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Review

Anaerobic Co-Digestion of Wastes: Reviewing Current Status and Approaches for Enhancing Biogas Production

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Abstract: Anaerobic digestion is one of the technologies that will play a key role in the decarbonization of the economy, due to its capacity to treat organic waste, recover nutrients and simultaneously produce biogas as a renewable biofuel. This feature also makes this technology a relevant partner for approaching a circular economic model. However, the low biogas yield of traditional substrates such as sewage sludge and livestock waste along with high installation costs limit its profitability. Further expansion of this technology encounters several barriers, making it necessary to seek improvements to attain a favorable financial balance. The use of co-substrates benefits the overall digestion performance thanks to the balancing of nutrients, the enhanced conversion of organic matter and stabilization, leading to an increase in biogas production and process economics. This article reviews the main co-substrates used in anaerobic digestion, highlighting their characteristics in terms of methane production, kinetic models commonly used and the synergistic effects described in the literature. The main process parameters and their influence on digestion performance are presented, as well as the current lines of research dedicated to improving biogas yields, focusing on the addition of hydrogen, bioaugmentation, supplementation with carbon compounds and nanoparticles, the introduction of bioelectrodes and adsorbents. These techniques allow a significant increase in waste degradation and reduce inhibitory conditions, thus favoring process outcomes. Future research should focus on global process efficiency, making particular emphasis on the extrapolation of laboratory achievements into large-scale applications, by analyzing logistical issues, global energy demand and economic feasibility.

Keywords: methane kinetic models; synergistic effects; bioaugmentation; conductive materials; process parameters; reactor performance

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1. Introduction

Organic waste has been traditionally treated by biological processes such as composting and anaerobic digestion. Composting requires a supply of air to keep microbial metabolisms active, whereas anaerobic digestion lacks these oxygen requirements resulting in a less exigent energy demand. Digestion technology has been applied worldwide because the process can deal with high organic loading and generates biogas, which can be easily valorized for producing heat or electricity. The excellent capacity for treating a wide variety of wastes makes anaerobic digestion a technology capable of reintroducing low-quality materials into the production chain, attaining their transformation into energy, organic amendments or any other type of goods. Therefore, wastes can be used to generate new products and should be considered as "renewable resources" [1].

Digestion technologies have a relevant role in transforming the linear economy model by integrating circularity. Biogas is the main energetic product obtained, which is composed of methane (CH_4) and carbon dioxide (CO_2) as its majority constituents. Digestate is also derived from this process and contains anaerobic biomass, partially degraded organic materials and residual components which are recalcitrant to the degradation route. Digestate is rich in humic, fulvic substances and nutrients making it suitable as raw material to produce organic fertilizers [2]. Recently, digestate is also being considered as an organic soil improver, growing medium or organic non-microbial plant biostimulant [3].

Digestion plants can also become an excellent ally for mitigating greenhouse gases (GHG). The treatment of organic waste avoids uncontrolled degradation and thus the release of methane into the atmosphere. Biogas obtained from this process is easily valorized for energy production (thermal or electrical) or upgraded to obtain a gaseous fuel with similar characteristics to that of natural gas. There is a rising concern regarding the effect of CO_2 concentration in the atmosphere and how the global climate responds to the continuous increase in CO_2 levels. Research efforts should focus on attenuating these changes, reducing the negative impact on the economy and searching for efficient ways of producing energy from renewable sources. The current energetic crisis needs urgent solutions provided by mature technologies capable of producing huge amounts of energy. Anaerobic digestion is capable of generating eco-friendly energy and, at the same time, addressing the waste management crisis [4]. However, several aspects are still pending a solution such as the profitability of the whole treatment system and the need to increase conversion efficiency to reduce installation costs.

Anaerobic digestion has been traditionally linked to the treatment of sewage sludge in large-scale wastewater treatment plants and the treatment of livestock waste. Both applications are characterized by the use of substrates with high organic content but lacking suitable nutrient balances. The addition of a co-substrate to any of these systems aids in balancing the C/N ratio and improving the global process performance allowing a better economic balance by increasing profits [5]. In the case of sewage sludge digestion, its composition based on primary and secondary sludge results in a mixture that would demand an excessive amount of energy if stabilization is carried out by aerobic treatment. In addition, secondary sludge or waste-activated sludge may need the application of pre-treatments for facilitating microbial degradation under anaerobic conditions, but this increases the energy demand. On the other hand, when dealing with livestock wastes, it is usually accepted that the methane yield of these organic materials is not high enough to make digestion attractive. Nitrogen-containing compounds may cause inhibition leading to poor performance. Therefore, adding a co-substrate capable of balancing the C/N ratio and trace element content will significantly increase methane production [6,7] and energy valorization.

Improving the efficiency of anaerobic digestion is of great relevance when considering this process to be a suitable alternative for energy production. This is a key aspect if this technology is to play a relevant role in decarbonizing the economy. The valorization of organics into energy and valuable end-products allows the reduction of the carbon footprint of different human activities. Low-quality resources are in this way re-integrated into the global economy as energy, nutrient cycling or valuable organics. However, not all attempts to increase the efficiency of anaerobic digestion are to be considered adequate. A careful evaluation of the energy demand of the whole process should be carried out. The transformation of organics into biogas may require additional equipment based on co-substrate characteristics and introducing pre-treatment units, which would translate into further energy demands [8], probably making the whole treatment chain unfeasible. There are several pre-treatment options for improving the degradation of organics and enhancing the hydrolysis stage. Still, not all of these alternatives find commercial applications due to their excessive energy demand, the limited capacity for recovering energy and the detrimental effect of some chemical compounds generated during the pre-treatment.

Major achievements recently attained in anaerobic digestion deal with new technologies capable of accelerating the hydrolysis stages (thermal, mechanical pre-treatments, ultrasound application, additions of chemicals). Novel techniques have been developed such as the application of pulsed electrical fields, high-voltage pulsed discharges and electrooxidation [9–11]. The success of the industrial implementation of these technologies keeps a close relationship between biogas production improvement and the energy demanded during pre-treatment. Other alternatives for improving anaerobic digestion are the supplementation of carbon conductive materials, adsorbents, nanomaterials and trace elements to enhance organic degradation [12–16]. However, any type of material added to the system may be subsequently released into the environment, creating interactions with biota which may result in adverse effects due to the presence of co-contaminants [17].

Although anaerobic digestion is a widely extended technology, several factors prevent the number of installed units from growing worldwide at a higher pace. These are related to high installation costs and operational complexities. The economy of scale favors large industrial plants, but this option is not always possible due to social opposition and constraints due to substrate transport. Recently, several reports have been published in the literature regarding the costs associated with these treatment plants, the efficiency of the process and the enhancement of biogas production in an attempt to increase economic feasibility [18–20]. There is vast experience at a large scale in co-digestion of sewage sludge and livestock farm wastes and extensive literature regarding research work also dealing with this subject [21–23]. However, better performance and faster conversion rates are still needed to improve plant financial balance and search for configurations that allow the finding of a mid-point between process conversion efficiency and plant operating costs. For this reason, great hope is set on the addition of supplements capable of attaining these objectives, such as conductive carbon materials and low-cost adsorbents [24,25].

The present manuscript provides a description of the substrates suitable for the digestion process. A brief review of the application of different kinetic models for predicting cumulative methane production under batch tests is also included. The main goal of this manuscript is to present an assessment of the different parameters affecting co-digestion process performance and highlight the relevance between microbial interactions and reactor operating conditions. Finally, an analysis of the current alternatives for increasing biogas productivity is presented, setting a special focus on reactor dynamics and conversion efficiency. The present review connects the current state of the art regarding data obtained under laboratory experimental conditions with the implications expected under large-scale performance, setting a special focus on process efficiency and treatment capacity. The novelty of the present document is establishing a link between the findings obtained under laboratory scale conditions and the implications at large-scale plants.

2. Common Substrates Used in Anaerobic Digestion

Animal manures are residues characterized by high nitrogen (N) and organic content. Ammonia is released during the degradation of proteins, reaching a high concentration in the reactor that may inhibit methanogens. This reason explains this fact for the extended application of co-digestion in livestock farms. The high ammonia content reached in reactors also affects the equilibrium between different chemical species such as carbonates and volatile fatty acids (VFAs) derived from the sequential transformation of organics. Ammonia in the digester liquor is present as free ammonia (NH_3) and ionized ammonium (NH_4^+), with the first being considered the most toxic form [26]. The buffering system created by the presence of these compounds produces an environment where pH is kept at levels higher than 6.4 units, ensuring suitable acid–base environments for methanogens [27].

