

Review

Impacts of Anaerobic Co-Digestion on Different Influencing Parameters: A Critical Review

Mohammed Kelif Ibro ¹, Venkata Ramayya Ancha ¹ and Dejene Beyene Lemma ^{2,*}

¹ Faculty of Mechanical Engineering, Jimma Institute of Technology, Jimma University, Jimma P.O. Box 378, Ethiopia; mohamedkelifa@obu.edu.et (M.K.I.); dra.venkata@ju.edu.et (V.B.A.)

² Faculty of Civil and Environmental Engineering, Jimma Institute of Technology, Jimma University, Jimma P.O. Box 378, Ethiopia

* Correspondence: dejene.beyene@ju.edu.et

Abstract: Lignocellulosic feedstocks are year-round, available bio-residues that are the right candidates for counteracting the energy crises and global warming facing the world today. However, lignin leads to a slow hydrolysis rate and is a major bottleneck for biogas production via anaerobic digestion. Anaerobic co-digestion (AcoD) is an economical method available, which overcomes the limitation of a single feedstock's properties in an anaerobic digestion process. This paper critically reviews the impacts of co-digestion on lignocellulosic biomass degradation, process stability, various working parameters, and microbial activities that improve methane yields. A combination of compatible substrates is chosen to improve the biomethane yield and conversion rate of organic matter. AcoD is a promising method in the delignification of lignocellulosic biomass as an acid pretreatment. Ultimate practices to control the impact of co-digestion on system performances include co-feed selection, in terms of both carbon-to-nitrogen (C/N) and mixing ratios, and other operating conditions. A detailed analysis is performed using data reported in the recent past to assess the sensitivity of influencing parameters on the resultant biogas yield. For the investigators motivated by the basic principles of AcoD technology, this review paper generates baseline data for further research work around co-digestion.



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Keywords: biomethane potential (BMP); synergistic effect; biodegradability; anaerobic co-digestion; lignocellulosic biomass; operating parameters

1. Introduction

Bioenergy is a key component in biorefinery, with bio-residues from other green economy sectors being used as raw material in bioenergy conversion processes. In this respect, biomass is one of the most promising renewable sources of energy generation. It offers an opportunity to switch to renewable energy, making bioenergy (biogas) cost competitive. Biogas plants are the most competitive technology for energy generation through organic waste management and an expected, respectable applicant for the future of renewable energy supply [1] under the umbrella of circular biorefinery. Anaerobic digestion (AD) is the microbial degradation of complex organic matter in oxygen-limited conditions [2], as shown in Figure 1. Several studies were conducted on the AD process using sole feedstocks for biogas production [2–4]. However, feeding a single substrate to a biodigester does not produce sufficient methane yields due to several problems such as the stubborn nature of lignocellulosic material and lack of diversified microbes [4], the low and imbalanced C/N ratio [5], and the effect of operating conditions.

The concept of co-digestion to improve the methane yield using different substrate combinations is called anaerobic co-digestion (AcoD); it may create the best synergisms in the biodigester. There have been various pieces of research performed on the AcoD of lignocellulosic feedstocks with other different organic biomasses, such as animal manure [6,7],

food waste, aquatic plants, and algal biomass, to improve the methane yield [8–10], as shown in Figure 2.

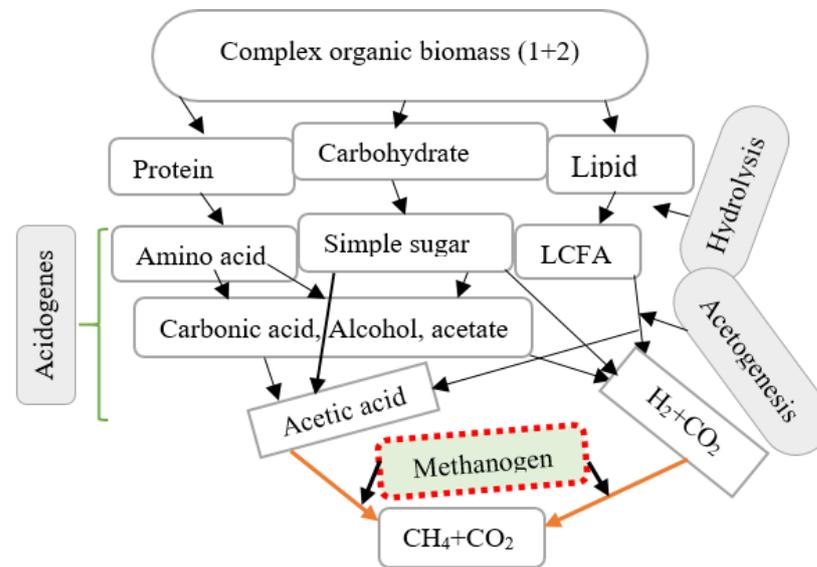


Figure 1. Microbiology of organic material in the anaerobic co-digestion system (adapted from [1]).

AcoD may deliver significant advantages, including improved stability of the system [11], neutralization of toxic compounds [12], encouragement of a multiple microbe society, and better nutrient balance (appropriate C/N ratio and delivery of trace elements) [13], providing the needed amount of moisture for substrates [14], increasing the organic loading rates (OLRs) [15], and improving the rate of organic degradation in the digester [9]. As per one review, the beneficial aspect of an enriched biogas yield of 400% was reported for co-digestion compared to digestion of a sole feedstock [1]. In addition, regardless of the substrate to inoculum ratio, the methane yield increased by 120% with co-digestion of vicuñas (VM) and amaranth (AS) compared to anaerobic digestion of a single substrate, which was higher compared to other studies on co-digestion of animal byproducts with an increment in volatile solid (VS) conversion of about 75% [16]. In this regard, this review paper details the impacts of a co-substrate on system performance, various operational parameters, microbe status, and recent achievements. In addition, the status of the AcoD process, current progress, and the future trend for further enhancements are discussed.

2. Microbiological Pathways in Anaerobic Co-Digestion Condition

The science behind the AcoD process is complex as the biological degradation of organic matter is performed by groups of anaerobic microorganisms through a multi-step process in an oxygen-limited environment. An anaerobic condition involves hydrolysis, acidogenesis, acetogenesis, and methanogenesis as the methane-producing last step (Figure 1). The first step is a deliberately critical rate-limiting stage due to the barrier of lignin at the start of the AD process [1]. The AcoD system makes the first step stable by combining substrates with high and slow hydrolysis rates [2]. The second stage is called the fermentation process, in which all products of hydrolysis are converted into alcohols, volatile fatty acids (VFAs), H₂, acetates, and CO₂ with byproducts such as H₂S and NH₃ [17]. In the third stage, acetogenesis, microorganisms convert organic acid into acetates, H₂, and CO₂, which are utilized by methane-producing groups of microbes [18]. In the last stage, methanogen archaea use acetates, H₂, and CO₂ to form methane [19].

