



REVIEW ARTICLE

OPTIMIZATION STRATEGIES, SYNERGISTIC EFFECTS, AND EMERGING
TECHNOLOGIES FOR ENHANCED BIOGAS PRODUCTION THROUGH
ANAEROBIC CO-DIGESTION: A REVIEW

U.T. Abba*, A.B. Muhammad, M.B. Maina, M. Shuwa, S.S. Dumbulwa, I. Yahaya

Department of Mechanical Engineering, Faculty of Engineering, University of Maiduguri, P.M.B 1069,
Maiduguri, Nigeria

*Corresponding author's email: umartijjaniabba@gmail.com

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ABSTRACT

Anaerobic co-digestion (AcoD) has been found to be one of the most promising and technically feasible approach for maximizing the production of biogas from various organic feedstocks. AcoD allows simultaneous digestion of two or more feedstocks, thus improving the complementary nutrient profile, balancing the carbon to nitrogen (C/N) ratio, and enhancing the microbial syntrophy, all of which results in biogas outputs consistently greater than mono-digestion. This review aims to analyse the state-of-the-art in anaerobic co-digestion technologies with a focus on optimization strategies, synergistic effects and new technologies. In particular, the review covers: (i) the selection criteria and characterization parameters for feedstock that are important to ensure the compatibility of the substrate; (ii) the biochemical and microbiological mechanisms that underlie synergistic interactions in co-digestion; (iii) the mixing ratio of co-substrates and its effect on methane production; (iv) the optimization parameters of the co-digestion process such as temperature, pH, hydraulic retention time (HRT), and the organic loading rate (OLR); (v) advanced pre-treatment technologies before the co-digestion process; and (vi) new digital and biotechnological innovations, such as machine learning for process control, bio electrochemical systems, and multiple-stage reactor configurations. This review systematically synthesizes peer-review findings from 51 selected articles, key findings mostly published between 2018 and 2025, including pilot-scale, bench-scale, and full-scale operations. Key findings indicate that optimum methane production can be achieved with a C/N ratio of 20:1 to 30:1, mesophilic temperature (35-38°C), and strategic pairing of co-substrates such as food waste and sewage sludge, animal manure and agricultural residues that can raise methane output by 30-120% above the mono-digestion baselines. Innovative technologies such as biochar addition, ultrasound pretreatment, and catalysts with nanoparticles have opened a new window of hope for even further enhancing the process efficiency. The review highlights the main research gaps and outlines prospective pathways to make AcoD processes sustainable and economically viable in a circular bioeconomy.

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1.0 Introduction

Concerns about climate change, declining fossil fuel resources and the imperative for sustainable waste management are concurrently fueling a significant overhaul of the global energy paradigm. Among these drivers, the biogenic origin AD has emerged as a major focus of research, industry and policy offering a renewable energy solution to enhance organic waste valorisation and curtail greenhouse gas emissions (Anukam et al. 2019; Hagos et al. 2017). Biogas – its main product – consists of a mixture of methane (CH₄; 50-70%) and carbon dioxide (CO₂; 30-50%) and can be harnessed for heat and power, injected into gas grids as biomethane and used as a vehicle fuel, thereby lending itself as a rather flexible renewable energy vector (Ryue et al. 2020; Sol-Bund et al. 2019).

However, the use of mono-digestion of a single feedstock can often be hampered by inadequate nutrient ratio, process instability caused by ammonia inhibition, poor biodegradability of lignocellulosic feedstocks and the economical unviability of transporting low-concentration or bulky feedstocks to centralized biogas plants (Xu *et al.* 2018; Koch *et al.* 2017). Anaerobic co-digestion (AcoD) (Figure 1), namely the co-digestion of two or more types of organic feedstocks, has been widely demonstrated to overcome these constraints by the complementary pairing of feedstocks with distinct physico-chemical properties (Mata-Alvarez *et al.* 2014; Nges *et al.* 2012). For instance, combining nitrogen-rich substrates such as animal manure or sewage sludge with carbon-rich materials such as food waste, crop residues, or algae can correct unfavourable C/N ratios, dilute inhibitory compounds, introduce additional trace elements, and increase the overall organic loading to the digester (Hagos *et al.* 2017; Dumas *et al.* 2011).

