

Integrated assessment of agro-industrial biomass for sustainable methane production: Physicochemical and nutrient synergies in anaerobic co-digestion

Razia Begum^{1*}, Shaheen Aziz¹, Suhail Ahmed Soomro¹,
Tooba Naveed², Abdul Qadeer Laghari¹ , Abdul Sattar Jatoi³

¹ Department of Chemical Engineering, Mehran University of Engineering and Technology, 76062, Jamshoro, Sindh, Pakistan

² Karachi Laboratories Complex, Pakistan Council of Scientific and Industrial Research, Karachi, Pakistan

³ Department of Chemical Engineering, Dawood University of Engineering and Technology, 75500, Karachi, Sindh, Pakistan

* Corresponding author's e-mail: razia.begum.memon1@gmail.com

ABSTRACT

Anaerobic digestion (AD) is a well-established process for converting organic waste into renewable bioenergy. However, optimizing methane potential remains a challenge due to feedstock variability and process limitations. This study presents a comparative and integrative evaluation of four agro-industrial biomasses: neem deoiled cake (NDC), mango kernel (MK), waste maize flour (WMF), and cow dung (CD) by assessing their physicochemical profiles, calorific values, and essential trace metals. The objective is to investigate substrate synergies in co-digestion systems to maximize methane production. NDC displayed the highest calorific value (5219 kcal/kg) and a favorable C:N ratio (12:1), while CD, despite its lower calorific value (3000 kcal/kg), provided superior buffering and microbial support due to high moisture and moderate nitrogen levels. WMF and MK offered high organic content but required co-substrates for process stability. Essential trace elements such as Fe, Zn, Cu, and Mn were detected in varying concentrations, influencing microbial metabolism and methane potential. The findings underscore the significance of balanced substrate combinations over individual calorific contributions and advocate for nutrient-based feedstock engineering for improved biomethane productivity.

Keywords: anaerobic digestion, co-digestion, biomass, trace metals, methane potential, substrate synergy.

INTRODUCTION

Global shift in energy consumption, driven by fossil fuel depletion, population growth, and rising environmental pollution, has accelerated the search for renewable and sustainable energy alternatives (Parkash et al., 2025; Hussain et al., 2025). Among these renewable or sustainable alternative energy resources, bio-methane is a promising and revolutionary option. The utilization of high calorific value biomass substrates offers significant benefits for biomethane production. Biomethane is a purified form of biogas mainly composed of methane, which has gained significance as an eco-friendly alternative to conventional fossil fuels

(Dębowski et al., 2020; Jarwar, Laghari, et al., 2023). In the process of biomethane, the impact of high-energy biomass substrates has been a pivotal area of research due to the anticipated potential of increasing productivity and efficiency. The calorific value of biomass varies based on the type of biomass being considered. Calorific value is the total amount of energy obtained from a substance. It is denoted in kilojoules per kilogram (kJ/kg) or megajoules per kilogram (MJ/kg), which shows the amount of heat released when a certain amount of biomass is burnt. This value is different based on the substance, the moisture content, variety, or composition (Dębowski et al., 2020). In case of higher moisture, the calorific value is usually

lower, as during combustion, some energy evaporates water (Dragusanu et al., 2022). Sustainable waste management is another global concern that implies the adoption of methods that can generate energy from waste (Esteban-Lustres et al., 2022). Moreover, it calls for capitalizing on energy resources that are cheaper than fossil fuels, from the disposal of lignocellulosic waste while harnessing its energy content (Lee et al., 2020; Olatunji et al., 2023) addressed the challenges associated with lignocellulosic materials like *Arachis hypogea* shells during anaerobic digestion and employed thermal pretreatment before the digestion process. Varying temperatures, such as 90 °C, 100 °C, 110 °C, and 120 °C, were used for the substrate for durations of 30 and 60 minutes. These substrates underwent digestion at mesophilic temperature for 30 days in a batch digester. For physicochemical analyses, scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR) were conducted. The analysis revealed that there was an enhanced porosity with increased temperature and time, while XRD analysis indicated a reduction in the crystalline index, supporting the breakdown of the crystalline structure. Similarly, FTIR analysis depicted the elimination of hemicellulose and lignin. The highest biomethane potential, increased by 23.96%, was observed at 100 °C in 30 minutes. Temperatures exceeding this threshold led to a decreased biomethane yield, ranging between 16% and 44%. The study found that through conventional thermal pretreatment at 100 °C for 30 minutes, there was an optimal improvement in biomethane yield during the anaerobic digestion of *Arachis hypogea* shells, which is viable for commercial use (Olatunji et al., 2023). The effect of using microalgae biomass from *Arthrospira platensis* and *Platymonas subcordiformis* in common agricultural biogas plant feedstock (i.e., cattle manure, maize silage) on methane production was evaluated by Dębowski et al., 2020. Anaerobic biodegradability tests were conducted using respirometry reactors with initial organic loading rates, temperatures, and retention times set at 5.0 kg VS/m³, 35 °C, and 20 days, respectively. A systematic increase in biogas production efficiency was observed as the proportion of microalgae biomass in the feedstock rose from 0% to 40% (%VS). Cattle slurry-based biogas experimental study evaluated the impact of feedstock screening on biogas yield from Napier grass and cattle slurry under mesophilic conditions in batch digesters. According

to the results, the highest methane content was 64.4% and combining Napier grass with common cow farm slurry increased reaction rates, efficiency, and methane content (Souvannasouk et al., 2021). Biomethane, a renewable and sustainable energy source, is produced through anaerobic digestion (AD) of organic materials, offering an environmentally friendly alternative to conventional fossil fuels. As concerns over climate change intensify, the demand for clean energy solutions has surged, and biomethane production has emerged as a promising strategy to reduce greenhouse gas emissions while addressing waste management challenges. Biomethane production primarily involves the breakdown of complex organic substrates by microbial communities under anaerobic conditions, which generates biogas composed predominantly of methane (CH₄) and carbon dioxide (CO₂). Among the factors influencing biomethane production, the type and quality of the feedstock, particularly its calorific value, play a pivotal role (Jarwar, Aziz, et al., 2023).

