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Review

A Review on Anaerobic Digestion of Lignocellulosic Wastes: Pretreatments and Operational Conditions

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Abstract: Anaerobic digestion (AD) has become extremely popular in the last years to treat and valorize organic wastes both at laboratory and industrial scales, for a wide range of highly produced organic wastes: municipal wastes, wastewater sludge, manure, agrowastes, food industry residuals, etc. Although the principles of AD are well known, it is very important to highlight that knowing the biochemical composition of waste is crucial in order to know its anaerobic biodegradability, which makes an AD process economically feasible. In this paper, we review the main principles of AD, moving to the specific features of lignocellulosic wastes, especially regarding the pretreatments that can enhance the biogas production of such wastes. The main point to consider is that lignocellulosic wastes are present in any organic wastes, and sometimes are the major fraction. Therefore, improving their AD could cause a boost in the development in this technology. The conclusions are that there is no unique strategy to improve the anaerobic biodegradability of lignocellulosic wastes, but pretreatments and codigestion both have an important role on this issue.

Keywords: feedstock and degradation pathway; AD systems; pretreatment technologies; process stability; codigestion

1. Introduction

Organic wastes are produced in large amounts worldwide. Mismanagement of such wastes will lead to various problems for both human beings and the environment. Among the different technologies used to treat this type of waste is the anaerobic digestion process (AD) [1]. This process is a biological treatment that allows the decomposition and stabilization of a wide spectrum of organic wastes, from complex lignocellulosic materials to easily degradable food waste, while simultaneously producing renewable energy, recovering fibers and nutrients for soil amendments, and offsetting greenhouse gas emissions [2,3].

The AD process is a well-established technology that transforms the organic fraction into renewable fuels, such as methane. However, the process is recognized as a complex one, and many research studies have indicated that hydrolysis and methanogenesis can be the rate-limiting steps in AD because of the accumulation of undesirable volatile fatty acids (VFA) [4–6]. Nevertheless, controlling and optimizing the major operational parameters can improve the process efficiency and obtain stable methane generation. Recently, numerous studies have been performed to enhance the biogas production and to mitigate the inhibition effect caused by various compounds and situations. In this regard, pretreatment methods of feedstock, including physical, chemical, biological, thermal, and combined approaches, were investigated to overcome kinetic disadvantages and increase process potential, mainly for lignocellulosic biomass, as this kind of waste represents a promising source of alternative energy since it has high potential for biogas production [7,8].

Monodigestion of substrates has been applied in AD, and this approach proves to work effectively for a narrow range of generated wastes, especially for easily degradable fraction waste. However, introducing a mixture of different substrates together, which is known as codigestion, is able to improve the process performance and make it the more attractive alternative in waste management hierarchy. In this context, codigestion provides a great chance for recycling a wide spectrum of wastes, supporting the synergic effects of microbial activities through improving nutrient balance (mainly carbon/nitrogen (C/N) ratio), and consequently, the enhancing the process stability in general [9]. Various mixtures of agricultural wastes, municipal wastes, industrial wastes, sewage sludge, etc., have been investigated by researchers using the codigestion approach and satisfactory results have been obtained [10]. Importantly, selecting an adequate co-substrate and mixing ratio, organic loading rate, co-substrate characteristics, and co-substrate-prompted inhibitions are essential factors to be considered, as they may greatly influence the process performance [11].

This current review provides a comprehensive overview of the process of AD, which is considered to be one of the most viable options for recycling the organic fraction of solid waste. Feedstock and degradation pathways, AD systems, pretreatment technologies, process stability, and codigestion for lignocellulosic materials are introduced and discussed thoroughly in this review.

2. Overview of AD Process

2.1. Feedstock for AD

A wide variety of feedstock can be processed through AD. However, lignocellulosic biomass, as it has a high potential for biogas production, represents a promising source for alternative energy [8]. This type of feedstock is abundantly available and can be found in different wastes, such as agricultural residues (crop residues), wood, and grass. About 181.5 billion tons of lignocellulosic biomass are produced annually worldwide [12]. Nevertheless, process inhibition is commonly noticed during the digestion of such materials caused by the complexity of their structure, which contains about 10–25% lignin [13]. Accordingly, pretreatment is often needed before use in AD for biogas production, or alternatively it can be codigested with other feedstock [14]. Solid-state AD (ss-AD) digesters are ideally suited for such feedstock [15]. Consequently, more attention is being directed to this process, as it provides promising solutions either for waste management or for introducing a renewable energy source.

2.2. Feedstock Degradation Pathway

AD plays a key role in the recovery of renewable energy in the form of biogas and nutrients from waste materials. The degradation process of feedstock passes through sequential and distinct phases or steps, as shown in Figure 1. During these phases, the rate and characteristics of the generated gas reflect the microbial processes taking place in the reactor. The four main phases successive phases are set out below.

2.2.1. (a) Hydrolysis

Macromolecules, which include proteins, carbohydrates, and lipids, are the main components of organic substrates, and under anaerobic conditions they are initially broken down to monosaccharides, namely amino acids, long chain fatty acids, and glycerol, respectively. The efficiency of this step is based on the presence and active action of hydrolytic and fermentative microbes (e.g., *Clostridium, Proteus Vulgaris, Bacillus, Bacteriodes, Micrococcus, Staphylococcus*) to excrete extracellular enzymes. The presence of a variety of extracellular enzymes, including amylase, cellulase, protease, and lipase, which are exerted by bacteria, are mandatory as catalyzers for the decomposition of each macromolecule. For instance, hydrolytic enzymes (e.g., cellulase, β-glucosidase, xylanase) or complex enzyme systems (e.g., cellulosome) attack polysaccharides, protease degrades proteins, and lipase is suitable for breaking down lipids [16,17]. Hydrolysis is known to be a rate-limiting step, especially for lignocellulosic substrates [18], because of the presence of lignin, which along with cellulose and hemicellulose units forms a rigid three-dimensional complex compound. This physical barrier protects the biomass from the enzymatic attack. Thus, the existence of microbes with augmented enzymatic activity is mandatory for an efficient decomposition, especially in the case of lignocellulosic materials [19].