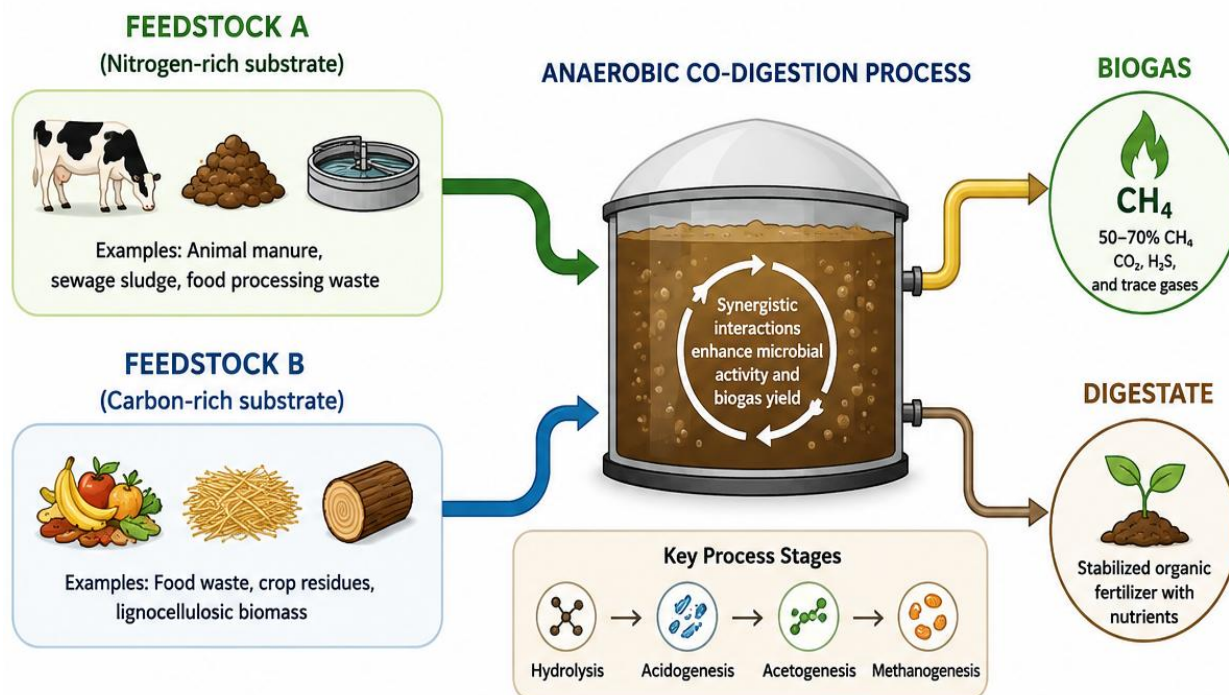


Figure 1: Conceptual framework of anaerobic co-digestion

Synergy in co-digestion does not mean just a dilution effect of nutrients. Reduced pH fluctuations, increased microbial diversity, improved substrate hydrolysis kinetics and cross-feeding between the hydrogenotrophic and acetoclastic methanogens are considered as the possible synergistic enhancements of CH₄ production (Ebner *et al.* 2016; Ngu *et al.* 2023). Such interactions are substrate specific, and are extremely process parameter dependent, and thus need to be optimized systematically to achieve the best performance of AcoD systems.

In the last 20 years, a significant amount of literature has been published regarding AcoD. According to a bibliometric analysis done by Mata-Alvarez *et al.* (2014), there were 10-fold more AcoD-related publications in the period from 2003 to 2013. The trend is ongoing in the 2020s as research has expanded to include the use of novel feedstocks, such as microalgae, food processing waste, and pharmaceutical sludge, as well as the development of pretreatment processes, process intensification, and digital optimization algorithms, including machine learning and real-time process monitoring (Poulsen *et al.* 2019, Hassaan *et al.* 2023). Nevertheless, although there is considerable body of literature, there are still gaps of critical knowledge. These are: (i) lack of understanding of mechanisms behind synergistic effects at the microbiome level for each substrate; (ii) limited scale-up of optimization results from lab-scale to full-scale; (iii) insufficient studies on the integration of new digital technologies like machine learning and real-time sensor systems; and (iv) lack of techno-economic and life cycle studies of new co-digestion solutions.

The motivation for this review is to bring together the progress made in AcoD optimization, and critically assess new developments since 2018 through 2025. The aims of the paper are: (1) To review the selection, characterization and compatibility assessment of the feedstocks used in AcoD; (2) To look at the biochemical and microbial mechanism of the synergy; (3) To survey the process parameter optimization strategies; (4) To evaluate the role and effectiveness of pretreatment of substrates in improving the co-digestion performance; (5) To look at new and innovative technology with transformative potential.

2. Method

2.1 Literature Search Strategy

The present review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework adapted to narrative reviews of engineering literature (Moher *et al.* 2009). A search was made in several peer review bases such as Scopus and Google Scholar. The main search terms used were: “anaerobic co-digestion”, “biogas optimization”, “methane enhancement”, “synergistic effects co-digestion”, “C/N ratio optimization”, “pretreatment anaerobic digestion”, “emerging technologies biogas”, and “machine learning anaerobic digestion”. Search terms were combined and refined using Boolean operators (AND, OR).

2.2 Inclusion and Exclusion Criteria

Inclusion criteria for the study were (i) published in peer-reviewed scientific journals or in conference proceedings, (ii) using data on single- or co-digestion in biogas production, but publishing data on biogas or methane production as a primary variable, (iii) a publication in the English language, (iv) providing quantitative information on the digestion performance, (v) published between 2010-2025, with a focus on publication after 2018. Exclusion criteria were: (i) single-digestion studies only, unless an actual comparison was made to co-digestion studies; (ii) reviews without original data or enough methodological information to match data, (iii) failure to report enough data to evaluate quality or performance. In the initial literature search 312 publications were retrieved, of which 238 publications were selected for full-text screening, and 51 publications met the quality criteria to be included in the review (full detail can be seen in 2018, which is a separate section on this database). Figure 2.2. Graphical representation of the review process.