The calorific value of biomass refers to the amount of energy that can be obtained from its combustion, typically measured in terms of MJ/kg or kcal/kg. Biomass with a higher calorific value generally contains more energy-rich organic compounds, such as lipids, proteins, and complex carbohydrates. These organic components, when subjected to anaerobic digestion, are converted into biogas. High calorific value (HCV) substrates are expected to yield higher amounts of methane per unit mass of feedstock, making them an attractive option for enhancing biomethane production. Several studies examined biomass substrates for the high potential of Biomethane. However, a gap persists in conducting a comprehensive optimization of pretreatment techniques, evaluating long-term stability and efficiency, exploring novel biomass substrates, integrating multiple feedstocks, and conducting rigorous techno-economic and environmental assessments. Enhancing biomethane yields using high-calorific-value biomass substrates presents a promising strategy for improving the efficiency and sustainability of anaerobic digestion processes. By integrating biomass sources with higher energy content, such as agricultural residues, food waste, and energy crops, it is possible to significantly increase methane production while optimizing the utilization of available resources. The careful selection and pretreatment of these substrates can further accelerate biodegradation rates and enhance microbial activity within

anaerobic digesters, leading to more robust and efficient biogas production systems. However, challenges remain in terms of substrate variability, process stability, and optimizing the balance between high calorific value and digestibility. Future research should focus on refining pretreatment technologies, improving microbial consortia, and exploring the synergistic effects of mixed substrates to maximize bio-methane output. As the global demand for renewable energy and waste-to-energy technologies continues to rise, the utilization of high-calorific-value biomass substrates in biomethane production has the potential to play a key role in the transition to a more sustainable and circular bioeconomy. The present study adds to the existing literature on biofuel and bioenergy to address the challenge of accelerating renewable energy resources, as well as highlighting the critical role of advanced bio-based solutions in mitigating environmental challenges while meeting escalating energy demands

MATERIALS AND METHODS

Sample collection

Four biomass substrates were selected for this study due to their high calorific value and local availability. Neem kernel de-oiled cake (NKDC) was sourced from a nearby oil pressing mill, where neem oil was mechanically extracted. Ripe mango seeds were collected from a mango pulp processing unit; the seeds were separated, and the kernels were retained as highlighted in Figure 1. Waste maize flour (WMF), a by-product of flour milling, was obtained from local flour mills. Additionally, fresh cow dung (CD) was collected from a cattle colony to serve as both an inoculum and co-digestion material in the anaerobic digestion process.

Sample preparation

Each substrate underwent specific preparation procedures to ensure uniform particle size and enhanced digestibility. Neem seeds were first de-hulled to extract the kernels, which were then processed in an oil expeller to produce neem kernel de-oiled cake. Mango seeds were manually split to retrieve the kernels, which were air-dried before further processing. The maize flour waste was sieved to eliminate coarse impurities. All substrates were subsequently crushed or ground

using a mechanical grinder and passed through sieves to achieve a consistent particle size suitable for anaerobic digestion trials. The prepared biomass materials were stored in airtight containers at ambient conditions until further use in experimental assays (Chhandama et al., 2022; Mohanty et al., 2022; Roy et al., 2025).

Analysis

To evaluate the biomethane potential of the selected biomass substrates, comprehensive physico-chemical analyses were performed (Chhandama et al., 2022; Mohanty et al., 2022). Parameters such as pH, moisture content, odor, and color were initially assessed to determine the general characteristics and stability of the samples. Key compositional metrics, including total solids (TS), volatile solids (VS), fixed carbon, total carbon, and chemical oxygen demand (COD) were measured to estimate the organic load and degradability of each substrate. The carbon-to-nitrogen (C: N) ratio was also calculated, as it plays a critical role in microbial activity during anaerobic digestion. Additionally, proximate analysis included the determination of moisture and ash content along with total nitrogen concentration. The calorific value of each biomass type was measured to assess its energy potential. Furthermore, essential trace metals such as ferric (Fe), zinc (Zn), copper (Cu), manganese (Mn), and boron (Br) were quantified, as they influence enzymatic functions and microbial metabolism during the anaerobic digestion process.

Physico-chemical characterization

The substrates were analyzed for their basic physical parameters, including moisture content, color, odor, and pH. Moisture content was determined by using the oven-drying method at 105 °C until a constant weight was achieved. Color and odor were recorded through visual and sensory observations, respectively. pH was measured by preparing a 10% slurry of each sample in distilled water and using a calibrated digital pH meter.

Proximate and ultimate analysis

Standard analytical procedures were followed to determine TS, VS, ash, and fixed carbon content. TS and VS were measured using gravimetric analysis as per APHA guidelines. Ash content was

estimated by combusting the sample in a muffle furnace at 550 °C. Fixed carbon was calculated as the residue after subtracting volatile and ash content. Total carbon and total nitrogen contents were measured using a CHNS analyzer. The C:N ratio was calculated accordingly.

Calorific value and COD

The Calorific Value of each substrate was determined using a bomb calorimeter, which provides the energy content in kcal/kg. COD was assessed using the closed reflux titrimetric method (as per APHA standards), which estimates the amount of oxygen required to oxidize organic matter in the substrate.

Essential metal analysis

Concentrations of Zn, Cu, Fe, Mn, and Br were determined using atomic absorption spectroscopy (AAS). For this, samples were digested using nitric-perchloric acid digestion and then analyzed to quantify the essential trace elements crucial for anaerobic digestion.

RESULTS AND DISCUSSION

The comparative evaluation of neem de-oiled cake (NDC), mango kernel (MK), waste maize flour (WMF), and CD for their potential

in anaerobic digestion (AD) revealed significant distinctions in their physicochemical characteristics, elemental profiles, and methane yields. These results offer insight into the individual and synergistic performance of biomass feedstock's for biogas production, highlighting the need for a strategic substrate selection and co-digestion framework.

Physicochemical characteristics of biomass substrates

Initial proximate and ultimate analyses revealed stark contrasts in moisture content, total solids (TS), volatile solids (VS), ash, and fixed carbon content across the four biomass types. WMF exhibited the highest TS content (97.4%), indicating minimal water presence and a highly concentrated organic matter, which is advantageous for increasing the volumetric methane productivity in digesters. NDC and MK also demonstrated high TS values of 94.7% and 91.4%, respectively, while CD had a much lower TS content of 17.22% (Figure 1).

Figure 2 reflects its high moisture content, a common feature in livestock waste. While high TS content is typically desirable, substrates with excessively low moisture can hinder microbial transport and mass transfer within digesters. Thus, WMF and MK may require dilution or co-digestion with wet biomass like CD to maintain optimal hydrolytic and acidogenic phases.

