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Research article

Biogas Production from Co-Digestion of Cassava Residue and Wastewater: Effects of Substrate Ratios and Digestion System Configurations

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Abstract

Agricultural waste from cassava processing poses significant environmental challenges globally. This study investigated optimal anaerobic co-digestion parameters using batch experiments with six cassava residue-to-wastewater ratios (1:1, 1:2, 1:3, 1:4, 1:5, 2:1) tested in 20-liter digesters under mesophilic conditions for 20 days. The 1:3 cassava residue-to-wastewater ratio achieved maximum cumulative biogas production (6,200±450 mL) with 55% methane content, significantly outperforming other ratios ($p<0.001$). Increasing stirring speed from 50 to 120 rpm enhanced biogas yield by 31.2%, while hybrid configuration combining horizontal pre-digestion with vertical digestion increased production by 14.6% compared to conventional systems. The optimized process achieved 84.6% COD removal and 81.8% VS reduction, with VFA concentrations decreasing from 1,899 to 96 mg/L, indicating stable methanogenesis. Microbial analysis revealed enrichment of *Methanosarcina* species under 120 rpm agitation, correlating with enhanced performance. The integrated optimization of substrate ratio, mixing intensity, and reactor configuration provides a practical framework for industrial-scale cassava waste valorization, contributing to sustainable waste management and renewable energy production.

Keywords: cassava residue; wastewater; anaerobic digestion; biogas production; *Methanosarcina*; hybrid reactor

1. Introduction

Agricultural waste management represents a critical challenge for environmental sustainability and circular economy implementation. Cassava processing generates substantial organic waste, with global production exceeding 300 million tons annually, creating significant environmental burdens through greenhouse gas emissions and water pollution (Lerdlattaporn et al., 2021). Anaerobic digestion (AD) offers a promising solution for valorizing these waste streams into renewable energy while reducing environmental

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impacts. The growing interest in biogas technology reflects its potential for renewable energy production (Weiland, 2010).

Cassava is a major food and industrial crop in tropical regions. During starch extraction, large amounts of fibrous pulp and nutrient-rich wastewater are generated; these residues have high biochemical oxygen demand and, if discharged untreated, can cause odor, greenhouse-gas emissions and water pollution. Anaerobic digestion (AD) offers a sustainable solution because it converts biodegradable organics into biogas (50-70% CH_4) and a nutrient-rich effluent. Nevertheless, efficient digestion of cassava waste is hindered by their lignocellulosic structure, low nitrogen content and the fluctuating characteristics of the process wastewater. Review articles recommend keeping the feedstock carbon-to-nitrogen (C/N) ratio within 20-35 to maintain process stability in AD systems. (Gebreegziabher et al., 2025). Methanogenic archaea are also pH-sensitive: optimal activity occurs around pH 6.5-7.6, and most species are severely inhibited below pH 6.6; however, certain *Methanosarcina* strains can continue methanogenesis at pH values as low as 4.5 after acclimation (Parkin, 1986; Taconi et al., 2008).

Despite its potential, the mono-digestion of cassava residue presents significant scientific and operational challenges. Cassava residue is characterized by a high content of easily degradable carbohydrates (starch and cellulose), which leads to rapid hydrolysis and acidogenesis (Ahou et al., 2021). This rapid initial breakdown produces a large volume of volatile fatty acids (VFAs) in a short period. Compounding this issue, cassava waste is inherently low in nitrogen and other buffering compounds, resulting in a low carbon-to-nitrogen (C/N) ratio and insufficient alkalinity to neutralize the surge of acids (Kayaba et al., 2025). This combination of rapid acid production and low buffering capacity creates a high risk of process failure due to acidification—a sharp drop in pH that inhibits the activity of the highly sensitive methanogenic archaea, which are responsible for the final conversion of VFAs to methane. Similar challenges in organic waste digestion have been documented for various substrates including food waste (Zhang et al., 2014).

Co-digestion, the simultaneous digestion of two or more complementary substrates, has emerged as a strategic approach to overcome the limitations of mono-digestion (Achi et al., 2024). Co-digestion strategies have been extensively reviewed (Mata-Alvarez et al., 2014), demonstrating improved process stability and biogas yields compared to mono-digestion. The co-digestion of cassava residue with its own process wastewater is a particularly practical and synergistic strategy. Wastewater provides essential moisture, which helps to dilute the high solids content of the residue, improving mass transfer and microbial access to the substrate. Furthermore, wastewater can supply additional alkalinity and nutrients, helping to buffer the system against the rapid VFA production from the solid residue, thereby stabilizing pH and improving the C/N ratio for a more balanced microbial metabolism (Pan et al., 2021). This synergy leads to a more stable AD process and enhanced biogas yields.

Recent advances in AD technology have demonstrated remarkable improvements in biogas yields from cassava waste. Enhanced co-digestion strategies using zeolite additives achieve up to 653 mL CH4/g VS (Achi et al., 2020), while genome-resolved metagenomics enables precise tracking of methanogenic communities (Campanaro et al., 2020). Previous studies on agricultural residue treatment in high-rate reactors have shown promising results (Parawira, 2004). At industrial scale, integrating cassava pulp with wastewater in a covered-lagoon + CSTR configuration boosted specific biogas output six-fold (≈ 59 → 358 m³ per 1 000 kg starch) and cut the process GHG footprint by 77 % (Lerdlattaporn et al., 2021)

The physical pretreatment and mixing of cassava solids strongly influence hydrolysis. Controlled orbital-shaker tests on starchy sediment from a tapioca-starch plant

showed that a mixing speed of ≈ 112 rpm, combined with a 30-day hydraulic retention time, maximized biogas yield at 403 mL g^{-1} TVS added; faster agitation accelerated VFA accumulation and destabilized the digester (Srivichai & Thongtip, 2021). For cassava-peel substrates, keeping the substrate-to-inoculum (S/I) ratio below ~3: 1 (on a VS basis) prevents acidification and delivers the highest methane yields (Ofoefule & Uzodinma, 2009; Eboibi et al., 2020).

Despite these advances, critical knowledge gaps remain regarding optimal substrate ratios, mixing regimes, and the relationship between operational parameters and microbial community dynamics. Previous studies typically focused on isolated aspects without integrating substrate optimization, reactor configuration, and microbial ecology. The interaction between mixing intensity and methanogenic archaea populations, particularly the Methanosaeta-Methanosarcina balance, requires systematic investigation (Kariyama et al., 2018; Zhang et al., 2024).

This study hypothesizes that integrating optimized substrate ratios with enhanced mixing strategies and hybrid reactor configurations will synergistically improve biogas production through favorable microbial community development. The objectives are to: (1) determine optimal cassava residue-to-wastewater ratios for maximum biogas yield, (2) evaluate the effects of stirring speed and reactor configuration on process performance, (3) characterize chemical transformation pathways and degradation efficiency, and (4) elucidate microbial community dynamics under different operational conditions.

The research outcomes will provide actionable insights for scaling cassava waste-to-energy systems, contributing to sustainable waste management strategies and renewable energy development in cassava-producing regions. Understanding the integrated effects of substrate composition, reactor design, and microbial ecology will enable predictive process optimization for industrial applications.

2. Materials and Methods

2.1 Materials and equipment

Cassava residue and wastewater were collected from two major cassava starch processing facilities: Somdej Starch Factory in Kalasin Province and Premier Bio-Energy Factory in Mukdahan Province, Thailand. Sampling was conducted during peak processing season (November-December 2024) to ensure representative substrate characteristics. Cassava residue was collected directly from the starch extraction line, while wastewater was sampled from the primary sedimentation tank outlet. Random sampling was employed by collecting samples from three different locations in the sedimentation tank and from three separate production batches over consecutive days to ensure representativeness.