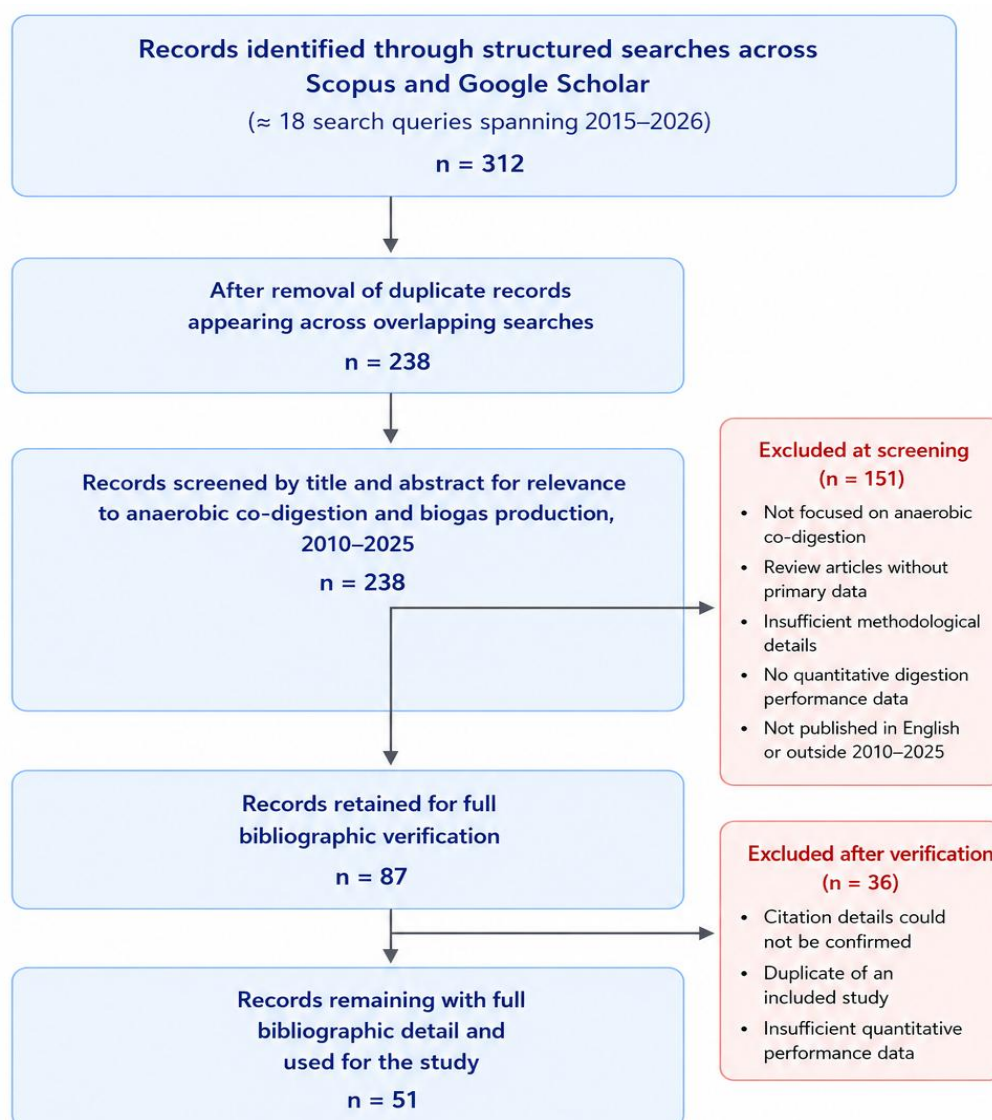


Figure 2.1: Review steps followed in selecting the papers

2.3 Data Extraction and Synthesis

Information obtained from each studies using an extraction template for each study comprised the following: feedstock type(s) and mixing ratios, digester type and operating temperature, HRT, OLR, biogas yield and methane yield (L/kg-VS added or removed), pH, ammonia, and reported synergy indices. These data were described narratively and in a table. For those papers with similar experimental conditions, typical values from each were compared and contrasted.

3. Synthesis and Critical Evaluation of Co-Digestion Literature

3.1 Feedstock Characterization and Substrate Selection for Anaerobic Co-digestion

3.1.1 Physicochemical characterization of common co-substrates

A successful co-digestion strategy is based on a careful physicochemical characterisation of candidate substrates. Total solids (TS), volatile solids (VS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), total phosphorus (TP), C/N ratio, lipid content and biodegradability expressed as the biochemical methane potential (BMP) are key parameters (Holliger *et al.* 2016). Table 1 summarises a comparison of physicochemical characteristics of frequently co-digested substrates from recent literature.

Table 1: Physicochemical characteristics and biochemical methane potential (BMP) of common co-digestion substrates

Substrate	TS (%)	VS/TS (%)	C/N Ratio	BMP (L CH ₄ /kg VS)	Reference
Cattle manure	8–12	75–82	14–25	150–280	Ngu <i>et al.</i> 2023
Swine manure	3–8	70–80	6–10	200–300	Xu <i>et al.</i> 2018
Sewage sludge	2–5	60–75	5–9	180–270	Ryue <i>et al.</i> 2020
Food waste	15–30	85–95	14–20	350–550	Koch <i>et al.</i> 2017
Corn stover	85–90	80–88	57–80	170–250	Hagos <i>et al.</i> 2017
Wheat straw	88–92	82–90	80–100	130–220	Solé-Bundó <i>et al.</i> 2019
Microalgae	5–20	70–85	5–10	100–400	Poulsen <i>et al.</i> 2019
Fruit/veg waste	10–25	85–95	20–35	300–480	Hagos <i>et al.</i> 2017
Chicken manure	20–35	65–75	5–10	170–250	Anukam <i>et al.</i> 2019
Slaughterhouse waste	15–25	80–92	5–15	350–600	Hassaan <i>et al.</i> 2023

Table 1 shows a wide range of C/N ratios for common feedstocks, with low ratios (5–25) for animal manures and sewage sludge and high values (57–100) for lignocellulosic agricultural residues.

3.1.2 Biochemical methane potential as a feedstock selection tool

The BMP (biochemical methane potential) assay is regarded as a standard laboratory method for assessing the feasibility of feedstock and predicting co-digestion efficiency, as set out by Holliger *et al.* (2016). The BMPs are performed in batch at inoculum-to-substrate ratios of ISR from about 2: 1 to 4: 1 (on a VS basis) at mesophilic temperature (37°C) with biogas collected daily or semi-daily until production becomes less than 1% of total production.

To evaluate digestion performance and kinetic behavior across studies, previous researchers frequently utilize mathematical models such as the modified Gompertz equation (Zwietering *et al.* 1990; Koch *et al.* 2017):

$$G(t) = P \exp\left\{-\exp\left[\frac{R_m e}{P}(\lambda - t) + 1\right]\right\} \quad (1)$$

In this established kinetic framework, $G(t)$ represents the total methane production (mL CH₄) that takes place during the time t (days), whereas P , R_m , e , and λ stand for the methane potential (mL CH₄), the maximum methane production rate (mL CH₄/day), the number e (approximately equal to 2.718), and the lag phase duration (days), respectively. Similarly, the assessment of co-digestion synergies relies on the calculation of theoretical additive methane yield (TAMY), which is defined by the theoretical methane yield obtained through averaging of BMP values of individual substrates. In case the obtained value of BMP of the mixture (BMP_{mix})

is higher than the value of TAMY, then it proves the occurrence of synergy during the co-digestion process (Ngu *et al.* 2023).

3.2 Synergistic Mechanisms in Anaerobic Co-digestion

The key factor contributing to better performance of AcoD compared to mono-digestion pertains to the occurrence of synergistic processes that result from the interaction of complementary substrates and the wide range of microorganisms involved in organic material degradation. Such synergistic processes occur throughout the anaerobic digestion process and are associated with nutrient balance, higher microbial diversity and syntrophic cooperation, buffering capacity towards changes in pH, detoxification of inhibitory compounds, as well as more efficient interspecies electron transfer. Overall, these processes form an environment that is more favourable in terms of microbial activity and is responsible for better stability of the process, rapid substrate degradation and methane production. Figure 3 shows stages of anaerobic co-digestion process and presents major types of synergies that take place during this process.