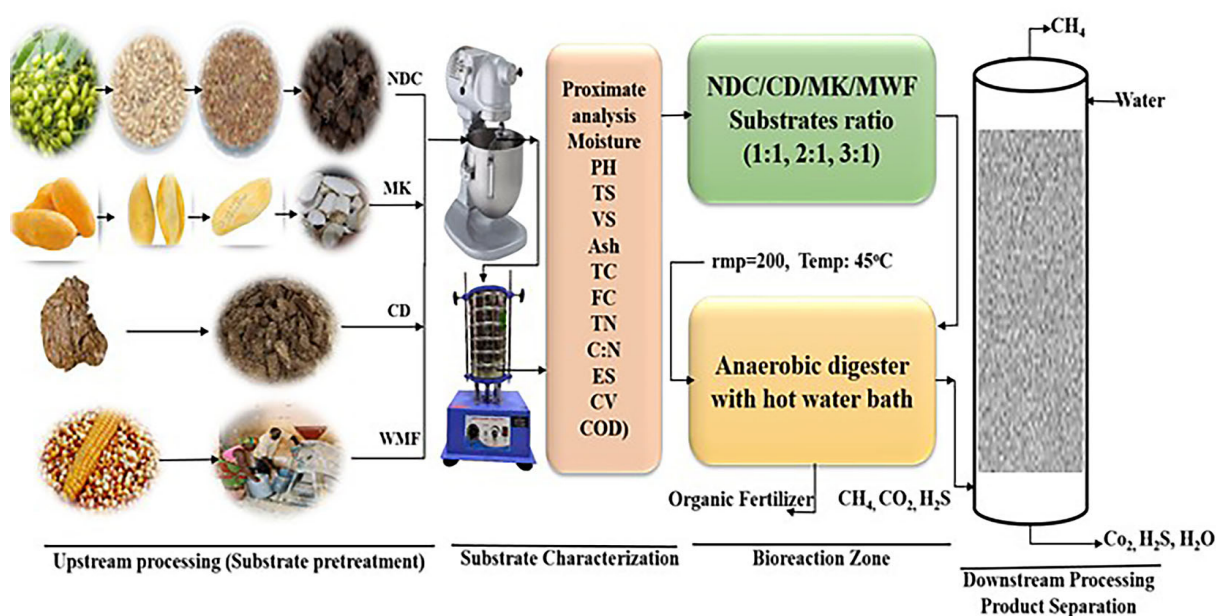


Figure 1. Schematic diagram of bio-methane production using various substrates

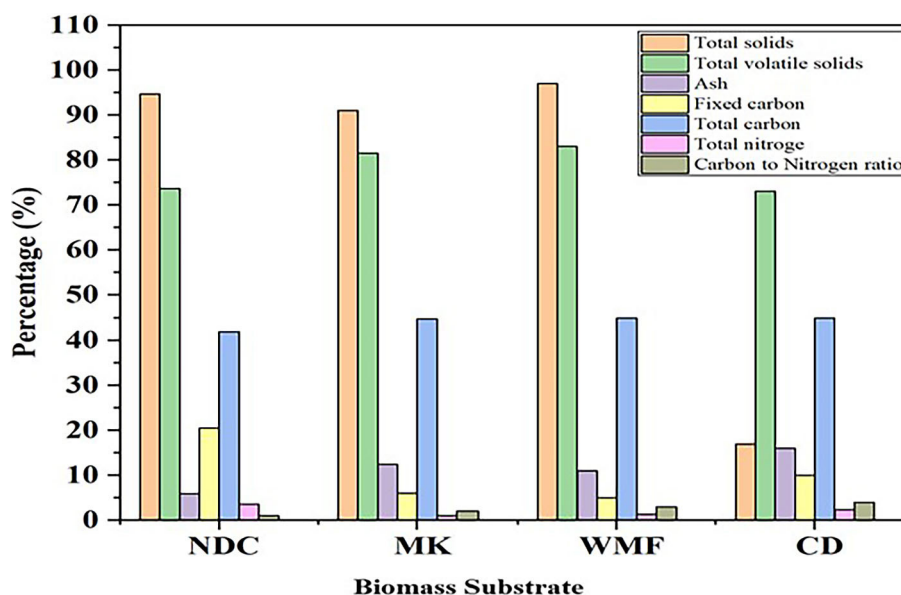


Figure 2. Physicochemical analysis of various biomass substrates

Volatile solids, an indicator of biodegradable organic content, were highest in WMF (83.24%) and MK (81.58%), suggesting their significant potential to contribute to biomethane formation. In contrast, NDC and CD recorded slightly lower VS values (73.6% and 73.61%, respectively), which, though still adequate, point to a relatively lower fraction of digestible matter. The ash content ranged significantly, with CD (16.39%) reflecting substantial inorganic residue, possibly from soil contamination or undigested dietary fibers. NDC had the lowest ash value (5.9%), implying a purer organic load conducive to biogas production and minimal post-digestion waste.

The C:N ratio is a pivotal parameter in anaerobic digestion, influencing microbial balance and methane generation. NDC had a C:N ratio of 12:1, slightly lower than the optimal range (20–30:1) but still indicative of good microbial support. In contrast, MK and WMF displayed higher C:N ratios of 44:1 and 35:1, respectively, which may lead to nitrogen deficiency and slower microbial activity if used alone. CD, with a C:N of 20:1, fell within the optimal range, making it an ideal co-substrate to balance the nitrogen content of carbon-rich feedstocks.

Calorific value and energy potential

The Figure 3 determines AD efficiency; digestibility, nutrient balance, and metal content must also be considered. Calorific values were consistent with the organic composition and fixed

carbon content of the substrates. NDC registered the highest energy content at 5219 kcal/kg, followed by MK (4210 kcal/kg), WMF (3610 kcal/kg), and CD (2500 kcal/kg). This aligns with their volatile and fixed carbon contents and emphasizes NDC's high energy density, making it a strong candidate for bio methane production. However, calorific value alone cannot determine the AD efficiency; digestibility, nutrient balance, and metal content must also be considered.

Trace metal composition

Micronutrients such as iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), and boron (B) are crucial for microbial enzyme function, particularly during methanogenesis. NDC showed remarkably higher concentrations of Fe (640 mg/kg), Zn (57 mg/kg), Cu (16.2 mg/kg), Mn (33.6 mg/kg), and Br (15.82 mg/kg) compared to other substrates (Figure 4). These metals play a central role in the enzymatic systems of methanogens, including ferredoxins and hydrogenases. Their abundance in NDC likely supports robust microbial metabolism, explaining its superior methane yield.

Conversely, MK and WMF were notably deficient in most trace metals. Boron was undetected in both, and Fe levels were relatively low (MK: 12.4 mg/kg, WMF: 46.6 mg/kg). CD, although moderate in Zn (36.8 mg/kg) and Cu (11.7 mg/kg), exhibited very low Fe content (1.2 mg/kg), which is somewhat unusual given its organic origin. These findings suggest that while CD contributes

