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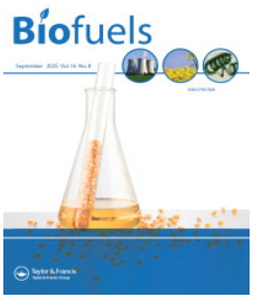

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Exploring potential synergies in the integration of anaerobic co-digestion with dark fermentation or microbial electrolysis to enhance methane output

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ABSTRACT

Anaerobic co-digestion (A-coD) of multiple substrates is a sustainable technique for converting mixed organic wastes to bioenergy. Research highlights A-coD's benefits, including improved treatability via synergistic effects, nutrient fulfillment, and enhanced process stability. Studies show methane production can increase by 20% to 170% using co-digestion versus mono-substrate digestion, depending on factors like substrate composition, organic loading rate, carbon-to-nitrogen ratio, and hydraulic retention time. Despite recent A-coD developments, integration with microbial electrolysis cells (MECs) and dark fermentation (DF) remains unexplored. However, studies indicate the integrated system could boost methane production by up to 80%, maintaining a 40.6% biological stability rate compared to control. This review analyzes co-substrate compatibility and their synergistic and antagonistic interactions during co-digestion. It also explores coupling A-coD to MEC and DF using co-substrates and their impact on biogas production. Conclusions and predictions aim to provoke the development of a bespoke substrate optimized for co-digestion compatibility.

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KEYWORDS
Anaerobic co-digestion; co-substrates; biogas production; microbial electrolysis cell; dark fermentation; renewable energy; microorganisms

Introduction

In the global effort to find sustainable and efficient renewable energy solutions, the integration of advanced bioenergy technologies has become a significant area of research. This review paper delves into the innovative synergies that can be realized by merging anaerobic co-digestion (A-coD) with either dark fermentation (DF) or microbial electrolysis, with a focus on their potential to increase methane production. As the world faces growing demands to lower greenhouse gas emissions and transition to cleaner energy sources, the development of optimized bioenergy production systems is more vital than ever [1].

A-coD has been a fundamental technology for transforming organic waste into biogas, which is mainly methane. This method not only tackles waste management issues but also offers a significant source of renewable energy (Figure 1). Nevertheless, the effectiveness of methane generation through A-coD alone is often limited by factors such as the composition of the substrate, the conditions of the process, and the dynamics of the microbial community [4]. In order to address these constraints and enhance methane production, scientists have been investigating the advantages of combining A-coD with other supportive technologies [5].

DF and microbial electrolysis are two promising technologies for boosting methane production. In DF, microorganisms break down organic materials without light, producing hydrogen and other valuable by-products. This process can generate a rich supply of substrates for further methane production through A-coD [6]. Conversely, microbial electrolysis is a developing technology that employs electroactive bacteria to transform organic substances into hydrogen or methane when an electrical potential is applied [7]. Combining microbial electrolysis with A-coD could potentially establish a synergistic system that enhances methane production while also boosting the efficiency and sustainability of the overall process [1].

This review offers an in-depth examination of the technical and biological components of these integrated systems, focusing on how synergies can be realized and the elements that affect their efficiency. Additionally, it aims to present a comprehensive

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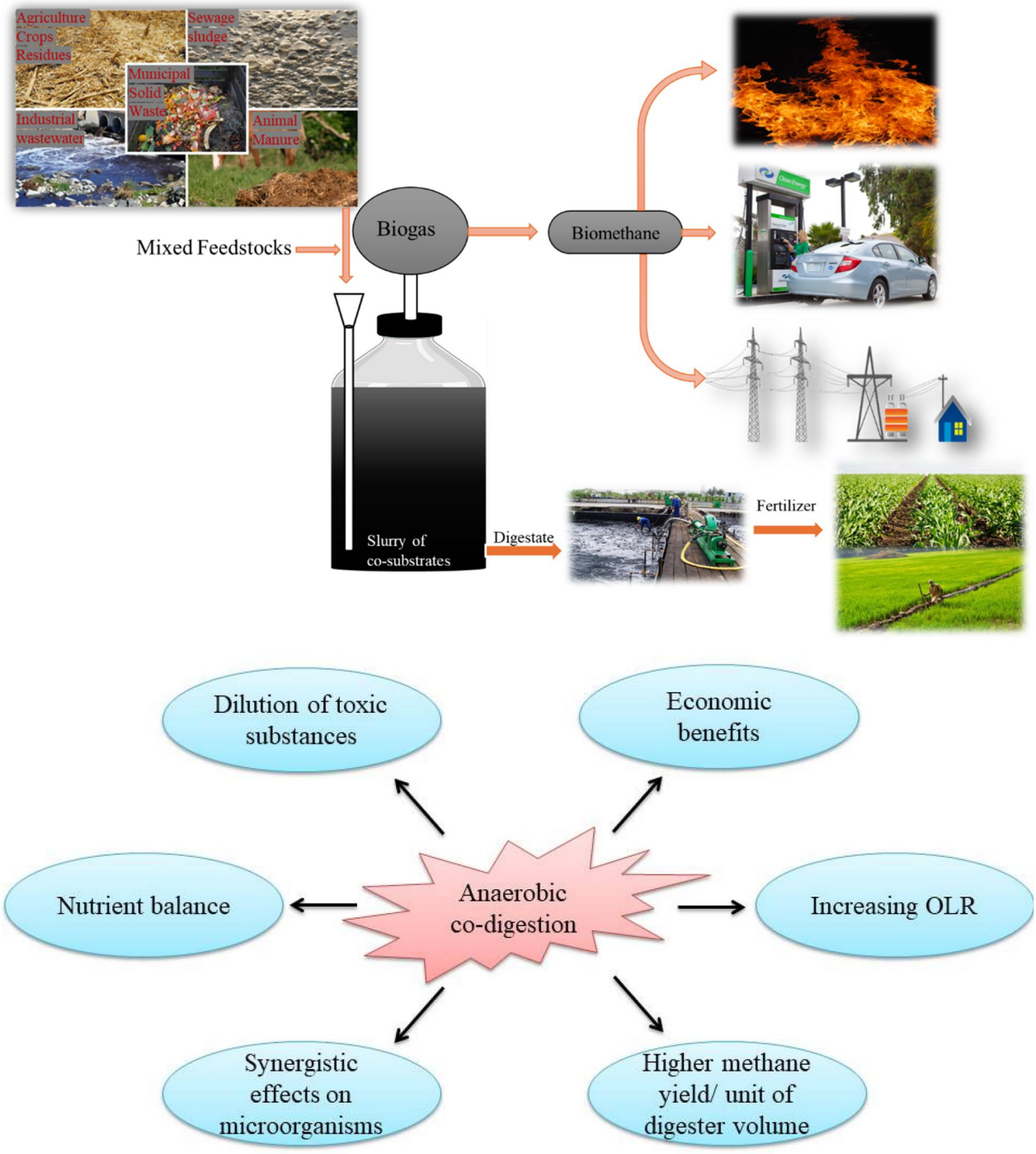

Figure 1. Diagrammatic representation of the anaerobic co-digestion of various organic wastes and the potential benefits for green energy recovery and environmental pollution reduction [2,3]. This figure depicts anaerobic co-digestion (A-coD) as a sustainable approach for generating bioenergy and enhancing waste value. A-coD facilitates the concurrent digestion of a range of organic materials—such as agricultural by-products, municipal solid waste, industrial discharges, sewage sludge, and animal manure—to yield biogas that is high in methane content (biomethane). The digestate, a secondary product of this process, can be applied as an organic fertilizer, supporting the principles of a circular economy.

narrative on research into the potential of compatible co-substrates for application in A-coD. By thoroughly reviewing the literature and analyzing experimental data, we strive to provide a detailed understanding of the potential advantages and obstacles linked to A-coD and its integration with DF or microbial electrolysis. Ultimately, this research endeavors to aid in the development of more efficient and sustainable bioenergy production methods, paving the way for a cleaner and more resilient energy future.

Synergistic impact of co-substrates on biogas production

Table 1 provides a summary of the use of multiple substrates in relation to the performance of A-coD and indicates that these, under anaerobic conditions,

Table 1. Performance of anaerobic co-digestion of different organic wastes (adapted from ref. [4] with copyright permission from Elsevier.