Sewage sludge is another common waste traditionally treated by anaerobic digestion. The treatment of urban wastewaters leads to a rejected stream with a high organic content and a significant amount of water. Sludge obtained from the primary settler receives the denomination of primary sludge. The aerobic treatment of wastewater by the waste-activated sludge process also gives rise to a sludge stream mainly containing microbial biomass. The rapid growth of this biomass makes the extraction from the biological system imperative, thus producing a secondary sludge or a waste-activated sludge (WAS). Large-scale wastewater treatment plants (WWTPs) generally have a sludge line dedicated to the exclusive treatment of sewage sludge, which is composed of a mixture of the above streams. Thus, the digestibility of sludge depends on the characteristics of WAS, which is recognized as having a limited degradation because cellular material needs to be hydrolyzed prior to the release of its internal content to make it accessible to the anaerobic microflora.

Anaerobic digestion of food wastes or the organic fraction of municipal solid wastes (OFMSW) has gained popularity in recent years, with several plants being installed for treating this material in urban waste treatment centers. Source-sorted separated material is usually preferred for its higher quality due to the lower presence of inert components. On the other hand, mechanically separated food waste generates a lower quality material, needing several additional pieces of equipment to handle the slurry produced. In this latter case, grit and contaminants contained in the feed need to be removed before introducing the slurry into the digester. In addition to these inconveniences, the seasonal fluctuation of this type of waste should be noted, which highly influences biogas production [28]. The presence of heavy metals is another factor that may also add complexity to pre-treatment operations. The difficulty encountered when attempting the removal of inert materials and the risk of obtaining digestate with undesirable levels of toxic compounds make this digested slurry not suitable for agronomic use.

The application of anaerobic digestion to the conversion of crop wastes and agro-industrial wastes is another field where this technology finds excellent results. However, when considering this type of substrate, the seasonal availability should be carefully evaluated along with the lignocellulosic content and high C/N ratio, which translates into excessively long digestion times and incomplete degradation. In addition, the low nitrogen levels and the lack of enough trace nutrients may hinder the successful performance and proper development of the anaerobic microflora.

Given the different characteristics of these individual substrates, co-digestion of the above materials becomes the obvious solution for balancing nutrients and reducing the disadvantages associated with mono-digestion. The mixture of different wastes and biomasses allows the adjustment in nutrients, improves the stabilization and conversion of the organic matter, and results in cost-effective use of installations because a single plant is used for treating a diversity of organics obtaining higher methane yields from the feeding mixture [29,30]. However, the composition of substrates is not the only factor influencing the global performance of a digestion plant; other parameters such as seasonal availability, transport distance and collecting costs have great relevance in the final decision for considering whether a material is a suitable co-substrate.

Biogas yields from the co-digestion of food wastes have a range of 0.31–0.88 L/g vs. (volatile solids) with methane contents in the range of 53–70%, whereas these values are usually lower for the single digestion of manures [31]. The improvement in process efficiency is expected to be in the range of 25 to 400%, thanks to the increase in organic loading and the enhanced degradation of volatile solids [32,33]. The composition of substrates significantly affects reactor performance. Carbohydrates, proteins, cellulose, hemicellulose, lignin and lipids present different degradation rates and releases of intermediary compounds exerting in some cases negative effects in fermentation development. Figure 1 presents a schematic description of the different substrates frequently used in digestion plants.

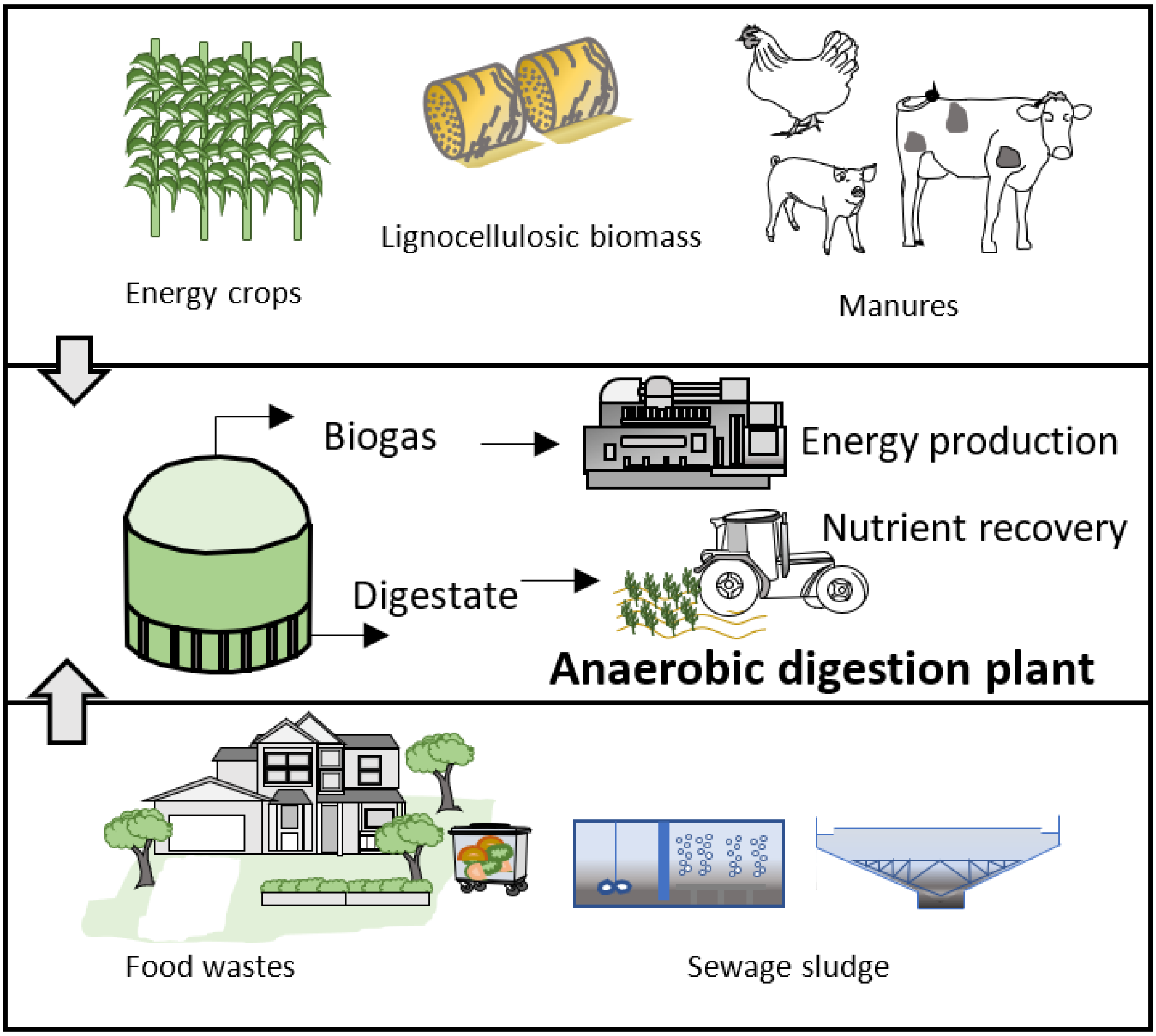

Figure 1. Schematic representation of different substrates suitable for anaerobic co-digestion and valorization of main process products (biogas and digestate).

2.1. Carbohydrate-Rich Substrates

Food wastes, wastes from the food processing industry, catering wastes and source-separated wastes from residential homes are characterized by a high carbohydrate content. Saccharides and disaccharides are the main components of fruit and vegetable wastes. These compounds are easily converted into fatty acid intermediaries by the anaerobic microflora, giving rise to pH changes if the accumulation of these acids overcomes the buffer capacity of the fermentation media [23]. The VFA imbalance may adversely affect the production rate of biogas. Accumulation of these intermediaries is commonly observed during the anaerobic conversion of easily degradable wastes. Wastes from the food processing industry, such as cheese whey or fruit wastes, also have a low nitrogen content leading to poor buffering characteristics of the fermentation liquor. The summation of these features results in inhibitory levels of acetic and propionic acids. When severe digestion imbalances are present, higher carbon chain (C_4–C_5) acids and iso-forms can be measured in the fermenting slurry.

The anaerobic digestion of cheese whey has been studied under different reactor configurations such as up-flow anaerobic sludge blanket (UASB) reactors and sequencing-batch anaerobic reactors (SBR) [34,35]. Cheese whey is a high organic content stream with soluble sugars, which are derived from cheese manufacturing. The digestion of this substrate has proven challenging due to the lack of sufficient nutrients to keep a balanced microflora and the low buffering capacity of the digestion system. In addition, the presence of soluble sugars aggravates the reaction imbalance, with acidification outcompeting the subsequent degradation stages. The application of high organic loading is attained by retaining anaerobic biomass inside the system. Mesophilic and thermophilic digestion of this single substrate have been studied by Treu et al. [36] and Fernández et al. [37], indicating the accumulation of VFA and proposing two-stage systems as a way of overcoming the acidification problems. Another solution proposed for stabilizing the fermentation is the addition of different nitrogen-containing substrates such as manures and sewage sludge [38,39].

