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A Review of the Impact of Co-Digestion Substrates on the Methane Yield

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ABSTRACT

This review highlights the impact of anaerobic co-digestion (ACD) on improving energy recovery from biogas production systems. Various factors from selected papers were reviewed to figure out their influence on ACD performance. Such factors include Carbon/Nitrogen (C/N) ratio, biodegradability of feedstock, microbial diversity, activity, buffering capacity, and trace element concentrations. Findings show ACD significantly enhances process stability and increases methane yield by 20% to 65% compared to mono-digestion. The process shares more insights on mechanisms for addressing environmental pollution challenges as it offers alternative approaches for reducing greenhouse gas emissions. Despite promising achievements in ACD systems, several limitations of the process still exist, requiring the attention of future studies to explore the full potential of technology. Specific areas include optimizing the mixing ratio of substrates to prevent acidification and ammonia toxicity risks that may occur during the process, hence affecting the system efficiency. Research should focus on process design and proper feedstock selection, considering innovative approaches such as bioaugmentation, supplementation with carbon compounds and nanoparticles, to improve microbial activity, process efficiency, and stability. Also, there is a need to develop predictive models that will accurately incorporate C/N ratio effects on digestion kinetics and nutrient transformation. Current models are complex, which hinders their scalability; thus, the use of machine learning could enhance model accuracy.

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1. Introduction

Anaerobic digestion (AD) is an important technology for producing a renewable energy source called biogas and addressing significant environmental challenges. Produced biogas is primarily composed of methane as its main energy-dense product. The characteristics and composition of the feedstock play critical roles in determining the production of methane as well as the working stability of biogas reactors (Czubaszek et al., 2022). The process further strengthens energy security, lowers greenhouse gas emissions, and offers practical approaches for a circular bioeconomy (Alengebawy et al., 2024). Mono-digestion, single feedstock type anaerobic digestion (AD), is widely used due to the simplicity of the process, stable supply chain, and simplicity of process control. However, the process is hampered by several limitations, including nutrient imbalance, low buffering capacity, and susceptibility to inhibitory compounds. Inhibitory, compounds produce excessive volatile fatty acids (VFAs), which reduce pH, and eventually affect both methane production and reactor stability (Ren et al., 2018; Wei et al., 2023). Without a buffering agent, pH reduction leads to process failure or reactor upset (Manyi-Loh et al., 2013). Due to an imbalance in nutrients and inhibition, mono-digestion is less likely to yield more methane than co-digestion systems (Xu et al., 2023).

The literature reports mono-digestion of several substrates, their respective effects on the system, and methane production efficiency. This includes mono-digestion of food waste, which generates volatile fatty acids rapidly, leading to a drop in pH that creates a toxic environment to methanogenic archaea, the microorganisms responsible for producing methane. Mono-digestion of meat waste, which has high protein, may generate ammonia toxicity by causing deamination of proteins (Yenigün & Demirel, 2013). Fat-rich substrates may lead to an accumulation of long-chain fatty acids (LCFAs), which coat microbial cells and interfere with substrate transport, thus slowing down microbial processes. Once a mono-substrate is used, the system lacks the complementary components to counteract the inhibitory effects, thereby exposing the reactor to the likelihood of instability (Ryue et al., 2020). A monosubstrate system supports a less variable microbial population dedicated to specific feedstock (Mignogna et al., 2023). This makes the system less resistant to environmental fluctuations, substrate alteration, and shock loading, which compromises the long-term sustainability of the process (Nazir et al., 2023; Sun et al., 2025). Most mono-substrate digestion operates outside the optimal C/N ratio boundary of 20 to 30, leading to slow microbial activity, low methane yield (Figure 1), and poor process stability (Paranjpe et al., 2023). Such substrates include food waste, which has high carbon but low nitrogen contents (Salangsang et al., 2022), and animal manure contains high amounts of nitrogen, leading to ammonia formation when digested individually (De Moura Zanine et al., 2015). Moreover, when high biogas-potential substrates are applied, the lack of synergistic effects through multi-substrate systems decreases the overall energy recovery (Saha et al., 2020). Table 1 presents different substrates employed in mono-digestion systems along with their methane yields and potential limitations.

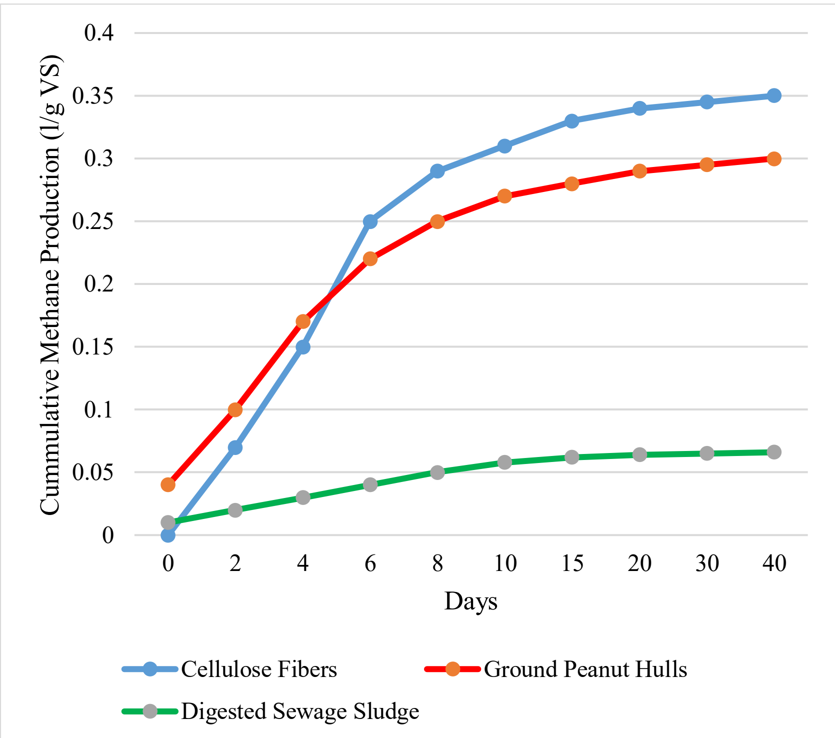

Figure 1: Cumulative Methane Production from Anaerobic Digestion of A Single Substrate (Hamilton, 2016)