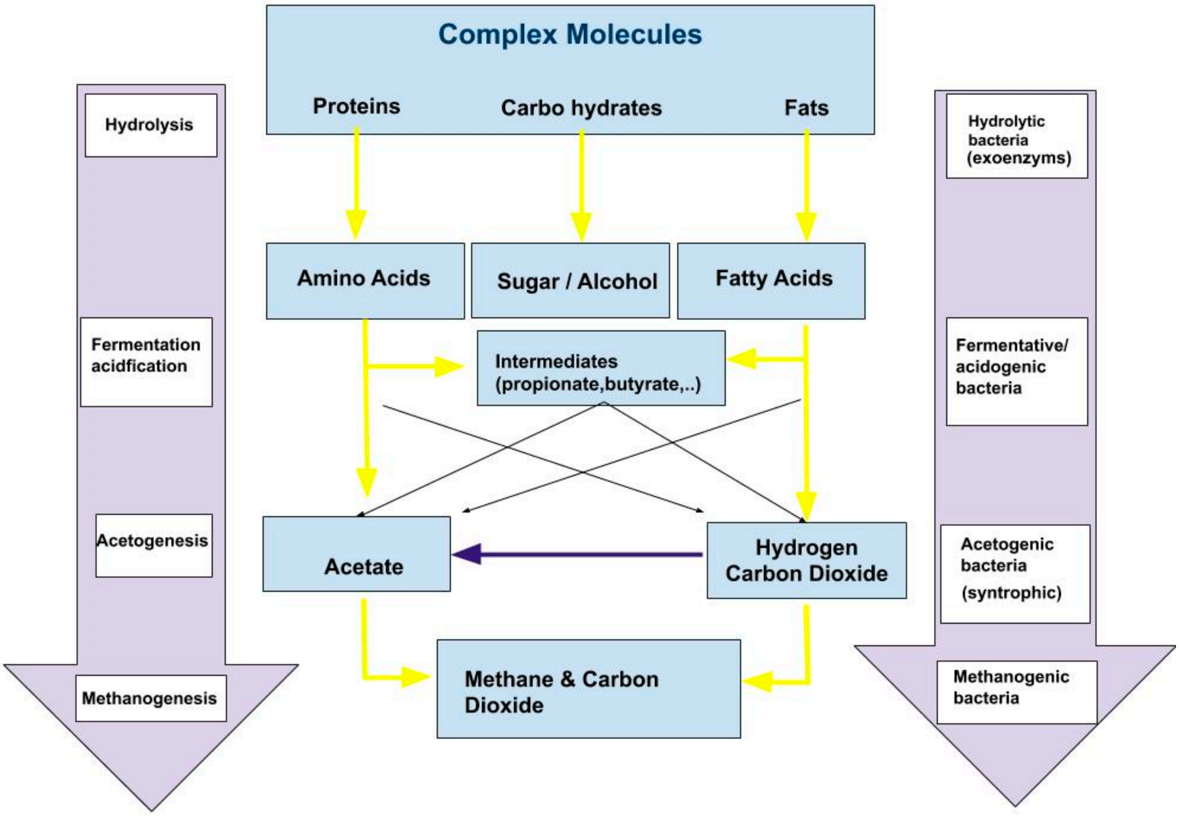

Figure 1. Schematic diagram of substrate decomposition through anaerobic digestion.

2.2.2. (b) Acidogenesis

Acidogenesis is the second step of AD, where the hydrolyzed products of macromolecules are subjected to the fermentation step following different metabolic pathways. Different facultative and obligate anaerobic bacteria (*Lactobacillus, Escherichia, Staphylococcus, Bacillus, Sarcina, Veillonella, Desulfobacter, Desulforomonas, Eubacterium limosum, etc.*) are involved in this step to produce volatile fatty acids (VFA), hydrogen, carbon dioxide, and alcohols. Sugars and amino acids are the main substrates of this step. Results of glycerol fermentation are propionate production and biomass generation [19,20]. Acidogenic microorganisms produce high concentrations of hydrogen during this phase, which may cause inhibition of the production of acetate.

2.2.3. (c) Acetogenesis

During acetogenesis, different syntrophic oxidation reactions convert the longer fatty acids into acetate (by homoacetogenic microorganisms) and hydrogen and carbon dioxide, and products of acidogenesis are utilized by hydrogen producing acetogens, using carbon dioxide and hydrogen ions as electron acceptors (e.g., *Syntrophomonas wolfei*). This bioconversion process is not exergonic, and thus, a syntrophic relationship with methanogens (as they remove hydrogen during the production of methane) is mandatory to keep the hydrogen partial pressure low in order for acetogenic reactions to be energetically favorable [19,21].

2.2.4. (d) Methanogenesis

In this final step, acetotrophic and hydrogenotrophic methanogens (*Methanobacterium, Methanobrevibacerium, Methanoplanus, Methanospirillum, Methanosaeta, Methanosarcina*) work on converting acetate (by acetoclastic methanogens) and hydrogen and carbon dioxide (carbon dioxide-reducing and hydrogen-oxidizing methanogens) into methane and carbon dioxide. The larger portion of methane is derived from the conversion of acetate and the rest is primarily produced from H_2 and CO_2 [20]. Alternatively, hydrogen and carbon dioxide may be produced through syntrophic acetate oxidation coupled with hydrogenotrophic methanogenesis. Extended methane production can be conducted via the hydrogenotrophic pathway based on process characteristics [22,23].

Generally, maintaining a synchronized state between different degradation steps is crucial throughout the process and any fluctuation or decrease in the activity of one or several microbial groups could severely influence the process performance and efficiency, and could even lead to process failure.

2.3. Anaerobic Digestion of Substrates under Thermophilic and Mesophilic Conditions

In the AD process, temperature is recognized as one of the key important factors that affects the entire process performance [20]. As general rule, the performance of the process is increased with the increase in temperature [20,24]. For instance, the rate of anaerobic degradation processes, and particularly enzymatic and coenzymatic activities responsible for the rates of hydrolysis and methanogenesis, are highly affected by the dominant temperature. Additionally, some other factors, such as gas transfer rates and settling characteristics of biosolids, are significantly affected by the temperature [25,26].