Co-substrates	Substrate ratio	Operating conditions	C/N ratio	OLR kg VS/ m^3.d	HRT (days)	SMY (mL/gVSadded)	VSRemoval (%)	Observations	Ref.
Cassava pulp: Swine manure	77:33 (w/w)	Semi-continuous 35 °C	35	6	23	380	82	Accumulation of low cyanide (0.5 mg/L)	[8]
Cassava pulp: Swine manure	3:2 (VS)	Semi-continuous 35 °C	33	3.5	15	3.6	61	SMY increased by 159% in co-digestion compared with single digestion of cassava pulp	[9]
WS:Micro-algal biomass	1:1 (VS)	Continuous stirred tank reactor 35 °C	13	1	20	240	48	Compared to mono-digestion of wheat straw, 15% higher SMY resulted in co-digestion	[10]
Vinase: Sugarcane press mud cake	73.27(VS	Semi-continuous reactor 35 °C	N/A	2.2	24	366	N/A	174% enhancement in SMY in co-digestion compared with single digestion of sugarcane press mud cake	[11]
Liquid hydrolysate of wheat straw	1:1 (COD)	Upscale anaerobic sludge bed reactor (UASB) 35 °C	N/A	6.6 (kg COD m^{-3}	3	220 mL g^{-1} COD	96 ($COD_{removed}$)	pH: 6.9 TAN: 0.1 g N/L	[12]
Sugarcane filter cake: Bagasse	7:3 (w/w)	Continuous stirred tank reactor 35 °C	41	3	28	176	N/A	Compared to mono-digestion 31% increased SMY was observed in co-digestion	[13]
FW: Dewatered sludge	1:0.4 (VS)	Lab scale	10–13	12.5	12	352	65	Low accumulation of VFA and TAN but increased FAN was observed	[14]
FW: SS	7:3 (TS)	Pilot scale	9–10	2.4	25	449	64	No process upset	[15]
FW: Fruits and vegetable waste and SS	2:1:1 (w/w)	Continuous stirred tank reactor (pilot scale) 35 °C	N/A	4	33	421	>60	The rate-limiting step was methanogenesis, but no inhibition occurred at OLR of 8 kgVS/m^3.d,	[16]
FW: SS	7:1 (N/A)	Pilot-scale	N/A	8.7 (kg COD/m^3.d	24	289	70	No process upset	[17]
FW: Chicken manure	2:1	Continuous stirred tank reactor (lab scale)	7–14	2.5	35	508	N/A	The SMY was not significantly higher in co-digestion compared to mono-digestion	[18]
FW: Chicken manure	7:3 (VS)	Continuous stirred tank reactor (bench scale)	20	4	17	660	63	SMY increased by 88% in co-digestion compared with mono-digestion of FW	[19]
FW: CM	2:1 (N/A)	Continuous stirred tank reactor (lab scale)	16	12	NA	388	N/A	Nearly 4-fold increased SMY observed in co-digestion	[20]
SS: Grease trap sludge	7:3 (VS)	Pilot scale	N/A	2.5	10	344	58	SMY enhanced by 27% together with VS reduction co-digestion of sewage sludge	[21]
SS:Grease trap waste	7.3 (VS)	Lab scale	N/A	3.5	16	463	67	SMY increased: 67% higher in co-digestion than single digestion of SS	[22]

(*continued*)

Table 1. Continued.

Co-substrates	Substrate ratio	Operating conditions	C/N ratio	OLR kg VS/ m^3.d	HRT (days)	SMY (mL/gVSadded)	VSRemoval (%)	Observations	Ref.
SS: Grease trap waste	22:3 (VS)	Lab scale	N/A	1.9	10	349	50	Co-digestion improved SMY by up to 93% compared to mono-digestion of SS	[23]
SS: Grease trap waste: OFMSW	4:3:3 (VS)	Lab scale	N/A	1.8–2.2	NA	547	80	20% increase in SMY resulted in co-digestion	[23]
SS: OFMSW	4:1	Full scale	N/A	0.8–1.0	20	600–890 mL (biogas yield)	81	Electrical energy (by 130%) and heat production (by 55%) are enhanced by co-digestion compared with mono-digestion of sewage sludge	[24]
Chicken manure: FW: WS	3:1:1 (N/A)	Semi-continuous	21.9	2.0 (kg TS/m^3.d	20	351	42	In co-digestion, a 93.4% increase SMY was observed when compared to single digestion of chicken manure	[25]
Cattle manure: Cheese whey	1:1 (V/V)	Two-phase	N/A	1.7 (kg COD/m^3.d	20	258	83 (sCOD)	About a 2-fold increase in SMY was observed in two phase compared with one phase	[26]
Swine manure: Corn stover (pretreated at 5% NaOH	1.2:1 (N/A)	CSTR	25	2.0	21	282	N/A	Increased methane yield in co-digestion compared with mono-digestion of swine manure	[27]
Chicken manure: Apple pulp	2:1 (N/A)	Semi-continuous	18.5	4.8	25	340	N/A	Inhibition caused by accumulation of VFA and TAN in mono-digestion	[28]
Swine manure: Olive mill wastewater	3:2 (N/A)	CSTR	N/A	4.4	30	373	71	Increase in phenol concentration did not cause inhibition in co-digestion	[29]

SMY: specific methane yield; TAN: total ammonia nitrogen; VFA: volatile fatty acid; VS: volatile solids; CSTR: continuous stirred tank reactor; OLR: organic loading rate; HRT: hydrothermal pretreatment; FW: food waste; CM: cattle manure; WS: waste sludge; OFMSW; organic fraction of municipal solid waste; SS: sewage sludge; COD: chemical oxygen demand; N/A: not available.

can exert synergistic impacts to enhance the overall digestion process [30,31]. The enhancement of methane output during A-coD is associated with the type and amount of organic matter used as feedstock and the effective dilution of any inhibitory components present [32]. For instance, by using multiple substrates and co-digestion, inhibitory compounds like ammoniacal nitrogen, phenolics such as eugenollignin-derived phenolic acids, and furans were successfully reduced in laboratory-scale experiments [33,34]. These compounds negatively affect microbial activity in anaerobic reactors, resulting in decreased bioconversion of organics into carbon dioxide and methane.

Synergistic (i.e. CPI $<$ 1, CPI $=$ 1, CPI $>$ 1, where CPI $=$ co-digestion performance index) impacts of co-substrates in the A-coD process can be evaluated by finding the CPI using Equation (1). The CPI is defined as the specific methane yield (SMY; determined from Equation (3)) generated during the co-digestion process as a function of the average SMY generated during mono-digestion and is equivalent to the volume of methane generated per unit of volatile solids (VS) of the substrate.

$$CPI = \frac{MYg}{MYw} \tag{1}$$

where MY_g is the measured methane produced during the co-digestion of co-substrates and MY_W is the combined calculated methane yield of the co-substrates produced during the mono-digestion of each substrate and their composition. The MY_W value can be calculated using Equation (2):

$$MYw = MYi \times Pi + MYj \times \mathrm{Pj} \tag{2}$$

where MYi and MYj are the experimental methane production of feedstock i and feedstock j, respectively, in mono-digestion, expressed as L.g^{-1} VS; and *Pi* and *Pj* are the respective percentages of feedstock i and feedstock j in the co-feedstock per unit VS. The optimal mixing ratio of co-substrate in the co-digestion should be identified using SMY (L CH_4.g^{-1} VS) and CPI (g^{-1}.VS) values. Still, CPI should only be used as a performance indicator, as a high CPI is no guarantee of a high SMY [35].

$$SMY = \frac{Biogas\ yield \times Methane\ content}{VS} \tag{3}$$

One study reported that mono-digestion of food waste (FW) gave a high SMY [4]. Unfortunately, when co-digested with cellulosic substrates like toilet paper, residuals from root extract preparations of *Sophora flavescens*, and spent coffee grounds, CPI values seldom reached 1.05 to 1.30 compared to co-substrates like pig manure, corn stover, cucumber residue, etc. [36,37]. Moreover, when co-digested with cellulosic materials, cattle manure and sludge gave CPI values of around 2.0. Although CPI is considered an important tool, it has only been used and assessed in batch experiments. However, the synergistic effects of co-substrates may be better understood by determining CPI than by conducting continuous experiments. And digesting multiple but compatible substrates anaerobically should improve digester stability through mitigation of the inhibitory effects typical of single substrate systems.

Organic waste compatibility in A-coD

The effectiveness of A-coD depends on choosing appropriate co-substrates with compatible characteristics [38]. However, to expand the full-scale co-digestion application, screening of new and potential substrates is essential. To determine substrate value, researchers should conduct preliminary studies to find the substrate of choice and then fabricate a set of criteria for substrate selection based on an initial assessment of characteristics of the influent solutions. However, the factor assortment, divergence in practices, and differences in the structure and composition of the substrate can obstruct or constrain the final understanding of the researcher's objective [39]. Furthermore, the most critical thing in the feedstock is the C/N ratio and the relative proportions of S and P [40,41]. Apart from the essential stoichiometric ratios, there is a slight compromise for demanding proportions. The synergistic effects of the substrate aggravated by the complexity of biological processes hinder the identification of ideal substrates and substrate concentrations [42]. Hence, further feedstock optimization related to the composition of the primary substrate becomes more critical. An alternative might be to alter the microbiome within the A-coD reactor by enrichment with appropriate hydrolyzing bacteria and perhaps by the addition of novel archaea.