To sustain the activities of the acidifying and methane-producing bacteria, the methanogenesis stage should be carried out at a pH above 6.6, ideally between 6.8 and 7.2 [20]. Caruso et al. [21] identified some conditions that may block microbial activity, including a shortage of nutrients and the existence of barrier chemicals, such as sulfide, which cause a

drop in pH and excess VFA accumulation [21]. Thus, co-digestion provides the micro- and macronutrients for microbial growth [22]. Generally, the AcoD process is a promising strategy for keeping the optimum pH constant and permitting digester stability by buffering the extreme acidification/alkalinity conditions for groups of archaea [23].

3. Principal Parameters and Factors Affecting Microbial Activity during AcoD

3.1. Temperature

Based on the set-up condition, the optimum temperature of the AcoD route is classified as thermophilic (55 °C) [24], psychrophilic (20 °C) [25], or mesophilic (35 °C) [26]. In thermophilic conditions, the anaerobic process improves pathogen reduction, achieves greater conversion rates, and achieves a shorter digester hydraulic retention time [27]. However, thermophilic conditions are harder to control and demand more energy to heat to keep the AD at ideal condition [28]. Thermophilic groups are more sensitive to changes in operating conditions and environmental fluctuations than mesophilic conditions [27]. In addition, temperature influences digester performance when it is less than optimum and reduces the substrate consumption in the digester (i.e., causes substrate turnover), microbial growth rates, microbial diversity [29], and methane production rate, while higher temperatures increase the degradation, VS removal rate, and process stability [27]. Uma et al. [30] combined food waste (FW) and switchgrass (SG) at different mixing ratios, focusing on the effect of temperature on the process performances. They achieved maximum methane yields of 267 mL/g and 234 mL/g (VS) from FW:SG (1:1) under 35 °C and 55 °C, respectively. They revealed that the mesophilic temperature (35 °C) showed the highest methane yield with better performance.

3.2. Total Solid (TS) and Volatile Solid (VS)

The volatile solid (VS) and total solids (TS) content are useful indicators of biogas conversion rates [31]. The TS of a feed indicate how much moisture is available in the substrates. The higher the TS content, the more volatile fatty acids (VFAs) are accumulated [32]. Similarly, reactors containing lower TS content showed higher methane yield in mono-digestion of dairy manure [25]. Combining two or more substrates may improve the degradability of TS in organic waste due to moisture increments in the digester [32]. VS content allows evaluation of the AD performance, biogas potential approximation, and biomass decomposition rate. For instance, Elsayed et al. [33] investigated the conversion efficiency of organic matter at different mixing ratios of primary sludge (PS), fruit, and vegetable wastes (FVW) through batch mesophilic conditions. The highest VS removal rates of 73% and 70% were observed at mixing ratios of 70:30 and 50:50, respectively. However, the maximum cumulative methane yield was produced at PS:FVW 50:50. Klarosk et al. [34] defined VS as the amount of organic matter that may be converted to biogas. The greater the VS amount, the better the anaerobic digestion process (higher biogas production). VS removal efficiency indicates the synergistic effect of co-digestion due to a blend of supplement substrates [35]. Considering this benefit, Hamrouni [36] digested food waste with sewage sludge (SS) at different mixing ratios. They revealed that a co-substrate improved the VS removal efficiency for SS.

3.3. Carbon to Nitrogen Ratio

The archaea are highly sensitive to the C/N proportion that indicates deficient nutrient levels of feedstocks. Assessing C/N balance is the method for minimizing or avoiding the issue of ammonia in the anaerobic digestion process [37]. When the C/N ratio is greater than the ideal (Tables 1 and 2), it means deficient nitrogen (or underuse of carbon), which is consumed rapidly by methanogens and leads to low biogas production [38]. Nitrogenous substrates, such as animal manure and chicken droppings, might be required to boost biogas generation in this situation. A low C/N ratio indicates insufficient carbon content or nitrogen underuse, resulting in ammonia buildup, low pH, and the presence of phenolic chemicals [39]. Carbon-rich substrates, such as kitchen waste and energy crops, might

be required to improve biogas production [38]. As a result, the digestion of energy crops alone may result in significantly low CH₄ yield (i.e., CO₂-rich biogas with poor CH₄) if an optimum C/N ratio (20–30:1) is not achieved [40].

The AcoD process is more suitable for optimizing the value of the C/N ratio than single substrate-based mono-digestion [1]. Substrates with the best carbon to nitrogen ratio may ensure the desired nutrition for the microbes' activities. In a previous study, a C/N ratio of 25 resulted in the best biogas yields when rice straw and *Hydrilla verticillata* substrates were co-digested [41]. In other experiments, biogas yield was boosted by 100.2% over the control with a C/N ratio of 25:1 over the value of 9 [42]. When the ratio of food waste to rice straw was tuned at C/N 30, pH 7.32, and F/M 1.87, the CH₄ yield increased by 94.41% over rice straw mono-digestion [10]. Contrastingly, the oily biological sludge's C/N ratio is lower than the ideal 20–30 ratio required by anaerobic digestion technology for desired biogas yield [37]. The C/N proportions of various single feedstocks are shown in Table 1. However, determining the appropriate C/N ratio for co-substrates is difficult because many parameters, such as substrate type, trace element content, chemical components, and biodegradability, can all influence the best value. When the C/N ratio deviates from the ideal, the system becomes unstable, and biogas output suffers.

Table 1. Different substrates characterized by C/N ratios lower and higher than the optimum value.

Comparatively Lower C/N Value Materials			Comparatively Higher C/N Value Materials		
Substrates	Lower C/N Value < 23	References	Substrates	Higher C/N Value > 24	References
Chicken manure	9.27	[43]	Corn stover	42.92	[43]
Vicuñas (VM)	15.40	[16]	Olive mill solid waste (OMSW)	31.4	[44]
Rugulopteryx okamurae	15.2	[44]	R. Okamurae—OMSW	27.4	[44]
CCF	13	[8]	Raw llama dung	26.8	[45]
Pig manure	11.70	[46]	Buckwheat hull	43.8	[47]
Raw dromedary dung	22.2	[45]	Cardboard (CB)	163	[48]
Palm oil mill effluent	9.7	[49]	Corn stover	40.8	[46]
Slaughterhouse waste	13.7	[47]	Brewery trub	33	[47]
Cucumber residues	14.76	[46]	Fruit wastes	44.7	[47]
Sewage sludge	8.5–12	[2,47]	Sophora flavescens residues	65.64	[2]
Dairy manure	22.5	[47]	Coffee husk	86	[20]
Water hyacinth	19.5	[50]	Cactus	27.9	[51]
MSW	18.4	[50]	Decanter cake	49.54	[49]
Llama manure (LM)	17.40	[16]	Food waste	24.6	[2]
Microalgae	15.3	[52]			
Empty fruit bunch	12.86	[49]			

3.4. Retention Time

The average time it takes slurry to transfer between the digester's entrance and exit is hydraulic retention time (HRT). Another retention time is solid retention time (SRT), described as the total length of time bacteria spend in the digester [25,53]. Microorganisms require sufficient retention time to convert organic substrates into desired products [54]. Long HRT improves the effluent quality [55], but, with longer residence time, the reaction rate decreases. If HRT is less than the optimal value, there is an accumulation of VFAs that inhibit bacterial activity, resulting in low biogas yields [56]. The proliferation of acetic acid is the main cause of the process disturbance observed at short HRTs [24]. In addition, low C increases the risk of biomass removal from the bioreactor, which may negatively affect the constancy of the entire system with VFA accumulation and increasing alkalinity [25].