For the AD process, two main temperature regimes are usually applied—mesophilic (37 °C) and thermophilic (55 °C). Mesophilic bacteria are supposedly more robust and can tolerate higher changes in environmental parameters, including temperature. Because mesophilic digestion takes place at a lower temperature, digestion in this temperature regime requires longer retention time and yields less biogas; however, mesophilic digesters remain attractive because of their stability and lower heating energy costs compared to thermophilic digesters [14,24]. On the other hand, thermophilic digestion operates at a higher temperature, and consequently reaction rates and kinetics are enhanced, leading to the possibility of higher loading rates, thus increasing biogas production [14,27,28]. Moreover, thermophilic digestion is known to have higher levels of pathogen removal [29]. Nevertheless, this method, is more sensitive to toxic substances and changes of operation parameters [24].

2.4. Solid-State (ss) and Wet-State Anaerobic Digestion

Wet AD and solid-state AD (ss-AD) are considered the two main types of anaerobic technologies that have been employed to digest solid organic matter for biogas production. The classification between the two technologies is based principally on the particular content of total solids (TS) concentration at below or higher than 15%, respectively [30,31].

2.4.1. Wet-State AD

In wet digestion processes, the feedstock is slurried with the addition of water to the mixture in order to be within the desired level (TS less than 15%), and normally this can be achieved by adding water either by recirculation of the liquid effluent fraction or by codigestion with wetter wastes. The latter is more preferable, as it provides a chance to mix various waste streams, such as sewage sludge or manure and OFMSW [32–35]. The less complicated mechanical equipment needed and the opportunity to reduce the inhibitory effect of some substances as they become diluted by water are considered advantages when applying this process. On the other hand, complicated pre-treatment, high consumption of water and energy for heating, and the reduction of working volume caused by the sedimentation of inert materials remain as obstacles in this process [36–38]. Continuous stirred tank reactors (CSTR) are generally used in wet digestion processes, in which mechanical mixers or a combination of mechanical mixing and biogas injection are employed [37].

2.4.2. Solid-State AD (ss-AD)

Solid-state anaerobic digestion (ss-AD) provides the ability to process high-solid-content substrates; typically, with a solid content above 15%. The process is recognized as robust and advantageous compared to wet AD because it requires less energy for heating, less water, smaller digester volume, minimal material handling, and because of its low moisture content, the digestate of ss-AD is more coherent and can be used as fertilizer or pelletized fuel, which is much easier to handle than the effluent produced by wet AD [14,34,35]. These advantages contributed to the large industrial development of ss-AD, with the rapid emergence of full-scale plants [31]. Nonetheless, it should be noted that regardless of the various advantages of ss-AD technology and advancements in system design, there are still a number of aspects that need to be deeply investigated and improved for further commercialization of the technology.

Water content is noted as a key factor in deriving microbial activity, and low content of this parameter in ss-AD would lead to accumulation of the microbial end products, and thus inhibition of methanogenesis [38]. In this regard, and because of slow mass transportation, longer retention time (up to three times) has been documented for ss-AD compared to wet AD [38–40]. According to [41], water availability presents two distinct forms: (i) “free water”, which can act as a solvent for soluble compounds and salts; and (ii) “bound water”, which is unable to act as a solvent, as it has more structural bonding (chemical and physical interactions) than liquid or free water. The variation in the composition and structure of the organic matter has a major role in determining the dominant form of water and consequently the rate of degradation. In this context, it should be considered that when the TS content increases, the quantity of free water decreases; thus, rheological behavior of the substrate is modified and the diffusion of metabolic compounds within the substrate at a macroscopic level is lowered [42,43], meaning the transport of soluble content within the substrate can become a limiting factor [43].

As illustrated in the literature, substrate degradation and consequently biogas production are reduced by increasing the TS content [38,39,42]. One study [44] demonstrated that an increase from 20% to 30% in TS content was associated with a decrease in organic waste degradation by 17%. Also, another study [45] reported reduced methane yields and cellulose hydrolysis rates of lignocellulosic biomass in ss-AD compared to those of wet AD. The limitation of ss-AD might be explained by the slow diffusion of dissolved inhibitory products inside the organic matrix causing local accumulation at the microbial scale [46–48]. In a recent study [38], methanogenesis inhibition was observed at high solid content levels, which was attributed to gas transfer limitation, and more particularly by a local accumulation of gases (H_2 and CO_2) leading to VFA accumulation. In this context, and because of thermodynamic reasons, anaerobic oxidation (acetate formation) can only take place at low partial hydrogen pressure, meaning the balance between the hydrogen-forming and hydrogen-consuming microorganisms is very important. In order to avoid stress in the acidogenic bacteria, the partial pressure of the hydrogen gas should always be less than 1–4 atm [49].

Solid-state AD under mesophilic and thermophilic conditions has been investigated, and mesophilic AD is considered more stable and better established than thermophilic AD [14]. However, for lignocellulosic wastes, thermophilic conditions usually show better initial performance than mesophilic AD, since high temperatures improve the hydrolysis of substrates, being “the major rate-limiting step” [14]. It was found that VFA generation and feedstock degradation were faster in a thermophilic digester than in a mesophilic digester, and that 95% of the methane yield potential was achieved in 11 and 27 days under thermophilic and mesophilic conditions, respectively [50]. The fermentative microbial activities are enhanced under thermophilic ss-AD; therefore, more care must be taken, as this behavior may cause accumulation of VFAs and result in inhibitory effects on the AD process [51].

High energy input for heating under thermophilic ss-AD should be considered, as it may offset the higher methane production yields and rates [7,52]. Accordingly, net energy production and the performance of ss-AD under different temperatures should be evaluated and optimized for feasible process operation. In this regard, even though thermophilic ss-AD of switch grass showed greater methane yield than mesophilic ss-AD, the latter had higher net energy production than thermophilic conditions because of reduced initial heating requirements after inoculation [52].