After the initial substrate characterization, these can also be further characterized in co-digestion to assess their potential and suitability as feedstock alongside other substrates. Based on their specific function in the co-digestion process, substrates can usually be separated into two groups: first, those that enhance biogas generation; and, second, those that facilitate long-lasting stability of the process [43–45]. Therefore, a balance of inhibitory components in organic wastes is mandatory, by diluting the contaminants, manipulating the stoichiometry of the substrates, or optimizing factors like alkalinity.

Different wastes used as co-substrates

Food waste

FW is generated in households and restaurants and as a by-product of catering services, food processing, and food production [46]. If not adequately treated, FW may begin to biodegrade immediately and can affect the environment by releasing hazardous gases into the atmosphere, spreading foul odors, and leaching pathogenic bacteria [47,48]. FW should be considered the most easily accessible organic waste for biomethane production due to its relative ease of biodegradability and rich nutrient content, which is estimated to be 12–74% carbohydrates, 14–18% proteins, and 4–34% lipids by dry weight. Usually, FW generates an SMY within a broad range (maximum 460 mL/g VS added) because of disparities in operational conditions and substrate composition [49]. Nevertheless, one estimate is that every kg of VS will produce 384 L methane [50], resulting in an estimated methane production from 1 ton of FW of $\sim$100 m^3. Methane production from FW during mono-digestion is inhibited by several factors, including an inadequate supply of trace metals, which are obligatory for efficient microbial function and include Co, Fe, Ni, Mo, and Se [51,52]. Second, the rapid hydrolysis of FW also leads to massive volatile fatty acids (VFA) accumulation which inhibits the metabolic activities of methanogens [49].

Co-digestion of FW with other organic wastes is becoming an increasingly hot topic for research due to its enormous benefits in digester stability. For instance, due to a low C:N ratio in animal manure, accumulation of ammoniacal-N can suppress activity in single digestion systems. However, mixed with FW at a specific ratio, it can lessen the inhibitory effects of ammoniacal nitrogen and enhances methanogenic activity by achieving a suitable C:N ratio. Generally, manure can also increase the buffering capacity in the digester to maintain pH neutrality and provide essential nutrients that are either lacking or limiting in FW to the microbial communities [34].

Moreover, the substrate ratio in the co-digestion reactor can also affect process stability. In one study, mixing FW with more than 60% cattle manure resulted in a 250% (2000 to 7000 mg/L) increase in the ammoniacal-N concentration, severely suppressing the methanogenesis [53]. In contrast, a study conducted by Kim and Oh (2011) showed that A-coD of FW with animal manure at a proportion of 50:50 (VS: VS) and oreganic loading rate (OLR) 3.6 kg VS/m^3.d enhanced anaerobic digestion (AD) synergistically with reduced weight and volume of digestate [54]. These studies reveal that determining the optimum concentration of co-substrates in the co-digestion process is a critical factor in enhancing methanogenic activities.

Many studies also confirm that high ammonia-N concentration in the leachate can prevent digester acidification [55]. However, the co-digestion of FW and leachate can increase the buffering capacity to decrease the inhibitory effects of ammonia-N and keep the environment suitable for methanogenic bacteria [56]. Leachate also provides trace metals like Fe, Co, Mo, and Ni during its co-digestion with FW, which microbial communities require to enhance their activities [57].

The use of sewage sludge as co-substrate

Combinations of many organic wastes have been assessed in the co-digestion process for high biogas production and stable operation (Table 1). For instance, a commonly studied substrate in the literature is sewage sludge [58,59]. This traditional substrate is used in AD due to its easy accessibility and related solid reduction necessities. These properties make it one of the most suitable co-substrates for use in A-coD and for the dilution of inhibitory components that occur in this substrate [58,60].

The use of sewage sludge as co-substrate offers various benefits such as its high moisture content, high alkalinity, optimum C:N ratio, and availability of macro- and micronutrients [61]. Sewage sludge can dilute the inhibitory effects of Na^+ and K^+ as it increases short-chain VFA production in sufficient amounts, which can be used as a substrate by methanogens [62]. Sewage sludge co-digested with FW has been found to recover hydrolytic enzymes of high value, improving anaerobic digestion efficiency and providing potential energy for municipal wastewater treatment plants and to assist in meeting carbon neutral operation goals [63]. However, challenges still exist in maximizing the economic value of biogas and developing cost-effective utilization processes. Nonetheless, the potential for co-digestion remains promising for sustainable wastewater treatment.

Carbon-rich substrates

A combination of substrates rich in carbon can speed up biogas production. Generally, carbon-rich substrates containing cellulose originate from paper mills, textile mills, and cardboard factories. In these wastes, a high C:N ratio ranging from 173:1 to >1000:1 is typical, whereas the suggested C:N ratio required for AD is 20:1 to 30:1 [64,65]. Carbon-rich wastes balance the C:N ratio when used with a substrate that has a high ammonia concentration and mitigate the risks of ammonia inhibition. Studies on combining cellulosic substrates with substrates

containing low amounts of carbon are more common in the literature [65]. For instance, the cyanobacterium *Arthrospira platensis* is considered to have low carbon content when co-digested with carbon-rich substrates such as brown seaweed and resulted in stable anaerobic digestion at a high OLR equivalent to 4.0 gVS $L^{-1}.d^{-1}$ [66]. Thus, carbon-rich wastes could be considered as, potentially, the best co-substrate for anaerobic digestion of substrates containing high nitrogen content to adjust the C:N ratio.

Municipal solid waste (MSW)

Municipal solid waste (MSW) is another commonplace but favorable carbon-rich organic material that can be used in A-coD as a co-substrate. Because of its easy availability in large amounts, the organic content of MSW has attracted much attention for balancing the C:N ratio of feedstock for A-coD. Many studies have demonstrated the practical use of MSW as a co-substrate with nitrogen-rich substrates [67]. Furthermore, fruits and vegetables have sometimes been separated from MSW and are also a good co-substrate. These materials contain high VS concentrations that can be easily degraded at high OLR [68]. Wastes with high buffering capacity like sewage sludge (SS), animal manure, and vegetable and fruit wastes have been considered the most attractive co-substrate [69].

Energy crop residues

Residue from energy crop cultivation and processing is used commonly as a feedstock in A-coD. Included are residues from sunflower, maize, rapeseed, rice, wheat straw and sugarcane [70]. Among the energy crops, maize and sugar beet have the highest gross energy potential [40]. Moreover, the SMY from these crops is affected by their chemical composition, which changes with plant developmental stage. In one study, and as might be expected, about 37% more methane was produced when maize seeds were harvested at the milk ripe stage (i.e. while the endosperm in the seed kernel is liquid and the pericarp is still tender) than at the full maturity stage [71].

The C:N ratio of these crops is high due to their high hemicellulose, cellulose, and lignin contents, all regarded as potential substrates for biogas production. In these crops, the high lignin concentration is the main challenge in anaerobic digestion due to its hardness which makes it difficult for anaerobic microbes to degrade [72]. Lignin acts as a shield, cross-linking the cellulose strongly, and prevents enzyme and microbial access to the cellulose. Lignin contributes up to 40% of the energy content and 30% of the mass of these materials. A significant limitation of lignin bioconversion is that native lignin is non-degradable without oxygen, whereas anaerobic conditions are necessary for producing valuable products by fermentation [73]. Naturally, lignin can be degraded by enzymes or biochemical reactions for which aerobic conditions are required [74]. Lignin-rich plant material can be pretreated chemically or biologically to enhance its organic matter bioavailability for optimal anaerobic digestion results [75,76]. Typical biological approaches include the use of isolated enzyme preparations such as laccase or lignin peroxidase or the use of microbes [77]. Apart from the lignin problem associated with energy crops, these are still considered the best source for bioenergy production as they have been used for this purpose for several decades due to their significant carbon content. One possible way to overcome the above-associated problems with energy crops, and to avoid pretreatment which is costly, is their co-digestion with other substrates. In a study conducted by Cahyono et al. (2021), when sugarcane bagasse was co-digested with chicken manure, biogas production was increased by up to 36% [78]. However, lower methane yield has also been reported during the co-digestion of straw and manures [79]. But another investigation reported high SMY during co-digestion of 40% wheat straw and cattle manure [80].

