation in goat manure-rich substrates. Therefore, the results indicate that anaerobic digestion **effectively reduces the C/N ratio and enhances substrate stability**, with the extent of change depending on the proportion of beer sudge and goat manure. Conversely, ammonia buildup might result from an extremely low carbon-to-nitrogen ratio, increasing the pH of the slurry and potentially inhibiting bacterial growth [109]. Because of increased levels of free ammonia, a high carbon-to-nitrogen ratio during the digestion of plant waste increases the likelihood that microorganisms may be exposed to dangerous conditions, thereby lowering biogas yield [110]. Therefore, maintaining an optimum carbon-to-nitrogen ratio among 20 & 30 is critical for efficient biogas production and microbial stability in anaerobic digesters [111]. These findings highlight the importance of substrate composition in optimizing nitrogen retention and carbon utilization during AD, which is essential for maximizing bioenergy generation and improving digestate quality for potential use as fertilizer.

3.1.4. Comparative evaluation of total solids & volatile solids in anaerobic digestion (AD)

Analysis was done on all substrates' volatile solids (VS) and total solids (TS) both before and after AD (Table 4). Prior to AD, 100% goat dung (GM) had the highest TS concentration, measuring 93.40 ± 0.01%, whereas the lowest TS was recorded for 100% beer sludge (SB) at 91.04 ± 0.03% (Table 3). Comparable patterns were noted for volatile solids, with GM exhibiting the highest VS content. The high proportion of total and volatile solids in GM indicates that a large fraction of this substrate is biodegradable, demonstrating its applicability as a feedstock for methane yield. In contrast, SB, with lower TS and VS content, may contribute less organic matter to the digestion process. Therefore, goat manure can be considered a more effective substrate for biogas generation compared to SB, particularly when used alone or in combination with SB in co-digestion systems.

After anaerobic digestion, the greatest decline in TS & VS was detected in digestion setup (treatment) T4, which consisted of 25% beer sludge (SB) and 75% goat manure (GM) (Table 04). In this study, the highest decreases in TS and VS corresponded directly with maximum biogas production, indicating efficient transformation of the substrate into biogas during AD [112]. It is commonly acknowledged that TS and VS destruction are crucial metrics for assessing anaerobic digestion effectiveness and serve as reliable indicators of biogas yield [113].

Table 04: Comparison of %TS & %VS variation during AD

Digester setup	TS (%) BAD	TS (%) AAD	VS (%) BAD	VS (%) AAD
T_1	91.04 ± 0.03^{Aa}	81.22 ± 0.21^{Bb}	33.48 ± 0.03^{Ca}	31.92 ± 0.07^{Eb}
T_2	92.90 ± 0.10^{Aa}	80.69 ± 0.32^{Bb}	64.85 ± 0.15^{Ba}	62.34 ± 0.01^{Ab}
T_3	92.36 ± 0.32^{Aa}	77.59 ± 0.29^{Cb}	54.55 ± 0.17^{Ca}	50.50 ± 0.00^{Cb}
T_4	92.84 ± 0.15^{Aa}	73.12 ± 0.11^{Db}	49.66 ± 0.14^{Da}	45.54 ± 0.11^{Db}
T_5	93.40 ± 0.01^{Aa}	84.67 ± 0.16^{Ab}	76.65 ± 0.17^{Aa}	72.67 ± 0.18^{Bb}

Values represent mean ± standard deviation (n = 3). BAD= before anaerobic digestion, AAD= after anaerobic digestion. Different uppercase superscript letters within the same row indicate significant differences between parameters ($p < 0.05$), while different lowercase superscript letters within the same column indicate significant differences among treatments ($p < 0.05$).

Nevertheless, when measuring TS and VS, it is useful to reflect on the potential impact of some acids such as volatile fatty acids (VFAs) present in the substrate. VFAs can volatilize during heating, potentially leading to underestimation of TS and VS values, which may in turn produce inaccurate estimates of biogas potential [114]. Therefore, careful interpretation of TS and VS measurements is necessary to accurately assess substrate degradation and biogas production efficiency.