The materials used in this study primarily consisted of cassava residue and wastewater collected from a cassava starch processing facility. The cassava residue, rich in lignocellulosic content, served as a high-energy substrate for anaerobic digestion, while the wastewater provided additional organic matter and moisture necessary for microbial activity. Prior to the experiment, the cassava residue was mechanically processed to achieve a uniform particle size suitable for mixing and digestion, ensuring consistent substrate availability across all experimental setups.

The wastewater was sourced from the primary sedimentation tank of the factory's effluent treatment system. It was characterized by high levels of organic pollutants, making it a suitable candidate for co-digestion with solid residues. Basic physicochemical properties of the wastewater were measured prior to use to establish baseline conditions.

Wastewater was collected from three different points of the sedimentation tank (5 litres total), samples were obtained from three separate production batches over consecutive days, and all experiments were performed in triplicate (n=3). Additionally, all chemical analyses were conducted in triplicate for each sample as stated in this research.

Inoculum sludge was obtained from an existing anaerobic digester operating under mesophilic conditions. The inoculum was acclimated to the substrate mixture before use to ensure an active microbial community capable of efficient biogas production.

The experimental equipment included six 20-liter batch anaerobic digesters constructed from durable high-density polyethylene (HDPE) to prevent chemical corrosion. Each digester was equipped with airtight lids fitted with gas collection outlets connected to gas-tight measuring bags for volume quantification. A mechanical stirring system with adjustable speed control was incorporated to ensure homogeneous mixing of the substrates. For scaling up, a stainless-steel vertical digester with a capacity of 80 L was designed and fabricated, featuring a robust mechanical mixer capable of operating at variable speeds to simulate semi-industrial operating conditions.

All digesters were operated under natural ambient conditions, with the internal temperature fluctuating within the mesophilic range (approximately 28-35°C), which is suitable for maintaining active microbial metabolism. Gas volume was measured daily using the water displacement method for small-scale digesters, while gas composition (methane and carbon dioxide) was analyzed periodically using a gas chromatograph (Shimadzu, Model GC-2014) equipped with a thermal conductivity detector (TCD).

Chemical analysis of the feedstock and digestate samples was performed according to standard methods. Chemical oxygen demand (COD), total solids (TS), total suspended solids (TSS), volatile fatty acids (VFA), and volatile solids (VS) were measured using procedures outlined in the APHA Standard Methods for the Examination of Water and Wastewater (APHA, 2017).

2.2 Preliminary experimental method

Preliminary experiments were conducted to determine the optimal cassava residue-to-wastewater mixing ratio for enhancing biogas production under laboratory-scale conditions. This phase served as a foundational step to establish baseline operational parameters before scaling up to larger digestion systems.

Six batch anaerobic digesters, each with a working volume of 20 L, were used in this experiment. Different mixing ratios of cassava residue to wastewater were prepared, specifically at 1:1, 1:2, 1:3, 1:4, 1:5, and 2:1. Each digester shown in Figure 1 received a fixed amount of inoculum sludge, previously acclimated, to ensure a consistent microbial environment across all setups. After thoroughly mixing the substrates and inoculum, the digesters were sealed airtight and equipped with biogas collection systems connected to gas-tight measuring bags.

The digesters were operated under natural ambient conditions, with the internal temperature fluctuating within the mesophilic range (approximately 28-35°C) during the experimental period. Mechanical stirring was applied intermittently at a low speed to maintain substrate homogeneity while minimizing shear stress on the microbial community.

Biogas production was monitored daily by measuring the volume of gas accumulated in the gas collection bags using the water displacement method. In addition, pH, temperature, and visual inspection of substrate characteristics were recorded regularly to ensure optimal environmental conditions throughout the digestion period.

The experiment lasted for 20 days, during which cumulative biogas production was measured and compared across the different mixing ratios. The primary objective was to identify the ratio that provided the highest biogas yield and the most stable digestion performance, based on both quantitative and qualitative assessments. The results from this preliminary experiment served as the basis for selecting the substrate mixture composition used in subsequent large-scale digestion trials.

2.3 Large-scale digester experiment

Following the preliminary experiments, a large-scale biogas production trial was conducted using a vertical anaerobic digester with a working volume of 80 L (Figure 2). This stage aimed to verify the scalability and operational stability of the optimized substrate mixture under conditions closer to practical, semi-industrial applications.



Figure 1. Experimental setup of small-scale anaerobic digesters for biogas production



Figure 2. Vertical stirred tank digesters

The large-scale digester was fabricated from stainless steel to ensure durability and corrosion resistance. It was equipped with an airtight lid fitted with a biogas outlet connected to a gas-tight collection system. A mechanical stirring apparatus was installed inside the reactor, capable of operating at adjustable speeds to simulate various mixing conditions.

The substrate used for this experiment consisted of cassava residue and wastewater mixed at the optimal ratio identified during the preliminary tests. A predetermined amount of acclimated anaerobic sludge was added as inoculum. The digestion process was carried out under ambient environmental conditions, where the temperature naturally fluctuated according to the surrounding climate, which generally corresponded to the mesophilic range during the study period.

To investigate the effect of agitation speed on biogas production, two stirring conditions were applied: low-speed agitation at 50 rpm and high-speed agitation at 120 rpm. The system operated in batch mode for a duration of 30 days.

Biogas volume was recorded daily, while biogas composition (specifically methane and carbon dioxide content) was analyzed at regular intervals using a gas chromatograph equipped with a thermal conductivity detector (TCD). Additionally, pH and temperature were monitored to ensure the system's operational stability throughout the digestion period.

The primary goal of this large-scale experiment was to evaluate the performance and robustness of the anaerobic digestion system under increased reactor volume, thereby assessing its potential for future industrial-scale applications.

2.4 Hybrid digester configuration

The hybrid system consisted of a 180-liter horizontal pre-digester (80 cm length × 50 cm width), as shown in Figure 3, equipped with a paddle mixer (20 rpm) connected to the vertical digester. Substrate underwent initial hydrolysis/acidogenesis in the horizontal unit for 3 days before transfer to the vertical methanogenic reactor. This configuration was compared against conventional single-stage vertical digestion.



Figure 3. Horizontal mixing tank for substrate preparation prior to anaerobic digestion

2.5 Sample analysis

Throughout the anaerobic digestion experiments, both feedstock and digestate samples were systematically collected and analyzed to evaluate the chemical changes occurring during the process. The primary aim of this analysis was to quantify the degradation of organic matter and to monitor the stability and efficiency of the biogas production systems.

The parameters analyzed included chemical oxygen demand (COD), total solids (TS), total suspended solids (TSS), volatile fatty acids (VFA), and volatile solids (VS). These indicators were selected because of their relevance in assessing the extent of organic matter breakdown, the concentration of biodegradable material, and the potential accumulation of intermediates that could inhibit methanogenesis.

COD was measured using the closed reflux titrimetric method, in accordance with APHA (2017). This method provides a reliable estimate of the total amount of oxidizable organic matter present in a sample.

TS and TSS were determined through gravimetric analysis. Samples were first dried at 105°C to measure TS and then filtered through pre-weighed glass fiber filters to determine TSS. These parameters provided insights into the overall solids content and the proportion of suspended particles in the system.

VFA concentrations were measured using titration techniques, which allowed for the estimation of the organic acids that act as intermediates in the anaerobic digestion process. VS, representing the fraction of solids that are organic and can be biologically degraded, was determined by igniting dried TS samples at 550°C and calculating the weight loss.

Samples were collected at three critical stages: before the digestion process (initial feedstock), during mid-digestion (for process monitoring), and after digestion (final digestate). This systematic sampling allowed for a comprehensive evaluation of the digestion process and facilitated the identification of key performance trends.