Table 1
Different Substrates Used in Mono-Digestion Systems, With Their Methane Yields and Challenges

Substrate	Methane Yield	Key Challenges	Source
Food waste	~450 mL CH_4/g of Volatile Solids (VS)	VFA accumulation, pH drops.	(Joolaei et al., 2025)
Cow dung	~200 mL CH_4/g VS	Low degradability, potential ammonia.	(Veerabadhran et al., 2021)
Poultry manure	300–350 mL CH_4/g VS	Ammonia toxicity.	(Hosseini et al., 2025)
Rice straw	100–150 mL CH_4/g VS	Poor C/N, low degradability.	(López-Escobar et al., 2024)
Slaughterhouse waste	~600 mL CH_4/g VS	LCFA inhibition, ammonia.	(Rhee et al., 2024)
Sewage sludge	150–250 mL CH_4/g VS	Low biodegradability, long Hydraulic Retention Time (HRT).	(Hosseini et al., 2025)

The discussed inherent limitations of anaerobic mono-digestion have triggered recent studies to focus much of their investigations on anaerobic co-digestion of multiple organic substrates. Studies on co-digestion demonstrate significant enhancement of methane yield, digestibility of feedstock, process stability, and balanced nutrient value (Kadam et al., 2024; Karki et al., 2021). Co-digestion promotes diverse microbial consortia that contribute to a superior methane yield of up to 65% compared to mono-digestion (Rabii et al., 2019). According to Rajaonison et al. (2020), anaerobic co-digestion provides a more friendly mechanism for waste management, making it an attractive alternative for fossil fuel dependence. Figure 2 illustrates the significant improvement achieved when multiple substrates are digested together compared to mono-digestion.

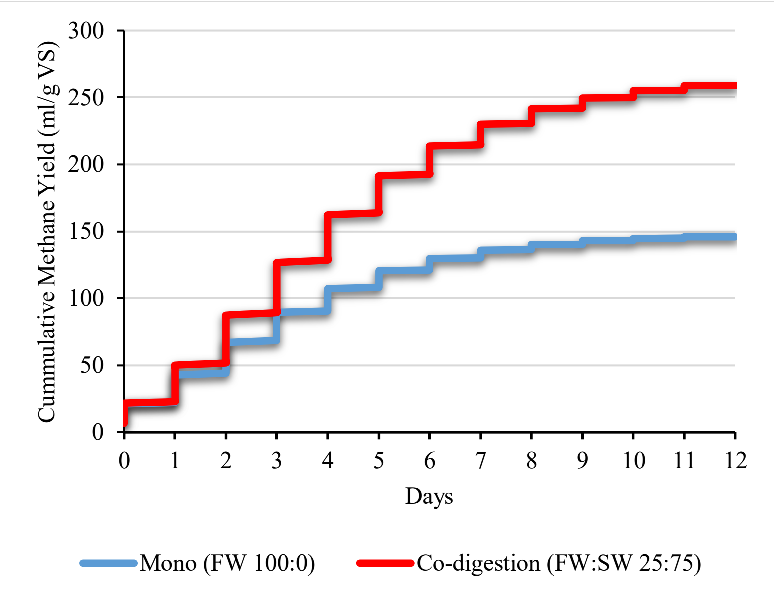

Figure 2: A Comparison of Methane Yield from Anaerobic Mono-Digestion of Food Waste Alone (FW, 100%) Versus Co-Digestion of Food Waste With Swine Wastewater (SW) Under A Thermostatic Bath At A Constant 35 ± 2 °C (Sousa et al., 2024)

The limitations of mono-digestion and the significant advantages of anaerobic co-digestion suggested the importance of this review. This manuscript provides a thorough discussion of works published from 2013 to 2025 to enrich the understanding of both advantages and limitations of anaerobic co-digestion, and thus, identifies areas that require further studies to enhance the usability of the technology on a commercial scale. Papers published in various indexed journals using keywords like anaerobic digestion, co-digestion were selected. This manuscript is organized as follows: Firstly, we discussed the concept of anaerobic digestion, the disadvantages and limitations of mono anaerobic digestion, and the potential advantages of anaerobic co-digestion. Secondly, the manuscript discusses anaerobic co-digestion, its various factors that influence the process, and their respective limitations. This is followed by the prospects and challenges of co-digestion, and finally, the conclusion.

2. Anaerobic Co-Digestion Systems

Co-digestion of two or more substrates is superior to mono-digestion in ensuring maximum methane production and stability of the process (Cardona et al., 2019). It is rooted in synergistic relationships between feedstock types, which mitigate the shortcomings of individual substrates. According to Mudzanani et al. (2022), co-digestion of different substrates may improve methane production by 20 – 50% compared to mono-digestion (Hamzah et al., 2022). Co-digestion gains are largely because of complementarity among substrate characteristics, e.g., carbon-to-nitrogen (C/N) ratio, biodegradability, and trace element concentration (Osman et al., 2023). Enhancing methane production through co-digestion can be achieved through different approaches, such as C/N ratio optimization, improving biodegradability, buffering capacity, and pH stability, enhancing trace elements and micronutrients concentration, etc.

2.1. Carbon/Nitrogen Ratio Optimization

C/N ratio is an important parameter of anaerobic digestion that regulates microbial growth and metabolic efficiency. A properly balanced C/N ratio delivers sufficient nitrogen for microbial protein synthesis without excessive ammonia production (Qian et al., 2025). High C/N ratios lead to nitrogen deficiency, which lowers microbial activity and development (Hashim et al., 2022). When it is excessively high (over 35:1) results in ineffective substrate decomposition, slowing down microbial reproduction that leads to lower methane output (Raja Ram & Nikhil, 2022). Low C/N ratios may cause ammonia accumulation, which is harmful to methanogens (Samadi et al., 2022). Ammonia (NH_3) is produced when the C/N ratio is too low (below 15:1). The microbial ecosystem is upset by ammonia buildup, which results in acidification and the suppression of methane synthesis (Bhusal, 2024).