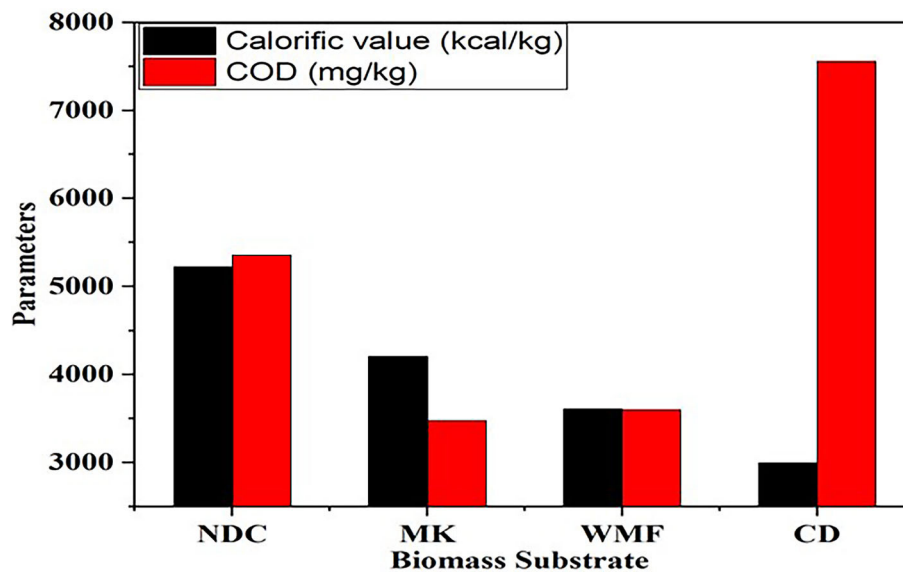


Figure 3. Calorific value and chemical oxygen demand of biomass substrate

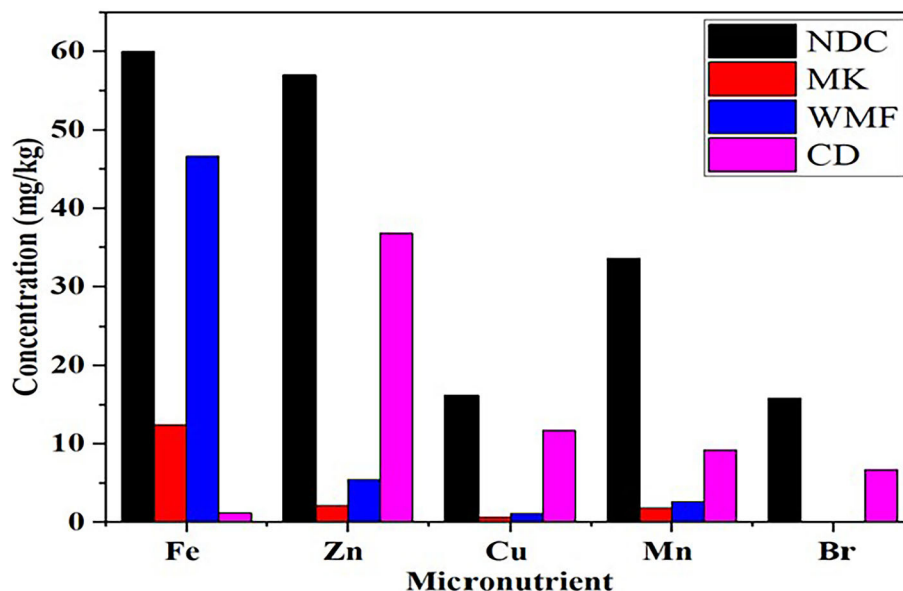


Figure 4. Nutrient concentrations in different biomass substrates

to buffering and microbial inoculation, it may require supplementation for optimal methanogenesis when used as a primary feedstock.

Parametric investigation

Effect of a single substrate on biomethane potential

Methane yield profile on 30 days demonstrates varying levels of biodegradation of Neem Deoiled Cake (NDC), mango kernel (MK), waste maize flour (WMF), and cow dung (CD) destruction with the MK performing the best on sustained

coverage and CD performing the lowest in overall yield, as shown in Figure 5. The start value of NDC, MK and WMF is also relatively high (textsup1620;640 N ml/g VS), showing these soils have been decomposed rapidly and the material easily accessible to the biodegrader, whereas CD starts lower (textsup1460 N mL/g VS), indicating a distinctly high level of fiber content and a low volatile solids composition. In week 1, all substrates experience an acute decrement, which is sharpest in CD and nearly equals ~200 N mL/g VS after day 7, whereas MK, NDC, and WMF exhibit a higher intermediate output. On days 7–15,

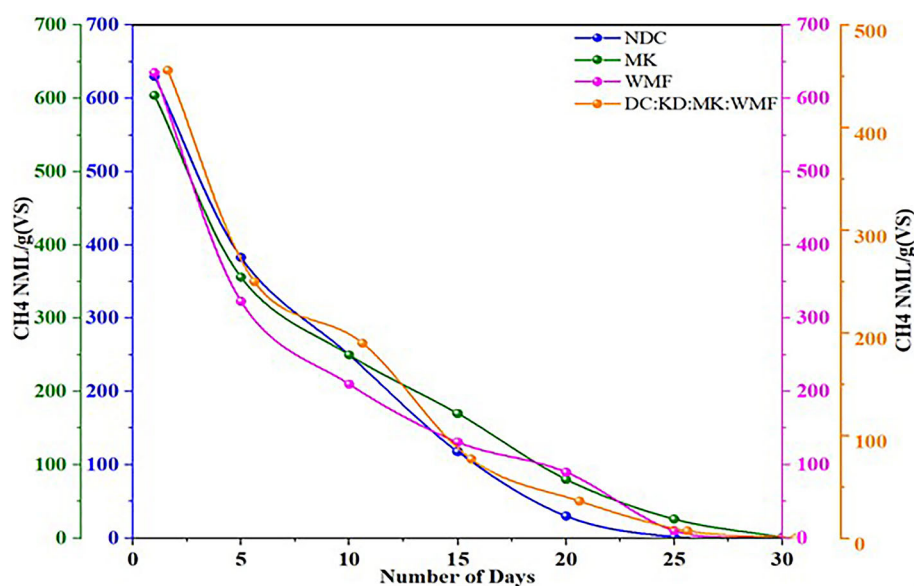


Figure 5. Biomethane potential in single-substrate NDC, MK, WMF, and CD

MK outpolls the others consistently, presumably because it is in a balanced carbohydrate/lipid mixture, creating prolonged methanogenesis, and NDC/WMF exhibits comparable and slightly poorer protracted generations. Biomethane potentials overlap one another by day 20 to low rates, but CD approaches zero faster, suggesting faster depletion of biodegradable matter. The trends indicate MK as the most effective substrate to generate long-term methane, and NDC and WMF as fairly effective ones, whereas CD is the least efficient to produce high-potential biomethane.

Effect of the ratio 1:1 on the substrate for biomethane potential

The methane production patterns of the co-digestion mixes reveal that the addition of NDC with other substrates has a stark effect on the kinetics of biogas production, as highlighted in Figure 6. The NDC:CD blend achieves the peak methane yield (~800 N mL/g VS) the soonest (day 1) and the most dramatic decrease, to almost ~300 N mL/g VS by day 4, and nearly negligible yields by day 20, with the implication that the partly accessible organics are consumed quickly with little ongoing activity. The initial rate of NDC:MK is slightly lower, but subsequently, it remains much higher during the middle phase (5 measures to day 20), showing that mango kernel has a well-balanced nutrient composition and can induce long-lasting methanogens. NDC:WMF has a comparable albeit slightly

lower sustained output than NDC:MK, perhaps because of protein and lipid content differences. Three-substrate mixture (NDC:MK:WMF) begins at a moderate level and falls steeply after day 5 with low yields thereafter, possibly a sign of nutrient imbalance, or greater utilization of digestible pools when three supplements are mixed. In general, it appears that NDC:MK is the most stable and long-lasting methane production potential, and NDC:CD is the one that delivered high initial yields but pathetic performance over an extended period.