Moreover, Lehtomäki et al. (2007) studied the effect of crop-to-manure ratio for the production of methane by mixing different energy crops such as grass silage, sugar beet tops, and oat straw, based on 40% VS content and co-digested with cattle manure in a continuous stirred tank reactor [81]. The highest SMY was produced by grass silage (268 L CH_4/$kgVS_{added}$) followed by sugar beet (229 CH_4/$kgVS_{added}$) and oat straw (CH_4/$kgVS_{added}$).

Fats, oils and grease (FOG)

Lipid-rich substrates have a maximum theoretical gas yield when compared to protein and carbohydrate-rich substrates [64]. Lipid-rich wastes are commonly characterized as fats, oils, and greases (FOG), originating from food processing factories or slaughterhouses, and as MSW [82]. Besides possessing high methane potential, the substrates mentioned above have slow production rates, and inhibition can occur. Sun et al. (2014) found that the digestion of lipid substrates causes inhibition with 65% lipids beyond the VS concentrations [16]. At high lipid proportions, microorganisms are encapsulated by lipid particles, presumably liposome-like, decreasing the anaerobic digestion potential by clogging, and blocking mass transfer.

Moreover, flotation of biomass caused by the attachment of lipid can lead to high energy losses because of biomass foaming and washout which decrease operational efficiency [75]. These problems may be solved by co-digesting FOGs with other organic waste [83]. In an investigation evaluating the co-digestion of FOGs and FW at various ratios, the highest methane yield of about 800 L/kg was obtained at 70% FW and 30% FOG [84]. Moreover, adding FW with waste activated sludge (WAS) increased methane production by 18.4%, while with FOG, it increased by 21.1% [85]. Still, VFA accumulation is considered a limiting factor in the process even at different co-substrate concentrations. A suitable co-substrate with different characteristics is essential for sustained overall process efficiency [86]. As discussed earlier, searching for a suitable substrate with low lipid content and easy degradability is crucial for successful anaerobic digestion of FOG to limit the total lipid load. The C:N ratio is directly influenced by the relative concentrations of lipids, carbohydrates, and proteins (LCP). By adjusting these concentrations, the C:N ratio can be modified to optimize different phases of the AD process, accelerating improved microbial activity and digestion process. Yet tuning and controlling the LCP ratio to achieve the optimum balance of nutrients for anaerobic microbes is essential. This matter has rarely been investigated, whereas attention has been given to the impact of the C:N ratio, mainly on the general chemical composition of the substrate, in many studies. Therefore, finding a suitable feedstock with low lipid, moderate protein, and high carbohydrate content for A-coD with FOG is of great importance to achieve a balanced LCP in the digester.

Advanced techniques used to improve the A-coD process

Anaerobic co-digestion-coupled microbial electrolysis

A-coD of various wastes (such as crop straw, FW, MSW, wastewater, vegetable residues, and fruit peel) is an accepted technique with excellent performance due to the substrates' synergistic impacts on each other. This technique can provide an appropriate C:N ratio, fulfill the nutrient needs of syntrophic microbes and maintain the system's buffer [34]. Even so, rather than address the merits of A-coD, it is necessary to improve the A-coD process to digest more diverse bio-wastes [87]. Researchers have recently coupled anaerobic digestion to microbial electrolysis cells (MECs) to improve biogas yield and speed up the biodegradation of organic wastes.

The MEC is a novel bioelectrochemical system that can efficiently convert organic matter into chemicals of interest and is used for biogas production from various industrial and domestic organic wastes (Figure 2) [89,90]. MECs can cause an upsurge in direct interspecies electron transfer (DIET) among exoelectrogens and methanogens, accelerate ion transfer in high complex solution, and reduce the build-up of CO_2 at the cathode [91,92]. DIET takes place through outer membrane-localized cytochromes found in many microorganisms [93]. Generally, in MEC, CO_2, electrons, and protons can be produced due to the decomposition of organic matter by electrochemically active bacteria (EAB) on the anode. *Geobacter* and *Shewanella* are two widely reported bacteria containing this delicate network of outer membrane cytochromes capable of performing direct electron transfer. Other microorganisms do not have any inherent potential to facilitate electron transfer due to the inability to contact with electrode surfaces. A redox mediator acts as a carrier by gaining electrons from bacterial cells and transferring these electrons to the anode, becoming oxidized in the process. The oxidized mediator can perform subsequent electron transfer. A good mediator should (a) possess positive redox potential to facilitate electron transfer, (b) exhibit good solubility in the anolyte, (c) readily cross the microbe cell membrane, and (d) be non-toxic toward bacteria [93].

Furthermore, an external circuit could transfer these electrons to the cathode, while CO_2 and protons would reach the cathode by bulk solution in the digester. On the cathode, electrons, protons, and CO_2 are used by hydrogenotrophic methanogens to synthesize methane (Figure 3). The reactions occurring at the anode and cathode are shown in Equations (4–6).

Anode:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \quad (4)$$

Cathode:

$$\left(8\left(H^+ + e^- \rightarrow \frac{1}{2}\ H_2\right)\right) \quad (5)$$

Final reaction:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + H^+ + 4H_2 \quad (6)$$

Zhi et al. (2019) compared the performance and efficiency of an integrated MEC-AD system to electrochemically transform FW and SS as co-substrates to methane. These researchers showed that highest production of methane (enhanced 2.8-fold) occurred in the MEC-AD system and at a ratio of 0.2:0.8 for FW and SS. Also, MEC-AD considerably increased the removal of ammonia and improved digestate dewaterability compared to AD [89]. Although this example is not a true MEC-coupled A-coD but rather an MEC-AD, it is the former system that needs to be explored further. For example, in the MEC-A-coD

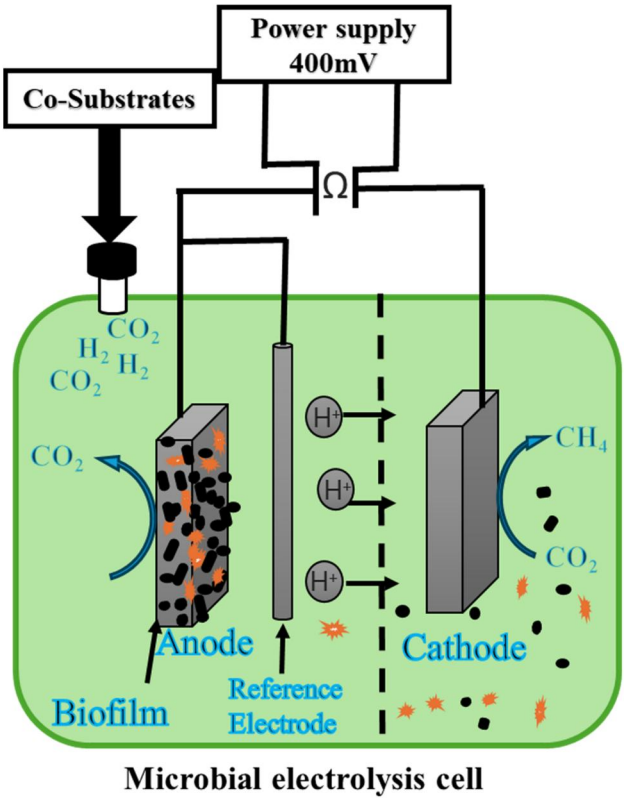

Figure 2. Schematic diagram of MEC consisting of an anode on the left attached to electroactive microorganisms, a reference electrode (to maintain stable electrode potential) and a cathode on the right, where co-substrate oxidation occurs on the anode, releasing CO_2, H^+, and VFAs utilized by methanogens to produce methane [88].