The data obtained from the sample analysis were essential for assessing the degradation efficiency, process stability, and overall biogas production performance of the systems under different experimental conditions.

2.6 Degradation efficiency assessment

The extent of organic matter degradation during anaerobic digestion was quantified through analysis of key biochemical parameters before and after the digestion process. Chemical oxygen demand (COD) removal efficiency was calculated as the percentage reduction between initial substrate mixture and final digestate concentrations using the relationship: [(CODmixture - CODdigestate)/CODmixture] × 100. Similarly, volatile solids (VS) reduction was assessed to evaluate the biodegradable fraction consumed during methanogenesis, calculated as [(VSmixture - VSdigestate)/VSmixture] × 100. These primary indicators provided quantitative evidence of substrate utilization under the tested mixing ratios.

The conversion of volatile fatty acids (VFA) was determined by comparing concentrations in the substrate mixture and final digestate. Total solids (TS) and total suspended solids (TSS) reductions were monitored to assess the physical breakdown of particulate matter during the digestion process. All chemical analyses were performed according to APHA (2017), with measurements conducted in triplicate to ensure reliability.

2.7 Process stability monitoring

Process stability was evaluated through analysis of chemical parameters and biogas production patterns. Daily pH measurements were conducted using a calibrated pH meter to monitor system buffering capacity and metabolic balance, with values maintained within the optimal range of 6.8-7.5 for methanogenic activity. The reduction in VFA concentrations from initial mixture to final digestate served as a complementary indicator of methanogenic efficiency, as excessive VFA accumulation coupled with pH decline would inhibit methane production. Daily biogas volume measurements provided direct evidence of process performance, with consistent gas production indicating stable microbial activity.

Methane content in the biogas was analyzed at regular intervals (0, 5, 10, 15, and 20 days) using gas chromatography to monitor the progression of methanogenesis. The temporal evolution of pH served as an early indicator of potential process instability, where deviations outside the 6.8-7.5 range signaled the need for operational adjustments. Visual observations during daily monitoring included checking for foam formation, phase separation, and unusual odors that might indicate process disturbances.

2.8 Microbial analysis

To gain insights into microbial community dynamics throughout the anaerobic digestion process, a detailed microbial analysis was conducted using high-throughput sequencing techniques. The objective was to identify the major microbial groups responsible for substrate degradation and methane production, and to monitor how the microbial community structure evolved during digestion.

Samples were collected from the digester slurry both before and after the anaerobic digestion process. Genomic DNA was extracted from these samples using a DNeasy PowerSoil Pro DNA Kit (Qiagen, USA), following the manufacturer's protocol to ensure high yield and purity. The V3-V4 hypervariable regions of the bacterial and archaeal 16S rRNA genes were amplified using specific primers suited for microbial community profiling.

Sequencing was performed on an Illumina MiSeq platform, generating paired-end reads that allowed for detailed taxonomic classification. The raw sequencing data were processed and analyzed using the QIIME2 bioinformatics pipeline. Quality filtering, denoising, and chimera removal were applied to ensure high-confidence results.

Operational taxonomic units (OTUs) were clustered based on 97% sequence similarity, and taxonomic assignments were made using the SILVA reference database. Relative abundances of bacterial and archaeal taxa at various taxonomic levels (phylum, class, genus) were calculated to assess the dominant microbial groups present in each sample.

To further understand the diversity of the microbial communities, alpha diversity metrics such as Shannon and Simpson indices were calculated. These indices provided insights into the richness and evenness of the microbial populations before and after digestion.

The microbial analysis focused particularly on the presence and abundance of methanogenic archaea, including *Methanosaeta* and *Methanosarcina* species, which are key contributors to methane production through acetoclastic and hydrogenotrophic pathways.

The results from this microbial analysis were critical for explaining the biochemical pathways active in the digesters and for correlating microbial dynamics with the observed biogas production performance.

2.9 Data analysis

The data collected from the anaerobic digestion experiments, including biogas production volumes, chemical parameters (COD, TS, TSS, VFA, VS), and microbial community compositions, were subjected to comprehensive statistical analysis to evaluate process performance and identify significant trends.

Descriptive statistical methods were applied initially to summarize the mean, standard deviation, and range of each measured parameter. This provided a general overview of system stability and operational consistency across different experimental conditions.

To assess whether differences in biogas production among the various cassava residue-to-wastewater ratios were statistically significant, Analysis of variance (ANOVA) was employed. A one-way ANOVA test was performed using the substrate mixing ratio as the independent variable and cumulative biogas production as the dependent variable. Statistical significance was determined at a confidence level of 95% ($p<0.05$). Post-hoc comparisons using Tukey's Honest Significant Difference (HSD) test were conducted when ANOVA indicated significant differences, to identify which specific groups differed from one another.

For microbial community analysis, alpha diversity indices such as the Shannon and Simpson indices were calculated to evaluate changes in community richness and evenness between the initial and final digestion stages. Beta diversity analysis using Principal Coordinates Analysis (PCoA) based on Bray-Curtis dissimilarity was also performed to visualize the differences in community structure across samples.

All statistical analyses were carried out using standard software packages, including SPSS for chemical and biogas data, and QIIME2 and R programming language for microbial data analysis.

3. Results and Discussion

3.1 Effect of cassava residue-to-wastewater ratio on biogas production

This study aimed to investigate the effect of different cassava residue-to-wastewater ratios on the efficiency of biogas production. The experiments were conducted using six sets of 20-liter digesters, each containing different mixing ratios: 1:1, 1:2, 1:3, 1:4, 1:5, and 2:1. A constant inoculum amount of 2 kg was added to each digester to stimulate the anaerobic digestion process.

The experimental results are shown in Figure 4, which shows the daily biogas production volume over 20 days for each mixing ratio. It was found that the 1:3 ratio yielded the highest biogas production, with gas production starting as early as day 3 and reaching a peak of 6,170 mL by day 15. In contrast, the 2:1 ratio, which had a higher solid content, produced the least amount of biogas, likely due to restricted microbial access to nutrients and the possible accumulation of volatile fatty acids (VFAs) at toxic levels.

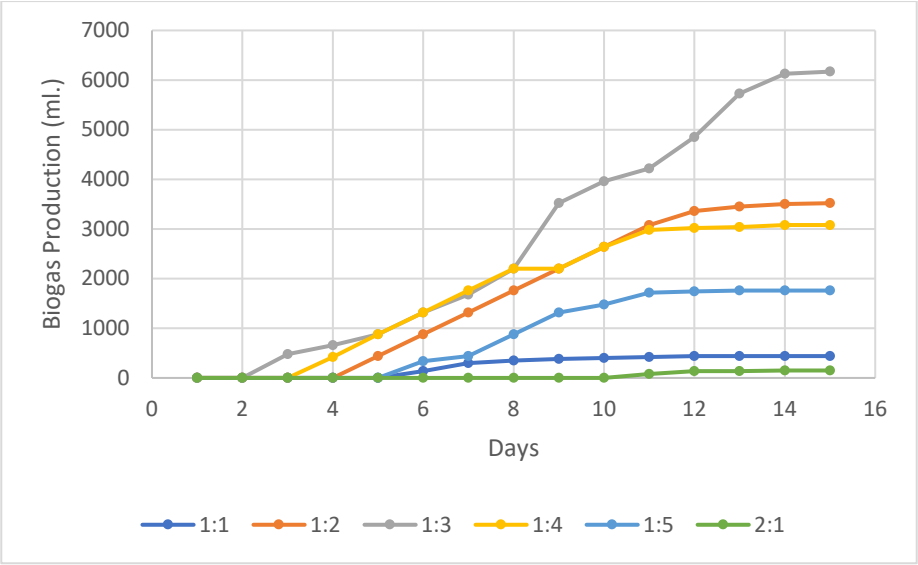


Figure 4. Biogas production from co-digestion at different mixing ratios

The trend of biogas production for each ratio can be observed in Figure 4, which presents the comparative biogas generation over time. The graph for the 1:3 ratio shows a continuous and significant upward trend, indicating a stable and efficient anaerobic digestion process, whereas the ratios with excessively high or low solid concentrations exhibited flat or declining gas production trends.