3.1.5. Average daily and total biogas yield estimation from substrate co-digestion and solo digestion

Fig. 2 and 3, respectively, provide an examination of the daily mean and total biogas output from the separate and combined digestion of various substrate mixtures. The results revealed that the combined substrates (T2, T3, and T4) generated higher biogas yields compared to the solo substrates (T1 and T5), which is consistent with previous studies showing that co-digestion promotes synergistic microbial interactions and enhances biodegradability [115, 116]. The improved biogas production in co-digested treatments can be ascribed to a nutrient composition that is more balanced, like optimal carbon-to-nitrogen ratios, & the combination of complementary substrate properties high fiber content from beer sludge and high nitrogen from goat manure which together accelerate microbial process & the decomposition of complex organic matter. In contrast, mono-digested substrates may lack such nutrient balance, limiting microbial efficiency and reducing gas yield, emphasizing the effectiveness of combined digestion strategies in increasing biogas production.

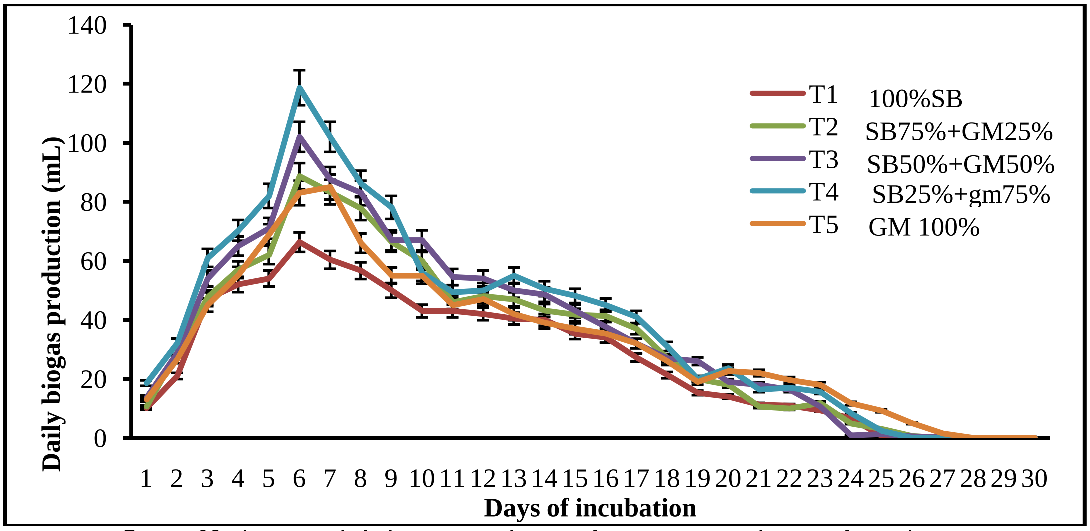

Figure 02: Average daily biogas production from various substrate formulation

Biogas yield was observed from the first day in all substrate types, suggesting the presence of active microbes in the added rumen fluid inoculum capable of rapidly degrading readily available organic matter. Over the course of 30 days, the overall biogas production was tracked until gas generation ceased. Peak biogas production occurred approximately one week into fermentation for all treatments, although the magnitude varied with substrate composition (Fig. 2). This early peak likely corresponds to the logarithmic growth phase of the microbial population, during which microorganisms actively metabolize easily degradable components of the substrates. The results highlight the benefits of co-digestion in improving substrate biodegradability and accelerating biogas generation. Following the first week of measurement, biogas production showed a varying drop before ceasing after 30th days of experimentation (Fig. 2). This decline may be attributed to the identification of easily degradable substrates following the initial period of rapid microbial activity [117, 118, 119], as well as to an increase in ammonium concentration, which can raise the pH and inhibit microbial metabolism [120]. Additionally, the accumulation of toxic metabolites resulting from high microbial activity in the digester may have contributed to the suppression of biogas production. Throughout the observation period, all substrate mixtures produced higher biogas than beer sludge (SB) alone. This is probably because goat dung (GM) contains a higher amount of biodegradable stuff, which provides a more readily available energy source for microbial growth and activity [121]. These observations indicate that biogas synthesis is strongly affected via the organic content and biodegradability of the feedstock, emphasizing the importance of livestock structure in optimizing AD performance [109].