Another high sugar-containing substrate is sugar molasses. The digestion of this material shows similar behavior to that of cheese whey. Therefore, two-stage configurations where acidification and methanogenesis, or hydrogen production and methanogenesis, have been proposed to overcome the problems associated with the rapid evolution of VFA and slow degradation of the acid intermediate stream [40–42]. The use of rich-carbohydrate substrates may be interesting in co-digestion systems, but the availability of these substrates is usually determined by their use in animal feeding. Therefore, an increase in the demand for these by-products will ultimately affect market prices and probably create adverse effects on the economy. Market distortions should be avoided either by the use of specific energy crops or by the application of specific policies intended to attenuate market deviations.

2.2. Lignocellulosic Biomass

Other relevant substrates treated by anaerobic digestion are crop wastes, energy crops and any type of high cellulosic-containing material, such as cellulose pulp mill effluent. The material conforming plant cell walls (cellulose, hemicellulose and lignin) is a complex structure with different levels of heterogeneity based on the biological function, age and type of tissue [43]. Cellulose is a component suitable for valorization through anaerobic digestion. However, substrates containing cellulose may also have a fraction of the lignin structure linked to the cellulosic material, making its access difficult to the anaerobic microflora. Therefore, this is the reason for denoting this biomass as lignocellulosic material.

Cellulose is an insoluble polymer with a high molecular weight having a main structure formed of D-glucopyranose units linked by β-1,4-glycosidic bonds and cellobiose repetitive units. Two forms of cellulose are generally considered, a crystalline and an amorphous structure, which are easier to degrade by enzyme complexes [44]. For cellulose to be assimilated by microorganisms, the degradation should be initiated exocellularly, either completely extracellularly with the aid of specific enzymes or in association with the outer cell envelop layer [45]. Anaerobic bacteria possess cellulosome, which is an extracellular multi-enzyme complex. This complex attaches to the cell envelope and the substrate, starting the degradation of cellulose [46].

Hemicellulose is the other main component of lignocellulosic biomass, with a lower molecular weight than cellulose. Hemicellulose forms together with lignin in a covering structure of cellulose fibers. Hemicellulose is a polysaccharide containing different types of sugars linked by β-1,4- and, less frequently, by β 1,3-glycosidic bonds [47]. Hardwoods and straw contain xylans as the predominant hemicellulose constituent, whereas galactomannans are the largest hemicellulose fraction in softwoods [48]. Cellulose and hemicellulose can be degraded by anaerobic microflora resulting in the accumulation of recalcitrant aliphatic molecules [49].

The degradation of cellulose was studied by Yamazawa et al. [50] and Li et al. [51]. Its degradation produces short-chain components, such as acetic and propionic acid, which are mainly metabolized by clostridial species. However, this conversion takes a long time (40–50 days) compared with that of carbohydrates under the same anaerobic conditions [48]. This is the main reason for proposing the application of pre-treatments when biogas production is intended [52]. Thus, accelerating the initial stages of this degradation process leads to a significant reduction in digester volume and therefore in plant installation costs.

Spectroscopic techniques have been used as a tool for evaluating the degradation of different substrates under anaerobic digestion [53,54]. Techniques such as nuclear magnetic resonance (NMR) and Fourier transform infrared (FTIR) spectroscopy allow for the evaluating of the fate of the process and characteristics of digestates in order to study its adequacy as an organic amendment and act as a soil improver when analyzing agronomic benefits [55,56]. Under anaerobic conditions, a preferential degradation of carbohydrates, cellulose and hemicellulose takes place, thus concentrating chemically recalcitrant aliphatic structures [57]. Aromatic structures originally present in the substrate may be partially degraded, causing also the accumulation of this material. The previous features translate into large digester volumes and therefore high capital costs. The accumulation of recalcitrant materials affects the final amount of digestate to be disposed of and becomes a problem if there is not enough land nearby.

Higher methane yields have been reported for cellulose, but a faster conversion was found for hemicellulose under mesophilic conditions [58,59]. Lignin structures, on the contrary, are scarcely affected during anaerobic digestion, hardly experiencing small changes in their native structure when extended digestion studies were performed [60]. Due to the recalcitrance of lignin structures, lignocellulosic biomass is usually used as a structuring agent during solid-phase fermentation under percolating leachate configurations. The poor degradation rate of lignocellulosics under anaerobic conditions here becomes an advantage since the porosity of the percolating bed is desirable to allow the circulation and homogenization of soluble compounds by leachate recirculation. However, if this type of biomass is added as a co-substrate, then pre-treatments are recommended to facilitate access to the microflora. Several value-added products can be obtained from the fractionation and conversion of this raw material by means of a concatenation of different processes capable of a sequential transformation, always keeping in mind the global efficiency of the production line.

The coupling of different processes leads to new developments integrated into the biorefinery concept, where a set of conversion platforms are available for obtaining green chemicals and recovering energy. Second-generation biofuels, such as biogas from lignocellulosic biomass, have great potential because of their plentiful abundance, offering no interference with other commercial activities such as animal feed or crops for human consumption. Still, the heterogeneous structure of this material and its recalcitrant nature adversely affect its use as a substrate in biogas plants [61]. Pre-treatment stages considerably increase the energy demand of the installation. Careful analysis should be performed regarding improvement obtained in biogas production after pre-treatment application and the energy required in the process.

Thermal pre-treatments are widely extended at an industrial scale due to the experience gained in pre-treating sewage sludge and the unique feature of recovering energy from high-quality lateral streams. The hydrolysis of hemicellulose produces oligosaccharides such as pentose (xylose and arabinose), hexose (glucose, mannose, and galactose), acids (acetic acid, formic acid, and levulinic acid) and furans (furfural and 5-hydroxymethylfurfural). Insoluble humins are also obtained as products under harsh hydrolysis conditions [62]. However, the high temperature and high pressure under which hydrolysis is carried out may release some compounds that can behave as inhibitors [63]. Recalcitrant inhibitory substances, such as furfurals and hydroxyl methyl furfural can be produced at high temperatures [64,65], thus introducing new complexities into the valorization process due to the additional stages necessary for removing these toxic compounds. Another relevant fact that should be noted is the high installation and operating costs associated with pre-treatment units, which sum up to the already high capital investments of digestion plants.

Table 1 reports on the different methane yield values found in the literature for a variety of substrates. Some of these results present a wide range of variability since methane yields are highly dependent on the characteristic of the substrate, experimental conditions and the presence of inhibitory compounds. Another parameter of relevance is the time needed for degrading the organic material, which translates into high retention times and therefore large digester volumes.

Table 1. Methane yields reported by different authors when evaluating individual substrates.

Organic Substrates	Methane Yield	References
Sewage sludge	0.13–0.45	[66–72]
Food wastes	0.33–0.5	[69,72,73]
Pig, swine manure	0.3–0.5	[19,74–78]
Poultry manure	0.03–0.11	[19,74,78,79]
Chicken manure	0.52	[80]
	0.053–0.75	[19]
Cattle manure	0.11–0.54	[12,81]
Slaughterhouse waste	0.2–0.8	[82,83]
Brewery waste	0.3–0.51	[84,85]
Residual glycerine	0.56	[86]
Corn stover	0.3–0.4	[75,87]
Sunflower crop wastes	0.2–0.4	[88,89]
Rapeseed crop wastes	0.25	[75]
Wheat straw (steam explosion pretreatment)	0.25–0.35	[90,91]
Rice straw	0.26	[92]
Grass: Napier grass, Canary grass, King grass	0.15–0.60	[93–95]
Meadow grass	0.39	[96]
Microalgae *Chlorella* sp.	0.23–0.26	[77,97]
Microalgae *Nannochloropsis oculata*	0.3–0.35	[98]

2.3. Protein-Rich Substrates

Proteins are also abundant in organic substrates, particularly in those derived from animal wastes. Slaughterhouse wastes, pig, cattle, chicken manure and any other type of manure from livestock farms are residues with a high protein content. When dealing with this material, ammonia accumulation may cause problems in the reactor performance if an equilibrating carbon source is not added to balance the C/N ratio of the feeding recipe. Another residue that has been studied recently as a suitable co-substrate is animal carcasses. This waste is subject to strict regulations, but livestock farms must confront a significant risk associated with the transport of animal carcasses due to the possible cross-contamination that may take place because of the route the transport truck must follow during collection operations, with a risk of failure in decontamination when traveling from one farm to the other always existing. This risk could be reduced if alternatives are allowed in situ in compliance with Regulation (EC) 1069/2009 and (EU) 142/2011 for animal by-products, so these farms could safely pre-treat this material to make it a suitable co-substrate [99].