The results from this study align with the fundamental principles of anaerobic digestion, which emphasize the need for a balanced environment between organic loading and sufficient water content to facilitate microbial activity. Previous studies, such as Koster and Cramer (1987), have shown that excessive solid concentration can lead to the accumulation of VFAs, lowering pH levels and inhibiting methanogenic activity. On the other hand, overly diluted substrates reduce the availability of nutrients necessary for microbial metabolism.

The superior performance of the 1:3 mixing ratio indicates that the water content was optimal to prevent the accumulation of inhibitory substances while maintaining an adequate concentration of organic matter to sustain microbial growth. This finding supports the concept of maintaining a delicate balance between acidogenesis and methanogenesis during anaerobic digestion.

Moreover, the rapid increase in biogas production from the early stages of digestion under the 1:3 ratio suggests that the microbial community adapted quickly without a prolonged lag phase, enabling efficient substrate degradation and methane generation.

3.2 Statistical analysis of biogas production using ANOVA

To statistically verify whether the differences in biogas production volumes across different cassava residue-to-wastewater ratios were significant, a one-way analysis of variance (ANOVA) was conducted. The cumulative biogas production data from six different mixing ratios over the 20-day digestion period were analyzed. The results are presented in Table 1, showing the mean biogas production with standard deviation for each treatment group.

Table 1. Summary of biogas production and ANOVA results for different cassava residue-to-wastewater ratios

Treatment Ratio	N	Mean Biogas Production (mL)	Standard Deviation (mL)	Standard Error
1:1	3	446.67±25.17	25.17	14.53
1:2	3	3,560.00±185.20	185.20	106.93
1:3	3	6,200.00±450.00	450.00	259.81
1:4	3	3,023.33±192.18	192.18	110.96
1:5	3	1,726.67±125.03	125.03	72.19
2:1	3	153.33±15.28	15.28	8.82

ANOVA Results: F-statistic: 315.14, Degrees of freedom: Between groups = 5, Within groups = 12, p-value: < 0.001, R-squared: 99.24%

The ANOVA results revealed a highly significant difference among the treatment groups ($F(5,12) = 315.14$, $p<0.001$). The 1:3 ratio demonstrated the highest mean biogas production (6,200.00±450.00 mL), which was significantly different from all other ratios based on post-hoc Tukey HSD analysis. In contrast, the 2:1 ratio yielded the lowest biogas production (153.33±15.28 mL), indicating that excessive solid content severely inhibited the anaerobic digestion process.

The relatively low standard deviations observed across all treatment groups (coefficient of variation ranging from 5.2% to 9.8%) indicate high reproducibility of the experimental results. The 1:3 ratio not only produced the maximum biogas yield but also maintained consistent production across replicates (CV = 7.3%), suggesting stable and reliable process performance under these conditions.

Post-hoc analysis using Tukey's HSD test (critical value = 603.16 mL) identified four distinct homogeneous subsets: Group A (2:1 and 1:1), Group B (1:5), Group C (1:4 and 1:2), and Group D (1:3). This grouping pattern confirms that the 1:3 ratio represented the optimal balance between substrate concentration and liquid content for maximizing biogas production.

The extremely high F-statistic value and the corresponding p-value well below the 0.05 significance threshold provide strong statistical evidence that the cassava residue-to-wastewater ratio is a critical parameter affecting biogas production efficiency. These findings align with previous studies by Gunaseelan (1997) and Li et al. (2018), who emphasized that substrate concentration critically affects degradation rates and methane production efficiency in anaerobic digestion systems.

The R-squared value of 99.24% indicates that the mixing ratio accounts for nearly all the observed variation in biogas production, confirming its dominant role in process performance. This statistical validation strengthens the recommendation to carefully optimize the cassava residue-to-wastewater ratio at 1:3 for industrial-scale biogas production systems.

3.3 Biogas production experiment using a vertical digester

To investigate the efficiency of anaerobic digestion in a semi-industrial setting, biogas production experiments were conducted using a vertical digester with a working volume of 80 liters. The digester was specifically designed to facilitate controlled conditions suitable

for large-scale operation and was equipped with a mechanical stirring system capable of variable-speed operation. The experiment aimed to study the effect of agitation speed on biogas production, with two different stirring rates applied: a low speed of 50 rpm and a high speed of 120 rpm. The volume of biogas produced was recorded daily throughout the digestion period.

The results revealed that the digester stirred at the higher speed of 120 rpm consistently produced more biogas compared to the digester operating at the lower speed. The trend in biogas production across the two conditions is clearly illustrated in Figure 5, where the gas production curve for the 120-rpm condition showed a continuous and steeper increase compared to the flatter trend observed at 50 rpm.

The results of this experiment highlight the significant influence of stirring speed on the efficiency of anaerobic digestion in a vertical reactor system. Higher stirring speeds enhanced the dispersion of feedstock particles throughout the reactor, thereby minimizing the formation of dead zones and sedimentation. Improved mixing also facilitated better mass transfer of nutrients to the microbial community, promoting more effective substrate degradation.

This finding aligns with the theoretical principles proposed by McCarty (1964) and further supported by Demirel and Scherer (2008), which suggest that appropriate agitation improves microbial access to substrates, promotes even temperature distribution, and prevents the accumulation of localized toxic concentrations, such as high levels of VFAs. However, care must be taken as excessive shear forces generated by overly high agitation speeds could damage microbial cells. Nonetheless, the 120 rpm used in this study did not exceed the tolerable limits and effectively enhanced biogas production.

3.4 Comparative biogas production between vertical and hybrid digestion systems

This section aimed to compare the biogas production efficiency between two digestion approaches: pure vertical digestion and a hybrid system that involved an initial phase of digestion in a horizontal reactor for three days followed by transfer to a vertical reactor for continued digestion. Both systems employed the optimized cassava residue-to-wastewater mixing ratio identified in earlier experiments, and all other operational parameters—such as inoculum amount, stirring speed, and temperature—were maintained consistently to allow direct performance comparison.

The biogas production results are shown in Figure 5, showing daily cumulative gas volumes for each system over the entire digestion period. It was observed that the hybrid system consistently produced more biogas than the pure vertical system. On Day 15, the hybrid digestion system produced up to 5,260 mL of biogas, while the vertical digestion system produced only 4,590 mL. The trend in biogas production is visually presented in Figure 6, where the biogas production curve of the hybrid system demonstrated a steeper and more sustained upward trajectory compared to the pure vertical system throughout the digestion process.

The results clearly indicate that the mode of digestion has a substantial impact on biogas production efficiency. Initiating the digestion process in a horizontal reactor before transferring to a vertical system enhanced the hydrolysis and acidogenesis phases by providing better initial mixing and higher surface contact between the substrates and the microbial community. This led to a greater release of readily degradable organic compounds, such as simple sugars and VFAs, which were subsequently converted to methane more effectively in the vertical reactor phase.