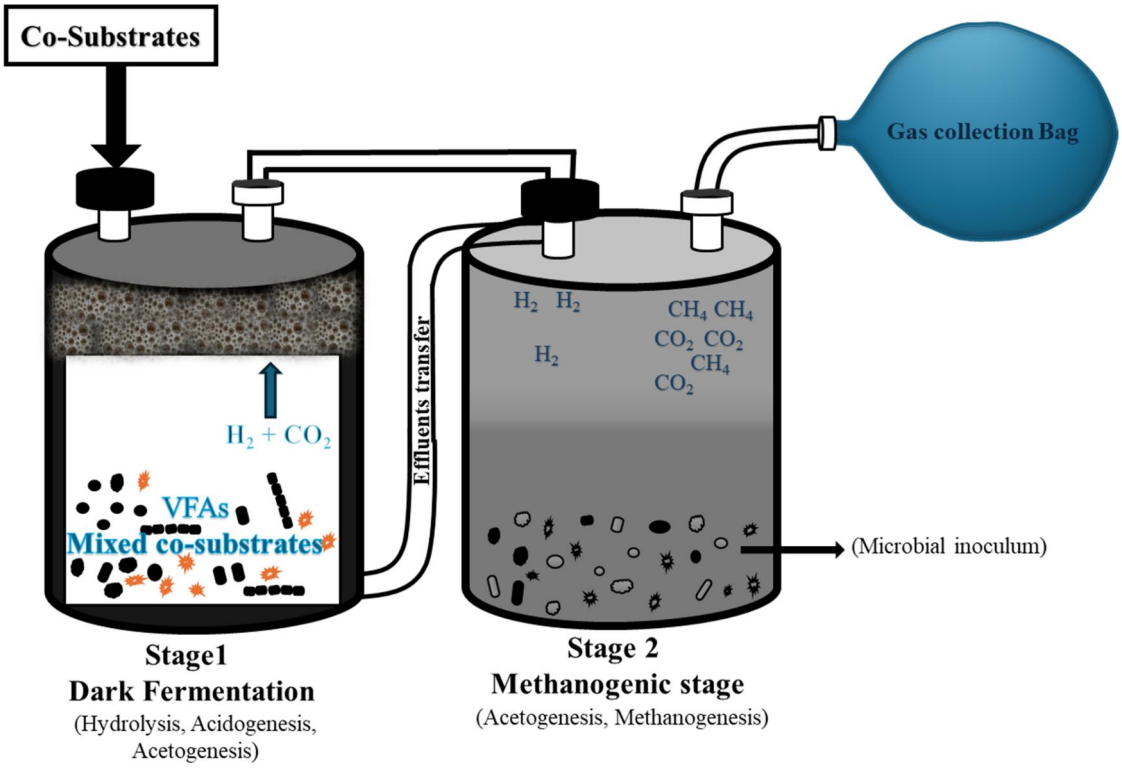

Figure 3. Diagram of integrated dark fermentation and anerobic digestion system. In the first stage, dark fermentation, the hydrolysis and acidogenesis of multi-substrate substrates occur, producing VFAs, hydrogen, and CO_2. Then, in the second stage, methanogens utilize these by-products for methane production [94].

reactor, coconut-shell-derived biobased carbon (CBC) was used to accelerate the A-coD of aloe waste and cattle manure, which resulted in an 80.2% increase in biogas yield and a chemical oxygen demand (COD) removal rate of 58.33% versus the control group [87]. An et al. (2023) reported that MEC-A-coD

has the highest methane yield at about 242.1 mL/g VS and the lowest carbon dioxide yield at 97.6 mL/g VS, against the control group (141.2 mL/g VS, 146.9 mL/g VS) [95].

In MECs, the driving force for the transportation of electrons and protons exerts voltage (i.e. acts as a power source) and plays a significant role in generating methane. The optimum voltage may fluctuate in a particular system and depends on the properties of the substrate, reactor conformation, and electrode material. For instance, voltages from 0.3 to 1.5 V were observed in the anaerobic digestion of WAS and caused a 76.2% increase in methane and a 26.6% rise in removal of VS compared to zero voltage [96]. In addition, Choi et al. (2017) found a 30.3% higher methane production at 1.0 V than in the reactor with no voltage applied [97]. Dairy manure was digested in mesophilic MEC-AD and, as shown by Qu et al. (2020), using voltages from 0.3–0.8 V an increased biodegradation of lignin and cellulose at 0.5 V with high methane production was observed [98,99]. These studies indicated that MEC coupled with either AD or A-coD can improve the process stability in terms of methane yield and waste management compared to AD alone or A-coD.

Concerning substrate characteristics, electroactive microorganisms in the MEC can utilize a wide range of substrates but with varying energy efficiency. For instance, a study reported by Zheng et al. 2020 used a single-cell MEC reactor with different substrates such as glucose, sodium acetate, sodium propionate and sodium butyrate, where sodium acetate-treated reactors showed higher electrochemical activity [100]. Substrate concentration and type are the most critical factors affecting the MEC-A-coD regulating the process efficiency and methane yield [101]. In MEC-A-coD, various types of substrates are used for methane production. These substrates could be divided mainly into three types, i.e. non-fermentable substrates, fermentable substrates, and various kinds of wastewater. For instance, acetic acid is widely used as a non-fermentable substrate, because it is the end product of the hydrolysis, acidogenesis, and acetogenesis stages of the AD process [102]. Moreover, glucose as a fermentable substrate can be easily transformed into VFA which can be used by electrogenic bacteria and methanogens to produce methane [103]. Different kinds of organic matter are present in wastewater which should be treated before release to the environment. Varieties of wastewater such as dairy wastewater, domestic wastewater, industrial wastewater, leachates, sewage sludge, FW, landfill, etc. have been treated in MEC reactors [104,105]. For instance, Li et al. [106] reported that compared to landfill leachate and swine wastewater, domestic wastewater could be a more appropriate MEC because of its lower toxicity to anode biofilms.

In comparison to classical anaerobic digestion of different substrates, MEC integrated with AD has presented high stability due to the absence of VFA accumulation during high organic matter degradation and due to the reduced effects of various toxic substances [105]. Moreover, MEC-AD has been found to have a methane production rate 1.7 to 4.0 times higher than the non-integrated anaerobic digesters due to faster degradation of organic matter [107]. For example, about 138 mL CH_4/L.d^{-1} of methane was produced from acetate in an MEC-AD reactor compared to control (46 mL CH_4/L.d^{-1}), which was about a 3-fold increase in methane yield [108].

Thus, the MEC process accelerates substrate hydrolysis to enhance biogas production. However, the structure and composition of the MEC microbial community can vary depending on the type of substrate used. Also, the impacts of operational changes on microbial community structure and composition during MEC-assisted A-coD are poorly understood. It is important to note that the enrichment of these microorganisms in an MEC requires a significant amount of time, although the process is faster than conventional A-coD methods. Despite the potential of MEC-assisted A-coD as a practical option for treating challenging and complex wastes, it has predominantly been studied at laboratory scale. Moreover, using MEC-A-coD may involve additional costs, such as the need for pretreatment of waste or adding other chemicals to optimize the process. Further research is needed to explore scalability.

Dark fermentation-coupled anaerobic co-digestion

One of the most popular approaches for improving the AD process for biogas production is to couple A-coD to DF (Figure 3) [109]. The acidogenic disintegration of organic compounds in an anaerobic environment without light to generate H_2 and CO_2 with the release of ethanol and acetic, propionic, butyric, and malic acids as end products is known as dark fermentation (DF). And effluents containing these end products can be used directly as feedstock for biomethane production [110].

After the hydrolysis phase in DF, the acidogenesis step is initiated and the glycolytic metabolic pathway converts carbohydrate into pyruvic acid. Both facultative and obligate anaerobic bacteria consume different organic compounds to produce organic acids and H_2 [111]. Facultative anaerobes can convert pyruvic acid to acetyl-CoA and formate using the enzyme pyruvate formate-lyase and by producing hydrogen and then formate hydrogen lyase.

However, obligate anaerobes convert pyruvic acid to acetyl-CoA and CO_2 by a process known as pyruvate ferredoxin oxidoreductase-biocatalysis, where reduction of Fd is also required [112]. And the end-products from DF, such as ethanol, acetic acid, propionic acid, malic acid, and butyric acid, are then processed further in the second stage of AD for methane production. This two-stage anaerobic combination for retrieving more bioenergy from the end products of DF has been widely reported [113–115]. Notably, about three times more methane was produced in a two-stage digestion process of coffee seed waste than in the single-stage process [116]. Fruit and vegetable wastes, olive oil mill wastewater, waste-activated sludge, and cattle manure co-digestion in two-stage reactors yielded 0.73 $L^{-1}.d^{-1}$ of H_2 during DF and 0.6 to 1.86 $L^{-1}.d^{-1}$ of CH_4 from AD [117]. In addition, the biogas produced in DF is recirculated into AD, and the resulting biogas contains 63.5% CH_4, 28.5% CO_2, and 8% H_2. On the other hand, the highest biological stability (40.6%) of two-stage systems was observed, presumably due to better hydrolysis of organic matter compared to that occurring in a one-stage digester (6.5%) [118].