Arenas et al. [24] studied biogas production from animal carcasses, reporting a methane yield of 0.47 m^3 CH_4/kg vs. from biochemical methane potential (BMP) tests. Tápparo et al. [100] reported a doubling in gas production when studying the co-digestion of swine manure along with animal carcasses, and Xu et al. [101] proposed the optimization of the hydrothermal pretreatment of animal carcasses for increasing biogas production, given the regulation requirement already established for category 2 material.

Ammonium ions released from the degradation of proteins inhibit anaerobic activity at values close to 4.0 g/L [102]. However, several factors are relevant in the response of the microflora to ammonium. Acclimation is crucial for tolerating high levels of this cation in the reactor liquor, along with temperature and pH. Co-digestion of substrates, with different C/N ratios, is a proper strategy for enhancing degradation performance [103] and avoiding toxic ammonia concentrations. Thus, the use of grass, straw and lignocellulosic biomass in general, or micro-algae biomass as co-substrates are suitable options that have been evaluated under small laboratory conditions in many cases. However, the availability of these materials should be carefully considered, with this not being always possible due to the limitations imposed by transport distances. The use of energy crops as co-substrates is currently a feasible option in some European countries, with maize silage being widely used as a co-substrate. However, this option is not adequate for many countries and may be undesirable due to the rise of market distortions. Digestion technology should be integrated into the economic cycle without requiring additional incentives. Otherwise, the process would not become a sustainable alternative for energy production.

2.4. Lipid-Containing Materials

Lipid materials are a type of waste coming mainly from the food processing industries, slaughterhouses, palm oil industry and grease traps present in different industrial and commercial activities which have this type of collector in their sewage system. This substrate presents an extremely high biogas potential with a value of 1014 m^3/kg vs. [104], although its addition to a digester needs careful control of organic loading due to problems associated with stratification and the formation of long-chain fatty acids which inhibit methanogenic metabolism [105]. This is particularly true when short hydraulic retention times (HRTs) are applied [106]. Other problems are associated with technical constraints. Several authors have reported pipe clogging and foaming problems in digester gas pipelines along with severe fouling of these lines [107,108]. If all these problems are overcome, this material could significantly enhance biogas production, almost doubling methane yields with a small supplementation in the feeding mixture [109].

The digestion of lipids requires hydrolysis into long-chain fatty acids (LCFAs) and glycerol as a first stage. Glycerol is transformed into an intermediate compound (glyceraldehyde 3-phosphate) by a set of enzymes before entering the glycolysis pathway, with this route being more complex than that followed by glucose [110], causing, in some cases, the accumulation of propionic acid. LCFAs are subsequently degraded via β-oxidation, requiring an external electron acceptor for oxidation [111]. Pre-treatments of lipid substrates are considered necessary for enhancing degradation through the increase in surface area and reducing the formation of conglomerates which affects the hydrolysis stage in a negative way [112].

Several reports have dealt with the successful digestion of lipid-rich wastes, indicating increments in methane production between 10 and 200% [113–115] and methane yields as high as 999.2 mL CH_4/g vs. for slaughterhouse wastes [116] obtained from BMP tests. The use of lipids as co-substrates in anaerobic digestion and the application of different strategies to avoid operational problems, such as acclimatization of the microbial consortium and developments of new reactor configurations, could be a potential approach for maximizing the valorization of these streams for producing biomethane [117].

3. Modelling Cumulative Methane Production

Labatut et al. [118] studied the behavior of several substrates using BMP tests and reported differences in biogas evolution behaviors based on the ease of degradation of the substrate by the microflora. Edwiges et al. [119] also studied the biogas production from several substrates and the effect of their composition. It is reasonable to assume that the main substrate constituents will keep a close relationship with the methane yield obtained and the evolution of the gas production curve. However, factors such as accessibility to the degradable components and inhibitory conditions created during their transformation may alter the final outcome. Thus, carbohydrate-rich substrates are usually well-fitted to first-order decay models (Equation (1)) whereas, more complex substrates or those experiencing inhibitory conditions are fitted to different models capable of predicting these effects.

$$B_{(t)} = B_0\left(1 - e^{k \cdot t}\right) \tag{1}$$

where $B_{(t)}$ represents the cumulative methane yield at any time, t is the time of the batch assay, B_0 is the maximum gas produced by the substrate and k is the first-order constant. The value of the first-order constant gives an indication of the easiness of degradation. Thus, substrates rich in carbohydrates are characterized by high k values between 0.39–0.66 1/d [119]. This model gives curves with a fast evolution of biogas during the first days of the batch assay, usually ending the fermentation in a short time and reaching a plateau very soon, coinciding with the end of the degradation. However, this fast degradation may cause operational problems in digesters and also when evaluating BMP tests. The inoculum-to-substrate ratio used for starting up the tests greatly affects the ultimate methane production. When acidification is expected to become a problem, adding a greater amount of inoculum, and if necessary alkaline solutions, would aid in obtaining the desired results.

The addition of rich-carbohydrate substrates as feed to a reactor operating under a continuous mode may cause localized acidification resulting in pH excursions. Many reactors work using a feeding recipe where the substrate is introduced at specific hours of the day. These substrates may cause a temporal variation in methane content, increasing CO_2 levels in biogas and leading to a greater gas production rate right after feeding.

Special care should be taken regarding the type of reactor configuration based on operating conditions and substrate composition. Continuous stirred tank reactors (CSTRs) run under equivalent values of HRT and cell retention time (CRT). Therefore, imbalances may appear due to the lack of enough methanogenic microorganisms. The lower growth rate of these organisms compared with that of acidogenic bacteria may cause a washout of the first ones. Other configurations capable of retaining biomass in the reactor may be more suitable for dealing with substrates that are easily degraded, thus needing a shorter retention time such as UASB, anaerobic filters or anaerobic sequencing batch reactors (ASBR). Recent strategies for attaining biomass retention and improving degradation rates are the introduction of active filling to promote biogas production and attain higher-quality effluents thanks to the presence of magnetically active filling layers inside the reactor which are capable of reducing the nitrogen and phosphorus concentration [120]. The use of active filling containing metals (copper and iron) has also proven effective with similar results [121].

Table 2 presents the k values obtained by different authors under mesophilic conditions. There is a wide range of values reported in the literature for this parameter which is highly dependent on the characteristics of the substrate and the experimental conditions [122], such as substrate concentration, inoculum-to-substrate ratio, temperature and particle size. As a matter of example, a decrease in the value of the disintegration rate constant was reported by Liotta et al. [123] with the increase in solid content and particle size of the substrate. Any increase in temperature leads to a rise in the reaction rate, whereas reducing particle size increases the specific surface area making it more accessible to the enzyme attack. Aldin et al. [124] studied the effect of particle size using casein as model protein material. These authors found an increase in the hydrolysis constant from 0.034 to 0.298 1/d by decreasing this parameter.

Table 2. Values of first-order decay constant available in the literature at mesophilic conditions.

Substrate	k (1/d)	Methane Yield (L/g VS)	Reference
Cattle, pig manure	0.106–0.149	0.217–0.287	[125]
Pig manure, swine manure	0.213	0.202	[86]
	0.11	0.161	[126]
Cattle manure	0.037–0.086	0.254–0.290 [1]	[127]
	0.069–0.278	-	[128]
	0.082	0.239	[129]
	0.19	0.238	[130]
Chicken manure	0.07–0.12	0.298–0.351	[131]
Grass	0.107	0.400	[129]
Two-phase olive pomace	0.054	-	[132]

Table 2. *Cont.*

Substrate	*k* (1/d)	Methane Yield (L/g VS)	Reference
Food wastes	0.55 0.2	- 0.524	[133] [126]
Organic fraction municipal solid wastes	0.0061	-	[134]
Fruit and vegetable wastes	0.34	0.350 [2]	[130]
Vegetable crop residues	0.094–0.167	0.094–0.147	[59]
Food waste leachate–sewage sludge	0.08	0.343	[135]
Corn stover	0.197 0.06–0.11	0.0008–0.0023 0.218–0.300	[136] [131]
Green corn stover	0.159	0.347	[137]
Air-dried corn stover	0.0624	0.319	[137]
Cellulose	0.123 0.32	0.348 0.353	[129] [130]

[1] Calculated from gas and vs. data reported. [2] Digitized from graph reported.

The composite structure of lignocellulosic materials translates into long degradation times, making inadequate the use of the first-order decay model to describe cumulative biogas evolution from batch tests. The slow degradation of cellulose and hemicellulose, contrary to what is observed in carbohydrates, makes the modified Gompertz model more suitable (Equation (2)). This model has demonstrated adequacy to evolved biogas data from substrates such as manures and agronomic wastes [138,139].

$$B_{(t)} = B_0 \cdot e^{-e^{\left(\frac{R_{max}\ e}{B_0}(\lambda - t) + 1\right)}} \tag{2}$$

In this model, two additional parameters are introduced with regard to the first-order one. R_{max} represents the maximum methane production rate. λ represents the delay associated with the acclimation of the microflora to a different substrate and environment. The *e* number (2.718) is also used in this equation. The modified Gompertz model presents the advantage of fitting biogas evolution from complex substrates or those containing partial inhibitory compounds. Thus, λ values describe the delay in biogas production during the initial stage of the fermentation. Other models containing a lag phase have also been evaluated with good results, such as the transfer function model, logistic function, cone model and Richards' model [140–142]. Diauxic metabolism has also been considered appropriate for the description of cumulative biogas curves using the modified Gompertz and the logistic model for describing each sequential degradation stage [143,144].