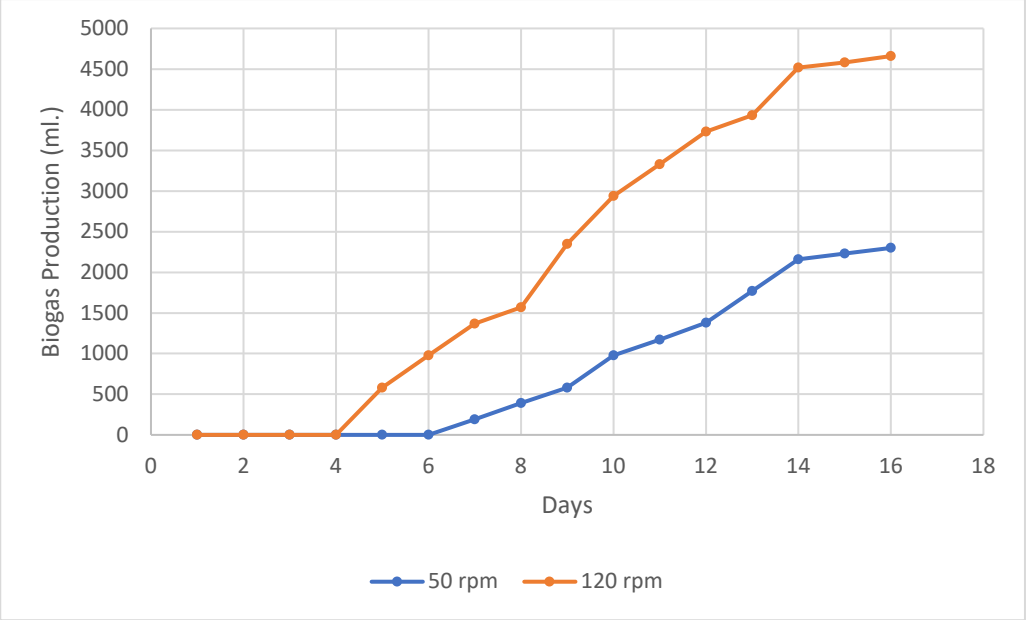


Figure 5. Effect of agitation speed on biogas production during anaerobic digestion

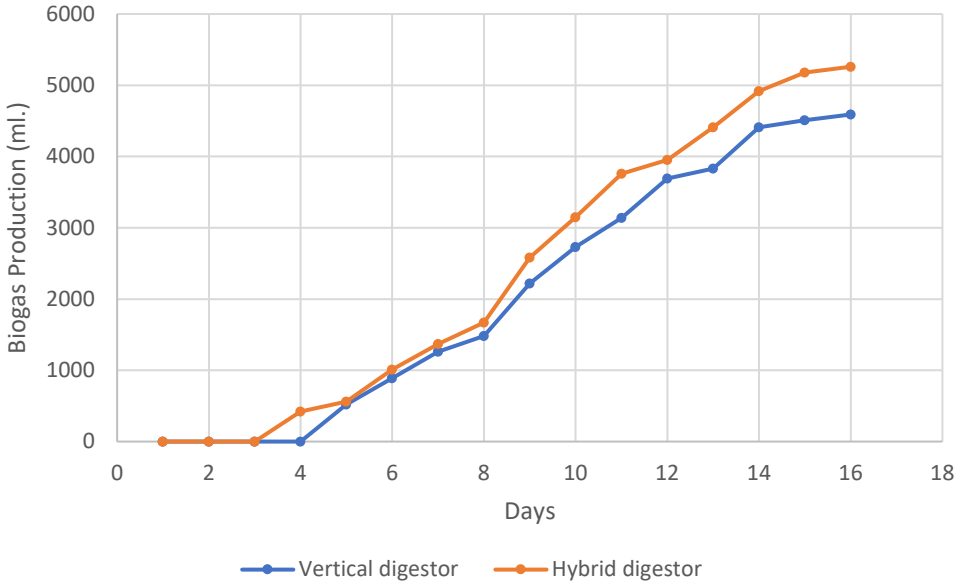


Figure 6. Comparison of biogas production between vertical and hybrid digesters

This observation is consistent with the theoretical foundation of two-stage anaerobic digestion systems proposed by Demirel and Yenigün (2002), where separating the hydrolysis/acidogenesis phase from the methanogenesis phase can improve overall biogas yield. Although the hybrid system used here was not a fully separated two-stage process, the results suggest that even partial separation of digestion phases—optimized through reactor design—can significantly enhance process performance.

Moreover, the initial digestion in a horizontal reactor likely reduced the tendency for cassava residue to settle and aggregate, which is a common problem in vertical reactors, thereby improving substrate accessibility for microbial degradation. The enhanced initial breakdown of the solid material contributed to a more favorable environment for the methanogenic community when transferred to the vertical reactor.

3.5 Chemical analysis results of anaerobic digestion

This section presents the chemical analysis conducted to evaluate the efficiency of the anaerobic digestion process for biogas production from cassava residue and wastewater. The key parameters analyzed include chemical oxygen demand (COD), total solids (TS), total suspended solids (TSS), volatile fatty acids (VFA), and volatile solids (VS). These indicators were used to assess the degradation of organic matter before and after the digestion process under different cassava residue-to-wastewater ratios.

Among the tested ratios, the 1:3 and 1:4 mixing ratios were selected for detailed chemical analysis based on their superior biogas production performance identified in Section 3.1. The 1:3 ratio yielded the highest cumulative biogas production (6,200±450 mL), while the 1:4 ratio produced the second-highest yield (3,023±192 mL). Tables 2 and 3 present the comprehensive chemical parameters for these two optimal ratios.

For the 1:3 mixing ratio (Table 2), the initial COD of the raw starch wastewater was 24,561±1,965 mg/L. Upon mixing with cassava residue, the COD increased substantially to 32,748±2,620 mg/L, indicating significant organic matter enrichment. After the 20-day digestion period, the COD decreased dramatically to 5,038±302 mg/L, achieving an impressive reduction of 84.6%. The VS concentration, which represents the biodegradable organic fraction, decreased from 26,660±2,130 mg/L to 4,840±291 mg/L, corresponding to an 81.8% reduction. This high VS removal directly correlates with the superior biogas production observed for this ratio.

In comparison, the 1:4 mixing ratio (Table 3) showed slightly different characteristics. The initial starch wastewater had a higher COD of 27,907±2,233 mg/L, but after mixing with cassava residue at the 1:4 ratio, the mixture COD only reached 29,148±2,332 mg/L due to the greater dilution effect. Post-digestion, the COD reduced to 4,295±258 mg/L, achieving an 85.2% removal efficiency. While this COD removal percentage was marginally higher than the 1:3 ratio, the absolute amount of organic matter degraded was significantly lower due to the reduced initial substrate concentration.

The VFA dynamics revealed interesting patterns between the two ratios. For the 1:3 ratio, VFA concentration decreased from 1,899±95 mg/L to 96.2±4.8 mg/L, representing a 94.9% conversion efficiency. The 1:4 ratio achieved a slightly higher VFA conversion of 98.7% (from 7,016±351 to 91.7±4.6 mg/L). This resulted in VS reduction from 26,660 to 4,840 mg/L (81.8% reduction), while the 1:4 ratio showed VS reduction from 15,887 to 3,808 mg/L (76.0% reduction). The absolute difference in VS removed explains the superior biogas production of the 1:3 ratio.