Since the integrated DF-A-coD system (Figure 3) has many advantages, one of the significant advantages is the production of biohythane, which has a higher energy value than pure CH_4 and can be directly used as a fuel [119]. Hythane is a gas formed in a two-stage AD and comprises a mix of H_2 and CH_4 in which the hydrogen concentration ranges from 10% to 30% v/v. The increased H_2 content (25% to 75%) in hythane enhances the temperature of the gas flame by 0.6 to 4.5%. Moreover, a significant reduction of CO_2 emission has been observed in integrated processes when compared to those producing methane only. Yet, the implications and possible application of hythane have not been fully established. Major challenges when using an integrated system include sustaining microbial community composition in each stage and integrating the process on a large scale for continuous biogas production.

Methane and hydrogen are considered suitable energy sources to meet the EU's goal of 10% green energy in household transportation. Methane, typically produced through AD of organic wastes, can be transformed into other energy sources with low environmental impact [120]. The production of hydrogen via DF is not fully developed but remains a promising energy source with zero carbon emissions during combustion [121]. These renewable fuels could be further used in the chemical syntheses of ammonia, oil, hydrogenated fats and, most importantly, electricity [86]. Details of the key advantages and disadvantages of MEC, DF, AD, and their integrated system are summarized in Table 2.

Impact of co-substrates on microbial community structure

Anaerobic digestion is a process driven by microorganisms that proliferate by consuming organics and the reduction of CO_2 in an oxygen-free environment. The process is facilitated by four distinct but interconnected enzyme-catalyzed degradation processes delineated as (1) hydrolysis, (2) acidogenesis, (3) acetogenesis, and (4) methanogenesis, that occur synchronously. The complex reactions (Equations 7–19) in the above phases of anaerobic digestion are carried out by several microorganisms, including hydrolytic bacteria, hydrogen-producing, and hydrogen-utilizing acetogens, carbon-dioxide-reducing archaea, and acetoclastic methanogens (Table 3) [132]. The mechanisms facilitated by these organisms are shown in Figure 4. Still, there is a significant research gap in understanding the AD system's complex mechanism, which is why inhibition remains a challenge in process operation and optimization.

Microorganisms that carry out anaerobiosis occur naturally in the environment and function syntrophically in anaerobiosis [134,135]. Among these microorganisms are the phylogenetically diverse hydrolytic bacteria among which Firmicutes and Bacteroides are the two common phyla containing most of the hydrolytic bacteria used in anaerobic digesters. Generally, hydrolytic bacteria grow fast and are more resistant to environmental changes such as pH and temperature. The by-products of hydrolytic bacteria can be fermented by acidogenic bacteria to produce short-chain fatty acids such as acetic acid, propionic acid, butyric acid, valeric acid and isobutyric acid during the acidogenesis stage. Moreover, during acidogenesis, CO_2, H_2, ammonia, and sulfides are also produced. The major phyla containing acidogenic bacteria include Firmicutes, Bacteroidetes, Chloroflexi, Proteobacteria, and Atribacteria, which have been used extensively in anaerobic digesters. The acetogenic bacteria include both hydrolytic bacteria and fermentative bacteria lacking hydrolytic activity. These bacteria further ferment propionic acid, isobutyric acid, valeric acid, and isovaleric acid to acetate, CO_2 and H_2. On the other hand, acetotrophic and hydrogenotrophic methanogens convert these products into methane. Some of the hydrogenotrophic methanogens (e.g. species of *Methanobacterium* and *Methanospirillum*) and obligate acetotrophic methanogens (e.g. *Methanosaeta harundinacea* and *M. concilii*) can also accept and utilize electrons donated by electron-donating bacteria for methanogenesis [136,137].

Table 2. Advantages and disadvantages of MEC, DF, AD, and coupling systems of MEC-AD and DF-AD.

Process	Advantages	Disadvantages	Ref.
Microbial electrolysis cell (MEC)	a. Generates bioenergy directly from waste streams b. Needs less energy input to produce H_2 compared to ordinary water electrolysis c. Produces highly pure H_2 over other biological processes d. Generate value-added chemicals like NaOH, H_2O, and formic acid e. Can treat a variety of wastewater (i.e. industrial, agricultural, and domestic) f. In addition to hydrogen production, MEC can generate CH_4 and ethanol g. It can remove pollutants like trichloroethylene, and azo dyes h. Can recover resources such as copper, lead, ammonia, and other heavy metals i. It does not produce O_2	a. H_2 yield decreases over time due to several undesired electron sinks in various metabolisms, thus impeding CH_4 production b. MEC reactor setup is determined by reactor configuration: (1) the material used and (2) the type of substrate; actual setup of these configurations may vary from theoretical configurations, which can change the results c. For efficient MEC, it is challenging to thoroughly understand microbes and their associated behaviour d. Difficult to meet the standard set for the effluent discharge	[122,123]
Dark fermentation (DF	a. Ability to produce H_2 in the absence of light b. A variety of organic wastes can be used as a substrate c. Less energy input to achieve zero carbon energy d. Limitations of O_2 do not affect the process	a. Hydrogenase activity inhibited by O_2 b. More CO_2 in the produced biogas c. Increasing H_2 yield does not favor the process thermodynamically d. Accumulation of alcohols and VFAs inhibit the bacterial growth e. Needs the second stage to recover energy from by-products and their bioremediation	[124,125]
Anaerobic digestion (AD)	a. Produces carbon-neutral energy in the form of biogas b. Low biomass sludge produced compared to other processes, converting 10% to mud and the remaining 90% to biogas c. Residuals are rich in nutrients and can be used as fertilizer in agriculture d. Highly cost-effective compared with other biological processes to recover energy with low environmental impact	a. Microbial communities take a long time to stabilize in the digester b. Pretreatment required for complex materials c. Important to post-treat the generated waste before its release into the environment d. Necessary to continuously monitor the critical parameters like temperature, pH, OLR, and generation of VFAs	[126,127]
MEC+AD and DF-AD	a. Higher bioenergy yield than operating individually b. Coupling technology can produce 90% fewer GHGs emissions than individual c. MEC-AD or DF+AD exhibits high energy efficiency d. The integrated technology of MEC-AD enriches CH_4 content in biogas by up to 90% by utilizing CO_2 e. Biohythane produced by DF-AD has higher energy value than individual gas f. DF-AD reduces CO_2 emissions significantly g. Competitive biogas upgrading technique	a. Severe energy losses can occur in MEC+AD, including overpotential, ohmic loss, and diffusion limitation b. Unavoidable side reactions occur in MEC+AD c. The high capital cost of coupling MEC+AD mainly results from the electrode material d. Not feasible for long-term operation due to electrode corrosion and membrane fouling e. DF-AD requires two reactors because different processes increase the initial cost f. Energy loss in DF-AD due to switching from one stage to another stage	[128–130]

Table 3. Sequential biochemical equilibria underpinning microbial cooperation during the anaerobic conversion of biomass to CH_4 [131].

Microorganisms	Electron donor	Electron acceptor	Product	Reaction type
Hydrolytic bacteria	Organic carbon	Organic carbon	CO_2	Hydrolysis
Acidogenic bacteria	Organic carbon	Organic carbon	H_2	Acidogenesis
Acetogenic bacteria	Organic carbon/H_2	CO_2	CH_3COOH	Acetogenesis
Archaea	Organic carbon/H_2	CO_2	CH_4	Methanogenesis

More diverse microbial communities in the A-coD system than in mono-digestion systems have been evidenced because various microbes are continuously introduced by different substrates [138]. Exopolysaccharide hydrolase enzymes have also been shown to regulate microbial community changes during the co-digestion of sewage sludge and FW [139]. Thereby, new attachment sites are created for microorganisms due to compositional changes in the original substrate [140]. Changes in the digester's ambient parameters, like OLR, temperature, type of digester, and co-substrate composition, also impact microbial community structure [141]. During the co-digestion of green wastes like

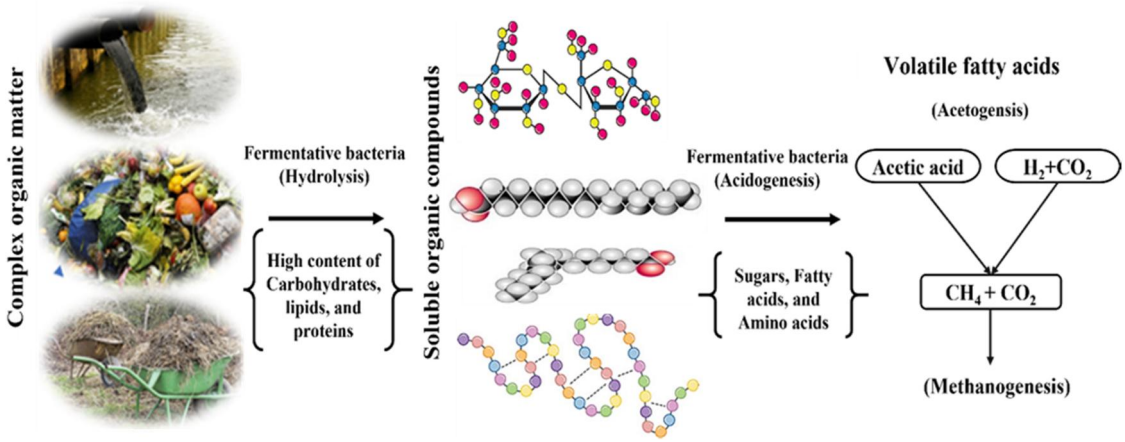

Figure 4. Schematic illustration of the mechanisms involved in the anaerobic digestion process [133].

the green alga *Enteromorpha*, and chicken manure, the most abundant bacteria detected were from the phyla Bacteriodetes, Synergistetes, Firmicutes, and Chloroflexi, representing 80% of the total microbial population. Firmicutes bacteria produce hydrolytic enzymes such as cellulases and proteases that degrade the substrate, whereas members of the Synergistetes group produces acetic acid and H_2 which are used by methanogenic bacteria for methane production [142]. Additionally, syntrophic VFA-oxidizing acetogens such as *Syntrophomonas* and *Syntrophobacter* have also been found abundantly in some co-digesters [143].