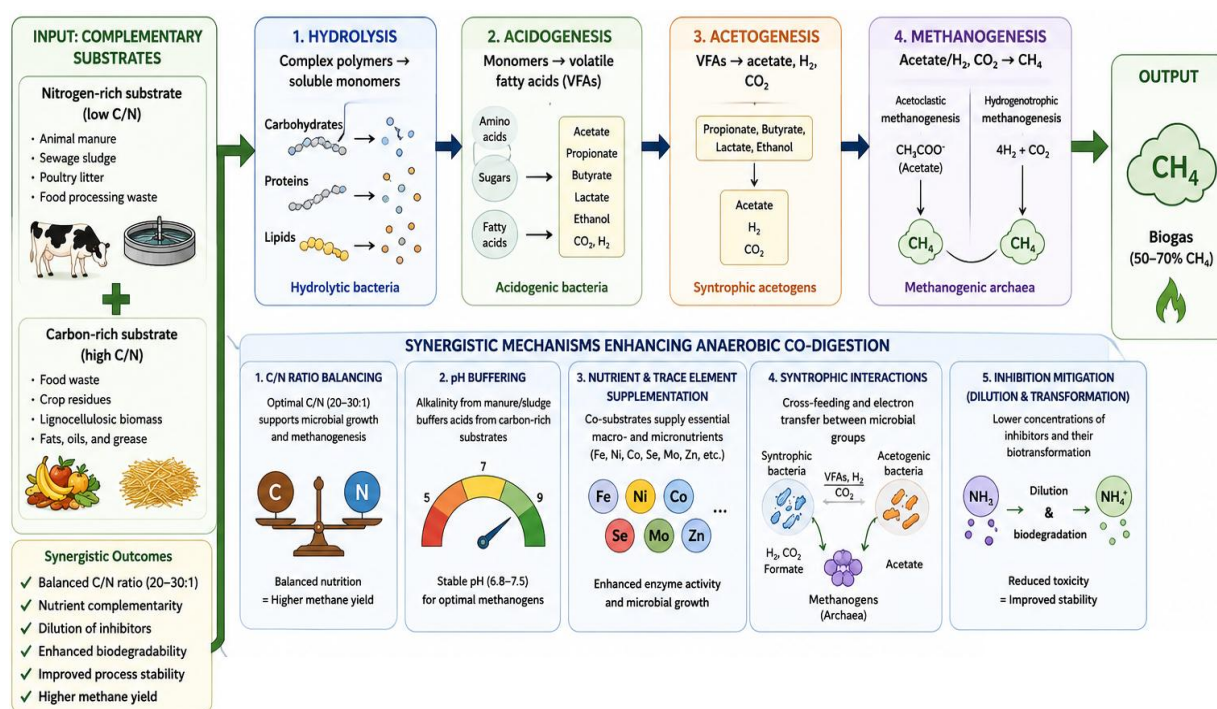


Figure 3: Anaerobic co-digestion process and synergistic mechanisms

3.2.1 Carbon-to-nitrogen ratio optimization

C/N ratio is one of the most relevant parameters influencing the synergy of AcoD. Methanogenic microorganisms need carbon for their energy requirements and nitrogen for the synthesis of cells, and the ideal C/N ratio for this to be achieved is 20:1 - 30:1 (Mata-Alvarez *et al.* 2014; Li *et al.* 2011). If the C/N ratio falls below 20:1, too much nitrogen is produced, which hampers the efficiency of methanogenic archaea and consequently lowers the methane production. If by contrast, the C/N ratio is more than 30:1, nitrogen deficiency will limit microorganism growth and generate prolonged lag periods (Hagos *et al.* 2017; Ebner *et al.* 2016). In the reviewed literature, the effective C/N ratio of a multi-substrate mixture is commonly evaluated and designed using Equation 2

$$(C/N)_{mix} = \frac{C_1m_1 + C_2m_2 \dots + C_nm_n}{N_1m_1 + N_2m_2 \dots + N_nm_n} \quad 2$$

where C_i and N_i represent the carbon and nitrogen contents (% VS basis) of substrate i , and m_i represents the mass fraction of substrate i in the co-digestion mixture. The effective C/N ratio of the mixed feedstock may be designed to fall into the ideal range based on the ratios chosen, and this can be done in spite of the C/N ratios of each individual feedstock. For instance, Xu *et al.* (2018) showed that co-digestion of swine manure (C/N = 8) and rice straw (C/N = 65) at 60:40 VS ratio finally resulted in the effective C/N of about 24, which meant that methane production was 41% higher than in case of processing manure alone. Likewise, Hagos *et al.* (2017) found that cattle manure mixed with corn stover at 70:30 VS ratio resulted in 38% improvement in methane yield and also overcame ammonia inhibition which occurred in case of manure only.

3.2.2 Microbial community dynamics and syntrophic enhancement

Reduced microbial community diversity and composition has wide-ranging ramifications in co-digestion systems especially in lipid-rich substrates (i.e. grease trap and slaughterhouse effluent) and under substrate imbalance conditions. Analytical advances such as 16S rRNA sequencing and metagenomics have demonstrated that the co-digestion enriches the following populations of syntrophic bacteria; Syntrophus, Syntrophobacter and Smithella together with highly hydrophilic methanogens (Westerholm *et al.* 2016; Ngu *et al.* 2023). Such an enrichment is significant in that the presence of the hydrogen transfer processes between the syntrophic organisms and the hydrogenotrophic methanogens leads to the establishment of the rate-limiting processes of anaerobic digestion under conditions of increased ammonia concentrations (Dumas *et al.* 2011; Westerholm *et al.* 2016).

Poulsen and the colleagues analysed microbial community shifts upon co-digestion of sewage sludge and food waste, using metagenomics to capture the details of the 2.3 times increased amount of *Methanoculleus bourgensis* at the most appropriate co-digestion ratio compared to single substrates. The researchers claimed that this enrichment was benefiting from the correctly balanced nitrogen sources and thus increased amount of partially fermented food waste (VFA production) resulting in the activation of hydrogen production process.

In lipid-based co-digestion systems (i.e., when lipid-rich substrates such as slaughterhouse effluent are used), the inactivation of the long fatty acid (LCFA) degradation process is predominant. In this way, the LCFA is going to be converted by the syntrophic microorganisms resulting in acetate, propionate and hydrogen generation, which have to be rapidly re-utilized by methanogens (Dumas *et al.* 2011). Co-digestion with manure or sewage sludge introduces sufficient biomass and buffering capacity to support this syntrophic partnership, enabling LCFA-rich substrates to be processed at loadings that would be inhibitory in mono-digestion systems (Hassaan *et al.* 2023).