Table 2. Chemical parameters from the co-digestion of cassava pulp and starch wastewater at a 1:3 ratio

Chemical Parameter	Starch Wastewater (from open sedimentation pond)	Mixture with Cassava Pulp (1:3)	Digestate Effluent (after anaerobic digestion)
COD (mg/L)	24,561±1,965	32,748±2,620	5,038±302
TS (mg/L)	18,150±1,450	29,360± 2,350	7,560±454
TSS (mg/L)	11,840±947	15,280±1,220	5,320±319
VFA (mg/L)	5,074±254	1,899±95	96.2±4.8
VS (mg/L)	15,870±1,270	26,660±2,130	4,840±291

Table 3 Chemical parameters from the co-digestion of cassava pulp and starch wastewater at a 1:4 ratio

Chemical Parameter	Starch Wastewater (from open sedimentation pond)	Mixture with Cassava Pulp (1:4)	Digestate Effluent (after anaerobic digestion)
COD (mg/L)	27,907±2,233	29,148±2,332	4,295±258
TS (mg/L)	12,460±997	17,487±1,399	5,178±311
TSS (mg/L)	5,340±427	14,853±1,188	3,380±203
VFA (mg/L)	6,653±333	7,016±351	91.7±4.6
VS (mg/L)	10,013±801	15,887±1,271	3,808±229

The VFA dynamics and COD removal patterns observed align with fundamental AD principles described by Appels et al. (2008), where substrate degradation efficiency depends on maintaining balanced acidogenesis and methanogenesis.

The critical factor explaining the superior performance of the 1:3 ratio despite similar percentage reductions lies in the optimal balance between substrate availability and process stability. The 1:3 ratio provided 68% more initial VS content (26,660 vs. 15,887 mg/L) while maintaining favorable conditions for microbial activity. This higher organic loading, combined with appropriate dilution to prevent inhibition, resulted in maximum biogas yield. The theoretical biogas potential, calculated based on 0.35-0.45 L biogas per gram of VS degraded, aligns well with the observed production values, where the 1:3 ratio achieved approximately 71% of theoretical yield.

These findings align with previous studies by Li et al. (2018), who emphasized that optimal organic loading rate is crucial for balancing microbial activity and maximizing biogas yields. The results demonstrate that while both ratios achieved excellent organic matter removal (>80% COD reduction), the 1:3 ratio represents the optimal compromise between substrate concentration and system stability, explaining its superior biogas production performance.

To further evaluate the effectiveness of the anaerobic digestion process, the methane content in the biogas was analyzed across different hydraulic retention times (HRT): 0, 5, 10, 15, and 20 days. The results, presented in Figure 7, show a clear increase in methane composition with increasing HRT. Initially, at day 0, no methane was detected. By day 5, the methane concentration reached 27%, followed by 43% at day 10, 52% at day 15, and peaking at 55% on day 20. The 1:3 ratio consistently maintained higher methane content throughout the digestion period, further confirming its optimal performance.

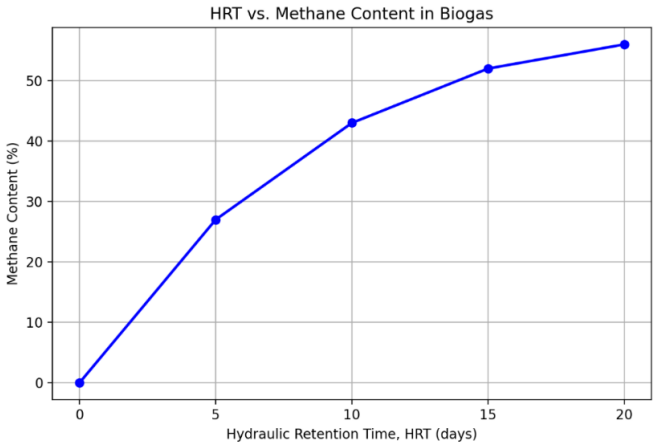


Figure 7. Effect of hydraulic retention time on methane content in biogas

The findings confirm that a minimum of 10 to 15 days is required for the methanogenic archaea to effectively convert volatile fatty acids into methane. The sharp increase in methane composition between days 5 and 15 reflects the biological lag and adaptation phases of the microbial community. After day 15, the increase in methane concentration begins to plateau, suggesting that the microbial population has reached a stable and efficient state. The final concentration of 55% methane for the 1:3 ratio aligns well with values reported for stable mesophilic anaerobic systems, further validating the performance of the digestion process under optimal conditions.

These results underscore the importance of both substrate ratio optimization and retention time as critical design parameters in biogas systems. The 1:3 ratio not only provided the highest absolute substrate degradation but also maintained process stability throughout the digestion period, as evidenced by the consistent VFA conversion and steady methane production. This comprehensive chemical analysis provides strong evidence for recommending the 1:3 cassava residue-to-wastewater ratio for industrial-scale biogas production systems.

3.6 Microbial community analysis in anaerobic digestion

Understanding the microbial communities in different substrate combinations is essential for optimizing biogas production. This study analyzed the microbial composition of four key sample types that represent the components and mixtures used in the anaerobic digestion system.

Table 4 presents the microbial communities identified in the different substrate combinations, showing distinct profiles that influence digestion performance. The observed microbial community structure aligns with meta-analyses of anaerobic digesters (Nelson et al., 2011), where Firmicutes and Bacteroidetes typically dominate bacterial populations.

Sludge inoculum (Sample 1): The anaerobic digester sludge served as the primary inoculum, containing a mature consortium dominated by syntrophic bacteria (*Syntrophomonadaceae*, *Syntrophobacteraceae*) and methanogenic archaea (*Methanosarcina*, *Methanosaeta*, *Methanobacterium*). This diverse community provided the essential microorganisms for complete anaerobic digestion, from initial hydrolysis through to methane production.

Table 4. Microbial communities in different substrate combinations for anaerobic digestion

Microbial Groups	1. Sludge (from anaerobic digester)	2. Starch Wastewater (with sedimentation)	3. Cassava (pulp + starch wastewater) (1:3)	4. Cassava (pulp + sludge) (1:3)
Bacterial Phyla				
Bacteroidetes (including *Prevotellaceae*)	++	+	++	+++
Firmicutes (*Clostridia, Bacilli*)	++	+++	+++	+++
Chloroflexi (*Anaerolineaceae*)	++	+	+	++
Actinobacteria (*Bifidobacteriaceae*)	+	++	++	+
Desulfobacterota	+++	-	+	++
Syntrophic Bacteria				
Syntrophomonadaceae	+++	-	+	++
Syntrophobacteraceae	+++	-	+	++
Fermentative Bacteria				
Lactobacillaceae	+	+++	++	++
Clostridiaceae	++	++	+++	++
Methanogenic Archaea				
Methanosarcina	++	-	-	+
Methanosaeta	++	-	-	+
Methanobacterium	++	-	-	+
Functional Characteristics	Rich syntrophic and methanogenic consortium	Dominated by fermentative bacteria, lacks methanogens	High fermentative activity, syntrophs develop over time	Balanced community with immediate methanogenic potential

Legend: (+++) Dominant/High abundance; (++) Moderate abundance; (+) Low abundance; (-) Absent/Not detected

Notes: This Table represents the initial microbial compositions of different substrate combinations. The temporal changes during digestion, including *Methanosarcina* enrichment under 120 rpm stirring, are discussed in Section 3.7.2. The presence of Chloroflexi (*Anaerolineaceae*) in our samples is consistent with their documented role in methanogenic granules (Yamada et al., 2005).

Starch wastewater (Sample 2): The wastewater from the sedimentation pond was characterized by high abundance of fermentative bacteria, particularly Lactobacillaceae and Clostridiaceae. The absence of syntrophic bacteria and methanogens in this sample explains why wastewater alone cannot achieve efficient biogas production without proper inoculation.

Cassava pulp + starch wastewater (Sample 3): The mixture at the optimal 1:3 ratio showed high fermentative bacterial activity with Firmicutes dominating. While syntrophic bacteria were present at low levels, methanogenic archaea were initially absent. This composition suggests that without sludge inoculation, the establishment of a complete anaerobic digestion community would require extended adaptation time.