Table 2
The Effects of Co-Digestion with Low-Ammonia Substrates with High-Ammonia Substrates

Substrates	Methane yield increase	Ammonia mitigation	C/N Ratio improved	Challenges	Source
Manure + food waste.	35%	~40% Total Ammonia Nitrogen (TAN) reduction.	Improved to ~25	Risk of acidification.	(Li et al., 2024)
Poultry litter + veg waste.	50%	~45% TAN reduction.	From 8 to 22	Hydrolysis limitations.	(Hanum et al., 2022)
Swine manure + corn straw.	~38%	~30% TAN reduction.	From 10 to 25	Pretreatment needed.	(Du et al., 2023)
Sludge + sugar beet pulp.	46%	Ammonia + metal toxicity reduced.	N/A	Sugar acidification risk.	(Adghim et al., 2021)

Co-digestion, which balances the carbon and nitrogen content of various substrates, is the most efficient method of optimizing the C/N ratio. It facilitates the optimization of C/N ratios by blending carbon-based and nitrogen-based substrates, such as food waste with animal manure or crop residues. Thus, it enhances methane yields and reduces the risk of inhibition by preventing ammonia toxicity and nitrogen (Xu et al., 2023). A balanced C/N ratio enhances the growth and metabolism of the microbial community in the reactor (Zhang et al., 2022). Table 2 shows the effect of co-digestion for materials with low and high ammonia substrates. In addition, Table 3 and Table 4 present materials with high and low content of carbon and nitrogen, and their respective potential upon being co-digested.

Table 3
Suggested Substrate Mixes for the Best C/N Ratio

Carbon-Rich Substrates (C/N > 30)	Nitrogen-Rich Substrates (C/N < 15)	Source
Straw, Sawdust, Crop residues.	Animal manure, slaughterhouse waste.	(Reichel et al., 2018; Tumusiime et al., 2022)
Fruit waste, Food waste.	Wastewater sludge, poultry manure.	(Agrawal et al., 2024; Mapenzauswa et al., 2024)
Paper waste, Sugarcane bagasse.	Fish waste, Dairy manure	(Ghaleb et al., 2021; Vivekanand et al., 2018)

Table 4
The Effects of Co-Digesting Fruit and Vegetable Waste with Nitrogen-Rich Substrates

Co-Substrates	Methane Yield (mL CH_4/g VS)	Improvement	Findings	Challenges	Source
Fruit & vegetable waste + wastewater sludge.	~420 mL CH_4/g VS	~50% increase over mono-digestion of sludge	Fruit/vegetable waste improved the C/N ratio to ~25–30.	High moisture content of fruit and vegetable waste reduced solid retention time (SRT).	(Fonoll et al., 2015)
Fruit waste + dairy manure.	~390 mL CH_4/g VS	~45% increase over fruit waste mono-digestion.	Co-digestion prevented rapid VFA accumulation and pH drop.	Risk of over-acidification without manure buffering.	(Mlaik et al., 2024)
Vegetable waste + slaughterhouse wastewater.	~430 mL CH_4/g VS	~55% over mono digestion of vegetable waste.	Combined nitrogen-rich wastewater balanced the C/N ratio.	Risk of foam formation at high protein levels.	(Mozhiarasi et al., 2023)
Fruit & vegetable waste (FVW)+ poultry manure.	~460 mL CH_4/g VS	~60% higher than FVW alone.	Poultry manure corrected the low C/N ratio of fruit and vegetable waste.	Poultry manure produces ammonia and odor, requiring OLR control.	(Bres et al., 2018)
Vegetable waste + sewage sludge.	~400 mL CH_4/g VS	~40–50% higher than mono-digestion	Vegetable waste enhanced biodegradability and methane production.	High degradability of vegetables led to excess gas production, requiring gas flow management.	(Di Maria et al., 2015)

Despite realizing significant methane yield in the co-digestion by optimizing the C/N ratio, challenges presented in Tables 2 and 4 suggest that proper mixing of substrates is necessary to achieve an optimal C/N ratio. Future studies should therefore focus on how the effect of the C/N ratio interacts with other parameters such as organic loading rate, temperature change, and hydraulic retention time (HRT). Predictive models should also accurately incorporate C/N ratio effects on digestion kinetics, as most current models prioritize methane yield (Yang et al., 2025).

2.2. Enhancing Biodegradability

In anaerobic digestion (AD), biodegradability and microbial activity are essential components for optimizing methane production. Biodegradability is not the same in biogas feedstock. Some, like lignocellulosic biomass (e.g., straw), are slowly degraded due to the lignin content, while others, like cheese whey or kitchen waste, are easily biodegradable but may lead to acidification when digested alone (Papirio et al., 2020). Co-digestion increases the degradability of the feedstock mix (Molinuevo-Salces et al., 2013), provides a continuous feed of degradable organics, which sustains continuous microbial activity and gas generation (Zhao et al., 2024). It further helps in synchronizing methanogenesis, acidogenesis, and hydrolysis, reducing the lag between process steps (Wang et al., 2023). Degradation may be partial in mono-digestion, especially when lignocellulosic biomass or substrates with imbalanced carbon-to-nitrogen (C/N) ratios are used (Bher et al., 2022). Therefore, enhancing biodegradability in a co-digestion process compounded with increased microbial activity plays an important role in improving the efficiency of methane production. These synergies increase methane yield by up to 20 to 65% compared to mono-digestion. For instance, methane output increases from around 230 mL/g for mono-digestion to 320–330 mL/g for co-digestion when cow dung is digested with food or fruit waste. This is due to improved organic matter breakdown and microbial energy conversion (Harirchi et al., 2025).

It can be challenging to break down some organic materials, such as waste sludge and lignocellulosic biomass. Pretreatment techniques can improve methane output by increasing biodegradability (Olatunji et al., 2021). For instance, enhanced hydrolysis and microbial accessibility resulted in a 65% increase in methane generation from thermal and enzymatic pretreatment of lignocellulosic waste (Poddar et al., 2021). Co-digestion of lignocellulosic biomass with food waste, particularly which is high in proteins and carbohydrates, also significantly improves its biodegradability (Chen et al., 2023). Table 5 demonstrates how pretreatment enhances microbial access and biodegradability.