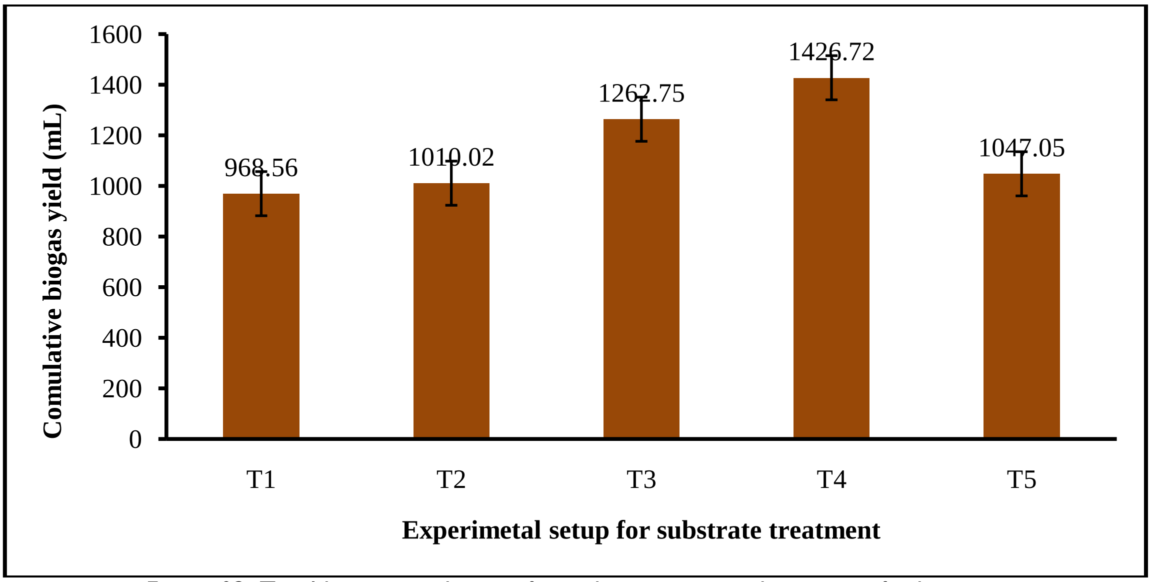

Figure 03: Total biogas production from the various combinations of substrates

There was a noteworthy variation ($p < 0.05$) in the total biogas production between the substrates (Fig. 3), with all co-digested treatments producing higher biogas than 100% beer sludge (SB). The total biogas yields for SB 100% is 1485 mL/g, SB 75% with GM 25% is 1946 mL/g, SB 50% with GM 50% is 2076 mL/g, SB 25% with GM 75% is 2329 mL/g, and GM 100% 1720 mL/g, with the highest yield recorded from the 25% SB + 75% GM mixture and the lowest from 100%

SB. The enhanced biogas production in the 25% SB + 75% GM mixture can be attributed to an optimal balance of nutrients and carbon-to-nitrogen ratio, which promoted microbial activity and efficient degradation of organic matter. Co-digestion combined the high fiber content of SB with the nitrogen-rich profile of GM, facilitating synergistic microbial interactions, whereas mono-digested SB had limited nitrogen and high lignocellulosic content, restricting microbial growth and slowing digestion [122]. These findings emphasize the effectiveness of co-digestion strategies in improving biogas yield and stabilizing anaerobic digestion, suggesting that appropriate substrate mixing ratios can optimize energy recovery in practical biogas systems.

4. DISCUSSIONS

The current study assessed how substrate properties and biogas generation were affected by thermal pre-treatment and co-digestion of goat dung (GM) and beer sludge (SB) under mesophilic anaerobic conditions. Substrate characterization, including pH, organic carbon (% OC), total nitrogen (% N), C-to-N ratio, TS, and VS, provided insights into the biodegradability and nutrient availability of the feedstocks. Initial pH values indicated that 100% GM (7.88) was within the optimal range for methanogenesis, whereas 100% SB (6.78) was less favorable. Co-digestion improved the pH of SB-containing mixtures, highlighting the buffering capacity of GM and the importance of substrate selection in maintaining stable conditions for microbial activity [102]. Post-digestion pH increases were attributed to ammonium production from protein degradation, reinforcing the role of nutrient composition in maintaining AD stability.