Furthermore, the time it takes anaerobic digestion to degrade any waste is determined by the feedstock and ambient factors such as temperature. Anaerobic mono-digestion of lignocellulosic material is difficult due to low moisture content and slow mass transfer within the blend of matter in the reactor and requires a long retention time to degrade. To compensate for the drawbacks of AD of lignocellulosic biomass, Wickramarachchi et al. [57] conducted three serial experiments on co-digestion of rice straw (RS) and cow dung (CD), recycling slurry from the first to the second and from the second to the third reactors as

inoculum under batch mesophilic conditions. In the second experiment, the lag phase dropped from 14 days to zero, with increments of methane yields of 104%. The optimum HRT for different temperatures is shown in Table 2.

Table 2. Operating parameters and optimum ranges for the anaerobic digestion process.

Operational Parameter	Optimum	Reference
pH overall	6–8.5 (ideal 6.8–7.2)	
Methanogenesis	6.8–8.0	[20,58]
Alkalinity	1000–5000 mg/L as CaCO ₃	
C/N	20–30:1	[37]
HRT	70–80 days at a psychrophilic temperature, 12–40 days at mesophilic temperature, and 15–20 days at thermophilic temperature	[53,59]
OLR	0.5–4.7 kg VS/m ³ d	[60]
Particle size	Less than 10 mm is recommended	[60]
Semi-dry, wet, dry	10–20%, ≤10%, ≥20%, respectively	[14]

3.5. Ammonia

Biodegradation of nitrogenous materials produces ammonia as a byproduct. Above threshold concentrations, free ammonia nitrogen (FAN), dependent on pH and temperature [61], is a significant inhibitor species of total ammonia in an anaerobic digester [62]. Thus, the excess accumulation of ammonia in the digester increases pH and finally causes the failure of a process [63]. Due to the synergistic effect, the AcoD process recovers the excess ammonia inhibition by combining nitrogen-rich and carbon-rich substrates. Pig manure (PM) is rich in ammonia that might affect anaerobic microbial activity. For example, pig manure was added to carbon-rich organic matter as a neutralizing agent to assist in optimizing the AcoD process via avoiding acidification through VFA neutralization in the digestion of olive mill waste that was poor in nitrogen [39]. In another experiment, Fadaïro et al. [64] mixed poultry litter and cow dung with water hyacinth (2:2:1) and achieved the highest biogas yield of 3.073 L/kg VS over sole digestion of water hyacinth without addition of any improving agent.

4. Effects of Anaerobic Co-Digestion on Different Parameters

4.1. As Chemical Pretreatment

To improve the hydrolysis rates of lignocellulosic biomasses, different pretreatment methods have been tried; however, pretreatment is always related to cost and high-energy requirements. In addition, some pretreatment leads to the formation of compounds toxic to microorganisms, resulting in low biogas production [65,66]. The anaerobic co-digestion system overcomes the problem of chemical pretreatment by simultaneously introducing two compatible substrates in a sole bioreactor (Figure 3). As acid pretreatment, food waste (FW) that may be able to acidify during the digestion of a corncob was explored for eight days under mesophilic conditions [67]. Specifically, the effects of FW as pretreatment on different parameters such as VFAs, pH, lignocellulosic organic matter, and strength were considered. The result demonstrated that the addition of food waste increased the amount of VFA in the digester and enhanced the activities of microbial enzymes. A hydrolysis efficiency improvement of 28% over the control substrate, reduction of crystallinity by 6.7%, and an increment of cellulose digestibility of 13.2% were achieved. In the methanogenesis step, the biodigester offered the best stability with better efficiency in hydrolysis with pH recovery in the optimum range (pH 6.3–7.2). In addition, the result of kinetics analysis revealed that the pretreatment of FW might assist cellulose digestibility [67].

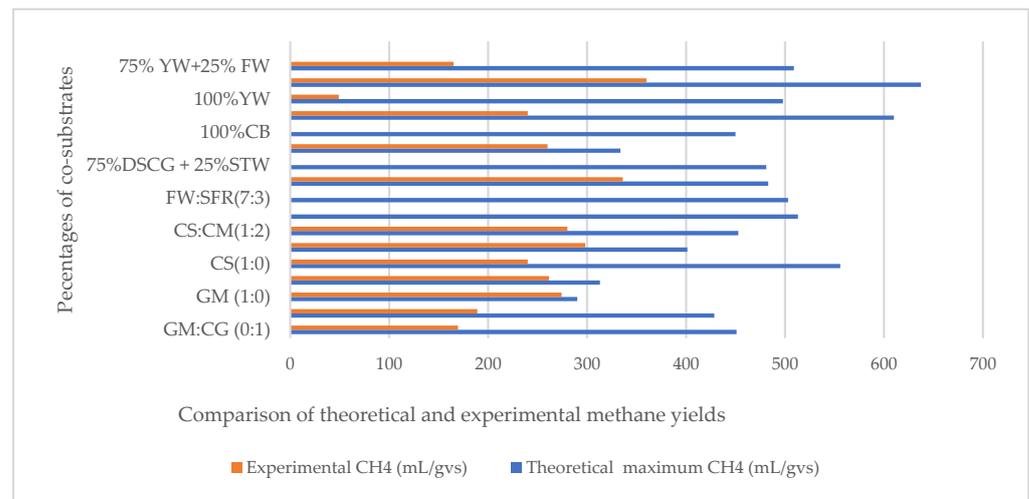


Figure 2. The best theoretical and experimental methane yields obtained from lignocellulosic biomass mono/co-digestion with food waste and animal manure versus mixing ratios (Table 3). Defatted spent coffee grounds (DSCG); macroalgae, MC (*Cladophora* sp.); glycerin (G); spent coffee grounds (SCG); spent tea waste (STG); chicken manure (CM); cotton gin trash (CG); goat manure (GM); corn stover; and *Sophora flavescens* residues (SFR).