Table 5
Enhancement of Microbial Access and Biodegradability via Pretreatment

Substrates	Pretreatment Type	Methane Yield Increase	Co-Digestion Role	Challenges	Source
Wheat Straw + Food Waste.	Thermal (120°C)	65%	stimulates hydrolysis and balances the substrate	Energy input	(Zafar et al., 2022)
Rice Straw + Manure.	Enzymatic	60–65%	Enhances enzymatic breakdown	Enzyme cost	(Ferdeş et al., 2020)
Corn Stover + Kitchen Waste.	Alkali + Thermal	~58%	Improves solubility and microbial access	Chemical handling	(Donkor et al., 2022)
Sewage Sludge + Fruit Waste.	Ultrasonic	45–55%	Boosts disintegration and balance	High energy	(Ruiz Espinoza et al., 2022)
Bagasse + MSW.	Steam Explosion	~62%	Increases biodegradability, stabilizes C/N	Mechanical clogging	(Karthikeyan et al., 2024)

Biodegradability significantly improves methane production; however, there is still insufficient understanding of how the biodegradability of one substrate affects the other during the co-digestion process, hence requiring the attention of future studies. Moreover, limited understanding exists of how biodegradability changes over time due to the storage and aging of feedstock.

2.3. Enhancing Reactor pH

For effective methanogens' performance, pH in the reactor must remain close to neutral (6.8 - 7.4), which is ideal for acidogenic bacteria and methanogens. Single substrates frequently have insufficient buffering capacity, leading to volatile fatty acids that contribute to pH drops (Qiu et al., 2023). Through co-digestion, alkalinity is increased by mixing acidic substrates with those that produce bicarbonates or ammonia, such as protein-rich manure (Yellezuome et al., 2022). This prevents acidification conditions, which in mono-digestion systems frequently result in reactor failure (Ibro et al., 2022). This also could be done by diluting inhibitors such as ammonia, long-chain fatty acids, or volatile fatty acids to prevent an excess of harmful substances that would otherwise limit microbial enzymes from functioning (Guo et al., 2021).

Studies on the role of pH during co-digestion have made substantial progress, but for further optimization of the process, future research could investigate how real-time control systems using sensors and automated feedback may monitor pH in the reactor, since most current studies rely on batch or periodic assessments.

2.4. Enhancing Trace Elements Concentration

Trace elements like iron, nickel, cobalt, and selenium are of critical importance to microorganisms engaged in anaerobic digestion (Šafarič et al., 2020). One or more vital micronutrients are frequently absent from mono-substrates. Through nutrient profile diversification, essential trace elements can be achieved by co-digesting different feedstocks, which encourages the development of a more varied microbial community, enhancing the system's resistance to shocks and variability (Lv et al., 2022). This lowers operating costs by eliminating the need for external trace element supplementation (Bardi et al., 2023). Trace elements and nutrients provided by co-substrates such as manure or industrial sludge further promote enzymatic and microbial processes, reduce inhibition, and maintain microbial consortia active and stable (J. Yang et al., 2024). Despite the discussed achievement, understanding of the impact of trace elements during co-digestion requires future studies to investigate how various digestion stages are affected by trace elements during the full lifecycle of AD with co-digestion substrates.

2.5. Enhancing Microbial Diversity and Activity

Anaerobic digestion-related microorganisms flourish in a variety of settings with a wide range of food sources (Wang et al., 2022). Combining many substrates makes microbial communities more resilient, flexible, and effective in decomposing complex organic material (Blair et al., 2021). The anaerobic digestion environment is improved by co-digestion in a number of ways, such as nutrient balance, microbial diversity, pH and buffering capacity, toxicity reduction, and enzyme activation and synergism (Shah et al., 2015). These approaches directly boost microbial efficiency as they support a wider variety of organic substrates, such as proteins, lipids, and complex carbohydrates. For example, co-digesting food waste with manure enhanced the hydrolysis rate by 35% and the methane output by 50% by increasing the activity of microbial enzymes (Wang et al., 2018). A co-digestion of sewage sludge with different materials and agricultural leftovers demonstrates an increase of up to 45% in hydrolytic and methanogenic activity, which raises methane yield by 30-60% (Neri et al., 2023). Sewage sludge promotes system resilience, while food waste increases the biodegradable organic content. Table 6 demonstrates the impact of different substrates on microbial activity.

Table 6
Impact of Co-Digestion on Microbial Activity

Co-Substrates	Microbial Activity Improvement	Key Findings	Challenges	Source
Sewage sludge + corn stalks.	~30%	Increased cellulase/hemicellulose activity	Pretreatment required	(Li & Huang, 2024)
Food waste + dairy manure.	~35%	Cellulase, Protease	VFA accumulation	(Abbas et al., 2023)
Sewage sludge + wheat straw.	25–35%	Boosted lignocellulosic degradation	Mixing difficulty	(Al-Da'asen et al., 2024)
Kitchen waste + swine. Manure.	~40%	Cellulase, Protease	Ammonia inhibition risk	(L. Zhang et al., 2019)
Sewage sludge + sugar beet pulp.	~40%	Fast hydrolysis; enhanced acetolactic methanogenesis	Acidification risk	(Borowski & Kucner, 2019)
Veg waste + cattle dung.	~30%	Endogenous Enzymes	Substrate inconsistency	(D'Silva et al., 2022)
Sewage sludge + food waste.	~45%	More amylase/protease activity	VFA accumulation	(Latha et al., 2019)
Sewage sludge + olive mill wastewater.	~30%	Enhanced lipid breakdown; more lipase activity	Polyphenol toxicity	(Al bkoor Alrawashdeh, 2019)
Food waste + cow manure.	~38%	Amylase, Cellulase	Temperature control	(Bi et al., 2020)

Despite a significant impact on microbial activity realized through anaerobic co-digestion, several issues require the attention of future research. Such issues include a limited understanding of how different substrate combinations dynamically influence microbial succession (Ferdeș et al., 2023). Also, there is limited research targeted at microbial inocula, such as microbial consortia tailored to specific substrate mixtures or operating conditions (Bhatia et al., 2024). Moreover, there is limited data on how operating parameters such as HRT, organic loading rate, temperature, etc., affect microbial dynamics during co-digestion (Zhang et al., 2023).