Table 3 shows a list of different values reported in the literature for λ and R_{max}. Values of λ are usually in the range of 1.5–9.4 days [145,146]. This parameter, as well as the maximum production rate, is affected by inhibitory substances either already present in the substrate or produced during the fermentation, as would be the accumulation of VFAs, long chain fatty acids and ammonia. Sánchez et al. [147] showed that an increase in the lipid fraction during anaerobic co-digestion of slaughter wastewater caused an increase in the lag phase. Similar results were also reported by Andriamanohiarisoamanana et al. [148] when evaluating the co-digestion of manure, slaughter wastes and glycerol. Increasing the content of glycerol in the mixture also caused a greater delay due to the fast initial conversion and accumulation of propionic acid.

Table 3. Values reported in the literature for different substrates under mesophilic conditions regarding kinetic parameters from the Gompertz model.

Substrate	λ (Days)	R_{max} (mL CH_4/g vs. d)	Methane Yield (L/g VS)	Reference
Swine manure	0 0.5	25.2 12.8	0.322 0.161	[149] [126]
Cattle manure	2.45 0	15.7 11.9	0.239 0.202	[129] [149]
Chicken manure	0.3–2.8 0	19.4–48.9 19.2	0.180 [1] 0.258	[136] [149]
Food wastes	0.5	72.3	0.524	[126]
Food waste leachate–sewage sludge	1.98	28.4	0.343	[135]
Waste activated sludge	5.4	19.2	0.253	[150]
Grass	1.94	34.5	0.400	[129]
Corn stover	0.9–1.9	16–32.1	0.218–0.300	[131]
Liquid effluent from Biorefinery (treating grass material)	3.9–10.2	44.7–66.3	0.459–0.505	[151]
Cellulose	2.93	42.0	0.348	[129]

[1] Digitized from graph reported.

4. Taking Advantage of Process Synergies

Digesting a mixture of several substrates is an efficient way of enhancing reactor performance and increasing methane yields. The main advantages of co-digestion, as already stated, are associated with the supply of nutrients lacking in single components, thus equilibrating the feeding recipe. Different values of biogas yields obtained from co-digestion experiments are listed in Table 4. The greater yields obtained for the mixture than from the individual digestion of substrates are usually explained by a better balance of nutrients and therefore are represented as a positive synergy. However, if this is not the case, then a summation effect is still of interest because of the increase in organic loading provided, which increases reactor productivity.

Table 4. Methane yields found in the literature for different co-digestion mixtures obtained from batch tests.

Digestion Mixture	Methane Yield (L CH_4/g VS)	Reference
Sewage sludge + food wastes	0.293–0.365	[72]
Waste activated sludge + organic fraction of municipal solid wastes	0.162–0.243	[152]
Sewage sludge + sludge from brewery	0.176–0.263	[135]
Sewage sludge + food waste leachate	0.233–0.344	[135]
Sewage sludge + maize straw	0.336–0.472	[71]
Sewage sludge + cattle manure	0.352–0.470	[71]
Swine Manure + glycerine	0.349–0.467	[86]
Pig manure + ESBP [1]	0.212	[153]

[1] ESBP: exhausted sugar beet pulp at 25:75 mixture ratio.

Some authors reported an additive effect in biogas production when studying the mixture of agricultural by-products and manures [75,96]. On the contrary, Li et al. [154]

reported a higher biogas production when co-digesting sewage sludge and leachate derived from food wastes in MSW incineration plants than from the individual substrates. In this latter case, the greater production was explained by the enhancement in solid removal which brings a higher methane evolution as a result. Similar performance was reported by Anjum et al. [155] and Ghaleb et al. [156] when studying co-digestion at different C/N ratios, indicating that the modification of the C/N ratio favors microbial activity and improves solid removal. The benefit associated with the addition of the co-substrate is considered as a priming effect by Insam and Markt [157] in resemblance to the enhanced organic matter decomposition that takes place in other habitats such as soils and sediments. This effect would explain the greater removal of volatiles frequently reported by several authors. However, the addition of a readily degradable substrate can affect the outcome of the digestion system; not always getting benefits.

Tambone et al. [49] indicated that anaerobic digestion proceeds through preferential degradation, accumulating complex structures in the remaining solids. Therefore, the co-digestion may lead to a degradation of carbohydrates contained in the co-substrate, hardly modifying the other components of the mixture [23]. In addition, not all BMP results can be directly extrapolated to a continuously operating system, as demonstrated by González et al. [86]. These authors indicated the successful performance of batch co-digestion tests under different co-digestion ratios but failures when attempting the semi-continuous operation of these same mixtures. Seekao and co-workers [158] set a mathematical connection between BMP and continuous operation using Monod kinetics. These authors indicated that although these tests give no clue regarding chronic toxicity, there is an evident link associated with microbial kinetics for both modes of operation which set the optimum operating conditions regarding the organic loading rate (OLR), HRT and methane production. Therefore, the different constituents of the feeding mixture may not be fully degraded if the OLR and HRT of the reactors are not in accordance with the microbial dynamics, although BMP tests predicted successful results.

Co-digestion allows the treatment of substrates that otherwise would not be possible or would lead to extremely low methane yields. This is the case in the study performed by Zahedi et al. [159] when co-digesting a mixture composed of chicken manure, sewage sludge and wine distillery wastewater. The results reported by Porselvam et al. [115] and Cuetos et al. [160,161] are similar, who studied the digestion of slaughterhouse wastes. The high lipid content in the first case and the high protein content in the second, where residual blood from slaughterhouses was studied, prevented the correct development of the fermentation when attempting the mono-digestion of these substrates.

The benefits of co-digestion are undeniable, but high installation costs along with the need for high-skill personnel for the operation and maintenance tasks are two important barriers to be overcome. Policies should focus on solving these issues, proposing solutions for small- and mid-size treatment waste systems which present serious difficulties in attaining proper waste valorization at a reasonable cost. Partial decentralization may become a practical solution if small digestion units are dedicated to treating local wastes, whereas raw biogas may be transported and upgraded at a centralized treatment plant. Figure 2 shows a schematization of different substrates commonly used in anaerobic co-digestion focusing on expected inhibitory effects.

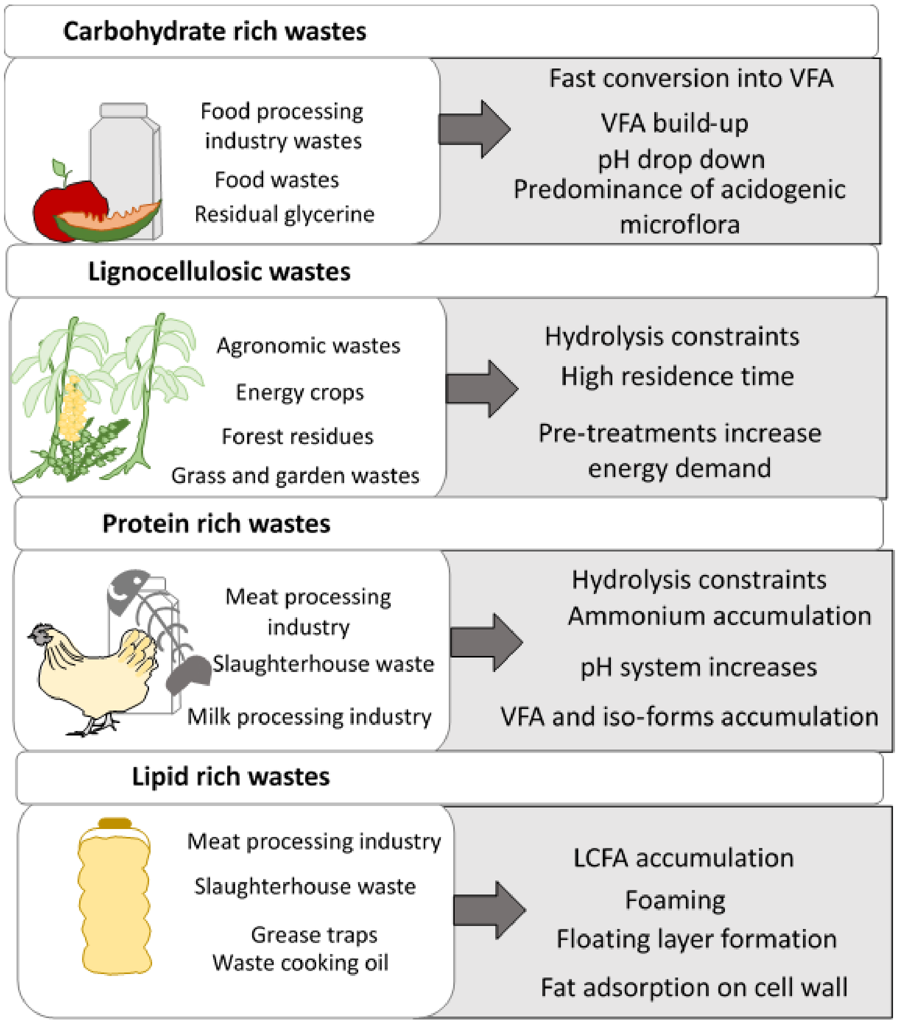

Figure 2. Co-substrates used in anaerobic digestion and expected problems in reactor dynamics.