Throughout the literature, the feasibility of two-stage reactors has been confirmed regarding energy balance. For instance, in a two-stage AD system treating OFMSW, better energy balance was obtained, where higher gross energetic potential was related to the higher performance in the methanogenic reactor rather than the hydrogen production from the first stage [1]. Furthermore, in a similar reactor treating thin stillage, optimizing the process was able to increase the energy balance by 18.5% [53,54], and better energy balance with a surplus of 2.17 kJ/day was also documented in a two-stage reactor treating sewage sludge as compared to a single-stage reactor [27].

3. Pretreatment of Feedstock to Enhance Biogas Production

Some wastes are found in large quantities in the waste stream, but they are difficult to degrade via microorganisms because of the complexity of their structure [13]. These wastes include lignocellulosic substrates, keratins, or high ammonia content materials. However, these wastes, when properly pretreated, can be a valuable source for biogas production. Appropriate pretreatment facilitates and increases the microbial accessibility to nutrients found within the mixture, thus speeding up biomass utilization during the anaerobic digestion process [55]. Different technologies or approaches have been applied in an attempt to overcome the problem and to increase the biogas production. According to [49], the selection criteria for any pretreatment technology should consider the following important points: (1) cost-effectiveness, (2) the microbial accessibility to nutrients, (3) any inhibition in biogas production, (4) energy requirements, and (5) eco-friendliness. In the following subsections, the main pretreatment technologies are discussed, and Table 1 provides a summary of research conducted in this field.

3.1. Physical or Mechanical Pretreatment

For lignocellulosic materials, physical or mechanical pretreatment methods are usually employed to promote microbial accessibility to hydrolysable polymers and to reduce the cellulose crystallinity and its degree of polymerization. This includes a change in the structure or appearance of the materials using different techniques [56,57]. The process includes crushing or breakdown of the feedstock particles, resulting in increased surface area that provides more contact between the substrate and the microorganisms, and presumably increases the process efficiency [57]. Comminution, such as ball milling, is a physical pretreatment method that increases the feedstock surface area and decrystallizes cellulose. Indeed, ball milling was found to be an effective pretreatment, with similar glucose yield and superior carbohydrate yield to steam explosion pretreatment. In this context, the effect of grinding on wheat straw was investigated, and the results showed that ball milling samples yield 46% total carbohydrates and 72% glucose as a result of the reduction in the cellulose crystallinity from 22% to 13% [58]. Ball milling was also applied to non-degraded digestate in order to feed it back into the digestion process [59]. Enhanced methane production of 9% was reported in the case of two-stage maize silage digestate, and an increase of 17% was detected when using two-stage hay/straw digestate. Irradiation is also an effective pretreatment method that disorganizes the structure of the biomass cell wall and decreases the crystallinity of the cellulose [60]. Solubilization of microalgae as a substrate for biogas production was evaluated using microwave pretreatment. The biogas production rate was increased by 27–75% at specific energy rates of 21.8, 43.6, and 65.4 MJ/kg TS.

The advantages of this method are that there is no risk of forming inhibitory compounds, and there is improvement in the methane yield in some cases caused by size reduction. However, the main disadvantage is the high energy requirements [55]. In some cases, this method is not viable as a stand-alone pretreatment for industrial applications, but it can be combined with other pretreatments that are more cost-effective.

3.2. Chemical Pretreatment

This method depends mainly on breakdown or destruction of organic compounds using acids, alkalis, and oxidants, and its efficiency is highly affected by the substrate characteristics [61]. Importantly, this type of pretreatment is less recommended for easily biodegradable substrates, as it can be associated with accumulation of VFAs, which ultimately leads to inhibition of the methanogenesis step [57].

During acid pretreatment, hemicellulose is hydrolyzed into monosaccharides, while the lignin condensates and precipitates [55,61,62]. The process is effective for substrates with high lignocellulosic content as it breaks down the lignin, and also because the hydrolytic microbes are capable of acclimating to acidic conditions [61]. Strong acidic pretreatment may result in the production of inhibitory by-products, such as furfural and hydroxymethylfurfural (HMF) [62–64]. The use of diluted acids combined with thermal methods is normally recommended to avoid the formation of such inhibitory products [57]. In this regard, and with mesophilic anaerobic digestion of wheat plant, diluted sulfuric acid pretreatment at 121 °C confirmed that methane yield could be increased by 15.5% higher than that of the untreated wheat plant after pretreatment for 120 min [65]. Also, in semi-continuous digestion experiments conducted for 12 days at 35 °C using HCl at pH 2 for subsequent digestion of waste-activated sludge (WAS), an increase of 14.3% in methane yield was achieved compared to untreated substrates [66].

The aim of alkali pretreatment is to enhance the enlargement of the solid particles, thus increasing the surface area, meaning the substrate becomes more accessible for microbial activities. Solvation and saponification are the main reactions during this process [55,57,62,63,67]. This process is considered more effective for materials with low lignin contents, such as agricultural residues, herbaceous crops, and hardwoods, than on softwood, which has high lignin contents [68]. Basic compounds, such as calcium oxide, ammonia, and sodium hydroxide, are used to solubilize lignin in this process [62]. The effect of alkaline (NaOH) pretreatment on ensiled sorghum forage was investigated in semi-continuous digesters. The researchers observed that pretreatment with 10 g NaOH/100 g TS increased the methane yield by 25% compared to untreated sorghum, without experiencing any inhibition of the process [69]. Additionally, the effect of trace element (TE) addition and NaOH pretreatment on the AD of rice straw was investigated in batch tests. Co, Ni, and Se were added to the raw rice straw at different dosages. The NaOH pretreatment was applied to the rice straw, both alone and in combination with the addition of TEs, in order to evaluate potential synergistic effects of the pretreatment and the TE supplementation on the biogas production yields. The obtained results showed that the alkaline pretreatment was more effective than the TE addition in increasing the cumulative biogas production, causing a 21.4% enhancement of the final biomethane yield, whereas the increase due to TE dosing was not statistically significant. The analysis of volatile fatty acids (VFAs) confirmed that the NaOH pretreatment resulted in a higher production of VFAs, indicating increased hydrolysis, while TE addition did not cause significant changes in the VFA concentrations [70].