2.6. Enhancement of Methane Yield through Substrate Synergies

Combining various substrates with complementary qualities during co-digestion frequently produces synergistic effects that improve methane generation and process stability (Eliasson et al., 2023). This is facilitated by improved nutritional balance, increased microbial activity, and optimized biochemical conditions (Zhang et al., 2023). Methane generation and microbial metabolism are improved by the distinct nutrition and degrading properties that various substrates offer. Complex polymers (cellulose, hemicellulose, and lignin) found in lignocellulosic materials like maize stover, straw, and wood chips are challenging for microbes to decompose (Bhatia et al., 2024). Unless they are pretreated (e.g., thermal, chemical, or enzymatic), these materials often have lower methane yields than simpler organic substrates.

Certain substrates might accumulate inhibitory substances like ammonia, volatile fatty acids, or long-chain fatty acids as a result of mono-digestion. Through diluting these inhibitors, co-digestion avoids process failure (Astals et al., 2021). An ideal balance, e.g., of lipids, proteins, and carbs can be achieved by co-digesting food waste with manure or lignocellulosic biomass (Mutungwazi et al., 2020). Compared to mono-digestion, combining manure, which is heavy in nitrogen, with food waste, which is high in carbs, boosts methane generation up to 40% (Table 7). This enhances substrate synergies since manure prevents ammonia inhibition by assisting in the regulation of the carbon-to-nitrogen (C/N) ratio (Pradeshwaran et al., 2024). Also, a co-digestion of dairy manure with lipid-rich food waste reduces (Table 8) ammonia inhibition and significantly increases methane production (Abomohra et al., 2022). Lipids increase energy content, and manure offers buffering capacity against acidity, leading to a methane increase of 35% (Egwu et al., 2021).

Table 7

The Effect of Combining Manure with Food Waste on Methane Production

Substrate	Methane Yield (Mono-digestion)	Methane Yield (Co-digestion)	Improvement	Challenges	Source
Food waste + cattle manure.	(Food Waste): ~310 mL CH_4/g VS	390–430 mL CH_4/g VS	~35% increase	Initial foaming required OLR control	(Baek et al., 2020)
Food waste + pig manure.	(Food Waste): ~320 mL CH_4/g VS	~450 mL CH_4/g VS	~40% increase	Ammonia accumulation, careful feedstock proportioning.	(Dennehy et al., 2016)
Food waste + poultry manure.	(Poultry Manure): ~350 mL CH_4/g VS	~460 mL CH_4/g VS	~30% higher yield	High risk of ammonia toxicity, periodic monitoring of free ammonia and pH.	(Chuenchart et al., 2020)
Food waste + dairy manure.	(Dairy Manure): ~220 mL CH_4/g VS	~400 mL CH_4/g VS	~80% higher than dairy manure alone	Settling and scum formation require agitation and periodic maintenance	(Jasinska et al., 2022)
Food waste + slaughterhouse waste.	(Food Waste): ~380 mL CH_4/g VS	~520 mL CH_4/g VS	~37% increase	High LCFA (Long-Chain Fatty Acid)	(Sánchez et al., 2021)

Co-digestion of several organic wastes increases methane output by improving substrate biodegradability and microbial diversity. Due to microbial synergy and increased hydrolysis efficiency, co-digesting food waste (high biodegradability) with lignocellulosic biomass (low biodegradability) increases methane output by 38% (Zhou et al., 2021). The effects of co-digesting food waste and lignocellulosic biomass are displayed in Table 9. A combination of various substrates and their respective impact on methane yield, as presented in Tables 2 to 9, is graphically demonstrated in Figure 3.

Significant impacts have been achieved through substrate synergies during anaerobic co-digestion. However, for optimal operations to enhance methane yield, several issues still need attention in future research. For instance, specific microbial pathways and interactions leading to synergy are not well understood. Long-term studies that consider changes in synergy over time are lacking, as most current assessments focus on batch experiments. Moreover, it is unclear how operating parameters such as temperature, HRT, loading organic rate, and pH affect synergy outcomes.

Table 8
Co-Digestion of Different Organic Wastes with Dairy Manure

Co-Substrates	Methane Yield (mL CH_4/g VS)	Improvement	Findings	Challenges	Source
Dairy manure + waste cooking oil.	430mL CH_4/g VS	~40% increase over manure alone	Lipids provided high-energy content; Manure offered buffering capacity.	At higher oil loading rates, long-chain fatty acids accumulated, inhibiting methanogenesis.	(Nogueira et al., 2019)
Dairy manure + cheese whey.	365mL CH_4/g VS	~30% increase	Cheese whey accelerates hydrolysis and acidogenesis.	High whey ratios caused acidification and VFA buildup.	(Casallas-Ojeda et al., 2024)
Dairy Manure + Slaughterhouse waste.	~500mL CH_4/g VS	~45–50% increase	Rich in lipids and proteins—high methane potential.	Risk of foam formation and scum layer due to fats.	(Chou & Su, 2019)
Dairy manure + grease trap waste.	450mL CH_4/g VS	~35–40%	Grease trap waste is highly energy-rich.	LCFA accumulation and poor mixing due to floating grease.	(Shakouri far et al., 2020)
Dairy manure + bakery waste.	390mL CH_4/g VS	~33%	Bakery waste was highly biodegradable, boosting hydrolytic activity.	Risk of rapid acidification if bakery waste is overused.	(Ebner et al., 2016)
Dairy manure + fish waste.	~480mL CH_4/g VS	~38–42%	Fish waste added methane-rich material.	Risk of ammonia toxicity	(Erraji et al., 2025)