Co-Digestion at Large Scale

Another relevant advantage of co-digestion is that it attains a better use of equipment and cost-sharing because existing facilities can be adapted, or new ones can be built to process multiple waste streams in a single unit [162,163]. The biogas production of existing plants can be increased by treating other materials from different industrial and agronomic sectors. However, implementing this approach for already-operating plants implies the introduction of several modifications in the facility to endow the system with the necessary flexibility for storing, pre-treating and feeding the co-substrate into the reactor. The plant must cope with the available co-substrates found in the surroundings, otherwise transportation costs would cancel out any benefit associated with the higher biogas production. The integration of co-digestion in WWTPs can be a solution for reducing the operating costs and also for increasing the electricity produced by the plant and the share dedicated for self-consumption.

Digestion plants have a delicate balance between waste treatment, energy production and economic feasibility, with the latter being dependent on the plant scale and revenues obtained from the different products. In recent years, the number of large-scale digestion plants installed has increased significantly, providing economic and environmental benefits [153]. There exist many literature reports about the successful performance of co-digestion with sewage sludge and manures under a laboratory scale [22,68,75,164]. Large-scale reports are less abundant, and therefore those found in the literature such as

Bolzonella et al. [165], Sembera et al. [166] and Koch et al. [167] represent a valuable source of knowledge.

The benefits of co-digestion are associated with greater energy production thanks to the higher treatment capacity, organic loading increase and higher methane yields [5]. The increase obtained in biogas production may cover the whole energy demand of the WWTP [168,169] and can be high enough, depending on the type and amount of co-substrate added, to become an efficient way of obtaining surplus energy and be considered as an eco-friendly and economically viable approach [170]. However, when attempting large-scale co-digestion strategies in already existing plants such as digesters in WWTP, operating problems become frequent as they are usually related to the high variability of co-substrate composition, changes in technical routines, maintenance and the installation of additional equipment to deal with these materials [171].

A co-digestion experience at the WWTP of Viareggio and Treviso (Italy) was described by Bolzonella et al. [165], where the source-sorted OFMSW was treated with sewage sludge, reporting a 50% increase in biogas production when increasing the organic loading of the reactor from 1.0 to 1.2 kg VS/m^3 d, and a five-fold increase in monthly biogas production in the second plant studied. However, not all reports present this significant of an improvement. At the Lansdowne WWTP in the municipality of Prince George, British Columbia, Canada, Park et al. [172] reported the results of a short co-digestion assay with source-sorted food wastes from supermarkets. The average daily biogas production was increased by just 8–10%, but several operational problems were highlighted and associated with this practice, such as the clogging of the hose connecting the chopper pump and the sludge recirculation line, needing manual maintenance for clearing up the line. Accumulation of fibrous scum was also reported near the digester floating roof and the visual presence of impurities in the biosolids was also indicated.

In another large-scale study, a two-year experience was reported by Mattioli et al. [173] using OFMSW separately collected for this aim. These authors performed their co-digestion study with sewage sludge at the Rovereto WWTP. The waste was submitted to a specific pre-treatment to remove any inert material and obtain a high-quality slurry. However, the accumulation of floating material was observed on the top layer of the digester and impurities were detected in dewatered sludge such as plastics, elastic bands and seeds. Even with these disadvantages, the authors also indicated that the addition of the co-substrate attained 85% electricity self-generation, whereas in the previous conditions, the electricity produced from single digestion of sludge only covered 50% of the WWTP energy demand. Table 5 present a list of different co-digestion studies regarding enhanced energy performance at a large scale. The homogenization of the results is not possible due to the different ways and measurement units the authors used for reporting results. However, the table contains the main characteristics of their studies.

Table 5. Benefits reported by large-scale co-digestion studies found in the literature.

Co-Digestion	Amount Added	Benefits	Disadvantage	Reference
SS * + food waste Grüneck WWTP (Munich, Germany)	5.5 t/d	16% increase in energy production	Poor dewaterability	[174]
SS + organic solid waste Zirl WWTP (Tyrol, Austria)	Increase in OLR from 1.17 to 2.18 kg VS/m^3 d	174% increase in biogas production. Energy obtained was 115% of the plant energy demand	33% increase in digestate production and nitrogen back load was doubled	[175]
SS + food waste WWTP Garching/Alz (Germany)	10% (*w*/*w*)	Enhanced methane yield reporting synergism (12% increase). Biogas production doubled	High nitrogen load in reject water. Reduced dewaterability	[167]

Table 5. *Cont.*

Co-Digestion	Amount Added	Benefits	Disadvantage	Reference
SS + organic waste from domestic refuse Velenje WWTP (Slovenia)	Increase in OLR by 25%	80% biogas increase. Increase in vs. degradation	No reported	[176]
SS + fat-waste Iława WWTP (Poland)	Variable amount-Added to set the OLR at a value of 4.8 g/L d as maximum	82% biogas increase 29% vs. removal enhancement Attain close to total energy consumption	No reported	[177]
Slaughterhouse waste + mixture of substrates Co-digestion plant operated by the company Svensk Biogas AB (SvB). Linköping (Sweden)	35–75% (*w*/*w*)	Energy savings, better odor control, higher gas quality and production	High ammonia load. Need addition of ferrous chloride and hydrochloric acid to increase process stability	[178]
SS + mixture (milk processing industry wastes and fat from grease traps) WWTP Moosburg (Germany)	186% OLR increase	300% CH_4 increase	Solid accumulation inside the digester. Nitrogen backload. Decrease in retention time and lower sludge dewaterability.	[166]
SS and mixture of food waste-garden waste (95:5% based on fresh mass) and grease trap sludge Grossache-Nord WWTP, Tyrol (Austria)	Amount of SS: 850 t/year Amount of co-substrate: 397 t/year	Increase in methane yield: PS: 302 m^3/t TS added WS: 133 m^3/t TS added Co-digestion: 627 m^3/t TS added (plant data) Benefit to cost ratio greater than one	Lower TS removal, higher amount of dewatered sludge and increase demand of flocculants. Sludge disposal represented 64% of overall costs (plant data)	[179]
SS + mixture of wastes (kitchen wastes and fats) WWTP Strass (Austria)	Kitchen waste added 329 g DM/m^3 treated SS Fat added 9 g DM/m^3 treated SS	Additional amount of electricity produced: 0.035–0.041 kWh/m^3 Energy self-sufficiency achieved for the WWTP	Higher nitrogen input, requiring a more efficient denitrification stage.	[180]

* SS: Sewage sludge; DM: dry matter, PS: primary sludge, WS: waste-activated sludge.

Co-digestion performed in large-scale plants offers several advantages. Nevertheless, digestate characteristics may be adversely affected and process modifications may be necessary, resulting in additional complexities in plant operation and maintenance. Research dealing with these aspects is necessary to properly balance the benefits and inconveniences, therefore, real practical solutions can be implemented without risking current process operation. Policies dedicated to favor the flexible treatment of wastes, thereby facilitating solutions to the final disposal of digestate derived from co-digestion systems are necessary.

5. Improving Reactor Performance

The increase in reactor performance is the best way for improving plant economic feasibility because of the greater capacity for treating biowastes and improving degradation rates, which bring along a significant enhancement in reducing the amount of digested material needing final disposal. Attaining a stable digestion process involves the control of biological parameters and reactor operating conditions. The dynamics of the process are very complex because the reactor configuration and feeding rate have a marked effect on microbial performance and, in turn, the predominant microflora greatly influences the process outcome. Table 6 presents a list of the main process parameters influencing the digestion process.

Table 6. Process parameter influencing anaerobic digestion performance.