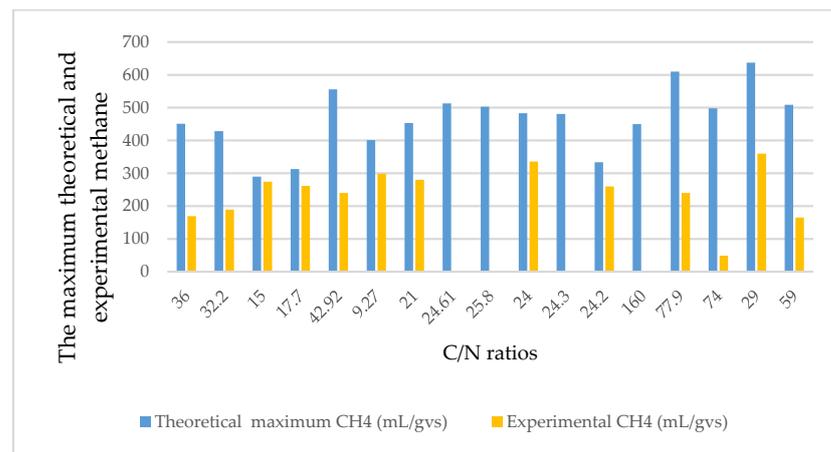


Figure 3. Comparison of the maximum theoretical and experimental methane of lignocellulosic biomass mono/co-digestion with food wastes and animal manure versus carbon-to-nitrogen ratio (Table 3).

In the classical anaerobic system, chemical buffering methods using chemicals such as sodium bicarbonate, calcium oxide [68], HCl, and NaOH, were reported as able to maintain the ideal pH setting [43,69]. Nevertheless, these agents are said to have a cost and may unfavorably hinder bioacid- and methane-forming microbes [68]. As a result, an organic co-substrate that primarily operates as a stabilizing agent to maintain pH during single-stage AD of FW is urgently needed. In light of this, the mesophilic anaerobic co-digestion of FW and grass clippings (GC) feedstock was examined as an environmentally beneficial neutralizing agent. The results showed that using GC to prevent pH decline in the biodigester helped to alleviate the redox environment and increase the chosen bioproducts, making the process more economical [68]. The acetic-acid-rich food waste assisted the derivation of lignin and depolymerization of cardboard without the help of the pretreatment step. In addition to focusing on expanding the potential of furfural wastewater as a low-cost acid pretreatment agent by substituting conventional acid pretreatment, Wang et al. [70] combined crop stalk and furfural wastewater at 20, 35, and 50 °C for 3, 6, and 9 days and conducted batch experiments for 25 days at 35 °C. The result showed that the maximum

total biogas product (196.68 mL/g VS) was produced by the treatment at 35 °C for six days, which was 59.28% greater than that produced by crop stalk without treatment. They revealed furfural wastewater as a feasible pretreatment agent for improving biogas in anaerobic co-digestion.

4.2. Synergistic Effect

4.2.1. Theoretical Biomethane Potentials

In the AcoD process, the biomethane potential (BMP) test may give the parameters to predict the theoretical BMPs of two or more co-substrates. Recently, several studies were performed on the effect of co-substrates on theoretical biomethane potentials (Table 3). For example, Kaur et al. [71] studied the effect of goat manure on the theoretical methane potential of lignocellulosic under different mixing ratios. The result indicated that the co-substrates did not enhance the theoretical methane of cotton gin trash in all mixing proportions. The maximum theoretical methane yield was obtained from mono-digestion of cotton gin trash with low biodegradability at a C/N ratio of 36. In addition, the co-digestion that obtained the maximum experimental methane yield did not show a similar trend for theoretical methane estimates [71]. In contrast, the maximum theoretical and experimental methane yield from mixtures of 25% yard waste (YW) + 75% FW [72] and combinations of 80% food waste and 20% cardboard [48] were attained (Table 3). Similarly, the better theoretical and experimental methane yield was obtained at the same mixing ratio [73].

Table 3. Theoretical and experimental methane yields of lignocellulosic biomass mono/co-digestion with food wastes and animal manure.

Mono/Co-Substrates	C/N	Mode Conditions	Theoretical Maximum CH ₄ (mL/g VS)	Experimental CH ₄ (mL/g VS)	References
Cotton gin trash (0:1)	36	BMP test, 36 °C ± 1	451.0	169.6	[71]
Goat manure:cotton gin trash (0.1:0.9)	32.2	BMP test, 36 °C ± 1	428.5	189.0	[71]
Goat manure (1:0)	15	BMP test, 36 °C ± 1	290	274.1	[71]
Goat manure:cotton gin trash (0.9:0.1)	17.7	BMP test, 36 °C ± 1	313.0	261.4	[71]
Corn stover (1:0)	42.9	Batch scale, 37 °C	555.81	240	[43]
Chicken manure (0:1)	9.27	batch scale, 37 °C	401.32	298.21	[43]
Corn stover:chicken manure (1:2)	21	Batch scale, 37 °C	452.82	280	[43]
FW (10:0)	24.61	Batch, 37 °C	513	nd	[2]
FW:Sophora flavescens residues (7:3)	25.8	Batch, 37 °C	503	nd	[2]
100% DSCG	24	Batch, 37 °C	483	336	[74]
75% DSCG:25% STW	24.3	Batch, 37 °C	481	231 ± 12	[74]
25% DSCG:75% MC	24.2	Batch, 37 °C	333.7	260	[74]
CB	160	Batch, 37 °C	450		[48]
80% FW and 20% CB	77.9	Batch, 37 °C	610	240	[48]
YW	74	BMP test, 37 °C	497.9	49	[72]
25% YW + 75% FW	29	BMP test, 37 °C	637.4	360	[72]
75% YW + 25% FW	59	BMP test, 37 °C	509	165	[72]

Comparatively, the maximum theoretical and experimental methane yields were achieved from co-digestion of yard waste and food waste (Figure 2). However, for most studies, the trend of theoretical and experimental methane yields was the opposite in the combination of two or more substrates. When theoretical methane yields decreased, the experimental methane yields increased for the different mixing ratios in the co-digestion of other substrates (Table 3 and Figure 2). In Figure 3, the effects of the carbon to nitrogen ratio on the production of theoretical and experimental methane yield are presented. This graph does not specify the effect of the C/N ratio on theoretical methane yield. The better theoretical methane yields were produced at the low or high values of C/N ratios. However, the maximum methane yields were produced at the optimum values of the C/N ratio (24 and 29) (Table 3 and Figure 3).