3.2.3 Quantification of synergy: the synergy index

Several mathematical frameworks have been proposed to quantify synergy in co-digestion experiments. The most widely adopted is the Synergy Index (SI) proposed by Ebner *et al.* (2016), defined in Equation 3:

$$SI = \frac{BMP_{mix}}{f_1 BMP_1 + f_2 BMP_2} \quad 3$$

where BMP_{mix} is the measured methane yield of the co-digestion mixture (mL CH₄/g VS), BMP_1 and BMP_2 are the mono-digestion methane yields of substrates 1 and 2, and f_1 and f_2 are the VS-based mass fractions of each substrate in the mixture ($f_1 + f_2 = 1$). An $SI > 1$ indicates positive synergy; $SI = 1$ indicates additive effects; and $SI < 1$ indicates antagonism. A threshold of $SI \geq 1.10$ (i.e., $\geq 10\%$ enhancement above additive) is commonly used to confirm meaningful synergy in published literature (Koch *et al.* 2017; Ngu *et al.* 2023). Table 2 summarizes SI values reported for selected co-digestion substrate combinations from recent studies, confirming that combinations involving food waste, sewage sludge, and animal manure consistently achieve the highest synergy indices.

3.3 Process Parameter Optimization

3.3.1 Temperature and its effect on methanogenic activity

Temperature is important to anaerobic co-digestion by controlling the rate of microbial metabolism, digestion of the substrate and thermodynamic balance between these reactions. Anaerobic digestion occurs over three temperature ranges: psychrophilic (<25°C), mesophilic (30 to 40°C) and thermophilic (50 to 60°C). The influence of temperature on the rate of the methanogenic reaction can be described by the Arrhenius equation:

$$k(T) = k(T_{ref}) \exp \left[\frac{E_a}{R} \left(\frac{1}{T_{ref}} - \frac{1}{T} \right) \right] \quad 4$$

where $k(T)$ is the rate constant at temperature T (K), $k(T_{ref})$ is the rate constant at reference temperature T_{ref} , E_a is the activation energy (kJ/mol), and R is the universal gas constant (8.314 J/mol·K). For mesophilic methanogens, E_a values typically range from 40–80 kJ/mol, implying that a 10°C increase in temperature approximately doubles the metabolic rate (Ngu *et al.* 2023). The mesophilic range (35–38°C) continues to be the temperature range most frequently implemented at full scale AcoD plants since it has lower energy inputs, improved process stability, and increased capacity to contend with organic overloading.2 On the other hand,

thermophilic operation (50-55°C) has been shown to provide increased degradation of difficult to digest feeds, enhanced pathogen destruction, and easier digestate dewatering.² Koch et al.² found a 23% higher methane yield from thermophilic co-digestion of food waste and sewage sludge compared to a similar process at mesophilic temperatures, but the system was more sensitive to ammonia toxicity and experienced more operational variability with sudden feedstock ratio alterations.² These performance dichotomies continue to dominate the scientific discourse around the operational limits of thermophilic AcoD systems since they provide faster reaction kinetics, but the narrow toxicity window with regard to free ammonia concentrations remains the primary obstacle for commercialization.² A major research gap still exists for designing adaptive, real-time control strategies coupled with advanced microbiome stability mechanisms that can circumvent catastrophic digestion failure under the operationally variable thermophilic regimes.

Table 2: Synergy Index (SI) and methane yield improvement for selected co-digestion substrate combinations

Substrate 1	Substrate 2	Mix Ratio (VS)	SI	Methane Increase (%)	Yield	Reference
Sewage sludge	Food waste	50:50	1.42	+42		Koch et al. 2017
Cattle manure	Corn stover	70:30	1.38	+38		Hagos et al. 2017
Swine manure	Rice straw	60:40	1.41	+41		Xu et al. 2018
Sewage sludge	Microalgae	75:25	1.29	+29		Poulsen et al. 2019
Chicken manure	Fruit waste	40:60	1.52	+52		Anukam et al. 2019
Cow manure	Slaughterhouse W.	80:20	1.35	+35		Hassaan et al. 2023
Sewage sludge	Grease trap W.	90:10	1.31	+31		Dumas et al. 2011
Food waste	Agricultural res.	60:40	1.44	+44		Ryue et al. 2020
Pig manure	Wheat straw	50:50	1.27	+27		Solé-Bundó et al. 2019
Municipal waste	Sewage sludge	70:30	1.33	+33		Ngu et al. 2023

3.3.2 pH and alkalinity management

Methanogenic archaea have a fairly narrow pH-optimum of 6.8-7.5, with maximum activity at near neutral pH (7.0-7.2) (Anukam et al. 2019). Hydrolytic and acidogenic bacteria are generally tolerant to wider pH ranges, active from 5.5 to 8.5, and when rapidly-fed this can cause pH imbalance and in some cases cause acidification (Anukam et al. 2019). Co-digestion with high-alkalinity feedstocks like animal manure or sewage sludge buffers against pH imbalance because of the bicarbonate/carbonate and ammonium bicarbonate buffering systems and markedly decreases the chance of acidification (Hagos et al. 2017; Xu et al. 2018).

Total alkalinity (TA) values of well-functioning digesters are usually between 2,000–5,000 mg CaCO₃/L, with the intermediate alkalinity (IA)/partial alkalinity (PA) ratio being a very sensitive early-warning indicator: should it fall below 0.3 the process is fine, if it goes above 0.4 then acidification has been detected (Mendez-Acosta et al. 2010).

Composting high-alkalinity materials with low-alkalinity waste like crop residue or food scraps safely buffers low-alkalinity systems and helps to maintain pH (Hassaan et al. 2023). However, researchers are increasingly finding there is a lot more to alkalinity than meets the eye. The early-warning cut-off threshold of 0.3 for the IA/PA ratio in typical systems has always been validated by classic models of volatile fatty acid (VFA) import and consumption processes, and so should be fairly reliable in standardized systems. Unfortunately in complex, multi-substrate systems it appears that there are a myriad of risks from obfuscated, localized acid drops to VFA spike events-and even with an apparently healthy stability-suggesting that the industry has a long way to go from basic mass alkalinity monitoring.