3.3. Biological Pretreatment

Biological pretreatments mainly depend on using microorganisms, such as bacteria and fungi, to degrade the lignin fraction [3,62,71]. Such pretreatment is gaining popularity because of its cost-effectiveness as a natural process that does not require chemicals or energy, in addition to being an environmentally friendly method. However, careful selection of the suitable microbial consortium for efficient pretreatment of biomass is a critical step [72]. Additionally, it is important to highlight that the process is slow and requires longer residence times, which is often not practical for large-scale applications [3,62,71,73].

The effect of bacterial-based biological pretreatment on liquefaction of microalgae *Chlorella vulgaris* with cellulose-secreting bacteria prior to anaerobic digestion was studied [74]. The results showed that bacterial pretreatment enhanced the bioavailability of biomass, and hence methane generation, with methane yield being almost double that of control. Furthermore, the BMP test to evaluate the potential effect of enzymatic pretreatment on pulp mill biosludge with protease from *B. licheniformis* found that an increase of 26% in biogas yield could be achieved under the studied conditions [75].

3.4. Thermal Treatments

This approach enhances the anaerobic process through hydrolyzing complex organic constituents, where the structure of the materials (mainly the cell membrane) is disintegrated under a certain temperature and pressure. Nowadays, different thermal pretreatment techniques using various temperatures (50–250 °C) are being studied and employed to improve the hydrolysis rate of AD feedstock. Among them is liquid hot water treatment, where the water is maintained as a liquid at high temperatures (160 to 230 °C) and under high pressures (>5 MPa) [55,62]. Another option is the use of thermal pretreatment as "an isochoric or constant volume process", where the material is placed in a sealed container and heated without applying extra external pressure [72]. On the other hand, steam treatment involves exposure of the biomass to high temperatures of up to 240 °C and pressure for a few minutes, thereby causing disruption in the structure of the material [55,73]. Another method is autohydrolysis, which is fundamentally based on hydrolyzing hemicelluloses using highly pressurized liquid hot water at 200 °C.

Besides high energy demand and operation at high pressure, the main disadvantage of these processes is the potential formation of inhibitors, such as furfural and soluble phenolic compounds, which inhibit the production of methane [55]. Concerning the lignocellulosic substrates, temperatures exceeding 160 °C cause not only the solubilization of hemicellulose but also the solubilization of lignin. The released compounds are mostly phenolic compounds that are usually inhibitory to anaerobic microbial populations [55]. The formation of inhibitors during liquid hot water pretreatment is relatively low because of the high water input [76]. In order to minimize the formation of these inhibitors, the addition of external compounds is needed to keep the pH in the range of 4 to 7 [55]. Thermal pretreatment at high temperatures (>170 °C) might lead to the creation of chemical bonds and result in the agglomeration of the particles [77]. One of the most known phenomena is the Maillard reaction, which occurs between carbohydrates and amino acids, resulting in the formation of complex substrates that are difficult to biodegrade. This reaction can occur in extreme thermal treatment at temperatures exceeding 150 °C, or with longer treatment times at lower temperatures (<100 °C) [1,53,54,78].

Table 1. AD pretreatment methods.

Pretreatment Process	Substrate	Conditions	Results	Ref.
Physical	Straw	Milling straw particles to different sizes of 0.25 mm, 1 mm, and 10 mm over 62 days.	Highest methane production for straw with 10 mm particle size (192 ± 25 Nm L/g VS), which was associated with straw biodegradability of 43%.	[40]
	Fruit and vegetable waste	Three sonication times of 9, 18, and 27 min, operating at 20 kHz and amplitude of 80 µm on the substrate.	Highest methane yield at 18 min sonication with specific energy if 2380 kJ/kg TS for 12-day batch period, while longer exposure to sonication led to lower methane yield.	[79]
	Olive mill solid residue	Microwave irradiation at a power of 800 W and temperature if 50 °C.	Maximum methane yield of 395 ml CH_4/g VS for an applied specific energy of 7660 kJ/kg TS.	[80]
	OFMSW	Extrusion pretreatment.	Biogas yield of 800 L/kg VS, containing about 60% methane content.	[81]

Table 1. *Cont.*

Pretreatment Process	Substrate	Conditions	Results	Ref.
Chemical	Cotton stalk residues	Alkali hydrogen peroxide (AHP), treatment at 37 °C for 61 days.	Highest methane yield of 192.4 mL/g VS after 3% AHP pretreatment, yield improved by 254.3% over the untreated waste.	[82]
	Agriculture straw	3% H_2O_2 and 8% $Ca(OH)_2$, treatment at 37 ± 1 °C for 35 days.	Highest methane yields of 216.7 and 206.6 mL CH_4/g VS using acid and alkaline, which are 115.4% and 105.3% greater than untreated waste, respectively.	[83]
	Sunflower oil cake	170 °C, 1% (weight) sulfuric acid concentration. Mesophilic method.	Biogas yield of 302 ± 10 mL CH_4/g VS at 170 °C), 50% greater than untreated waste.	[84]
Biological	Food waste and brown water	Composting or microaeration.	Microaeration pretreatment improved methane yield by 10–21%, caused by enhancement of hydrolysis.	[85]
	Chicken feathers	Aerobically pretreated for 2–8 days with *Bacillus* sp. *C4*, a bacterium that produces both α- and β-keratinases.	445 ml CH_4/g VS methane, 124% more than untreated feather.	[86]
	Napier grass	Microbial consortium: WSD-5.	CH_4 yield was increased by 49%.	[87]
	Organic waste	Soft rot fungi *Trichoderma viride*, 4 days treatment at 25 °C.	Up to 400% increase in methane production compared with controls.	[88]
	Paddy straw	White rot fungi *Fusarium* sp., 10 days treatment at 30 °C (70 % moisture).	53.8% increase in biogas production.	[89]
	Organic waste	Soft rot fungi *Trichoderma viride*, 10 days treatment at 22 °C (70% moisture).	More than two-fold increase in methane production.	[90]
Thermal	Wheat straw	Different thermal pretreatment temperatures of 120, 140, 160, and 180 °C.	Highest biogas yield of 615 ml/g VS (53% increase) and volatile solids reduction of 69% at 180 °C, compared to untreated material.	[91]
	Activated sludge	Steam explosion at 170 °C, autoclaved at 70, 100, and 125 °C.	A linear relationship was found between the thermal treatment temperature in the autoclave and biogas production of aerobic granules, but steam explosion was more effective.	[92]
	Hay	Steam explosion at 160–220 °C.	CH_4 yield increased by 16%.	[93]