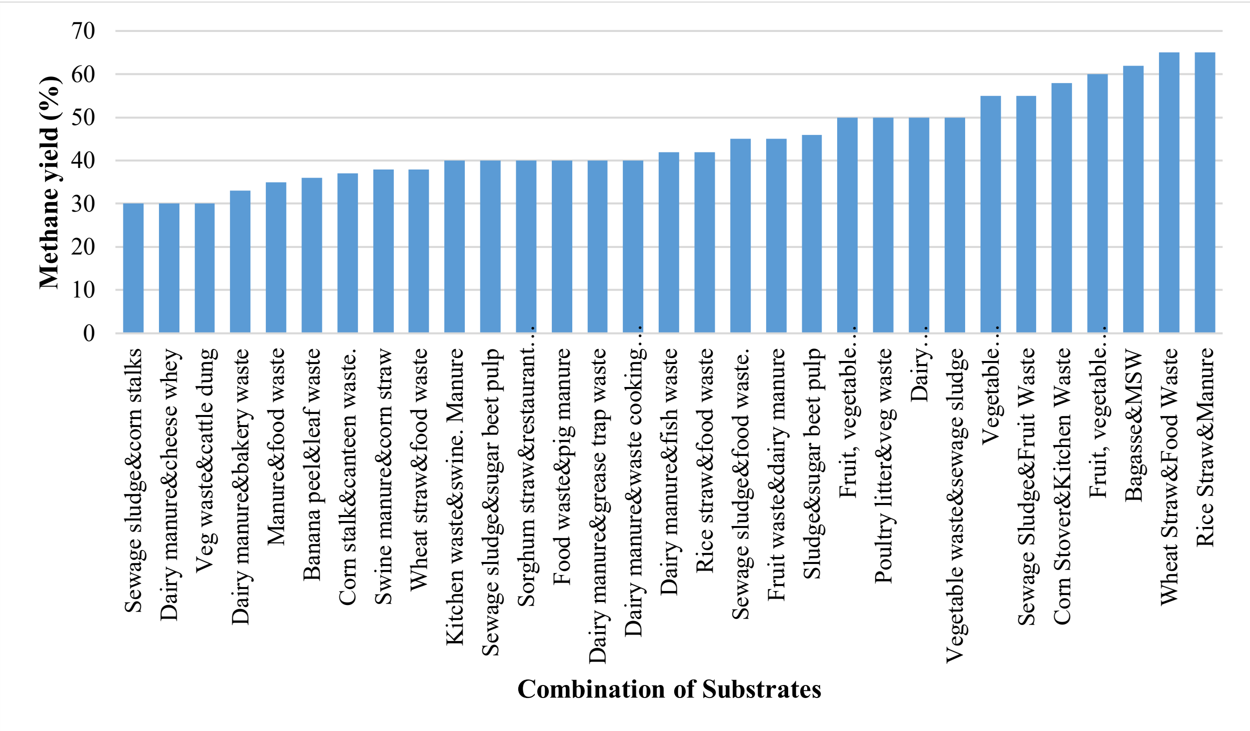

Figure 3: Impact of Co-digestion of Various Substrates on Methane Yield.

Table 9
The Effects of Co-Digesting Food Waste and Lignocellulosic Biomass

Substrates	Methane Yield Increase	Key Benefits	Challenges	Source
Wheat straw + food waste.	38%	Enhanced hydrolysis, microbial synergy.	Risk of acidification.	(Liu et al., 2025)
Corn stalk + canteen waste.	37%	Cellulolytic activation.	Pretreatment needed.	(Khaita et al., 2024)
Rice straw + food waste.	38–42%	Optimized C/N ratio, enzymatic boost.	Straw structure limits.	(Mohammed et al., 2024)
Banana peel + leaf waste.	~36%	Enzyme synergy (cellulase, protease).	Moisture imbalance.	(Ngabala & Emmanuel, 2024)
Sorghum straw + restaurant waste.	40%	Energy balance, microbial diversity.	LCFA inhibition risk.	(Nizzy et al., 2024)

2.7. Improved Volatile Solids (VS) Degradation Efficiency

The output of methane is also directly related to the breakdown of volatile solids (VS). More organic matter is turned into biogas when the degradation efficiency is higher (Atelge et al., 2018). When cow dung, crop residues, and food waste are co-digested, they may increase the elimination of volatile solids by 20–35%, leading to a greater methane output of up to 39% compared to mono-digestion (Leca et al., 2023). Table 10 illustrates the degradation efficiency of volatile solids.

Table 10
The Effect of Higher Degradation Efficiency of Volatile Solids (VS)

Co-Substrates	Methane Yield (mL/g VS)	VS Removal	Key Benefits	Challenges/ Limitations	Source
Food Waste + Cow Dung + Rice Straw.	315	~30%	Improved C/N, structured matrix.	Straw pretreatment needed	(Tamilselvan & Immanuel Selwynraj, 2024)
Veg Waste + Cow Manure + Wheat Straw.	328	~33%	Balanced degradability, stable microbes.	Mass transfer issues.	(Hasan et al., 2024)
Food Waste + Corn Stover + Cattle Dung.	310–330	25–35%	Synergistic hydrolysis.	VFA inhibition risk.	(Ihoeghian et al., 2022)
Food Waste + Bagasse + Cow Dung.	325	~28%	pH and nutrient stabilization.	Low digestibility of bagasse.	(Oladejo et al., 2020)
Canteen Waste + Paddy Straw + Buffalo Dung.	318	~32%	Avoids acid spikes, stable methane.	Variability in food waste, straw prep.	(Rahman et al., 2023)

To enhance understanding of the degradability of VS, future studies should develop predictive kinetic models that link volatile solids degradation rates, microbial population dynamics, and methane yield. Future research could also develop automated control systems to adjust loading rates or mixing based on volatile solids feedback (Nguyen et al., 2015).

2.8. The Impact of Co-Digestion Substrates on Operational Stability of Biogas Reactors

In order to maximize methane output and guarantee process stability, it is crucial to research how adding different co-digestion substrates affects the operational stability of biogas reactors (Rocha-Meneses et al., 2022). Co-digestion's primary advantages are its capacity to improve microbial diversity, balance nutritional ratios, and lessen any inhibition brought on by certain substrate components (Farghali et al., 2024). Choosing the right co-digestion substrates is essential to preserving the biogas reactor's stability (Shi et al., 2022). The organic content, C/N ratio, and biodegradability of the substrates must all be complementary to one another. When chosen carefully, co-digestion can lessen problems such as high volatile fatty acid concentrations, ammonia toxicity, and acid buildup (Liu et al., 2023).