Effect of the ratio 2:1 on the substrate for biomethane potential

Figure 7 shows the biomethane potential (BMP) of various mixes of substrates in ratio 2:1 with NDC, MK, WMF, and CD within 29 days of the anaerobic digestion process. Methane production was initially fast in all treatments with NDC:CD peaking highest about day 1 (~850 Nml/gVs), followed by NDC:MK (~740 Nml/gVs), NDC:WMF (~720 Nml/gVs), and NDC:MK:WMF (~730 Nml/gVs). But methane production reduced over time in all treatments as readily degradable organics were used. By day 5 see illustration, gaps between mixtures closed, with NDC:MK and NDC:WMF having relatively higher methane levels, and NDC:CD and NDC:MK:WMF having steeper losses. After day 10, the rate of production of methane decreased significantly as it stabilized at close to zero values

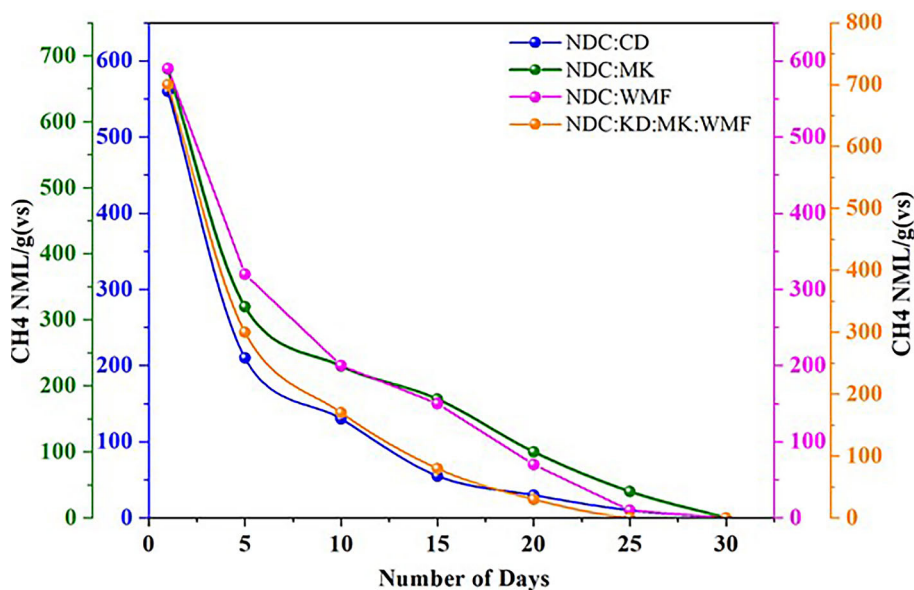


Figure 6. Biomethane potential in 1:1 ratio NDC, MK, WMF, and CD

toward the end of day 25, which represented the exhaustion of substrate and stabilization of microbes. In general, the findings indicated that co-digestion mixtures, e.g. NDC:MK, and NDC:WMF demonstrated more prolonged methane volatilization in comparison to the NDC:CD and ternary mixture, thus pointing to the synergistic impact of the substrate composition on the efficiency of methane generation. This highlights the fact that the embrace of compatible co-substrates is critical towards maximizing the generation of biomethane in anaerobic digester setups

Effect of the ratio 3:1 on substrate for biomethane potential

Figure 8 indicates the biomethane potential (BMP) expressed as CH₄ (Nml/gVS) on various co-digestion mixtures of NDC with MK, WMF, and CD in a ratio of 2:1 after 29 days of digestion. Initially, all mixtures produced methane quickly with NDC:CD showing a higher initial peak (~880 Nml/gVS) than either NDC:MK (~750 Nml/gVS), NDC:WMF (~740 Nml/gVS), or NDC:MK:WMF (~680 Nml/gVS). Nevertheless, a sharp drop in the production of methane was noted during the first week as labile organic part was consumed. NDC:MK continued to exhibit methane values that were significantly higher than other mixtures after day 5, indicative of improved biodegradability of the substrates and synergy. In contrast, NDC:CD declined significantly beyond day 3, demonstrating that

readily accessible substrates were quickly used up, and there could be an inhibitory outcome. NDC:WMF exhibited a moderate trend, whereas the ternary mixture (NDC:MK:WMF) displayed high, probably as a result of an uneven distribution of nutrients and competition in the microbial consortia. In addition to day 10, methane production in all treatments slowed down, and by days 25 to 29, it resembled zero, indicating digestion has proceeded. In general, the findings point out that although NDC:CD has the best initial methane peak, NDC:MK displayed better biomethane release over the long term, thus a more promising co-digestion associated with stable energy yields.

Biomethane potential in mono and co-digestion trials

Anaerobic digestion trials under mesophilic conditions revealed that methane potential closely followed the patterns observed in the physicochemical and trace metal analyses. NDC achieved the highest methane potential among the mono-digestion setups at 659 Nml CH₄/g VS, a direct result of its high energy density, balanced C:N ratio, and rich trace element content. MK and WMF achieved respectable potentials of 625 and 635 Nml CH₄/g VS, respectively, but nutrient imbalances and lower metal content limited their performance. CD, as expected, had the potential the lowest methane volume at 250 Nml CH₄/g VS, which, although modest, was improved in