Analysis of % OC, % N, and C:N ratios revealed complementary nutrient profiles of the substrates. SB contained higher carbon, while GM was richer in nitrogen. Co-digestion produced C:N ratios within the optimal range of 20-30, which is crucial for microbial enhance and methane production [108, 111]. Maintaining this balance prevents excessive nitrogen depletion or ammonia accumulation, both of which can reduce gas production or inhibit microbial activity [110, 123]. The nutrient balance in the mixtures likely contributed to higher biodegradability and enhanced biogas yields observed in co-digested treatments.

Total solids and volatile solids analyses showed that GM had higher TS and VS than SB, reflecting its greater biodegradability. Maximum reductions in TS and VS occurred in T4 (25% SB + 75% GM), corresponding to the highest biogas yield. TS and VS destruction serve as reliable indicators of AD efficiency, though care must be taken as volatile fatty acids may volatilize during measurements, potentially underestimating substrate degradation [113, 114]. Biogas production data indicated that co-digestion treatments (T2, T3, T4) outperformed single substrates, with maximum cumulative biogas achieved in T4 (2329 mL) and lowest in 100% SB (1485 mL). The activity of bacteria in the rumen inoculum operating on easily degradable material is reflected in the early biogas generation from day one, while peak production after one week coincided with the microbial log phase, followed by gradual decline due to substrate depletion and potential inhibitory metabolites.

The enhanced performance of co-digestion can be attributed to synergistic effects, including balanced nutrient composition, increased buffering capacity, and reduced toxicity from intermediates. While GM alone had higher TS, VS, and nitrogen content, its combination with SB provided optimal conditions for microbial activity and biogas generation [121]. The study demonstrates that thermal pre-treatment at 80 °C, combined with optimized SB-GM co-digestion, improves substrate biodegradability and biogas production. Maintaining optimal pH, C:N ratio, and nutrient balance is critical for achieving high methane yields, offering practical insights for sustainable biogas systems and waste management strategies. Furthermore, the findings highlight the potential for utilizing locally available agricultural residues and animal manures in tailored combinations to promote renewable energy production, minimize environmental waste, and support sustainable rural energy management. Optimizing co-digestion strategies can therefore provide both economic and ecological benefits, making anaerobic digestion a more efficient and practical solution for small and large-scale biogas systems.

1. CONCLUSION

This study showed that anaerobic co-digestion of goat manure and brewery sludge is a viable and efficient way to increase biogas production and enhance organic waste management. Compared to individual substrate digestion, the co-digestion method produced a larger biogas yield and more organic matter degradation due to the favorable nutritional balance and microbial synergy. In addition to promoting the production of renewable energy, the combined use of agricultural and industrial organic wastes reduces environmental contamination brought on by inappropriate waste disposal. Significant environmental and social advantages are also provided by the technique, including as lower greenhouse gas emissions, the creation of nutrient-rich digestate for use in agriculture, and the advancement of energy sustainability in rural and developing areas. Therefore, anaerobic co-digestion of goat manure and brewery sludge represents a promising renewable energy solution that contributes to circular bioeconomy development and sustainable environmental management.

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10. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this work.

11. INFORMED CONSENT

Not applicable, as this study did not involve human participants or animals.

12. ETHICAL STATEMENT

Ethical approval was not required for this study, as it did not involve human participants, animals, or the use of any sensitive or personal data

13. AUTHOR CONTRIBUTION

Meseret Desalegn (MD): Collection of data, experimental design, conducting experiments, data analysis, interpretation, and manuscript preparation. Meseret C. Egigu (MCE): Contributed to literature review, methodology development, and technical support during laboratory work and manuscript preparation. Yaecob Gebre (YG): Critical review, statistical analysis of the research data, checked references, and proofread the final manuscript. All authors have read and approved the final version of the manuscript and agree to its submission, confirm that the work is original, and take full responsibility for the accuracy and integrity of its content.

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