The aforementioned technologies have been applied as pretreatment methods in anaerobic digestion, and Table 1 provides some examples where these technologies have been successfully employed. However, it is worth mentioning that a combination of different technologies has been employed in many cases.

4. AD Stability or Inhibition

The anaerobic process is a sensitive process, where any variation or fluctuation in the optimum operational conditions could affect or even inhibit the process. In this regard, and in order to ensure stable conditions, it is important to achieve or maintain a balance between the acidogenic and methanogenic microorganisms. Usually, the inhibition of the anaerobic process is evidenced by the decrease in steady-state methane yield rates.

4.1. Effect of Ammonia

Ammonia, which is the end-product of anaerobic digestion of proteins, urea, and nucleic acids [94], plays a major role in the performance and stability of the anaerobic digestion process [6]. Optimal ammonia concentration ensures sufficient buffer capacity for the methanogenic medium, especially for nitrogen-rich organic feedstock. However, excess ammonia concentration is usually reported as the fundamental cause of digester failure when it exceeds the inhibition threshold levels [95–99]. Inhibition is caused by total ammonia nitrogen (TAN) concentration, which is a combination of free (unionized) ammonia nitrogen (FAN) and ionized ammonium nitrogen (NH_4^+) [6,100–102].

TAN concentrations between 1500–7000 mg N/L were found to be inhibitory for anaerobic digestion [98,103]. This broad range of inhibiting concentrations might be explained by different causes, including differences in substrates composition, inoculum origin, environmental and operational conditions (temperature, pH), and acclimation periods [97]. However, it should be noted that any probable inhibition by ammonia in the anaerobic digestion process should not only be directly attributed to the TAN concentration, but to the FAN levels, as this is believed to be the prime cause of inhibition of methanogenic microflora [104,105].

4.1.1. Mechanism of Inhibition by Ammonia

Microorganism growth and maintenance of an appropriate level of alkalinity within the AD reactor are both fundamentally based on ammonia concentration, and as such it is noted as a decisive element in the process stability [6]. The reason behind considering FAN as the main cause of inhibition is related to its high permeability to bacterial cell membranes [106]. The diffusion of FAN into microbial cells upsets the balance of the intracellular pH of methanogens, leading to lower levels or inhibition of enzymatic reactions and abnormal material transportation [107]. Another possibility is caused by the hydrophobic ammonia molecule, which may diffuse passively into bacterial cells, causing proton imbalance or potassium deficiency [108]. In general, and to avoid toxicity from ammonia, it is recommended that the concentration should never reach the range of 1500–3000 mg/L [109].

4.1.2. Mitigating Ammonia Inhibitory Effects

Even though the ammonia inhibition mechanism has been determined by different researchers, unfortunately until now there has been no direct approach to avoid ammonia toxicity, especially when it exceeds the threshold inhibition level [6]. However, some measures could be employed to mitigate ammonia inhibition, which are described below.

(a) Inoculum Selection and Adaptation of Microorganisms

The inoculum source and adaptation of methanogenic consortia to high ammonia levels have been recognized as effective and feasible tactics for enhancing the anaerobic digestion process. In this context, acclimation of acetoclastic methanogens to high concentrations of TAN reduce their susceptibility to any increase in TAN and improve their tolerance to a wider range of pH levels [96]. Methane was successfully produced in dry fermentation of chicken manure at about 8000 to 14,000 mg TAN/kg after adaptation time of 254 days at 37 °C and pH of 7.3–8.8. It was concluded that spontaneous acclimation of the methanogenic biomass to high concentrations of ammonia could occur and result in production of methane, even under a high TS content (25%) and high concentration of ammonia [6,35,96,97,110].

The research conducted in [111] investigated the effect of six different inoculum sources on the digestion of rice straw using BMP tests under the same conditions, and significant differences in the biogas yields were observed, caused by the different inocula sources. Methane yields were 180 mL/g VS, 160 mL/g VS, 75 mL/g VS, 125 mL/g VS, 15 mL/g VS, and 5 mL/g VS for dairy manure, swine manure, chicken manure, granular sludge, municipal sludge, and paper mill sludge inocula, respectively. Reactors inoculated with digested manure had higher methane yields compared to others, and this was attributed to the availability of macro- and micronutrients in manure, which enhanced the digestion of rice straw. One study [112] demonstrated that using diary manure digester effluent as the inoculum improved methane yields from corn stover up to 30% and 100% compared to inoculum obtained from food waste and sewage sludge digesters, respectively. This variation was attributed to the higher populations of hydrolytic microbes in dairy manure effluent. Also, best inoculum selection was able to reduce the lag phase from 20–30 days to 2–5 days [113].