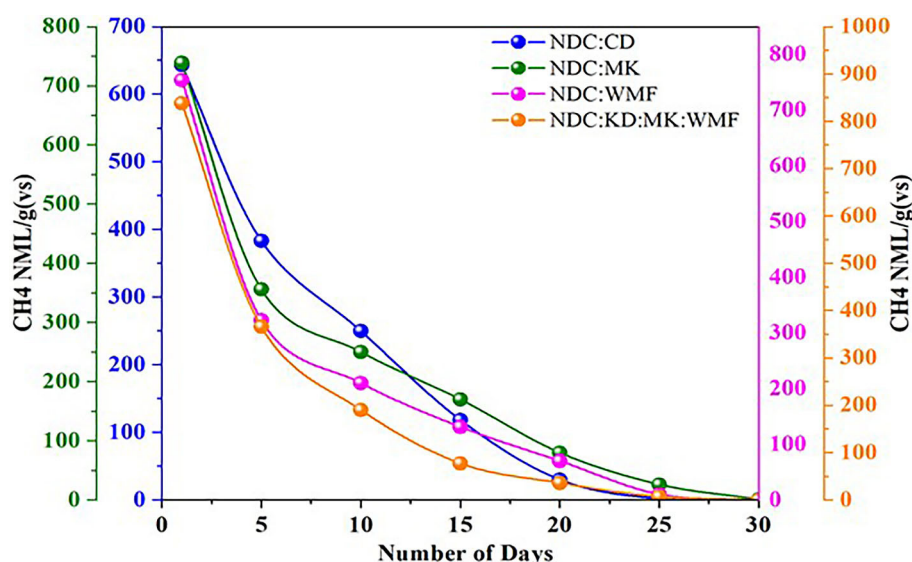


Figure 7. Biomethane potential in 2:1 ratio NDC, MK, WMF, and CD

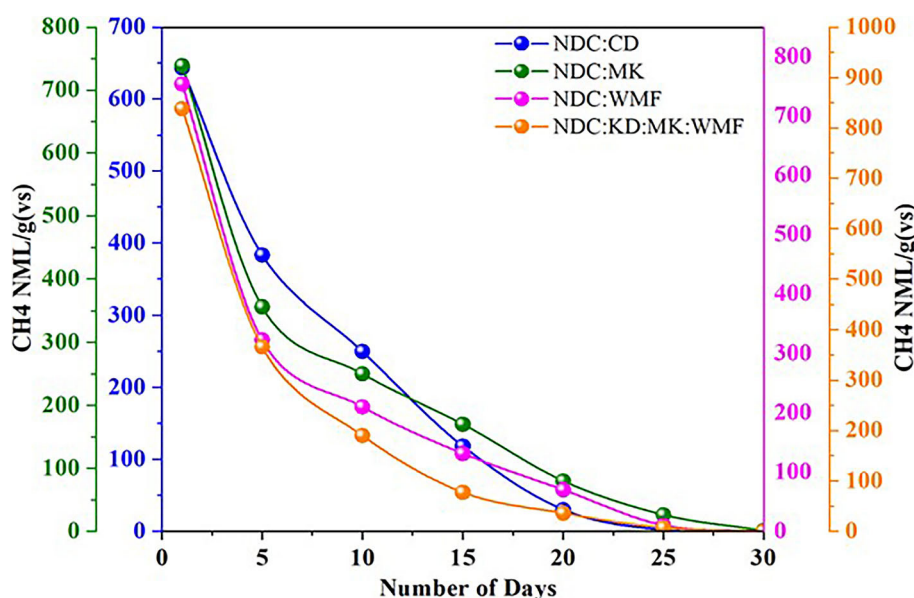


Figure 8. Biomethane potential in 3:1 ratio NDC, MK, WMF, and CD

co-digestion scenarios.in (Figure 9). Notably, when NDC was co-digested with CD in a 1:1 VS ratio, the methane potential increased to 659 Nml CH₄/g VS. This synergistic improvement highlights the benefits of -balancing CD's buffering and microbial load, complementing NDC's energy and metal profile, creating an optimal environment for methanogenesis. A similar improvement was observed in the WMF + CD co-digestion setup, where methane potential rose from 635 to 650 Nml CH₄/g VS. These enhancements confirm the hypothesis that co-digestion promotes higher methane productivity by compensating for the deficiencies of individual substrates.

Process stability and microbial considerations

The integrated assessment of agro-industrial biomass substrates – NDC, MK, WMF, and CD for anaerobic digestion reveals significant insights into their individual and synergistic performance in sustainable methane production in Table 1. Beginning with the physicochemical attributes, NDC demonstrated high total and volatile solids (TS and VS), a balanced carbon-to-nitrogen (C:N) ratio (~25), and stable pH behavior during digestion, which align well with optimal methanogenic conditions. This explains its superior mono-digestion methane yield of Nml871 CH₄/g

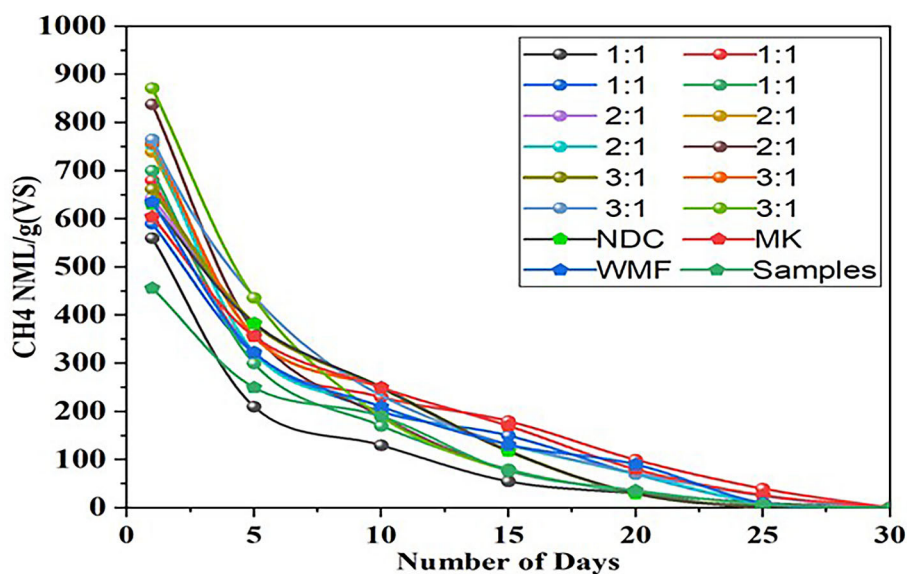


Figure 9. Trial experiments for biomethane production in mono and co-digestion

VS. In contrast, MK and WMF, though moderate in TS and VS, exhibited notably high C:N ratios (>30), indicative of carbon-rich substrates that often experience process imbalances due to nitrogen limitations. The experimental analysis of the biomethane potential of NDC co-digested with CD, MK, and WMF at different mixing ratios indicates the well-defined trends in volatile solids (VS), calorific values, methane potential, and methane enrichment of the co-substrates in the optimum utilization of anaerobic digestion of biomethane. At the initial 1:1 NDC:CD ratio, the final VS concentration was 6.36 percent with 19.08 g VS added, which translates to a methane potential of 659 Nml/g VS and methane purity of 80.5 percent, which indicates stable digestion at low yield of other substrates. Re substitution of CD with MK at 1:1 raised the VS content to 9.61 percent with 28.92 g VS, elevating methane yield to 713 Nml/g VS and methane concentration to 81.3 percent, suggesting that starch and lipid-rich MK supplied more readily available carbon than did CD. Likewise, NDC with WMF ratioed 1:1 also enhanced performance with VS of 9.71 and 29.13 g total VS that produced 716 Nml/g VS methane and methanogenesis biomethane 82.2 and calorific value 5150 kcal/kg confirming the synergistic effect of WMF and its high biodegradability. When the three substrates were used in equal proportions (NDC:MK:WMF, 1:1:1), the VS increased to 12.67%, the methane yield rose substantially to 807 Nml/Ml vs, and the methane purity rose to 85.4, taking its calorific value to