4.2.2. Biogas Yield

Lignocellulosic biomass is stubborn, it is difficult to degrade it without the aid of pretreatment, and microorganisms consume resources quickly, resulting in an imbalance between acidogenesis and methanogenesis during the first phase of AD. This fluctuation is owing to the excess formation of volatile fatty acids (VFAs) [19,75]. In the past, in AD, different wastes, such as municipal solid waste, food waste, kitchen waste, and animal manure, were used for biogas generation [50,76–78]. However, recently, attention has shifted to the co-digestion of lignocellulose material with kitchen waste, food waste, and animal manure to generate biogas due to various advantages [14,47,79]. The major drawback of food waste digestion is the fast hydrolysis rate and the resulting drop in pH (<5.5) because of the accumulation of volatile fatty acids [80]. Therefore, co-digestion of food waste with stubborn feedstocks can help to retard the hydrolysis rate and reduce the VFAs [81]. El et al. [81] co-digested agricultural waste, such as wheat straw and cow manure, with food waste in semi-continuous mesophilic AD and considered the effect of mixing ratio, C/N ratio, and variation of organic loading rates from 2 to 3.6 kg VS/m³d on the digester performance. The result demonstrated that the highest biogas yield increased by 119.97% for the organic load of 3.6 kg VS/m³d fed with the optimum mixing ratio of FW:CM (75:25) and a C/N ratio of 20.03.

As discussed before, the essential need for co-digestion is to achieve synergy between feedstocks in terms of yields of biogas and methane. This means that biogas obtained from digesting two feedstocks at once is higher than the amount produced by substrate mono-digestion ($AC > A + C$) [80,82]. The synergistic index (α) is used to assess the interaction between two or more feedstocks fed into a biodigester at the same time as co-substrates. If $\alpha > 1$, the synergistic effect occurs; if $\alpha < 1$, an antagonistic effect occurs. The antagonistic effect is caused when incompatible feedstocks are co-digested [72], and, if $\alpha = 1$, the interaction between co-substrates is unclear [43]. This parameter is used to indicate the performance of the process; however, it is not used to indicate the maximum methane yield, which means that the maximum synergistic index does not promise the highest methane yield [83,84]. Accordingly, both specific methane yield and the synergistic index can be measured to decide the optimum mixing ratio in AcoD. The best synergistic index, mixing ratios, and methane yields from several studies are shown in Figure 4 and Table 4. Furthermore, the approaches to improve the biogas yields of lignocellulosic biomasses based on co-digestion are shown in Figure 5.

Other important advantages attained with anaerobic co-digestion are the neutralization of inhibitory compounds, cost reduction through digestion of two or more substrates [85–87] and subsequent greenhouse emissions reduction [20]. Moreover, it decreases the hydrolysis rate [88]. Cow manure contributes to maintaining the digesters' optimal pH values, although its strong neutralizing power is unrelated to the C:N ratio. In another study, meadow grass and wheat straw digested with cattle manure in thermophilic (53 °C) conditions exhibited enhancement of biomethane by 20–24% over straw digestion alone. Furthermore, they achieved optimum co-digestion with a maximum yield of methane at 25%, the smallest lag of 6–7 days after 75% of organic matter was initiated from cattle manure, and the overall biodegradability, as compared to single feedstock digestion, was enriched and boosted methane yield [89].

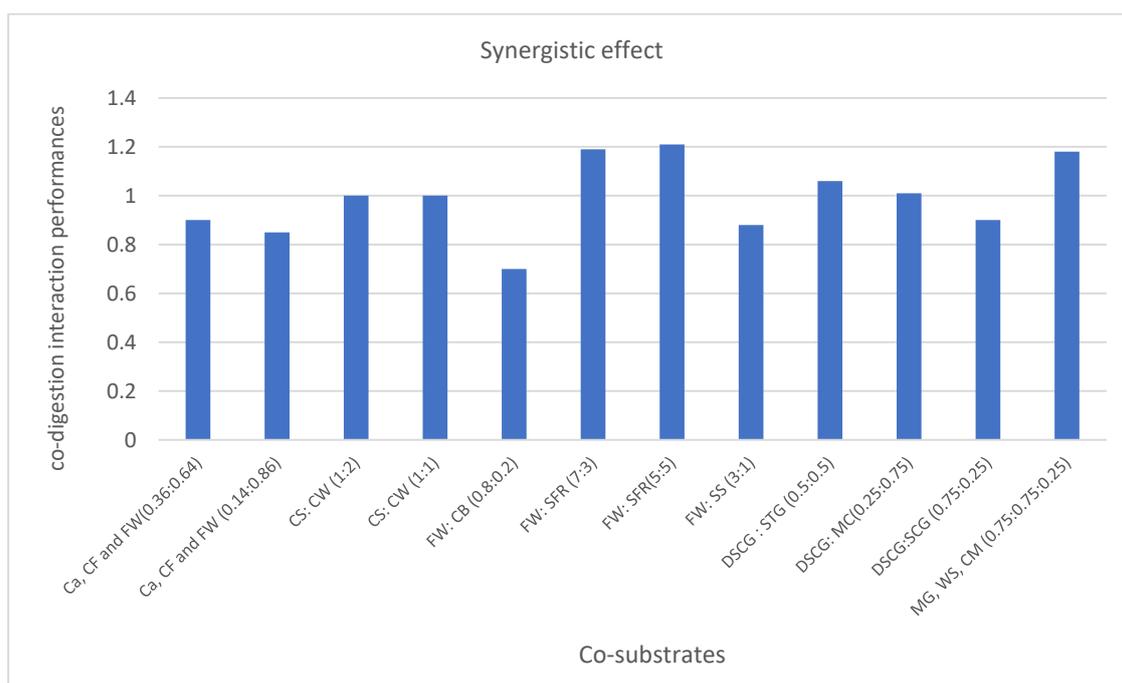


Figure 4. The co-digestion interaction index (α) of various co-feedstocks (Table 4).

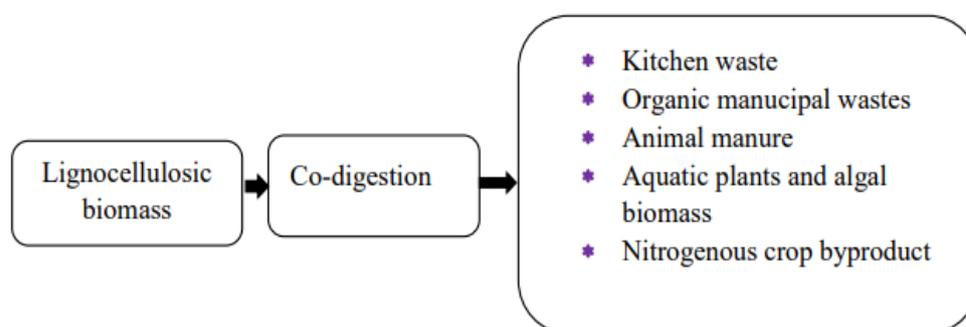


Figure 5. Innovative approaches based on co-digestion to improve lignocellulosic biomass via AD.

Table 4. Operating parameters for AcoD and their respective achievements in terms of biomethane yields with better biodegradability.