3.3.3 Hydraulic retention time and organic loading rate

Hydraulic retention time (HRT) and the organic loading rate (OLR) are sometimes called related variables because of their combined effect on the ratio of the conversion of the substrate to wash-out of the slow-growing methanogenic population. These relationships in a reactor with a continuous stirrer (CSTR) are as follows for HRT, OLR, and the volumetric biogas production rate (VBPR) and are given by following equations:

$$OLR = \frac{VS_{in}}{HRT} \quad 5$$

$$VBPR = MY \times OLR \quad 6$$

Here $[VS]_{in}$ refers to the concentration of volatile solids in the input (kg VS/m³), while HRT is expressed in days, MY is the methane yield (m³ CH₄/kg VS), and VBPR refers to the productivity of biogas per unit of

volume. In the case of mesophilic AcoD, the optimal values of HRT range between 15 and 25 days, and the OLR values range within 2 to 6 kg VS/m³ ·d depending on biodegradability of the substrate and the type of co-substrate used (Koch *et al.* 2017; Ngu *et al.* 2023). Ryue *et al.* (2020) studied the impact of OLR on co-digestion of municipal food wastes and sewage sludge using semi-continuous CSTR with a maximum calculated VBPR of 1.82 m³ per m³ ·d assuming an OLR of 4.5 kg VS/m³ ·d and a hydraulic retention time equaling to 20 days. By increasing the OLR to above 6 kg VS/m³ ·d, VFA have started to accumulate, resulting in a reduction in the methane yield by 28%, thus suggesting the inhibition of the process triggered by organic overload. This unstable process situation is generally avoided through gradual OLR progression and increase of the organic loading in small increments, normally from 10 to 20 percent for periods of several hydraulic retention times measured through the monitoring of the main indicators of the process like pH and the proportion of VFA in shares of alkalinity. Thus strategy helps develop the microbial population, and in particular methanogens to approach large organic amounts increasing the stability of the process and minimising the risk associated with acidification of the reactor during start-up or transition phases.

3.4 Pretreatment Technologies for Enhanced Co-digestion Performance

3.4.1 Mechanical pretreatment

The process of mechanical pretreatment process involves crushing, milling and ultrasonic cavitation; thus, the biodegradability potential of biodegradable materials can be increased by making their particle size smaller and the specific surface area higher, as well as breaking cell walls or lignocellulosic structures. The ultrasound pretreatment (20–40, 0.1–1 W/mL) is said to be more efficient with sewage sludge, providing something between 30 and 60% of the degree of disintegration and achieving an increase in methane yield from 15 to 40% (depending on the amount of energy used and attributes of the sludge (Poulsen *et al.* 2019; Anukam *et al.* 2019). The high-pressure homogenization is performed at 60–80 MPa, and it was observed during studies that the use of this method with food waste increases the methane yield by 18 to 25% during co-digestion with animal waste (Hassaan *et al.* 2023).

3.4.2 Thermal pre-treatment

Thermal pretreatment (60–170°C, 20–60 min) disrupts extracellular polymeric substances (EPS) in sludge, solubilizes particulate organics, and enhances hydrolysis rates of lignocellulosic materials. The thermal hydrolysis process (THP), commercially implemented as the CAMBI process (160–170°C, 25–30 min), has been widely adopted in full-scale sewage sludge co-digestion facilities, achieving VS reduction improvements of 15–25% and biogas yield increases of 20–50% compared to untreated sludge (Valo *et al.* 2004; Solé-Bundó *et al.* 2019). Thermal pre-treatment enhances co-digestion systems and rescues ammonia toxicity by improving the efficiency of hydrolysis and allowing for higher loading of volatile solids with the same load of the nitrogen (Ryue *et al.* 2020). However, thermal treatment beyond 180 °C contributes to the formation of negative substances such as furan, hydroxymethylfurfural (HMF), and Maillard reaction products that diminish methane production, which exhibits that optimization of temperature is a crucial factor (Hassaan *et al.* 2023).

3.4.3 Chemical pretreatment

Alkali pretreatment (NaOH, KOH, Ca(OH)₂; 0.5–5% w/w) is extensively applied to lignocellulosic substrates to saponify ester linkages in lignin-hemicellulose complexes, increase cellulose accessibility, and enhance enzymatic hydrolysis rates. Xu *et al.* (2018) reported that NaOH pretreatment (4% w/w, 24 h, 25°C) of rice straw prior to co-digestion with swine manure increased methane yield by 57% compared to untreated straw co-digestion, attributed to a 40% reduction in lignin content and a 2.3-fold increase in soluble COD. Acid pretreatment (H₂SO₄, HCl; 1–5%) selectively hydrolyses hemicellulose, releasing pentose and hexose sugars that are readily fermentable, and has been effectively applied to agricultural residues and energy crops (Anukam *et al.* 2019).

3.4.4 Biological pretreatment

Biological pretreatment methods employing fungal species (particularly white-rot fungi such as *Phanerochaete chrysosporium* and *Pleurotus ostreatus*) make use of unique ligninolytic enzymes (ex: laccases, manganese peroxidases, and lignin peroxidases) for the purpose of lignin degradation while maintaining cellulose and hemicellulose for later conversion into methane. It was observed that the time and temperature of pretreatment of wheat straw (21 days, 28°C) coupled with sewage sludge resulted in a 33% increase in

methane yield in comparison to the untreated wheat straw; however, the long pretreatment period presents a limitation for its industrial implementation.

On the other hand, enzymatic pretreatment using commercially available cocktails of cellulases, hemicellulases, and proteases is considerably faster and more manageable. According to Ngu et al. (2023), pretreatment of food wastes during the co-digestion with municipal sludge using cellulase (30 FPU/g VS) allows for additional 12 % reduction in VS and increases methane yield by 22% in comparison to non-treated substrate. The data summarizing the achievements of various pretreatment technologies in the context of co-digestion are presented in the Table 3 in below.