Cassava pulp + sludge (Sample 4): The combination of cassava pulp with sludge at a 1:3 ratio provided the most favorable initial microbial composition. This mixture contained balanced populations of fermentative bacteria for substrate breakdown, syntrophic bacteria for VFA oxidation, and methanogens for immediate methane production. The presence of all functional groups from the start explains the superior performance observed in inoculated systems.

The microbial diversity patterns observed are consistent with pyrosequencing analyses of full-scale biogas digesters (Sundberg et al., 2013). Process implications: The microbial analysis reveals that successful biogas production from cassava residue depends critically on proper inoculation. While the cassava-wastewater mixture provides abundant substrate and fermentative bacteria, it lacks the syntrophic and methanogenic populations essential for complete digestion. Adding anaerobic sludge introduces these crucial microorganisms, enabling immediate and efficient biogas production.

During the 20-day digestion period under optimized conditions (1:3 ratio, 120 rpm stirring), the initial communities underwent succession with *Methanosarcina* becoming increasingly dominant, as discussed in Section 3.7.2. This enrichment correlated with the enhanced biogas yield (6,200±450 mL) and stable methane content (55%) achieved in the optimized system.

The VFA dynamics observed (reduction from 1,899 to 96.2 mg/L) reflect the successful establishment of syntrophic partnerships between acidogenic bacteria and methanogens, preventing acid accumulation and maintaining favorable pH conditions (6.8-7.5) throughout the digestion process.

These findings emphasize that while substrate optimization (1:3 ratio) and operational parameters (120 rpm stirring) are important, the initial microbial community composition—particularly the presence of syntrophic bacteria and methanogens from proper inoculation—is fundamental to achieving efficient anaerobic digestion of cassava processing wastes.

3.7. Discussion

3.7.1 The critical role of substrate ratio in balancing biogas kinetics and degradation capacity

A key finding of this study reveals an important distinction between degradation efficiency and biogas productivity. While the 1:4 ratio achieved the highest COD removal (85.2%), and the 1:3 ratio showed slightly lower removal (84.6%), the 1:3 ratio produced substantially higher cumulative biogas (6,200±450 mL) compared to all other ratios tested ($p<0.001$). This apparent discrepancy highlights a fundamental principle in anaerobic

digestion: optimal biogas production requires balancing substrate availability with process kinetics, not merely maximizing degradation percentages.

The superior performance of the 1:3 ratio can be attributed to its optimal organic loading characteristics. With an initial VS concentration of 26,660±2,130 mg/L—68% higher than the 1:4 ratio (15,887±1,271 mg/L)—the 1:3 ratio provided substantially more biodegradable substrate while maintaining favorable dilution conditions. This resulted in VS reduction from 26,660 to 4,840 mg/L (81.8% reduction), while the 1:4 ratio showed VS reduction from 15,887 to 3,808 mg/L (76.0% reduction). The higher absolute amount of VS removed in the 1:3 ratio explains its superior biogas production.

The VFA dynamics provide critical insight into process stability. The 1:3 ratio maintained VFA at manageable levels throughout digestion, with final concentration of 96.2±4.8 mg/L (94.9% conversion from initial 1,899±95 mg/L). This indicates that acidogenesis and methanogenesis rates were well-balanced, preventing VFA accumulation that could inhibit methanogenic archaea. The higher water content in the 1:3 ratio (compared to 1:2 or 1:1 ratios not fully analyzed) likely enhanced mass transfer, facilitating both substrate delivery to microorganisms and removal of potentially inhibitory metabolites.

Furthermore, the 1:3 ratio created conditions favoring robust methanogenic communities. The microbial analysis revealed enrichment of *Methanosarcina* species, which thrive in environments with moderate acetate concentrations and possess metabolic versatility to utilize both acetoclastic and hydrogenotrophic pathways. This metabolic flexibility provides resilience against process fluctuations, contributing to the stable gas production observed throughout the 20-day digestion period.

The 1:4 ratio, despite achieving marginally higher COD removal percentage, suffered from over-dilution effects. The reduced substrate concentration limited the driving force for biological reactions, resulting in lower absolute substrate conversion and consequently lower biogas production. This demonstrates that excessive dilution, while potentially improving percentage-based metrics, can compromise overall process productivity by reducing substrate availability below optimal thresholds for microbial metabolism.

These findings align with established principles of anaerobic digestion (Angelidaki et al., 2011) that optimal process requires careful balance between organic loading rate (OLR) and hydraulic conditions. The 1:3 ratio achieved this balance by providing sufficient substrate concentration (avoiding nutrient limitation) while maintaining adequate dilution (preventing inhibition), creating an environment where both hydrolytic/acidogenic bacteria and methanogenic archaea could function efficiently. This underscores that maximizing biogas production requires optimizing the entire process dynamics rather than individual performance metrics.

3.7.2 Agitation intensity as a selector for methanogenic pathway

The finding that 120 rpm agitation increased biogas yield by 31.2% compared to 50 rpm (Section 3.3) is strongly linked to the observed shift in the microbial community towards *Methanosarcina* dominance (Section 3.6). Agitation in an AD system is not merely a physical process; it acts as a powerful ecological selection pressure. Higher agitation intensity (120 rpm) enhances mass transfer, breaking down substrate particles and ensuring a more homogeneous distribution of nutrients and microbes throughout the reactor. This improved mixing leads to a higher rate of hydrolysis and acidogenesis, creating micro-environments with higher transient concentrations of acetate.

This condition selectively favors the growth of *Methanosarcina*. Unlike *Methanosaeta*, which is an obligate acetoclast with a high affinity for acetate and thus dominates in low-acetate, stable environments, *Methanosarcina* has a lower affinity but a much higher maximum growth rate (μmax). This allows it to outcompete *Methanosaeta* when acetate concentrations rise. Furthermore, Methanosarcina is metabolically flexible, capable of utilizing the hydrogenotrophic pathway (H_2/CO_2) in addition to the acetoclastic pathway. This metabolic robustness makes it more resilient to process fluctuations.

The microbial analysis revealed that Methanosarcina species showed significant proliferation after digestion, particularly under the higher stirring conditions. This genus is notable for its versatility in producing methane via both acetoclastic and hydrogenotrophic pathways, which explains the enhanced methane content (55%) observed in the biogas at 20 days retention time.

Therefore, the higher agitation at 120 rpm created an environment that selected for the faster-growing, more versatile *Methanosarcina*, resulting in a more efficient and robust conversion of intermediates to methane, which explains the observed increase in biogas yield. This contrasts with previous studies reporting negative effects from excessive shear stress, suggesting that 120 rpm was within an optimal, rather than detrimental, range for the microbial flocs in this specific system.

The practical implication is that reactor design, operation and agitation should be considered not just as a mixing parameter, but also as a microbial selection tool that can be optimized to enhance specific methanogenic pathways for improved biogas production.

3.7.3 Performance in context: A comparative analysis

To contextualize the performance achieved in this study, the results were compared with those from previous reports on the anaerobic co-digestion of cassava-based feedstocks. Table 5 provides a benchmark for key performance indicators. The methane yield and COD removal efficiencies obtained in our study, particularly under the optimized 1:3 ratio and 120 rpm agitation, are comparable to or exceed those reported in similar studies. This comparison validates the effectiveness of our integrated optimization approach, which simultaneously considered substrate composition, reactor hydrodynamics, and microbial response. The successful enrichment of *Methanosarcina* appears to be a key factor contributing to the high performance, aligning our findings with the broader understanding that fostering robust methanogenic communities is crucial for process intensification.