Microorganisms work in a syntrophic relationship with each other in the anaerobic digestion process. For example, carbon dioxide and hydrogen produced by acetogenic bacteria could be used by hydrogenotrophic methanogens for CH_4 generation (Figure 5). These methanogens reduce the hydrogen partial pressure in the digester which indirectly promotes the growth and activity of other syntrophic bacteria such as *Syntrophomonas*, *Syntrophobacter*, and *Syntrophospora*. These bacteria utilize H_2 as an electron accepter to oxidize propionic and butyric acid. These microbes can only oxidize the above organic acids when the hydrogen partial pressure is low [52,145].

In addition, the growth of these syntrophic microorganisms is limited without association with other organisms like methanogenic archaea, as they consume the hydrogen produced by these bacteria. Therefore, syntropism refers to a process where organic compound degradation occurs in consert with two or more organisms, as none of the microorganisms is capable of it alone. Bacteria belonging to the genus *Syntophomonas* enable the oxidation of butyric, pentanoic, and caproic acid to acetate, H_2, and CO_2 [146]. Oxidation of propionic acid is also an essential step, which obligately desires the syntrophism sandwiched between acetogens and methanogens. The methanogens forcefully cause the oxidation of propionic acid, which can be made feasible by lowering the hydrogen and formate concentration in the system [147]. Furthermore, most propionate oxidizing bacteria belong to the *Syntrophobacter* domain within the *Deltaproteobacterea* [148]. These bacteria can accept an electron from sulfur for propionate oxidation. Furthermore, these bacteria can also grow during pyruvate fermentation and fumarate. Another species of the genus *Syntrophus* is known as *Smithella propionica* LYP, a gram-negative propionic acid oxidizer incapable of sulfate reduction and uses a different pathway for propionic acid oxidation [149]. Apart from gram-negative bacteria, gram-positive bacteria can also play a role in the syntrophic oxidation of propionate. Examples of gram-positive bacteria include *Desulfotomaculum thermobenzoicum* subsp. *thermosyntrophicum*. These bacteria can grow on several substrates and are also able to reduce sulfate, like *Syntrophobacter* strains [150].

Besides, methanogens can be classified into three categories depending on substrate and the metabolic pathways used: (i) acetoclastic (in which methanogens use acetic acid to produce methane); hydrogenotrophic (in which hydrogen-requiring methanogens use formate to reduce CO_2 to methane); and (c) methylotrophic (in which methyl group-requiring methanogens use methyl compounds to produce CH_4).

Recently, researchers have started using cutting-edge technologies (e.g. high-throughput sequencing and macroscopic gene sequencing) to help find the complex structure of microbial communities residing in the AD system and to explore the actual mechanisms driven by these communities to better understand A-coD [53]. However, other forces including fluctuations in digester operating mode, abiotic factors, and feed characteristics may also impact microbial community composition.

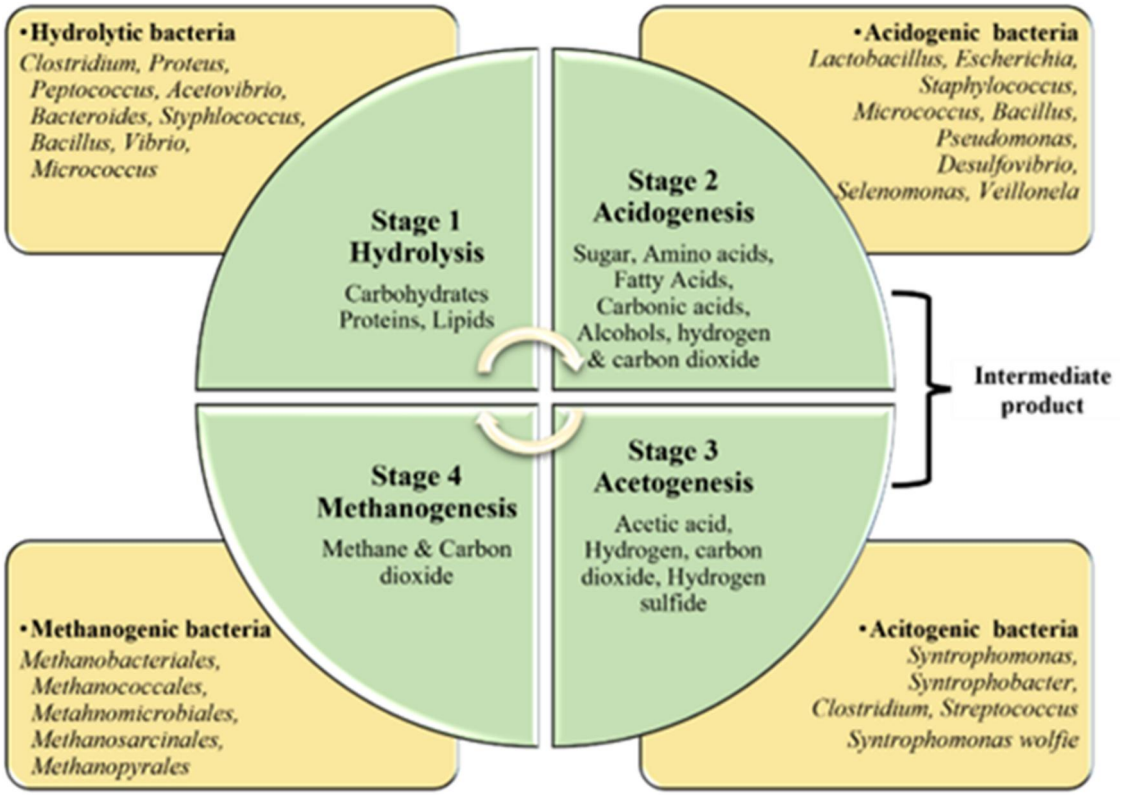

Figure 5. Microorganisms comprise different anaerobic digestion stages to produce methane and hydrogen [144].

Hydrolytic bacteria

$$(C_6H_{10}O_4)_n + 2H_2O \rightarrow C_6H_{12}O_6 + O_2 \quad (7)$$

Cellulose Glucose

$$(-RCH(NH_2)COD-)_n + (n-1)H_2O \rightarrow nRCH(NH_2)COOH \quad (8)$$

Protein Amino acids

$$(H_2COOC(CH_2)_n + (n-1)H_2O \rightarrow n(H_2COOC(CH_2)_nCH_3) \quad (9)$$

Fatty acids

Acidogenic bacteria

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (10)$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \quad (11)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + H_2 \quad (12)$$

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \quad (13)$$

Acetogenic bacteria

$$CH_3CH_2COO^- + 3H_2O \leftrightarrow CH_3COO^- + H^+ + HCO_3^- + 4H_2 \quad (14)$$

$$C_6H_{12}O_6 + 2H_2O \leftrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (15)$$

$$CH_3CH_2OH + 2H_2O \leftrightarrow CH_3COO^- + 2H_2 + H^+ \quad (16)$$

Methanogenic bacteria

$$CH_3CH_2OH \rightarrow CH_4 + 2CO_2 \quad (17)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (18)$$

$$2CH_3CH_2OH + CO_2 \rightarrow CH_4 + 2CH_3COOH \quad (19)$$

Techno-economic analysis (TEA) of the integrated A-coD with MEC and DF system

The complex nature of the integrated system demands careful optimization to ensure it operates stably and efficiently. Economically, the initial investment for the DF-MEC-AcoD system can be significant due to the need for specialized equipment for each process. Operating costs include expenses for feedstock, energy consumption, maintenance, and labor. However, using waste materials as feedstock can reduce costs, and income can be generated by selling hydrogen, biogas, and by-products such as fertilizers [129,151,152]. The economic feasibility of the system depends on its ability to generate biogas at a lower cost compared to other methods like steam methane reforming and electrolysis [151].