Resolving the inherent limits of each substrate type through synergistic interactions, co-digesting organic waste and agricultural waste greatly increases the operational stability of biogas reactors (Prasanna Kumar et al., 2024). Crop residues, straw, and husks are examples of agricultural waste that are lignocellulosic and are low in nutrients, whereas food trash, kitchen scraps, and fruit and vegetable waste are examples of organic waste that are highly degradable but may cause acidification (Gupta et al., 2022).

Since organic trash is rich in nitrogen (C/N ratio < 15), while agricultural waste has a high carbon content (C/N > 40), their combination improves microbial efficiency and biochemical balance by bringing the C/N ratio to the range of 20 to 30 (Zahan et al., 2018). This ensures that there is enough nitrogen for microbial growth and prevents ammonia toxicity due to excess nitrogen, resulting in a stable digestive environment (Beniche et al., 2021).

Operational stability of a biogas digester through co-digestion of different substrates could be achieved through other mechanisms, including: balancing the reactor pH by moderating acid accumulation (Chen et al., 2023), improving microbial activity and synergy that increases resistance to harmful substances and stress loading (Harindintwali et al., 2020). Dilution of inhibitory substrates through co-digestion with fibrous agricultural wastes improves reactor operation in the long term as it reduces volatile fatty acid buildup and microbial inhibition (Sheets et al., 2015). Table 11 presents some of the approaches that could be adopted to improve the operational stability of the reactor.

Table 11
Effects Of Different Co-Digestion Substrates on the Operational Stability of Biogas Reactors

Co-Digestion Substrates	Impact on Operational Stability	Source
Manure + food waste.	Improved reactor stability by lowering ammonia toxicity, balancing the C/N ratio, and decreasing VFA formation.	(Zhang et al., 2023)
Sewage sludge + food waste.	Improved stability by reducing ammonia inhibition, improving nutritional balance, and increasing methane production.	(Liang et al., 2021)
Used cooking oil + food waste.	Stabilised reactor conditions by preventing fat formation and improving lipid decomposition, ensuring ongoing microbial activity.	(Farghali et al., 2023)
Corn stover + food waste.	Improved reactor stability as a result of microbial breakdown of lignocellulosic material, which prevents acidification and VFA formation.	(Li et al., 2013)
Pig manure + food waste.	Prevented acidification and ammonia toxicity; co-digestion maintained a constant pH and lowered VFA levels.	(Xu et al., 2020)
Grass silage + food waste.	Improved stability by balancing the C/N ratio, lowering ammonia toxicity, and increasing microbial diversity.	(Himanshu et al., 2018)

Literature is rich in information regarding the influence of co-digestion substrates on the operational stability of bioreactors. However, several gaps in addressing the optimization of biogas yield could not be left unhighlighted. For example, the link between microbial diversity, resilience, and operational stress under limiting conditions such as shock loading, pH drops is still not clear, necessitating further investigations. In addition, most current studies are based on lab-scale systems, requiring testing at a large-scale setup.

3. Future Prospects and Challenges of Co-Digestion on the Methane Yield

Apart from enhancing methane yield, anaerobic co-digestion proves to have a significant impact on waste management policy makers. It offers an alternative to landfilling or incineration of organic wastes, thus reducing greenhouse gas emissions and supporting decarbonization goals (Alengebawy et al., 2024). The technology also presents an additional economic value of organic waste as it produces renewable energy and natural fertilizer that reduces reliance on synthetic fertilizer (Wang et al., 2024; X. Yang, M. N. Shafiq, R. Nazir, et al., 2024; X. Yang, M. N. Shafiq, A. Sharif, et al., 2024). Despite having several promising results, research gaps to further enhance anaerobic co-digestion still exist. Potential research areas for consideration include:

i) Tailored substrate blending: Future study could aim to optimize substrate combinations based on nutrient profiles, biodegradability, and synergistic effects. Precision co-digestion methods can be achieved by utilizing tools such as machine learning and simulation models.
ii) Inclusion of emerging waste streams: Agricultural leftovers, algae, and even industrial by-products are gaining popularity because of their high organic content and ability to increase methane production (Kaur et al., 2023).
iii) Further studies are required on pretreatment technology, such as emulsification, to improve lipid dispersion in the reactor (Eftaxias et al., 2021). Reports suggest that challenges with scum and foam formation from fats, particularly when mixing is inadequate, exist, requiring cautious feedstock ratio control to prevent lipid buildup (Raj & Ramamurthy, 2024).
iv) There is still insufficient understanding of how the biodegradability of one substrate affects the other during the co-digestion process, and how it changes over time due to storage and aging of feedstock.
v) Investigations are further required on how different substrate combinations dynamically influence microbial succession, on microbial inoculate tailored to specific substrate mixtures or operating conditions. Further studies are required to assess the link between microbial diversity, resilience, and operational stress under limiting conditions such as shock loading, pH drops. Future studies should also develop models that integrate substrate characteristics, reactor design, and microbial behavior to predict operational stability.
vi) Development of kinetic models that link volatile solids degradation rates with microbial population dynamics is necessary to predict methane yield. Moreover, the use of predictive models could work best if they accurately incorporate the C/N ratio effects on digestion kinetics and nutrient transformation.
vii) Current models are complex, hindering their scalability, and they focus most on methane yield. Machine learning offers opportunities for enhancing model accuracy and real-time optimization.

Table 12 demonstrates a summary of some potential substrate combinations and their respective impact on methane yield and limitations.