Co-Substrate	C/N	BD _{th} (%)	Mode and Condition	Synergistic Effect	Methane Yields	References
Cabbage cauliflower and FW (0.36:0.64)	45	98	BMP test at 37 °C	0.9	475 mL _{STP} CH ₄ /g VS	[8]
Cabbage and cauliflower FW (0.14:0.86)	56	85	BMP test, 37 °C	0.85	433 mL _{STP} CH ₄ /g VS	[8]
Corn stover:chicken waste (1:2)	21	70.60	Labscale, 37 °C	1	319.70 mL/g VS	[43]
Corn stover:chicken waste (1:1)	26	60.02	Labscale, 37 °C	1	287.28 mL/g VS	[43]
FW:CB (0.8:0.2)	60	39	Pilot scale, 37 °C	0.7	240 mL/g VS	[48]
FW:SFR (7:3)	25.8	58.83	Batch, 37 °C	1.19	640 mL/g VS	[2]
Food waste:Sophora flavescens residues (5:5)	27.3	58.11	Batch, 37 °C	1.21	629 mL/g VS	[2]
Food waste:sewage sludge (3:1)	28	40	Batch, 37 °C	0.88	452 mL/g VS	[36]
Defatted spent coffee grounds:spent tea grounds (0.5:0.5)	24.2	66.4	BMP, 37 °C	1.06	318 mL/g VS	[74]
Defatted spent coffee grounds:macroalgae (0.25:0.75)	24.2	77.9	BMP, 37 °C	1.01	260 mL/g VS	[74]
Defatted spent coffee grounds:spent coffee grounds (0.75:0.25)	24.8	64.3	BMP, 37 °C	0.9	306 mL/g VS	[74]
Meadow grass:wheat straw:cattle manure (0.75:0.75:0.25)	34	83	Batch, 53 °C	1.18	351 mL/g VS	[89]

4.2.3. Microbes Delivery

Different substrates contain microorganisms important for biodegradation, simultaneously sustaining various archaea through co-digestion and limiting the risk of microbe wash away. Co-digestion has the potential to ensure a microbial population with a more complex diversity than a sole substrate as varied microbes are constantly hosted from co-substrates [90]. Thus, anaerobic co-digestion of compatible substrates improves the stability of the bacteria population. In classical AD, it is mostly groups of phyla, such as *Firmicutes*, *Bacteroidetes*, *Fibrobacteres*, *Proteobacteria*, and *Actinobacteria*, that are dominant [91]. However, microorganism structures depend on the types of feed in a digester. For example, a sewage-sludge-fed AD system sustained *Microtrichaceae*, the mono-digestion of cellulose had a bacterial population dominated by *Ruminococcaceae* (79.20%), and, for hemicellulose, *Clostridiaceae* dominated by 84.57%. In the same study, the co-digestion of sewage sludge and lignocellulosic biomass was dominated by *Actinobacteria* (40%), *Proteobacteria* (14.38%), and *Chloroflexi* (23.89%), which showed improved bacterial population diversity over a sole substrate [91].

In another study, Zhang et al. [92] co-digested FW, cattle manure (CM), and corn straw (CS) in a mesophilic batch experiment focusing on the relation between synergy and microbes. The result showed that 65% FW + 35% CM digestion maintained the principal growth of hydrogenotrophic methanogens (68.9%) with maximum synergy because of dual-effect neutralizing inhibitory compounds. Different mixing fractions of feedstocks and working conditions also affect the microbe structure. For example, Shi et al. [29] studied the effects of working conditions and co-feed ratio on microbe structure and the stability of the system using the co-digestion of wheat straw and food waste under batch thermophilic and mesophilic conditions. The result indicated that, for a FW sole substrate, both digesters were disturbed. Further, an excess concentration of VFA failed in a mesophilic digester, and a thermophilic digester showed better stability than the mesophilic one. In addition, raising the fraction of FW supported the bacteria group of the phylum *Thermotoga* with the largest numbers in the thermophilic digester, whereas the phylum *Bacteroidetes* was the largest in the mesophilic digester [29]. It is worth noting that microbial diversity is not the only thing to examine when assessing the efficacy of AD processes; the community's functional status and resilience are also crucial. It is well acknowledged that co-feedstocks improve stability and overall digestibility by facilitating enzymatic activities through a better balance of micronutrients and trace minerals [90,93,94].

4.3. Biodegradability (BD)

Biodegradation involves microbiological degradation of biowaste by microbes under oxygen-deficient conditions for anaerobic digestion or oxygen-sufficient conditions for aerobic. Thus, it is used to realize the theoretical biomethane potential of biomasses. The realization of the theoretical potential is equal to the actual yields divided by theoretical potentials. Mathematically can be defined as

$$\%BD_{th} = \frac{\text{Actual yields}}{\text{Theoretical potentials}} * 100 \quad (1)$$

There are various physiochemical and natural aspects that affect the biodegradability of an organic substance, including bioaccessibility, temperature, pH, and moisture content [1]. There are several pretreatment methods, such as physical, chemical [95], biological [42], and physiochemical methods, and their combination, which are applied to improve the digestibility of lignocellulosic materials before the AD process to enhance the volume of biogas of sufficient quality by facilitating lignin removal and the destruction of the complex structure of organic biomass [96,97]. For example, as per reported studies on pretreatment approaches for biogas production, a 1200% boost in the yield of biogas was achieved with ionic liquid pretreatment of lignocellulose [98]. In addition, the pretreatment must be effective and economical and ideally meet the following requirements: expose lignin to enzymatic destruction, have a lesser effect on hemicellulose and cellulose destruc-

tion, minimize the production of an inhibitory compound for enzymes and fermenting microbes, and minimize cost and the energy requirement [97,99]. Each of these methods has individual negative and positive effects. As a result, one technique cannot apply to all kinds of lignocellulosic material. Thus, a single pretreatment technique that achieves all criteria for various types of feedstocks is still not available.

In the AcoD process, the biochemical methane potential (BMP), as in [2], may help to anaerobically evaluate the biodegradability and the quantum of organic matter in various feedstocks that change to biomethane during anaerobic digestion [100]. In addition, the BMP may be helpful for determining the organic content (VS) in the substrates that change to methane (biogas) in a given amount of time and remains for further management. It can also help researchers to figure out the best mixing ratios for the co-digestion process [47,95,101]. Hamrouni [36] co-digested Mediterranean FW and sewage sludge (SS) in both batch and semi-continuous experiments to predict the degradation using the BMP test. The report showed that FW had a higher biodegradability than SS. In line with this observation, Hamrouni [36] also found that, when mixed at SS:FW 1:3, the feed resulted in better biogas production and minimum biodegradability, which dropped the BD of FW to 40% (Figure 6).