Table 3: Summary of pretreatment technologies and their effect on methane yield improvement in anaerobic co-digestion systems

Pretreatment Type	Substrate	Co-substrate	Methane Improvement (%)	Yield (%)	Key Parameter	Reference
Ultrasonic	Sewage sludge	Food waste	+28		20 kHz, 0.5 W/mL	Poulsen et al. 2019
Thermal (THP)	Sewage sludge	Food waste	+35		165°C, 30 min	Solé-Bundó et al. 2019
Alkali (NaOH)	Rice straw	Swine manure	+57		4% w/w, 24 h	Xu et al. 2018
Acid (H ₂ SO ₄)	Corn stover	Cattle manure	+31		2% w/v, 121°C	Hagos et al. 2017
Fungal (P. chrysosporium)	Wheat straw	Sewage sludge	+33		21 days, 28°C	Solé-Bundó et al. 2019
Enzymatic	Food waste	Municipal sludge	+22		30 FPU/g VS	Ngu et al. 2023
High-pressure homog.	Food waste	Animal manure	+21		60–80 MPa	Hassaan et al. 2023
Microwave	Sewage sludge	Agri residues	+19		2.45 GHz, 700 W	Ryue et al. 2020
Ozonation	Sewage sludge	Food waste	+24		0.1 g O ₃ /g TS	Anukam et al. 2019
Combined (alkali+ultra)	Lignocellulosic	Manure	+67		Combined	Koch et al. 2017

3.5 Reactor Configuration and Design Optimization

3.5.1 Single-stage vs. two-stage reactor systems

In conventional single-stage CSTR systems, all four phases of anaerobic digestion (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) occur in one reactor. These CSTR systems are easy to operate and inexpensive; however, balancing the optimal conditions for the hydrolytic and acidogenic bacteria (pH 5.5–6.5) with those for the methanogenic archaea (pH 6.8–7.5) imposes limits and performance challenges especially when the feedstock is complicated or difficult to digest. Two-stage systems keep the acid producing phase separate from the methane producing phase, allowing for optimizing each phase (Ngu et al. 2023; Hassaan et al. 2023).

When it comes to the co-digestion processes, two-stage systems have shown some unique benefits in processing substrates which contain a lot of lipids. Dumas et al. (2011) demonstrated that the two-stage co-digestion process of slaughterhouse waste and sewage sludge in a thermophilic acidogenic CSTR (55°C, HRT=3 days) followed by a mesophilic methane-producing CSTR (35°C, HRT=18 days) achieves 62% higher COD removal and 48% more methane yield compared to the single-stage mesophilic co-digestion of the equally composed feedstocks. The authors link the improvements to the ability to efficiently destroy LCFA in the first stage while hindering their ability to inhibit methanogens in the second stage.

3.5.2 High-rate reactor technologies

Technologies in high-rate reactors like UASB reactors, EGSB reactors, and AnMBR make it possible to separate HRT from SRT, allowing for a high OLR and low HRT. While still having enough biomass concentration for efficient methanogenesis, the UASB reactor is able to run under conditions of OLR ranging from 5 to 20 kg COD/m³·d and as low as 4 to 8 hours in terms of HRT for easily decomposable waste materials, which is a major decrease in reactor size required compared with the CSTR technology (Ryue et al. 2020). The AnMBR technology, where anaerobic treatment is combined with membrane filtration to keep all

the biomass inside the reactor is considered effective in AcoD of dilute feed materials like municipal sewage, dairy wastewater, and pharmaceutical wastewater. Membrane saturation remains the biggest challenge for the operation of this type of reactor, which requires periodic cleaning of membranes with sodium hypochlorite and citric acid as well as relaxation cycles to restore membrane capacity. It has been illustrated by Solé-Bundó *et al.* in their AnMBR tests with microalgae and primary sludge that was effective in the combined digestion of these waste materials with SRT of 40 days and OLR of 1.8 kg VS/m³ d, while resulting in methane production of 340 mL/g VS, which is much higher than 210 mL/g VS obtained with CSTR systems under the same conditions.

3.6 Emerging Technologies for Enhanced Biogas Production

3.6.1 Direct interspecies electron transfer (DIET) and conductive materials

Unlike traditional hydrogen interspecies transfer, direct interspecies electron transfer (DIET) represents a new way of interaction between species. Doing DIET means that electrons are passed directly between methanogenic archaea and syntrophic bacteria through conductive connections. This means that instead of passing electrons through hydrogen molecules as in hydrogen interspecies transfer, bacteria are either connected by conducting pili or by some conducting materials that have been added into the digester. Studies suggest that magnetite nanoparticles, biochar and granular activated carbon are among the materials that might enhance DIET because those materials create surfaces that will lead to better flow of electrons.

Biochar addition has been very popular lately, because it is cheap and at the same time allows for several functions to be achieved, including DIET support. According to a meta-analysis carried out by Hassaan *et al.* (2023), the addition of biochar at the level of 5-20 g/kg could lead to the increase in methane generation of 17-43%.

3.6.2 Machine learning and artificial intelligence for process optimization

Now one of the most exciting new advancements to come to the base of AcoD process monitoring and control is the incorporation of artificial intelligence (AI) and machine learning (ML). The methane yield prediction from substrate properties, optimization of mixing ratios in real-time, early detection of process instability from sensors, and modelling of microbial community dynamics under varying operating conditions have been successfully employed using ML algorithms, such as artificial neural networks (ANN), support vector machines (SVM), random forests (RF), and long short-term memory (LSTM) networks (Poulsen *et al.* 2019; Ngu *et al.* 2023).

Poulsen *et al.* (2019) created a hybrid LSTM-ANN model, which was trained with data from an operational period of 18 months in a full-scale co-digestion facility that processes sewage sludge and food waste, and the model was used to predict methane yield with a root mean square error (RMSE) of 4.2% for 24-hour prediction of a methane yield. An online supervisory control and data acquisition (SCADA) system was introduced into the model for a 9% increase in the annual biogas production and a 15% decrease in process instability events compared to conventional proportional-integral-derivative (PID) control. To obtain optimal mixing ratios for multi-substrate co-digestion for all over the world, genetic algorithm (GA) optimization has been used with ANN models. Koch *et al.* (2017) used a GA-ANN system to optimize the co-digestion ratio of food waste, sewage sludge and agricultural residues in three-dimensional substrate space and found that the ratio of VS 45:35:20 yielded 11% increase in methane yield compared to that of the conventional optimum. All of these studies indicate that the use of ML-driven optimization may yield improvements not obtained by traditional one factor at a time experimental optimization methods.