5190 kcal/kg. This showed a high level of synergism since the mixture maintained an appropriate C:N ratio, besides a balanced nutrient input that favoured microbial syntrophy. Higher organic loading of NDC to a ratio of 2:1 with CD brought the VS to 9.32% and 27.98 g added, increasing methane yield to 665 Nml/g VS and content to 81.6% a little more than the 1:1 mixture. At the 2:1 ratio, the VS increased to 12.58 percent and 37.76 g added, the methane production was 739 Nml/g VS, methane purity was 82, and the calorific value was 5120 kcal/kg, showing that MK was more effective than CD in promoting digestibility. Similarly, NDC 2:1 WMF gave 12.36% VS and 37.08 g added, 725 Nml/g VS methane production, and 80.3% methane content, again a little lower than MK but better than CD. Even greater synergetic effect of the above triple mixture mixture of 2:1:1 led to an equalisation of stability and VS of 15.91% and 47.74 g added, yielding 838 Nml/g VS of methane besides 89 per cent of biomethane, a calorific value of 5275 kcal/kg, significantly better than the binary mixtures as well as demonstrating that a diverse combination shows better stability as well as methane-richness. With higher NDC proportions of 3:1, the NDC:CD mixture tested 12.24% VS, 36.81 g added to accomplish 680 Nml/g VS and 85% methane content, an improvement over 1:1 CD mixture but lower than the output of MK and WMF mixtures, where methane purity was higher. At a 3:1 ratio of MK, VS rose to 18.47% and 55.41 g was added with methane yield of 750 Nml/g VS and

methane content of 82% stabilizing that higher NDC loading with MK could indeed increase the yields but not necessarily the methane percentage above moderate levels. The NDC:WMF blend of 3:1 had equally uniform results, with 15.59 percent VS, 46.79 grams added, methane production of 765 Nml/g VS, and purity of 81 percent, showing that again methane production continues to be increased, but now the methane enrichment stays at a plateau. The most remarkable result was obtained with a tri-substrate mixture of 3:1:1, which showed the highest VS at 18.79 percent and 56.57 grams added, the highest calorific value at 5325 kcal/kg, and the highest methane potential at 871 Nml/g VS with 92 percent methane, which was the best score in the research. This shows that ternary mixtures not only offer better levels of degradable organic content but also offer the best conditions of microbial diversity, hydrolysis, acidogenesis, and methanogenesis, thus limiting the inhibitory effects of NDC on recalcitrant components. A comparison across the dataset showed several significant numerical trends: methane yields of CD-based mixtures were smaller than those of MK (713 to 750 Nml/g VS) and WMF (716 to 765 Nml/g VS) mixtures; tri-substrate mixtures yield consistently more methane than binary ones, which ranged between 807 and 871 Nml/g VS. Similarly, the least percent methane enrichment was in CD based systems (80.5–85%), moderate in MK and WMF mixtures (81–82.2%) and highest in ternary systems (85.4–92%). As these numerical comparisons indicate, the cow dung has the virtue of stabilizing digestion, but that MK and WMF contribute greatly to improving the yield and mixing all three substrates is best modulated in terms of yield against quality. What is more, the calorific values were within the interval between 5000 and 5325 kcal/kg, which envisages a high uniformity of energy in mixtures. Still, the highest energy content matched the best methane yield in the NDC: MK: WMF (3:1:1) system. It is clear that the significant increase in the performance of the mixtures rather than the additions of single substrates indicates the synergistic effect and not simple combinations of materials drives the superior results; e.g., the 1:1:1 (807 Nml/g VS, 85.4% CH₄), 2:1:1 (838 Nml/g VS, 89% CH₄), and 3:1:1 (871 Nml/g VS 92% CH₄) achievements in the mixtures comfort The experimental results, therefore, confirm that NDC can be digested in single- substrate a system, resulting in below-optimal digestion, co-digestion

with CD gains the advantage of stability but also low yields. In contrast, MK and WMF gain an efficiency advantage through improvements in both yield and quality, and tri-substrate formulations outperform all others in terms of efficiency, with potential uses in large-scale production and up-grading of biomethane.

In summary, the integrated Table 1. encapsulates the complex interdependencies among physicochemical composition, nutrient profile, and microbial ecology in determining the efficiency and stability of anaerobic digestion processes. While substrates like NDC demonstrate high intrinsic potential due to favorable C:N ratios and micronutrient density, their performance is further amplified when paired with substrates like CD that compensate for buffering and microbial deficiencies.

Comparative analysis of biomethane potential from different substrates

The BMP of experimental samples with currently used NDC, MK, WMF, and CD without husk is presented in a comparative Table 2 relative to the values later reported in studies with the usage of lignocellulosic co-substrates with husk. Methane yields obtained in the authors' study, viz. 659 Nml/gVS (NDC:CD, 1:1) to 871 Nml/gVS (NDC:MK:WMF, 3:1:1) are much higher than those reported in the literature, which in general lie below 400 Nml/gVS. An example is a 1:1 ratio of NDC:CD that yielded 659 Nml/gVS, nearly twice the methane emission of cow manure inoculated with fruit and vegetable waste (380 Nml/gVS; Callaghan et al., 2012). Likewise, n-decanoyl-choline:methanol:water mixture (NDC:MK:WMF) was ternary co-digested at 1:1:1 to get 807 Nml/gVS compared with cow manure amended with barley straw (4:1) that produced 160 Nml/gVS (Hills, 2015). Further increment in NDC proportion (2:1:1) increased the methane yield up to 838 Nml/gVS, which is more than tenfold greater than dairy manure and switch grass (207.8 Nml/gVS; Labatut et al., 2018). The resulting yield was maximum with the 3:1:1 ratio (871 Nml/gVS) performing better than buffalo manure with maize silage (358 Nml/gVS; Sesposito et al., 2020).

These findings are a clear indication that the biodegradability and energy potential of co-digestion mixtures, based on NDC are highly relative to lignocellulosic husk-rich feedstocks.

Table 1. Integrated assessment of agro-industrial biomass for anaerobic digestion