(b) Substrate/Inoculum Ratio (S/I)

Providing an optimal substrate to inoculum (S/I) ratio is considered to be important in AD process. However, this ratio may vary with types of substrates under consideration, because while the digestion of food waste may be susceptible to VFA accumulation because of its composition and high biodegradability, other substrates may have buffering capacities that reduce the potential VFA accumulation [114,115]. In ss-AD of lignocellulosic biomass, a wide range of S/I ratios have been investigated under both mesophilic and thermophilic conditions. Ratios between 2 to 3 (VS basis) were found to be relatively robust for ss-AD mesophilic conditions, whereas a relatively high S/I ratio was preferred under thermophilic conditions. For instance, the highest biogas yield for corn stover in mesophilic ss-AD was obtained with an S/I ratio of 2.43 (VS basis), while the highest yield under thermophilic conditions was achieved at an S/I ratio of 4.58 [7,116–118]. According to [115], and in accordance with the proposed guidelines by Holliger et al. [119], a minimum S/I of 0.5 is recommended for highly degradable substrates, such as food waste, while an S/I of 1 may be used for less degradable substrates, such as lignocellulosic waste. As will be illustrated in the following sections, other parameters, such as pH and C/N ratio, also directly affect ammonia toxicity [6,120,121]. Importantly, other different approaches have been studied to control ammonia inhibition during the AD process, for example anammox, which is fundamentally based on a reaction driven by a specialized group of planctomycete-like bacteria that convert (oxidize) ammonium to nitrogen gas, where nitrite is used as the electron acceptor under anoxic conditions [122]. Other approaches are struvite precipitation [123] and the use of zeolite as support media for the immobilization of microorganisms in different high-rate reactor configurations. Furthermore, zeolite has also been used as an ion exchanger for the removal of ammonium in anaerobic digestion due to the presence of Na^+, Ca^{2+}, and Mg^{2+} cations in its crystalline structure. Also, carbon fiber textiles (CFT) were used as supporting materials for better retention of microorganisms. Nevertheless, these approaches were reported to be expensive at large scale [124,125].

4.2. Effect of Organic Loading Rate (OLR)

Providing an adequate OLR helps to ensure a stable digestion process and an optimum biogas yield during the process [126]. Feedstock composition will determine the level of biochemical activities that will take place in the digester. Consequently, overload or undersupply will affect the process performance [6].

Anaerobic stability depends on the harmonic relations between acid formers and methane formers [127], and these two types of microorganisms differ widely in terms of physiology and nutritional needs [128]. In the case of easily degradable substrates with low buffering capacity, high loading rate will lead to formation of VFAs, pH will be dropped, and methanogenic bacteria will be inhibited [19,22,23,49,129]. For commercial biogas production, protein-rich feedstocks are preferred over other materials as they have high bio-methane potential (BMP) [130,131]. Unfortunately, high loads of such materials are often associated with process instability because of the release of ammonia nitrogen (NH_3-N) [6,97,132], causing inhibition of the process.

4.3. Carbon to Nitrogen (C/N) Ratio

In order to optimize biogas production, introducing substrates with an optimal C/N ratio is essential for the entire process and basically for providing sufficient and adequate nutrients levels for microorganisms [133]. Higher concentrations of ammonia are formed with low C/N values, and eventually this will result in hindrance of microbial growth. On the other hand, during the fermentative stage, a C/N ratio above the optimal value will lead to production of large amounts of VFAs. Furthermore, the C/N ratio can reduce the TAN level only if the TAN concentration is slightly more than the threshold inhibitory level; action should be taken promptly, before the inhibition of the process [6].

Table 2 provides the C/N ratios of different substrates. The optimal C/N ratios of various substrates attained from different AD processes will likely be different, and the AD process is more stable when the C/N ratio ranges from 20 to 30 [14,134]. During the anaerobic codigestion process, substrates are added to maintain the C/N proportion within digesters [135]. To unify a specific range, including the existing carbon of the easily degradable part and excluding the carbon that is not specifically affected by microorganisms, an available C/N ratio is proposed [126]. By maintaining the C/N ratio at 17:1 [136], enhanced methane generation by around 3.8- and 1.5-fold was achieved compared with perennial ryegrass alone and waste activated sludge (WAS) alone, respectively.

Table 2. C/N ratio of some substrates [137].

Substrate	C/N Ratio	Substrate	C/N
Kitchen waste	26:30	Grass cutting waste	11:15
Food waste	2:18	Rice straw	50:68
Cattle manure	15:26	Wheat straw	51:151
Poultry manure	4:16	Corn straw	51:57
Sheep manure	20:34	Sawdust	199:501
Vegetable waste	8:36	Algae	74:101
Slaughterhouse waste	21:36	Sugar cane waste	139:151

Additionally, it is worth mentioning that organic wastes used for biogas production are generally rich in lignocellulose-type resistant materials [14]. Thus, special pretreatments (Section 3) are required to utilize such wastes under short retention times with anaerobic organisms [138]. Therefore, significant amounts of “invalid carbon” in these materials affect the calculation of C/N. Thus, C/N calculation shows only the general characteristics of organic waste materials, not the actual substances utilized by anaerobic microbes.

4.4. pH Value

Growth and enzymatic activities of the microorganisms and solubilization of organic matter are affected by pH value [127,139–143]. Providing an optimal pH value during the different phases was the key reason some reactors were divided into two phases, with an acidogenic and methanogenic phase [144,145]. Moreover, pH affects the distribution of total ammonia nitrogen (TAN) between toxic NH_3 and innocuous NH_4^+ [146]. Optimum performance of the anaerobic microorganisms will be reached with neutral pH (6.8–7.2) [147]. Importantly, pH affects the distribution of total ammonia. It was noticed that at a higher pH of 8.5, unionized ammonia was highly (2473 mg/L) associated with poor biogas production, which confirms the ammonia toxicity, in particular for leather fleshing waste [148]. By contrast, it was reported that at pH of 4.5 the ammonia nitrogen was minimal (510 mg/L), but high VFA (26,803 mg/L) inhibited the methanogens [6].

4.5. Concentration of Ions

Some metal ions, including sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), ferric (Fe^{3+}), and magnesium (Mg^{2+}), are formed because of the breakdown of organic matter or introduced with the pH adjustment (alkali and acid reagents used in pretreatment). Excessive concentrations of such ions would lead to decreased microbial growth or would even cause toxicity, as they cause dehydration of bacterial cells due to osmotic pressure [149,150].