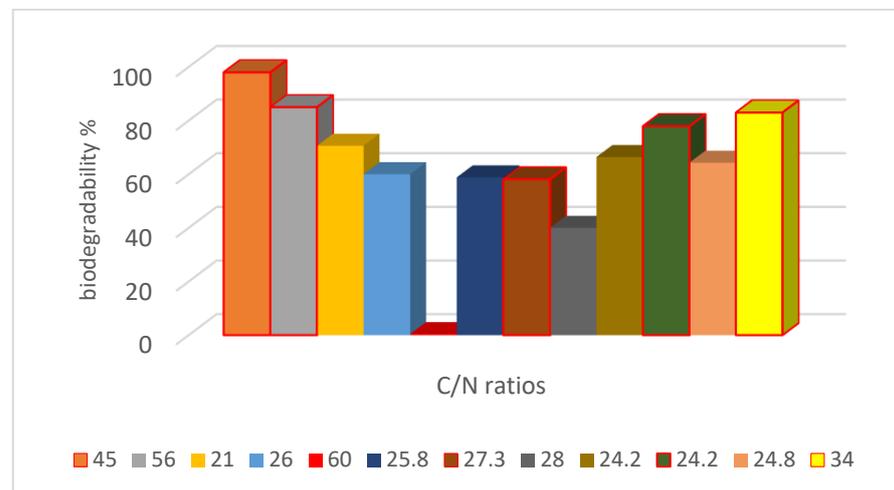


Figure 6. Realization of theoretical methane potential of organic waste co-digestion versus C/N ratios (Table 4).

Cardboard and acidified food waste were co-digested to improve digestibility under mesophilic conditions. The highest methane yield of 0.24 L/kg and biodegradability of 39% was obtained, with better stability without any pretreatment method. However, due to low moisture content and slow mass transfer within the reactor's blend of matter, solid anaerobic co-digestion of lignocellulosic material had limitations [48]. For instance, based on the benefits of AcoD, the balance of pH for acidogenesis and methane-forming archaea was studied under mesophilic conditions to improve system stability and yield. The findings demonstrated that adding 20% of food waste to garden waste enhanced methane and organic material elimination by 83% (VS) [34].

BMP tests may indicate the parameters to predict the degradation of organic matter. In the co-digestion role, combining different feedstocks might at least, improve the biodigestibility of one substrate as the balance of the BD of both. With this baseline, Atelge et al. [74] studied digestion of defatted spent coffee grounds (DSCG) without other feedstocks and co-digestion with other feedstocks, such as macroalgae (MC), spent tea grounds (STG), and spent coffee grounds (SCG), at batch mesophilic conditions for 49 days to improve the performance of the system. The result demonstrated that, for a sole feed, in the case of VS conversion, the better removal efficiency of 35.48% reflected that the biodegradability of organic biowaste improved with maximum methane yield from oil-extracted spent coffee grounds because of the organic composition of proteins, sugars,

and lipids. At the same time, oil extraction assisted as a pretreatment agent. For co-feed, they achieved the maximum yield with the best biodegradability from DSCG and STG (50%:50%); however, biodegradability was not the highest (Figure 7) [74]. However, Awais et al. [89] observed a better BD (83%) and maximum methane yield with mixed meadow grass, wheat straw, and cattle manure at a ratio of 0.75:0.75:0.25, with better performance of the system.

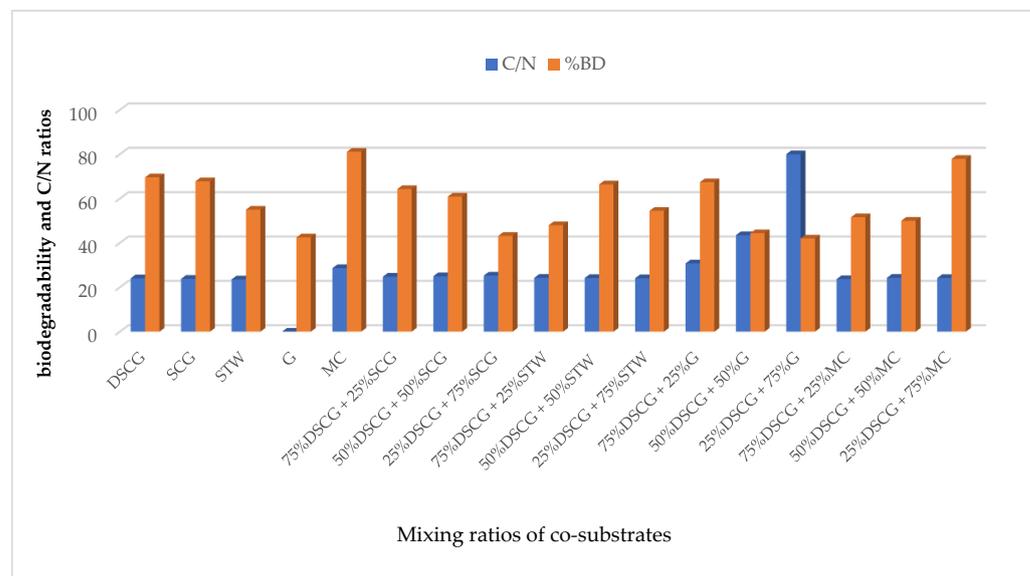


Figure 7. Comparing the biodegradability and C/N ratios to mixing ratios of different organic wastes in mono- and co-digestion (data from [74]). Defatted spent coffee ground (DSCG); macroalgae, MC (*Cladophora* sp.); glycerin (G); spent coffee grounds (SCG); spent tea waste (STG).

4.4. Moisture Contents

Moisture content determines the kind of biodigester based on the total solid composition in the organic matter added. Based on the moisture contents, the biodigester can be classified as a solid, semi-solid, or liquid anaerobic digester [14]. In line with this benefit, anaerobic co-feeding is the best potential pretreatment method that combines high and low moisture feedstocks into a bioreactor at the same time, ensuring proper growth and free mobility of microbes in the bioreactor, and elevating yields of the bioreactor. Animal manures contain high moisture content, which might be responsible for sufficient moisture for the formation of archaeal function [14] and create benefits in anaerobic co-digestion. Concerning this significance, they achieved the best yield of biogas ($307\text{-cm}^3 \text{CH}_4/\text{g}$ (VS)) from the digestion of pig manure, water hyacinth, and poultry droppings via a mixing ratio of 15:40:45 under mesophilic conditions [102].

4.5. Stability

4.5.1. pH

The pH value is a prominent operational parameter that strongly affects microbial activities and biomethane yields. Most microorganisms prefer a neutral pH in the biogas generation process. Although a different group of microbes plays a great role that needs varied optimum pH settings for their metabolism and anaerobic digestion, keeping a pH range of 6.3–7.2 is important to achieve a high biogas yield [23,39,67]. Methanogens are extremely sensitive to pH changes and prefer a pH of 7.0 [20], while acidogenic microbes require a pH range of 4–8.5. The co-substrate may permit operational constancy of the digester by buffering the extreme acidification/alkalinity condition. As per a reviewed study, in co-digestion, it is simple and easy to keep ideal pH settings constant during the period of the digestion process compared to during the digestion of a substrate alone [1,23].