The rising demand for green energy and the growing need for sustainable waste management solutions create a supportive market landscape for the DF-MEC-AcoD system [153]. This system is in competition with other technologies for renewable energy production and waste management, and its ability to compete will rely on how efficient and cost-effective it is [154]. For example, Liu et al. (2022) conducted a techno-economic analysis (TEA) of the integrated DF-MEC system using the Hydrogen Analysis Production Models (H_2A) model. Their findings reveal that both the capital cost of MEC and its current density significantly influence

the cost of H_2 production. With a recently demonstrated MEC current density of 20 A/m^2, the levelized cost of H_2 production is $8.6 per kg H_2, based on an 80% DF conversion rate (target) and a 90% MEC conversion rate (validated), along with an additional revenue of $50 per metric ton of CO_2 due to carbon capture and sequestration (CCS). Raising the MEC current density to 100 A/m^2 would lower the cost to $6.3 per kg H_2. Additional opportunities for cost reduction include continuously improving current density, utilizing zero-cost waste feedstock, decreasing electricity consumption in the MEC, and reducing NaOH usage in biomass pretreatment [155]. In another study, the economic feasibility of integrated AD-MEC technology was evaluated by calculating the return on investment (ROI), which was determined to be 13% in current wastewater treatement plant (WWTP) systems [156]. Furthermore, the most notable enhancement in overall ROI was observed when the investment cost for MEC decreased. A 20% cut in MEC investment expenses is expected to result in an ROI exceeding 16.5% [156]. Numerous studies have concentrated on creating cost-efficient electrode materials for MEC systems [157,158], indicating that the use of affordable carbon materials could significantly reduce MEC costs [159,160].

From an ecological standpoint, this integrated system promotes sustainability by making use of organic waste, cutting down on greenhouse gas emissions, and lessening dependence on fossil fuels [161]. This is in line with international initiatives to combat climate change and shift toward renewable energy. Nonetheless, additional research and refinement are necessary to tackle technical issues and enhance the system's economic feasibility [151].

Prospects and challenges

A-coD offers a prospective platform for improving renewable energy generation and sustainable waste management. A-coD of different organic wastes potentially enhances biogas production and increases resource recovery, containing valuable nutrients (e.g. N and P) for agricultural purposes. In other words, after A-coD, liberated C, N and P can be recovered by the application of integrated technologies appropriate to efficient energy use and recovery of resources. It is remarkable that A-coD, among all the available technologies, contributes so well to the mitigation of climate change by decreasing greenhouse gas emissions and that it may be useful in the development of an economical solution for organic waste management.

Despite its potential to enhance the digestibility of miscellaneous substrates for energy recovery and waste management, A-coD remains challenging, as it is intrinsically a complex process facilitated by diverse microbial groups under constant parametric differences. Each microbial group can survive abiotic extremes such as dramatic changes in pH, temperature, the existence of inhibitory compounds, and competition for nutrients [162].

For extensive application of co-digestion, challenges that remain include the sourcing and transportation costs of the co-substrates, extra pretreatment services, and the energy input for homogenization of wastes. Additionally, different substrates have different characteristics that affect the co-digestion process. Therefore, it is necessary to optimize the mixing ratio prior to A-coD of different substrates. Some substrates are seasonal and are unavailable during certain periods of the year (e.g. agricultural biomasses), which is an important difficulty for the continuous operation of A-coD. Ensuring a regular supply of agriculture biomass from different sources is a big challenge. Moreover, these may require pretreatment such as size reduction, chemical pretreatment (e.g. acid and alkaline pretreatment), or heat treatment to improve their digestibility. However, implementing such pretreatment methods can increase the overall costs and complexity of implementing A-coD.

To address the aforementioned challenges, substrate characterization and chemical composition should be evaluated to measure and establish their ideal mixing proportion based on C:N ratio. Additionally, TEA and life-cycle assessment (LCA) are vital in co-digestion investigations and their results will be subject to change based on the type of substrate, cost of substrate generation, pretreatment requirements, and logistics. Consideration of these aspects is also important in deriving recommendations for best-case biorefinery scenarios. Also, many features including biogas generation rate, reactor buffering capacity, microorganism stability, and balance of nutrients should be evaluated. Several studies have shown that pretreatments like alkali, thermal, and ultrasonic can enhance the efficiency of hydrolysis and acidogenesis processes [163–165]. These single pretreatment steps may not achieve the target goals. However, when integrated with neutral protease pretreatments, they can further promote the hydrolysis of proteins and enhance the digestibility and biodegradability of substrates [166]. Other novel techniques including pulsed electric field application, high-voltage pulsed discharge and electro-oxidation have been developed to disintegrate highly recalcitrant organic wastes [167,168].

In recent times, a variety of trends have emerged in scientific research concerning anaerobic technology. The advancement and widespread availability of

systematic technology have introduced a broader range of methods in this area. For example, over the last few decades, new techniques such as DF combined with A-coD and MEC in anaerobic technology have prompted scientists to concentrate on the bioconversion of complex substrates [169]. This integration leverages the combined benefits of these processes to enhance biogas production and overall efficiency. DF-A-coD enhances the hydrolysis and acidogenesis of co-substrates and provides substrates with high conductivity, which aids in resolving charge transfer challenges in MEC, thereby further boosting electro-methanogenesis [153,170,171]. Furthermore, the MEC system can increase P and N recovery, and enhance the degradation of refractory or harmful substances like humic compounds via cathodic reduction [172]. Moreover, this integration can reduce the inhibitory effects of H_2S on methanogenesis, and can increase VFA oxidation, resulting in enhanced CH_4 yield [173].

Energy loss occurs in anoxic digestion by switching the DF stage and transferring the products to another stage [174]. In the MEC stage, further energy loss can be a serious issue including electrode overpotential, ohmic loss, and limitation of diffusion due to mass transfer limitation [175]. To overcome these challenges, it is necessary to develop cost-effective electrodes with better electrochemical performance including high conductivity, low internal resistance, high surface area, better biocompatibility, and good chemical and physical stability. A solution might be to avoid using membranes in MEC-A-coD and instead optimize the system without them [129]. Moreover, supplying exogenous accelerants is an efficient strategy to overcome the limitations of MEC-A-coD. Different accelerants such as carbon conductive materials, trace metals, transition metal compounds, biochar, titanium balls, adsorbents, black phosphorus and nanomaterials at optimum concentrations accelerate direct interspecies transfer among syntrophic microbes, which can enhance the degradation of biomass, biogas production and the quality of digestate [95,176–178]. The interconnections between enhanced substrate degradation, biogas production, and shifts in microbial communities within MEC-AD systems remain uncertain and require further investigation. Focused efforts should be directed toward understanding the dynamics of microbial communities during this integrated process.

The DF-MEC-AcoD integrated system holds considerable promise for expansion from a laboratory setting to industrial applications. Studies have indicated that combining these processes can boost the production of hydrogen and biogas while efficiently handling organic waste [179]. The system's ability to handle a wide range of organic waste, including agricultural residues and food waste, makes it adaptable to various industrial settings [180]. However, expanding operations necessitates tackling issues like fine-tuning process parameters, maintaining consistent microbial populations, and controlling energy consumption [179]. For industrial applications, it is essential to develop strategies that accelerate start-up times and enhance process stability [179]. Governments and regulatory agencies can significantly influence the widespread use of these integrated systems by offering incentives, subsidies and supportive policies. Such measures can help to remove financial obstacles and speed up the adoption of these technologies.

Conclusion

As an alternative to fossil fuels, biogas production from various organic wastes by A-coD is a very promising and seemingly sustainable technology with the ability to increase methane production by up to 170%. Furthermore, the integration of A-coD with DF or MEC has gained much attention for its ability to enhance methane production by up to 80% compared to conventional A-coD process. This review explores the many complications linked to the co-digestion process and the current progress in developing integrated technology. The process is continuously improving with advances to lessen future challenges. However, it still needs further investigation to improve stability and for parameter optimization. Future studies should pay attention to new and emerging methods to help elucidate details of the complexity of co-substrates and enumerate the diverse hydrolysis rates or kinetic parameters. For this reason, the recent development of integrating DF and MEC into A-coD may indeed improve process stability and bioenergy production.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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