Process Parameter	Effect
Temperature	The increase in temperature accelerates degradation rates, fluid dynamics and settling characteristics of particles [181]. The improvement in microbial activity increases the reactor treatment capacity of organics reducing the digester volume needed [182].
pH and alkalinity	pH values should be close to neutral conditions. The stability of the digestion is closely related to the capacity of buffering acid intermediaries, the release of CO_2 and the presence of ammonia. The interaction between the ionic species and free forms attenuates pH deviations making the process more robust to organic loading fluctuations [27].
Organic loading rate (OLR)	Represents the amount of organic material entering into the digester with the influent. Increasing the volumetric flow or increasing the solid content of the feeding material leads to an increase in organic loading. Biogas production is directly associated with the amount of organics fed into the reactor, and any increment in OLR is usually associated with an improvement in the biogas production rate. The increase in solid content attained by adding a co-substrate in anaerobic digestion is one of the main reasons for obtaining a better volumetric efficiency of the reactor. However, an excess in OLR may also cause process imbalances due to the accumulation of acid intermediaries associated with disturbances in the acidogenic and methanogenic phases.
Hydraulic retention time (HRT)	Refers to the time the fluid spends in the reactor. This time is calculated as the ratio between the volume of the reactor and the volumetric flow applied. HRT and OLR are linked by the volumetric flow, thus increasing the incoming flow also leads to an increase in OLR and a decrease in HRT. The time needed for the substrate to be fully degraded depends on the characteristics of the material, complexity in the structure of organic compounds and the activity of the microflora. Co-substrates characterized by a limited hydrolysis phase will need a higher retention time in the anaerobic reactor. Inhibitory conditions lead to poor performance of the microbial activity, with the digestion system not being able to degrade organics in the time given by the HRT.
Volatile fatty acids (VFAs)	Short-chain fatty acids are produced as intermediary compounds during the anaerobic conversion of organics. Process imbalances lead to the accumulation of these acids, inhibition of methanogens, and therefore a decline in biogas evolution along with pH variations when the buffer capacity of the system is surpassed [183]. Process inhibition has been reported to occur at VFA concentrations in the range of 2000–4000 mg/L [184] depending on the type of substrate evaluated. However, co-digestion with high N-containing organics allows the maintenance of process stability even though high levels of VFA may be present. Stable performance was reported by Jiang et al. [185] when studying co-digestion of pig manure, reporting as inhibitory the VFA range of 16.5–18.0 g/L
Ammonium	This compound is derived from the conversion of protein-rich material. The toxicity of ammonia in the digester is linked to the level of free ammonia, which is dependent on the system pH. Nitrogen is an essential nutrient for the process, but excessive levels lead to methanogenic inhibitory conditions. The ammonium concentration found in the reactor liquor depends on substrate C/N ratio, HRT and OLR applied to the reactor, and the degradability of the substrates (hydrolysis performance). Ammonia also plays a relevant role in the buffer capacity of the system by attenuating pH drops through the equilibrium ammonia–ammonium reaction. However, a high concentration of ammonium ions may be detrimental to the anaerobic microorganisms. Moestedt et al. [186] reported that a threshold for stability is found at 1 g NH_3-N/L (free ammonia), irrespective of the OLR studied. Acclimation of the microflora to high ammonia levels may attain stable performance when treating high-nitrogen-containing wastes. Bi et al. [187] reported stable performance of a full-scale chicken manure digestion plant under ammonium-N levels of 6.2 g/L and Yan et al. [188] indicated that 8.5 g NH_4^+-N/L was the threshold for experiencing inhibitory conditions, with this value being associated with free ammonia nitrogen (FAN) values greater than 800 mg NH_3-N/L.

Several authors have proposed different alternatives for enhancing methane production; among these strategies is worth mentioning the addition of hydrogen gas into the reactor or introducing a hydrogen-producing culture favors higher levels of this gas in the reactor liquor [189]. It is widely known that the digestion process is a sequential one where a delicate balance between the different intermediary species is necessary. The great capacity for transforming hydrogen gas into methane as described by Martínez et al. [68] and Zhu et al. [190] has been widely reported, who indicated that the activity of homoacetogenic microbes was enhanced when continuously feeding hydrogen into the reactor, increasing the levels of acetate and subsequently favoring the acetoclastic pathway to end in an increased production of methane. This same idea can be applied to the conversion of syngas into methane, thus facilitating the treatment of this low-energy content gas and reducing syngas handling problems.

5.1. Bioaugmentation

Bioaugmentation has also been proposed as an alternative for improving the performance of methanogens. Ács et al. [191] studied the performance of digestion systems inoculated with *Enterobacter cloacae* cells. These authors obtained a 20% increase in biogas production after running the inoculated reactor for 6 weeks, using continuously stirred reactors under a fed-batch configuration. Kovács and co-workers [192] also studied the performance of mesophilic digestion systems with the inoculation of the same organism (*E. cloacae*) and that of a thermophilic reactor using, in this case, *Caldicellulosiruptor saccharolyticus*. However, they reported that inoculated microflora was washed out from the systems, with them being incapable of competing with the microbial consortium. Therefore, the improvements obtained were limited in time. Figure 3 shows several techniques available for improving anaerobic digestion performance.

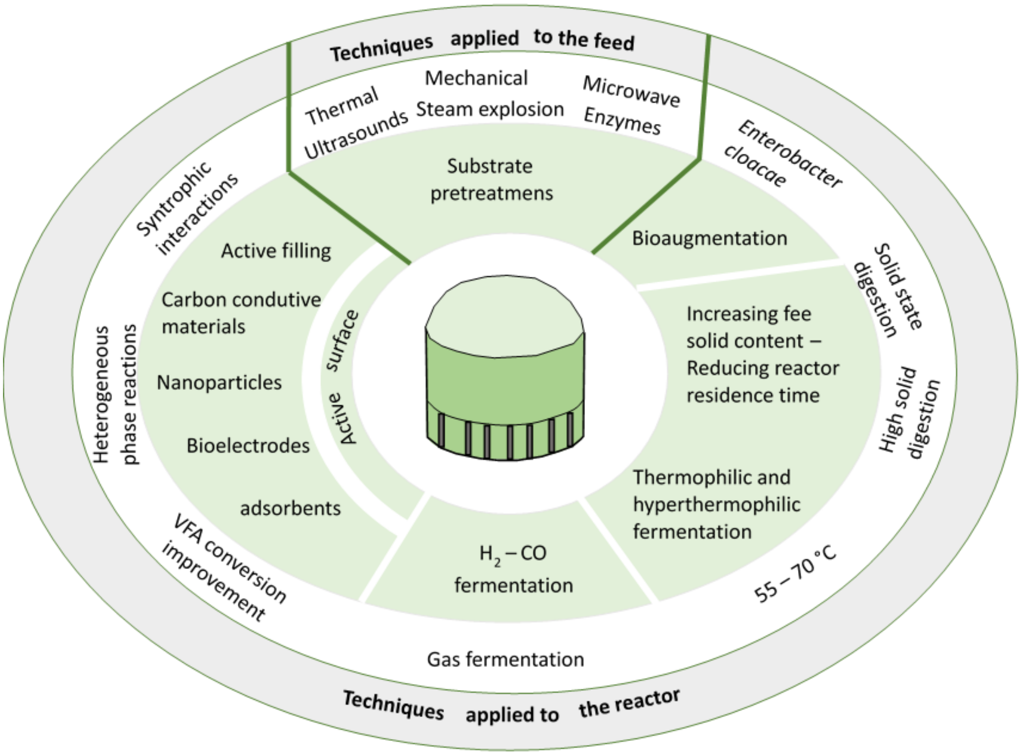

Figure 3. Techniques for improving anaerobic digestion performance.

5.2. Operating at a Higher Solid Content

The increase in solid content as a way of increasing organic loading, and therefore digester productivity, may cause a detrimental effect, which is linked to the delicate balance between VFA, ammonia release, pH and the lower dilution capacity of the system. High-solid anaerobic digestion and solid-state anaerobic digestion are two forms of carrying out the process at high organic loadings. This strategy has as a main advantage the downsizing of the biological system, but adverse effects may result from the lower water content. Solid-state digestion is a term used to describe digestion carried out at a solid content greater than 15% [193]. The term high-solid digestion is used when carrying out the fermentation at a solid content greater than 6%, a threshold for the appearance of diffusion limitations [194,195].

Xu et al. [196] reviewed the performance of digestion reactors treating sewage sludge under high-solid conditions. These authors reported that the main limitation of these systems was associated with process instabilities, high viscosity and high concentration of ammonia and acid intermediaries. The increase in solid content causes the accumulation of VFAs and ammonia, thus leading to a decrease in methane production [197]. Pastor-Poquet et al. [198] studied the digestion of the OFMSW under a high-solid configuration reactor, obtaining 40% less methane yield at a total solid (TS) content of 15.0% and an NH_3 concentration greater than 2.3 g N–NH_3/kg. The performance of the process could be improved by adding an inert material to exert a diluting effect and decrease the high localized nitrogen levels. It becomes evident that any attempt to increase reactor treatment capacity will need to deal with the attenuation of inhibitory conditions.