Reactor (substrate combination)	VS ratio (substrate: inoculum)	Final VS in Mix (%)	Total VS added (g)	Calorific value (kcal/kg)	Volume (substrate + water)	Methane potential (Nml/g VS)	Biomethane (%) CH ₄
NDC : CD (1:1)	1:1	6.36 ± 1.74	19.08 ± 1.04	5000 ± 11.84	300 mL	659 ± 4.50	80.5 ± 1.89
NDC : MK (1:1)	1:1	9.61 ± 1.35	28.92 ± 1.60	5100 ± 13.00	300 mL	713 ± 2.08	81.3 ± 1.25
NDC : WMF (1:1)	1:1	9.71 ± 1.00	29.13 ± 1.06	5150 ± 20.81	300 mL	716 ± 2.51	82.2 ± 1.21
NDC : MK : WMF (1:1:1)	1:1	12.67 ± 1.47	38.03 ± 1.01	5190 ± 10.00	300 mL	807 ± 3.60	85.4 ± 1.28
NDC : CD (2:1)	1:1	9.32 ± 1.25	27.98 ± 1.52	5050 ± 11.26	300 mL	665 ± 3.51	81.6 ± 1.92
NDC : MK (2:1)	1:1	12.58 ± 1.84	37.76 ± 1.15	5120 ± 10.01	300 mL	739 ± 3.05	82.2 ± 1.21
NDC : WMF (2:1)	1:1	12.36 ± 1.18	37.08 ± 1.57	5165 ± 11.01	300 mL	725 ± 3.00	80.3 ± 1.47
NDC : MK : WMF (2:1:1)	1:1	15.91 ± 1.37	47.74 ± 1.38	5275 ± 10.53	300 mL	838 ± 4.00	89.0 ± 1.52
NDC : CD (3:1)	1:1	12.24 ± 1.42	36.81 ± 1.10	5065 ± 12.50	300 mL	680 ± 2.08	85.0 ± 1.25
NDC : MK (3:1)	1:1	18.47 ± 1.80	55.41 ± 1.14	5135 ± 14.73	300 mL	750 ± 1.00	82.0 ± 1.04
NDC : WMF (3:1)	1:1	15.59 ± 1.36	46.79 ± 1.04	5190 ± 12.05	300 mL	765 ± 1.52	81.0 ± 2.00
NDC : MK : WMF (3:1:1)	1:1	18.79 ± 1.01	56.57 ± 1.20	5325 ± 10.50	300 mL	871 ± 2.08	92.0 ± 1.01

Unavailability of husk probably reduced recalcitrant lignin predisposing it to microbial stress and methane conversion efficiency. Furthermore, Table 2 explores the WMF and MK, as high starch and lipid substrates, which are good candidates for readily fermentable carbon sources that ferment synergistically with anaerobic consortia. However, literature studies of husk-containing substrates might have biases based on the structural rigidity of husk and a slow hydrolysis rate. In this way, the current results indicate that husk-free co-digestion of NDC with MK and WMF has the potential to enhance biomethane production as well as provide a more sustainable and efficient approach to producing bioenergy than standard manure lignocellulosic feedstock systems. all deficiencies. CD, despite its own limitations in energy content and trace metals like Fe, plays a pivotal role in stabilizing and optimizing digestion conditions, making it an ideal co-substrate. MK and WMF, though less promising in standalone digestion, respond positively to co-digestion strategies by leveraging CD's buffering and microbial assets. These findings strongly advocate for a tailored co-digestion approach in biogas projects, particularly in agricultural and agro-industrial contexts, where feedstock heterogeneity is common. Strategic blending based on complementary physicochemical and biological traits not only boosts methane productivity but also ensures operational resilience and sustainability.

The data reinforces the notion that successful anaerobic digestion is not solely about selecting high-yielding substrates but about orchestrating a balance of energy, nutrients, buffering agents, and microbial consortia to create a stable and efficient bioconversion system.

Future perspectives

Future perspectives on biomethane generation from agro-industrial residues such as NDC, MK, WMF, and CD focus on enhancing efficiency, scalability, and integration within circular bioeconomy models to meet rising energy and sustainability demands. Optimized feedstock combinations and pretreatment strategies (alkali treatment, steam explosion, enzymatic hydrolysis, microbial inoculation) can improve biodegradability and methane yields. Advances in metagenomics and synthetic biology may enable engineered microbial consortia to degrade lignin, hemicellulose, and proteins better, enhancing overall conversion. On the engineering side, approaches like two-stage digestion, high-solid anaerobic systems, and integration with biohydrogen or algal cultivation can create multi-product biorefineries while valorizing digestate as fertilizer or algal feed. Simulation and machine learning can support dynamic co-digestion ratios, minimizing inhibition from high-protein or lipid feedstocks. Digital monitoring tools such as IoT

Table 2. Comparison of previous work vs current work

Sample	Ratio	CH ₄ NML/g(vs)	References
NDC:CD (without husk)	1:1	659	Current study
NDC:MK:WMF (without husk)	1:1:1	807	
NDC:MKWMF (without husk)	2:1:1	838	
NDC:MK:WMF (without husk)	3:1:1	871	
Cow manure : fruit and vegetable waste (with husk)	4:1	380	(Sánchez Nocete and Pérez Rodríguez, 2022)
Cow manure : barley straw (with husk)	4:1	160	
Dairy manure : switch grass (with husk)	3:1	207.8	
Buffalo manure : maize slage (with husk)	3:1	358	

and AI-driven control systems will further stabilize yields under variable conditions. Large-scale deployment requires sustainability assessments of life-cycle emissions, energy balances, and soil impacts. Policy incentives, decentralized biogas models, and carbon credits will be vital for adoption in rural economies where residues are abundant but underutilized. Aligning NDC-based biomethane with the UN Sustainable Development Goals, clean energy, responsible production, and climate action, can maximize global impact. Finally, integrating biomethane into hybrid systems (solar, wind, bio-CNG) and coupling with CO₂ utilization technologies can diversify its applications, while cooperative models, skill development, and financing mechanisms will ensure socio-economic benefits and commercial feasibility.

CONCLUSIONS

The integrated assessment of agro-industrial biomass substrates for anaerobic digestion demonstrates that substrate selection and strategic co-digestion significantly influence methane yield, process stability, and nutrient balance. Among the mono-digested substrates, NDC exhibited the highest methane potential at 629 Nml CH₄/g VS, attributed to its high volatile solids, balanced C:N ratio (~25:1), and rich micronutrient content—Fe: 640 mg/kg, Zn: 57 mg/kg, Cu: 16.2 mg/kg, Mn: 33.6 mg/kg, B: 15.82 mg/kg. In contrast, MK and WMF biomethane potential 750 and 765 Nml CH₄/g VS, respectively, due to poor micronutrient profiles (Fe: 12.4–46.6 mg/kg; B: not detected) and high C:N ratios (>30), which resulted in process acidification risks. CD alone generated the lowest methane yield (250 Nml CH₄/g VS) due to low energy density and exceptionally low Fe content (1.2 mg/kg), but it excelled in buffering capacity

and microbial diversity. When co-digested, the CD enhanced system stability and biomethane output significantly. The NDC + CD combination achieved the highest methane yield at 680 Nml CH₄/g VS, reflecting a 13.5% improvement over NDC alone. Similarly, WMF + CD yielded 635 Nml CH₄/g VS, a 15% increase over WMF mono-digestion. These results confirm that co-digestion of energy-rich substrates with biologically active, buffer-rich co-substrates like CD can effectively mitigate nutritional and operational constraints. By leveraging complementary feedstock properties, co-digestion enhances microbial synergy, stabilizes pH within the methanogenic optimum (6.8–7.4), and maximizes methane output. This study underscores the importance of integrating physicochemical, micronutrient, and microbial assessments in feedstock selection for sustainable and efficient biogas production systems.

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