3.6.3 Bioelectrochemical systems integrated with co-digestion

Bioelectrochemical systems (BES), consisting of microbial electrolysis cells (MEC) and microbial fuel cells (MFC), are considered as the new technology that could help boost AD performance by stimulating microbial activity against the application of electrochemistry. MEC can be joined with AcoD to yield cathodic hydrogen production owing to electrical input, which depends on the interspecies hydrogen transfer, as well as hydrogenotrophic methanogenesis. On the other hand, it is also possible to apply even low voltage (0.2–0.8 V) completely to the co-digestion reactor, which is called bioelectrochemical co-digestion (BE-AcoD), helping with DIET pathways without hydrogen in gas phase (Ryue *et al.* 2020). Ryue *et al.* (2020) have conjectured the municipal food waste and sewage sludge BE-AcoD application relative to conventional AcoD at the voltage applied being 0.4 V and the result was the increase of methane production rate by 31% and VS removal by

18% accordingly. The enrichment of *Geobacter sulfurreducens* and *Methanosaeta harundinacea* at the cathodic surface was states to be 3.1 times according to 16S rRNA sequencing.

3.6.4 Nanomaterial supplementation

Nanomaterials such as zero-valent iron nanoparticles, magnetite nanoparticles, carbon nanotubes, and graphene oxide have been examined as additives to improve the performance of co-digestion through different methods. First, nZVI offers reducing equivalents for lowering the oxidation-reduction potential and creating suitable conditions to carry out anaerobic processes. Second, magnetite promotes DIET because of its semiconductor aspects and high electrical conductivity. Third, CNTs and GO provide conductive frameworks which increase electron transfer and promote biofilm formation (Hassaan *et al.* 2023). In their work, Anukam *et al.* (2019) state that introducing magnetite nanoparticles (1g/L) into the process reduced lag by 3.2 days, enhanced maximum methane production by 28%, and raised final methane production by 19% as compared to the control groups, which did not use nanoparticles. Nanotoxicity at high concentrations (more than 5g/L) is regarded as one of the major issues associated with nanoparticle use because it means that the dosages must be carefully optimized, and properties of nanoparticles in digestate investigated to provide safety.

3.6.5 Multi-stage and thermophilic-mesophilic cascade systems

Temperature-phased anaerobic digestion (TPAD) is a process that includes both thermophilic and mesophilic phases, with thermophilic bacteria being respected for their hydrolysis capacity, while mesophiles are recognized for their stability and efficiency in methane production. TPAD has been shown to be effective in pathogen removal (Class A biosolids quality), improving volatile solids reduction and producing more methane over time in comparison to processes which rely only on one temperature. Ebner *et al.* (2016) presented data that analyzed TPAD with co-digestion at 55 °C and 35 °C versus mesophilic co-digestion of food waste and sewage sludge and showed that TPAD produced 29 % more methane as well as completely eliminated *E. coli*.

3.7 Inhibition Mechanisms and Mitigation Strategies

3.7.1 Ammonia inhibition

Ammonia nitrogen exists in two forms in co-digestion systems: ammonium ion (NH_4^+) and free ammonia (FA, NH_3). Free ammonia is the primary inhibitory species due to its ability to penetrate cell membranes and disrupt proton gradients. The FA concentration is given by Equation 7 below:

$$FA = TAN \times \left[\frac{1}{1 + 10^{(pK_a - pH)}} \right] \quad 7$$

where TAN is the total ammonia nitrogen (mg N/L) and pK_a is the acid dissociation constant of NH_4^+ (approximately 9.25 at 35°C). Inhibitory FA concentrations of 150-700 mg N/L are typical for non-adapted communities, although adapted communities can withstand up to 1,100 mg N/L with adaptations leading to hydrogenotrophic methanogenesis (Westerholm *et al.* 2016). There are a variety of approaches to mitigating ammonia inhibition of AcoD including: (i) co-substrate dilution to mitigate N loading; (ii) pH reduction to modify the FA-TAN: NH_4^+ ratio; (iii) ammonia stripping from the digestate recycle stream; (iv) addition of NH_4^+ adsorbing media such as biochar or zeolite; (v) or bioaugmentation with ammonia-adapted consortia (Ngu *et al.* 2023, Hassaan *et al.* 2023). Among these, zeolite (clinoptilolite) supplementation at 20–40 g/L has demonstrated NH_4^+ adsorption capacities of 15–25 mg N/g zeolite and methane yield improvements of 12–27% in nitrogen-rich co-digestion systems (Xu *et al.* 2018).

3.7.2 Long-chain fatty acid (LCFA) inhibition

LCFAs, released during co-digestion of lipid-rich substrates, inhibit AD through multiple mechanisms: (i) adsorption onto cell membranes disrupting transport processes; (ii) inhibition of key enzymes including CoA transferases and acyl-CoA synthetases; and (iii) flotation of active biomass, reducing effective SRT (Dumas *et al.* 2011). Dumas *et al.* (2011) reveal that the critical long-chain fatty acid (LCFA) concentrations that produce 50% inhibition (IC50) in methanogens range from 1-2 mM for oleic acid (C18:1), while lauric acid (C12:0) produces IC50 at concentrations of between 0.1 to 0.5 mM. It will be noted that inhibition occurs with increasing LCFA concentration with atom length and degree of unsaturation being the primary factors.

Using intermittent feeding of lipids from grease traps allows for LCFA inhibition to be avoided. In a 4-day feeding cycle, Dumas et al. (2011) achieved LCFA concentrations below inhibitory levels, resulting in 22% more methane produced from treated material as compared to continuous feeding method. Due to periods of rest in between feeding times, organisms have the chance to degrade LCFAs and repair their membranes.

4. Conclusion

Anaerobic co-digestion is widely recognized as a robust, sustainable strategy for maximizing biogas production by leveraging synergistic interactions among complementary substrates, microbial communities, and optimized operating parameters. As demonstrated throughout this review, achieving optimal co-digestion performance requires an integrated approach encompassing careful substrate selection, balanced C/N ratios, rigorous parameter control, and effective mitigation of inhibitors. Furthermore, recent advancements such as pretreatment technologies, biochar-assisted direct interspecies electron transfer, machine learning-driven optimization, and bioelectrochemical systems hold substantial potential for intensifying process efficiency. Ultimately, successful deployment relies on simultaneously advancing biological, operational, and technological frameworks rather than addressing them in isolation. While these innovations demonstrate exceptional promise at the laboratory scale, bridging the gap toward pilot- and full-scale commercial validation remains a critical priority for future research.

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