5.3. Thermophilic Regimen to Increase Reactor Treatment Capacity

The increase in digestion temperature is also an evident way of increasing the degradation rate and therefore reactor productivity. Anaerobic digestion is strongly influenced by temperature [199]. Operating at thermophilic conditions and attaining stable performance may not be exempt from complications. Increasing the temperature of the digestion system from a mesophilic to thermophilic regimen allows for an existent installation to treat a significantly greater amount of organics. The biogas yield obtained under thermophilic conditions has been reported by some authors to be similar to that obtained under mesophilic conditions [200,201], but the main advantage resides in the lower time needed to complete the full degradation.

Thermophilic regimen may also bring, as a consequence, a lower quality of digested material. Thermophilic reactor liquor has been reported to contain higher levels of VFA and ammonia [202–204], which adversely affects its organic quality. Gómez et al. [205] studied the organic characteristics of cattle manure digestates, indicating a better quality than that obtained under mesophilic conditions. On the contrary, Provenzano et al. [206], studying the digestion of sewage sludge and municipal solid wastes, reported a better performance for the thermophilic system. The apparent inconsistency in results may be explained by the different characteristics of substrates and nitrogen content. The higher nitrogen content of manures leads to higher ammonia levels during digestion, having, therefore, a greater adverse effect on the process outcomes when the temperature is increased.

Yenigün and Demirel [207] performed a literature review regarding the effect of ammonia in mesophilic and thermophilic digestion. These authors explained the discrepancies found in numerous studies due to the different levels of free ammonia reached in the digestion assay, the values of which depend greatly on temperature, pH conditions and ammonium concentration. Thus, higher temperatures favor the degradation rate, reaching higher ammonium levels in a lower period, affecting the pH of the system and creating a toxic environment because of the high free ammonia content in the reactor. The increase in temperature may need specific adaptation protocols for the microbial biomass based on the intrinsic characteristics of the substrate and the OLR at which the reactor is expected to operate.

Takashima and Yaguchi [208] studied the digestion of sewage sludge under thermophilic conditions in a high-solid configuration (9–10% total solids). These authors included in the digestion system an ammonia stripping stage to remove the excess ammonia produced. This way, the digestion could proceed at low levels of total ammonium nitrogen of 1720 mg N/L (below the value of 2500 mg N/L reported as inhibitory by the authors) proving that this strategy is an efficient way of attaining higher gas production rates at high loadings.

5.4. Addition of Adsorbents, Conductive Materials and Nanoparticles

The addition of certain compounds to attenuate the negative effects associated with acid intermediaries and microbial products may aid in obtaining a better performance of high-solid and solid-phase digestion systems. Carbon conductive materials, bio-electrodes and adsorbents may provide alternative routes or protective sites for microorganisms, thus allowing anaerobic degradation to proceed under highly inhibitory conditions. Wang et al. [209] reported an outstanding capacity for thermophilic reactors working at high OLR when biochar was added. These authors attributed this excellent performance to the ability of char particles to favor VFA syntrophic oxidation thanks to the electron-accepting capacity of the carbon particles. Petracchini et al. [210] also studied high-solid digestion of food waste and cow manure. To prevent negative effects, natural zeolites were added to the reactor, thus obtaining a biogas yield in the range of 680 and 920 mL/g VS. The use of adsorbents in digestion reactors temporarily reduces the level of ammonia and total VFA allowing for the process to proceed steadily [211,212].

The experiments carried out by Cuetos et al. [12] clearly demonstrated this fact. These authors studied the digestion of poultry blood as a single substrate. In this study, the addition of activated carbon allowed the digestion to be completed, whereas in the control reactor with no adsorbent addition total inhibition took place. Adsorption is not the only mechanism justifying the better performance of the biological process, as the presence of the adsorbent creates protecting sites to the microflora and mass transfer limitations, thus microorganisms attached to these particles experience a lower concentration of inhibitory compounds. Several authors have also proposed the mechanism of direct interspecies electron transfer (DIET) as a phenomenon responsible for explaining the greater capacity of these anaerobic systems to degrade short-chain fatty acids and enhanced biogas production [213–216].

The addition of biochar particles and the presence of bioanodes in anaerobic reactors have similar effects to that reported for activated carbon and adsorbents [24,217,218]. Cui et al. [219] studied the behavior of digestion systems under high-solid conditions and biochar addition. The improvement in digestion obtained was attributable to the presence of biochar causing an enhancement in food waste hydrolysis thanks to the promotion of butyric acid degradation pathways. These authors demonstrated the involvement of the DIET mechanism by proving a relationship between the *Syntrophomonas* and *Methanosarcina* species. The introduction of bio-electrodes also provides a similar effect in digestion systems. Moreno et al. [220] described the improvements in VFA degradation thanks to the role played by soft-carbon-felt electrodes in overloaded batch reactors. However, adding just a conductive material to create a biofilm attached to its surface is an even more efficient way of increasing digestion performance as Baek et al. [83] were able to prove. These authors evaluated anaerobic digestion under fed-batch mode operation, introducing a large carbon brush device, electrodes and a combination of brushes and electrodes. As a result, it was observed that a completely different microbial community structure was formed in the large-size brushes, with *Methanothrix* being predominant in the biofilm. The reactor containing these brushes was highly effective in improving digestion performance, demonstrating a superior efficiency compared with the system using microbial electrodes with an applied voltage.

Nanoparticles have also been studied as a way for improving biogas production. Ali et al. [221] studied the addition of three different types of iron oxide nanoparticles reporting increments in biogas production, doubling the yields obtained from the conventional system. The combination of nanoparticles and pre-treatments or their use with bioelectrochemical systems has also reported promising results [222,223]. The addition of this material to co-digestion reactors enhances biodegradability, allowing digestion to proceed under conditions that otherwise would lead to upsetting results, as was demonstrated by Samer et al. [224]. These authors evaluated dry anaerobic co-digestion of manure and whey supplemented with photoactivated cobalt oxide nanoparticles. The control system showed a methane yield of 28.01 mL CH_4/g vs. whereas the fermenter containing nanoparticles gave a result of 169 mL CH_4/g VS. The relevance of these findings lay in the benefits associated with reactor downsizing due to the higher organic content of the feed and the greater capacity for avoiding acute acidification. Dry anaerobic digestion and high-solid anaerobic digestion are biological processes that must affront localized inhibitory levels of acid intermediaries. The study performed by Ajayi-Banji and Rahman [225] demonstrated that the use of magnetic nanoparticles (nFe_3O_4)—when evaluating batch digestion of pretreated corn stover and dairy manure also using a solid-state system—enhanced reactor stability by facilitating acid conversion, thus reducing the initial lag phase and increasing the degradation rate of the different substrate components. Therefore, higher methane yields were obtained in a much shorter period.

Although the use of nanoparticles may represent a promising technology for improving biological reaction rates, their final disposal and the possible interactions with microbial ecosystems should be kept in mind. These particles may finally be released in aquatic environments and on the soil matrix affecting the biogeochemical cycling of nutrients [226] (Donia & Carbone, 2019). Future applications of this technology should consider any negative impacts on the environment by monitoring long-term effects.

6. Conclusions

Anaerobic digestion is a technology capable of converting organic materials into energy, stabilizing organic matter and recovering nutrients. There is plenty of room to improve process performance and increase the economic feasibility of digestion plants. Co-digestion is an efficient way to increase reactor productivity, but factors related to operating at higher organic loads need to be addressed to improve process economics. Greater plant flexibility is needed, and economy of scale needs to be carefully evaluated to take advantage of process synergies and benefit from an enhanced removal of volatile solids.

The co-digestion process has been widely studied in the past and it is expected that future work will deal with bioaugmentation by inoculating anaerobic reactors with specific microbiota, allowing an increase in degradation rates and enhancing the removal of organic components under environments that may currently be considered as inhibitory. The addition of supplements such as carbon-conductive materials and nanoparticles, the introduction of bioelectrodes or the development of internal biofilms capable of increasing the degradation rate of acid intermediaries is another field of research with great expectation of implementation in the near-term given the significant improvement obtained in methane yields and reactor performance. More research activities are needed regarding the feasibility of extrapolating different methodologies that may prove successful on a small scale but implementation at a larger scale may give rise to serious doubts. Therefore, further research integrating global process efficiency, by considering the improvement in biogas yields, along with energy demand and logistical issues are needed. There is an urgent need to produce huge amounts of energy, which is being exacerbated by the current war crisis.

Production of bioenergy is imperative and attaining this goal is only possible if technologies involved present clear profitability. The productivity of digesters needs significant improvements if this technology is to play a relevant role in the development of circular economy models. Experimental research regarding laboratory conditions under batch tests and small scales is extensive. However, there is a lack of reports regarding the large-scale implementation and description of the necessary modifications of equipment and energy demand associated with the auxiliary equipment involved when co-substrates are added to conventional digestion units.

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