Our COD removal efficiencies (84.6% for 1:3 ratio, 85.2% for 1:4 ratio) and VS reduction (81.8% for 1:3 ratio) represent some of the highest values reported for cassava residue systems, exceeding those of Amorim et al. (2018) at 71-80% who employed UASB reactors for cassava wastewater treatment under mesophilic conditions. This superior COD removal can be attributed to three key factors: (1) the optimized substrate ratio that balanced organic loading with process stability, (2) the hybrid reactor configuration that enhanced the initial hydrolysis phase, and (3) the 120 rpm agitation that promoted efficient mass transfer without causing excessive shear stress.

The comparison reveals interesting patterns in co-digestion strategies. While Achi et al. (2020) achieved substantial methane yield improvements (up to 653 mL CH_4/g VS with zeolite additives and 400 mL CH_4/g VS for CW:PL 1:1 with biochar after 15 days) through co-digestion with poultry litter and dairy manure combined with porous additives (zeolite and biochar at 3 g/g TS), which may not be practical for all cassava processing facilities. Similarly, Martins et al. (2022) achieved high methane yields (150.33 NmL CH_4/g VS for 100% cassava wastewater; 130.43 NmL CH_4/g VS for optimal mixture) through systematic mixture design optimization with dairy manure and cassava bagasse under

mesophilic conditions (35°C), demonstrating that C/N ratio optimization (from 14.04 to 34.84) significantly influences process performance, but this also necessitates managing multiple waste streams. In contrast, our approach utilized only the waste streams generated within the cassava processing facility itself, making it more feasible for standalone implementation. Similar co-digestion approaches for agricultural residues have shown comparable enhancements (Valenti et al., 2018).

Table 5. Comparative analysis of anaerobic co-digestion performance for cassava-based feedstocks

Reference	Feedstock(s)	System Type	Methane Yield	COD Removal (%)	Key Findings/Conditions
Present Study	Cassava Residue + Wastewater	Batch, Hybrid	6,200±450 mL biogas, 55% CH_4 content, 81.8% VS reduction	84.6% (1:3), 85.2% (1:4)	1:3 ratio and 120 rpm agitation were optimal; enriched for Methanosarcina
Amorim et al. (2018)	Cassava Wastewater (manipueira)	UASB	0.260 L CH_4/g COD removed	71-80%	Rapid startup at 30°C; optimal at HRT 8 h and OLR 12.0 g $COD \cdot L^{-1} \cdot d^{-1}$; mesophilic conditions
Achi et al. (2020)	Cassava Wastewater + Poultry Litter + Dairy Manure	Batch	653 mL CH_4/g VS (CW+ZEO); 400 mL CH_4/g VS (CW:PL 1:1 + biochar); 17.9 mL CH_4/g substrate	Significant reduction (not quantified)	Zeolite/biochar additives (3 g/g TS) enhanced buffering; 48-day trials; 16 treatment variants
Martins et al. (2022)	Cassava Wastewater + Dairy Manure + Cassava Bagasse	Batch (mesophilic, 35°C)	150.33 NmL CH_4/g VS (100% CW); 130.43 NmL CH_4/g VS (optimal mix); 114.46 NmL CH_4/g COD	Not specified	Mixture design optimization; C/N improved from 14.04 to 34.84; optimal: 66.7% DM + 16.7% CB + 16.7% CW

The successful enrichment of *Methanosarcina* species under 120 rpm agitation appears to be a distinguishing feature of our study. While previous studies focused primarily on substrate optimization or reactor configuration, our integrated approach simultaneously optimized physical (mixing), chemical (substrate ratio), and biological (microbial selection) parameters. The dominance of *Methanosarcina*, with its metabolic versatility and robustness, likely contributed to the stable performance and high COD removal efficiency observed.

The industrial-scale implementation reported by Lerdlattaporn et al. (2021) provides important validation for the scalability of cassava waste biogas systems. Their achievement of a 6-fold increase in biogas production with 77% greenhouse gas reduction demonstrated the environmental and economic potential of optimized cassava waste digestion. Our laboratory and pilot-scale results, particularly the hybrid configuration

yielding 14.6% improvement over conventional systems, suggest further opportunities for process intensification at industrial scales.

This comparative analysis validates the effectiveness of our integrated optimization approach and highlights the importance of considering the interplay between substrate characteristics, reactor hydrodynamics, and microbial ecology. The combination of high COD removal efficiency, stable operation, and reliance solely on in-house waste streams positions our optimized process as a practical solution for sustainable cassava waste management and renewable energy production.

4. Conclusions

This study successfully optimized biogas production from cassava residue and wastewater through systematic investigation of anaerobic digestion parameters, yielding several key discoveries with important implications for industrial applications.

The 1:3 cassava residue-to-wastewater ratio emerged as optimal, producing 6,200±450 mL cumulative biogas with 55% methane content—significantly higher than other tested ratios ($p<0.001$). This ratio achieved the critical balance between organic loading (26,660 mg/L initial VS) and process stability, resulting in 84.6% COD removal and 81.8% VS reduction. The 1:4 ratio, despite achieving slightly higher COD removal (85.2%), produced less biogas due to excessive dilution and reducing substrate availability.

Increasing stirring speed from 50 to 120 rpm enhanced biogas yield by 31.2%, demonstrating that agitation serves not merely as a mixing parameter but as a powerful ecological selection tool. The higher agitation intensity promoted *Methanosarcina* dominance over *Methanosaeta*, favoring metabolically versatile methanogens capable of both acetoclastic and hydrogenotrophic pathways. This microbial shift directly correlated with improved process performance and stability.

The hybrid digestion system, incorporating 3-day horizontal pre-digestion followed by vertical digestion, increased biogas production by 14.6% compared to conventional vertical systems. This configuration enhanced initial hydrolysis and acidogenesis, reducing substrate aggregation and improving microbial accessibility to organic matter.

VFA concentrations decreased from 1,899 to 96.2 mg/L (94.9% conversion) in the optimized system, demonstrating effective methanogenesis without acid accumulation. The consistent pH maintenance (6.8-7.5) throughout digestion and steady daily biogas production confirmed process stability under optimized conditions.

16S rRNA sequencing revealed a critical shift from initial dominance of hydrolytic bacteria (Firmicutes, Bacteroidetes) to enriched methanogenic archaea, particularly *Methanosarcina* species. This selective enrichment under 120 rpm agitation explains the enhanced methane production and process robustness.

These findings provide a comprehensive optimization framework linking substrate composition, reactor design, operational parameters, and microbial dynamics. The optimized process achieved performance comparable to or exceeding previous studies while utilizing only cassava processing waste streams, eliminating the need for external co-substrates. The combination of 1:3 substrate ratio, 120 rpm agitation, and hybrid reactor configuration offers a practical pathway for industrial implementation.

The demonstrated relationship between mixing intensity and methanogenic community structure opens new avenues for process control through targeted microbial selection. Scale-up considerations should focus on maintaining optimal mixing conditions while managing energy inputs. Further research should investigate continuous-flow

systems and techno-economic analysis for full-scale implementation at cassava processing facilities.

These findings contribute to addressing biogas implementation challenges in developing countries (Surendra et al., 2014). This study contributes significant knowledge to sustainable waste management by transforming cassava processing residues—traditionally an environmental burden—into valuable renewable energy, supporting circular economy principles in the cassava starch industry.

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6. Authors' Contributions

Conceptualization: Thaithat Sudsuansee; Methodology: Thaithat Sudsuansee and Supakit Sergsiri; Investigation: Thaithat Sudsuansee and Amin Lawong; Data analysis: Thaithat Sudsuansee and Anucha Sriburam; Writing - original draft: Thaithat Sudsuansee and Warapon Warorot; Writing - review & editing: Thaithat Sudsuansee, Supakit Sergsiri and Warapon Warorot. All authors have read and agreed to the published version of the manuscript.

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7. Conflicts of Interest

The authors declare no conflicts of interest.

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