Mg^{2+} was found to be inhibitory to methanogenesis when it reaches 720 mg/L. Also, high concentrations (> 100 mM) of the magnesium ion can cause disaggregation of methanogens; thus, the conversion of acetate is inhibited [151]. Nevertheless, Ca^{2+} was moderately inhibitory to microorganisms in concentrations above 300 mg/L. However, excessive amounts of Ca^{2+} can cause precipitation of carbonates and phosphates, which results in scaling of the reactors, pipes, and biomass; thus, it reduces the specific methanogenic activity and results in a loss of buffer capacity [152–154]. K^+ concentrations above 3 mg/L are toxic to microorganisms [97], and Fe^{3+} was reported to inhibit 52–82% of methanogenesis activities with concentrations of 21 mg/L or above, because Fe^{3+} could deactivate enzymes of microorganisms by reacting with their functional groups [155].

4.6. Sulfide

In the anaerobic process, sulfate is reduced to sulfide by sulfate-reducing bacteria [156]. The produced sulfide could inhibit the process, either by toxicity from the competition for a common organic matter substrate that cause stress for methanogenesis, or through the toxicity of sulfide to various bacteria population [127,157,158].

5. Codigestion of AD

As discussed in the aforementioned sections, providing optimal operating conditions is a crucial point for an effective AD. It is well-known that the deficiency of some nutrients could lead to low performance or even the inhibition of the process [53]. In this regard, codigestion arises as an efficient and cost-effective way to overcome some of the difficulties during this process [14,134,135]. The codigestion process simply involves mixing two or more substrates together, which would improve the utilization of nutrients through balancing them (mainly the C/N ratio), enhancing the microbial diversity, improve buffer capacity, and dilute inhibitors; consequently, codigestion may achieve positive synergistic effects and lead to higher methane yield than monodigestion of substrates [53,159,160]. In this context, lignocellulosic biomass is categorized as a great prospect for anaerobic digestion due to its high content of cellulose and hemicellulose. However, its elevated C/N ratio (Table 1) and the complexity of its biomass structure (contains 10–25% lignin) inhibits the anaerobic digestion process when it is used individually [13,161,162]. On the other hand, animal waste, such as manure, contains high amounts of organic nitrogen [163,164], which would lead to accumulation of ammonia, and potentially inhibition of the microbial activities because of a low C/N ratio [134,148,163,165]. Therefore, monodigestion of lignocellulosic biomass or animal wastes would result in less stable performance and low methane production as a result of the inappropriate C/N ratio. Creating an optimum C/N ratio through codigestion of lignocellulosic biomass with appropriate animal waste can maximize methane production and make the AD process more stable [162,164].

Cellulose, hemicellulose, and C/N ratio potential values for rice straw, wheat straw, corn stover, and switch grass are 32%, 24%, and 47; 38%, 21%, and 60; 37%, 22%, and 63; and 38%, 26%, and 93, respectively. These values indicate the high potential of these substrates to produce biogas, however, they are still considered difficult to digest, as they contain a significant fraction of lignin, whereas the codigestion of these substrates with a highly nitrogen-rich substrate, such as manure, would result in a successful way to utilize these materials in an anaerobic digestion process, since the materials complement each other when used together and the potential for inhibition is reduced [166]. Additionally, for the codigestion of hay with soybean processing waste at 25:75 ratio under solid-state

AD process, the methane yield was 258 L/kg VS, which is 50% and 148% higher than the amount obtained by each substrate individually [117]. Co-digestion of barley straw with pig manure produced 233.4 L/kgVS methane, but it produced only 192 L/kg VS methane when it was codigested with cow manure [167]. Corn stover and dog food at an equal ratio (1:1) produced a methane yield of 304.4 L/kg VS, which led to an increase of 129% compared to corn stover alone and 9% compared to dog food digestion alone [116]. Rice straw with swine manure, wheat straw with chicken manure, corn stover with chicken manure, and crop silage with cow manure produced 350, 235, 223–298, and 249 L/kg VS methane, respectively, in an AD codigestion process with optimum C/N ratio. In this context, codigestion resulted in a 31% increase of the expected yield, which was calculated from the methane potential of the individual fractions [168]. Food waste codigestion with sewage sludge can produce a high yield of biogas of 708 L/kg VS [169]. In Table 3, other examples of codigestion experiments are resumed.

Table 3. Codigestion of lignocellulosic biomass with other feedstock for methane production under ss-AD.

Lignocellulosic Biomass	Co-Substrate	Mixing Ratio	Temp (°C)	CH_4 (L/kg VS)	Ref
Yard waste	Food waste	4:1	36	120	[46]
Swine manure	Corn stover	NA	35	350	[170]
Hay	Soybean processing waste	1:1.3	37	258	[117]
Agricultural wastes	Chicken manure	7:3	55	502	[163]
Cattle manure	Palm pressed	1:3	37	346.2	[171]
Cattle manure	Silage of cardoon	85:15	37	308	[172]
Cow manure	Rice straw	1:1	37	383.5	[173]
Cattle manure	Corn stover	NA	37	228	[167]
Cow manure	Kitchen waste	1:1	35	179.8	[174]
Cattle manure	Wheat straw	95:5	55	351	[175]

6. Conclusions

In summary, the results compiled in this review clearly demonstrated that lignocellulosic wastes can be important sources of renewable methane through anaerobic digestion. Among all the proposals used to overcome the problems of these wastes in this process, which are mainly related to the hydrolysis step, pretreatments and codigestion seems to be the more suitable strategies. Regarding the operational conditions, inhibition must be considered, but solid-state anaerobic digesters have proved to be a great alternative to classical AD stirred reactors. It is evident that the state-of-the-art of this approach is still not completely developed, but it seems to be the correct approach for lignocellulosic wastes. Finally, it is clear that these wastes are the most abundant sources of organic matter on earth, and therefore, all approaches to convert them into renewable energy are a step toward sustainability and climate change mitigation.

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Abbreviation

	Full Name
AD	Anaerobic Digestion
BMP	Biochemical Methane Potential
FAN	Free Ammonia Nitrogen
OFMSW	Organic Fraction of Municipal Solid Waste
ss-AD	Solid State Anaerobic Digestion
TAN	Total Ammonia Nitrogen
TS	Total Solids
VFA	Volatile Fatty Acids
VS	Volatile Solids

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