4.5.2. Organic Removal Efficiency

As the significance of AcoD rises, it is essential to find a method to assess the biodegradability of substrates and the biogas production performance. In light of this, the BMP test may give useful information to predict the overall performance of the biogas digester. In the AcoD system, VS/COD removal is very crucial since biogas yield, in terms of VS/COD removal, can provide an overview of the efficiency of the anaerobic process [103]. From this perspective, a product of biogas is informed in terms of VS/COD removal. COD balance indicates the synergistic effect of co-digestion due to a blend of supplement feedstocks. Food waste was confirmed as the best way to speed up AcoD for surprising biogas production due to the formation of high acidity in the initial stage [36,104]. In addition, Elsayed et al. [35] mixed cow manure, linen (Ln), and wheat straw, focusing on the effects of the mixing ratio on process performance in a mesophilic digester using inoculum (sludge) in a BMP experiment. They demonstrated that the maximum methane yield of 351 mL/g VS was obtained at a mixing ratio of 50:25:25 with VS removal efficiency of 72.87% [35]. Further, the better strategies to improve biogas yields and VS removal efficiency is shown in Figure 8.

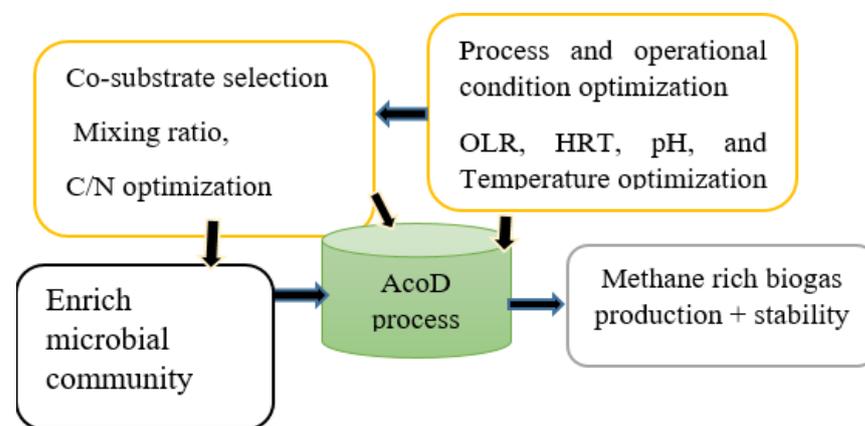


Figure 8. Relations among some factors affecting the performance of AcoD [103] and strategies to improve biogas yields.

4.5.3. Organic Loading Rates (OLRs)

The organic loading rates are one of the major parameters that indicates the capacity of the bioreactor. The real feeding rate of organic matter into the reactor depends on the types of waste. Increasing organic loading rates boosts microbe activities, which results in enhanced biogas production to some extent [105,106]. However, introducing a substrate into the digester without considering the optimum rate reduces the biogas volume. Overloading the digester may block the mobile microorganisms, cause the over-concentration of VFA, which mainly influences methane-producing bacteria, and result in low biogas yield [107]. Underload (introducing a small amount of organic substance) leads to the formation of alkalinity in the biodigester, resulting in low biogas yields [14]. In addition, increasing OLR exhibited a decrease in biogas yield by 168% even for the co-digestion process [15] and formed instability in the biodigester by influencing alkalinity [108].

The advantage of AcoD over mono-digestion is that AcoD has a higher organic loading and significant substrate composition variation [9,15,44,109,110]. In addition, methane yield is boosted with the optimum AcoD process through tolerable organic loading and a solid retention period, which may improve organic matter removal and VFA conversion. Further, it leads to a reduction of biosolids odorous releases [13]. Kesharwani et al. [15] explored the effect of OLRs on biogas at a pilot scale under ambient conditions. They achieved the highest biogas yield from co-digestion of food waste with cow dung compared to mono-digestion of cow dung (Table 4) without disturbance of inhibitory compounds. In the laboratory study, the methane production via mono-digestion of cow dung was higher

at lower OLRs, whereas the trend was the opposite in the co-digestion of cow dung and grass silage [25].

4.5.4. VFA, TA, and VFA/TA Ratio

VFA is a key indicator of the stability of an anaerobic digester. The excess formation of VFA can drop the pH in biodigesters, affecting active, methane-producing archaea and causing a disturbance of digester stability. The only balance for VFAs that leads to process stability is their removal, i.e., biogas conversion. High VFAs (28.88 g/L) cause long-term yield and methanogenic microbe suppression [105]. Therefore, anaerobic co-digestion is a promising method that ensures the achievement of higher yields in a short retention time with the addition of greater OLRs when compared with anaerobic mono-digestion of organic substrates.

Alkalinity (TA) is the acid-neutralizing ability of an aqueous solution. It is a more reliable indicator of digester imbalance than direct pH testing. The archaea produce alkalinity in the form of CO₂ and bicarbonate that offsets the pH drop. Pig manure (PM) contains a lot of ammonia that might repress the process anaerobically. PM coupled to assist the AcoD process as a buffering agent achieved a synergetic effect by avoiding acidification during co-digestion through VFA neutralization in olive mill wastewater, which is a low-nitrogen, low-pH substrate. As a result, a high biogas yield and stability were attained [39].

VFA/TA ratio is another parameter that measures the performance of the anaerobic digestion process that is more reliable than measuring the pH directly. In anaerobic conditions, depression in alkalinity or over-rising of VFA causes a quick drop in pH [30]. Thus, it is considered “the early warning indicator of process failure” within an optimum range of 0.2–0.4 [15]. It is a good parameter in the buffering of anaerobic process analysis [108]. VFA/TA ratios are used to assessing the effects of increasing the OLRs on the digester performance. For example, Mu et al. [72] experimented on SS, FW, and YW co-digestion under a BMP test and semi-continuous mesophilic reactor for 60 days. They achieved enhancement of the stability process and archaea/total microbe ratio by 17.1% from SS (25% vs. basis) in FW, which could be due to the delivery of trace metals by SS which buffered the inhibitory matter that affects microbe competition.

5. Conclusions and Recommendations for Future Studies

AcoD is a cutting-edge technology that is commercially and environmentally viable with novel approaches to increasing biomethane generation. The handling of AcoD technology requires a good understanding of the effect of different operating conditions. The effects of co-substrates on the performance of the AcoD process have been mentioned and detailed. Additionally, some recommendations for future work are worthwhile: The effects of co-digestion on theoretical biomethane production, volatile solid conversion rates, and the relation between theoretical biomethane, biodegradability, and volatile conversion rates need further investigation. Consideration of the effect of co-substrates on the start of the anaerobic digestion process and hydrolysis rate may also be required in future works. In addition, further studies may be necessary on the accompanying co-feed types with the groups of microbes and their activities.

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