Table 12
Potential co-digestion of substrates, and their respective limitations and impact on methane yield

Substrates	Methane yield (%)	Advantages/Significances	Limitations	Source
Sewage sludge & corn stalks	30	Increased cellulase/hemicellulose activity	Pretreatment required	(Li & Huang, 2024)
Dairy manure & cheese whey	30	Cheese whey accelerates hydrolysis and acidogenesis.	High whey ratios caused acidification and VFA buildup.	(Casallas-Ojeda et al., 2024)

Veg waste & cattle dung	30	Endogenous Enzymes	Substrate inconsistency	(D'Silva et al., 2022)
Dairy manure & bakery waste	33	Bakery waste was highly biodegradable, boosting hydrolytic activity	Risk of rapid acidification if bakery waste is overused	(Ebner et al., 2016)
Manure & food waste	35	Improved to C/N ratio ~25, mitigating ~40% Total Ammonia Nitrogen (TAN)	Risk of acidification	(Li et al., 2024)
Banana peel & leaf waste	36	Enzyme synergy (cellulase, protease).	Moisture imbalance	(Ngabala & Emmanuel, 2024)
Corn stalk & canteen waste.	37	Cellulolytic activation	Pretreatment needed	(Khaita et al., 2024)
Swine manure & corn straw	38	Improved C/N ratio from 10 to 25, Ammonia mitigation ~30% TAN reduction	Pretreatment needed.	(Hanum et al., 2022)
Wheat straw & food waste	38	Enhanced hydrolysis, microbial synergy	Risk of acidification	(Liu et al., 2025)
Kitchen waste & swine. Manure	40	Cellulase, Protease	Ammonia inhibition risk	(Zhang et al., 2019)
Sewage sludge & sugar beet pulp	40	Fast hydrolysis; enhanced acetolactic methanogenesis	Acidification risk	(Borowski & Kucner, 2019)
Sorghum straw & restaurant waste	40	Energy balance, microbial diversity	LCFA inhibition risk	(Nizzy et al., 2024)
Food waste & pig manure	40		Ammonia accumulation, careful feedstock proportioning	(Dennehy et al., 2016)
Dairy manure & grease trap waste	40	Grease trap waste is highly energy-rich	LCFA accumulation and poor mixing due to floating grease	(Shakourifar et al., 2020)
Dairy manure & waste cooking oil	40	Lipids provided high-energy content; Manure offered buffering capacity.	At higher oil loading rates, long-chain fatty acids accumulated, inhibiting methanogenesis	(Nogueira et al., 2019)
Dairy manure& fish waste	42	Fish waste added methane-rich material	Risk of ammonia toxicity	(Erraji et al., 2025)
Rice straw & food waste	42	Optimized C/N ratio, enzymatic boost	Straw structure limits	(Mohammed et al., 2024)
Sewage sludge & food waste	45	More amylase/protease activity	VFA accumulation	(Latha et al., 2019)
Fruit waste & dairy manure	45	Co-digestion prevented rapid VFA accumulation and pH drop	Risk of over-acidification without manure buffering	(Mlaik et al., 2024)
Sludge & sugar beet pulp	46	Ammonia mitigation: Ammonia + metal toxicity reduced	Sugar acidification risk	(Adghim et al., 2021)

Fruit, vegetable waste, and wastewater sludge	50	Fruit/vegetable waste improved the C/N ratio to ~25–30	High moisture content of fruit and vegetable waste reduced the solid retention time	(Fonoll et al., 2015)
Poultry litter & veg waste	50	Improved C/N ratio from 8 to 22, Ammonia mitigation ~45% TAN reduction	Hydrolysis limitations.	(Hanum et al., 2022)
Dairy Manure & Slaughterhouse waste	50	Rich in lipids and proteins—high methane potential	Risk of foam formation and scum layer due to fats.	(Chou & Su, 2019)
Vegetable waste & sewage sludge	50	Vegetable waste enhanced biodegradability and methane production	The high degradability of vegetables led to excess gas production, requiring gas flow management	(Di Maria et al., 2015
Vegetable waste & slaughterhouse wastewater	55	Combined nitrogen-rich wastewater balanced the C/N ratio	Risk of foam formation at high protein levels	(Mozhiarasi et al., 2023)
Sewage Sludge & Fruit Waste	55	Boosts disintegration and balance	High energy	(Ruiz Espinoza et al., 2022)
Corn Stover & Kitchen Waste	58	Improves solubility and microbial access	Chemical handling	(Donkor et al., 2022)
Fruit, vegetable waste, and poultry manure	60	Poultry manure corrected the low C/N ratio of fruit and vegetable waste.	Poultry manure produces ammonia and odor, requiring OLR control.	(Bres et al., 2018)
Bagasse & MSW	62	Increases biodegradability, stabilizes C/N	Mechanical clogging	(Karthikeyan et al., 2024)
Wheat Straw & Food Waste	65	stimulates hydrolysis and balances the substrate	Energy input	(Zafar et al., 2022)
Rice Straw & Manure	65	Enhances enzymatic breakdown	Enzyme cost	(Ferdeş et al., 2020)

4. Conclusion

This study presents the impact of anaerobic co-digestion of various substrates on methane yield and system stability. The review reveals that the nature and composition of substrates have a substantial impact on anaerobic digestion efficiency and long-term stability of the system. Co-digestion significantly improves methane yield and process stability compared to mono-digestion, hence presenting opportunities for enhancing energy security and reducing greenhouse gas emissions to address several environmental challenges posed by organic waste. Other key findings of the review include:

1. Mono-digestion is limited by nutritional imbalances, inadequate buffering capacity, and increased vulnerability to process inhibition. These result in lower methane yields and operational instability, especially when employing substrates with poor carbon-to-nitrogen (C/N) ratios or high quantities of inhibitory chemicals.
2. Co-digestion is a more resilient and adaptable technique for increasing biogas output. Optimizing substrate combinations enhance nutritional balance, resistance to changes

in organic loading rates and pH, resulting in synergistic microbial activity, reactor resilience, and enhanced biodegradation that improves methane outputs.

3. Future research should focus on optimizing co-substrate selection and process monitoring using advanced tools to maximize methane production. Large-scale demonstrations with techno-economic and life cycle assessment are necessary to validate the sustainability of anaerobic co-digestion.

Nomenclature

ACD: Anaerobic Co-digestion
C/N: Carbon/Nitrogen
VFAs: Volatile fatty acids
LCFAs: Long-chain fatty acids
HRT: Hydraulic Retention Time
FVW: Fruit & vegetable waste
TAN: Total Ammonia Nitrogen

Authors Contribution

Raphael Iddphonce: Conceptualization; Visualization; Writing – review & editing, and Project administration.
Joseph Matwani: Investigation; Writing – original draft; Methodology; and Data curation.

Conflict of Interests/Disclosures

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