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Review

A Comprehensive Review on Pretreatment Methods for Enhanced Biogas Production from Sewage Sludge

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Abstract: The treatment of municipal wastewater is considered a cornerstone for the protection of public health and environment. However, a major issue derived from this process is the large quantities of produced sewage sludge. Although anaerobic digestion is a widely applied method in Wastewater Treatment Plants (WWTPs) aiming to stabilize the sludge and to recover energy in the form of methane, it is usually limited due to the reduced decomposition efficiency and slow biodegradation rate of this recalcitrant substrate. For this reason, various pretreatment methods have been proposed aiming to modify the sludge structure, solubilize the organic matter, and decrease the crystallinity of sludge so as to accelerate hydrolysis and consequently enhance methane production. The current research is a comprehensive collection of recent advances in pretreatment technologies that can be potentially applied in wastewater treatment facilities. The critical review analysis presented herein reveals the several advantages and drawbacks, as well as the technical opportunities of the pretreatment methods and provides an assessment of their feasibility/applicability from an energetic, environmental, and economic point of view.

Keywords: pretreatment methods; sewage sludge; anaerobic digestion; methane production; bioenergy; sustainability; renewable energy sources

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1. Introduction

Currently, the need for new energy management strategies and technologies development is of vital importance, since significant inequalities in energy consumption and access to energy persist. As a result of rapid urbanization, rising energy demand has raised concerns associated with global climate change, energy safety, and the long-term consumption of natural resources. Consequently, the fact that the world is on an unsustainable path stimulated the obligation to establish new practices associated with the use of renewable energy sources and the exploitation of wastes for bioenergy production. Thus, the forthcoming transition in the sustainable energy generation through "Waste-to-Energy" facilities contributes to the meeting of global energy needs while eliminating the dependence on fossil fuels and avoiding exposing the world to a geopolitical risk.

The incessant escalation of populace is directly related to the gradual increase of Wastewater Treatment Plants (WWTPs) operating worldwide and thus, to the vast amounts of produced sewage sludge. The toxic substances presented in sewage sludge pose significant environmental threat, as well as odor and hygiene concerns; therefore, their proper disposal (after treatment) is crucial. Nonetheless, sewage sludge's treatment and subsequent disposal are quite expensive, representing almost the half of the total treatment cost [1,2]. The latter has attracted the attention in seeking sustainable and cost-effective techniques for sewage sludge management and treatment prior to their disposal in the final recipients. A promising technique fit for purpose is anaerobic digestion (AD). AD not only stabilizes sludge and removes odors and pathogens but also reduces the overall solids mass and offers solution to the increasing concerns of today's energy crunch through the production of renewable energy in the form of biogas [3]. To this end, sludge's treatment cost can be partially covered, while the reliance of humanity on fossil fuels is expected to be reduced. Subsequently, AD's contribution to environmental protection and renewable energy production makes this process an integral part of WWTPs, especially of medium-to-large size units.

It should be highlighted that the production of biogas from the anaerobic bioconversion of sewage sludge appears to be lucrative. This is attributed to the methane content in biogas, which ranges between 50–70% [4] and can be directly utilized as source of heat or electricity [5], while the produced digestate can be exploited as a substitute for mineral fertilizers [6]. Among the various 2nd generation biofuel feedstocks, AD of sewage sludge has been estimated that it can mitigate 75 to 100 Mt CO_2 eq. of GHG per year, mainly as a result of the fossil fuel-based energy replacement [7]. According to the World Biogas Association, it is calculated that capturing and treating all the available organic wastes through AD can potentially reduce the annual global GHG emissions by 10% by 2030 [8].

In view of these beneficial aspects, further research in AD process improvement has been promoted in order to enhance its efficiency, improve biogas production, and subsequently, increase methane production potential. However, sludge's complex floc structure and composition of extracellular polymeric substances (denoted hereafter as EPS) and hard cell walls obstruct the efficient performance of AD and lead to inadequate methane output. To enhance methane production, a variety of successful technologies for sludge pretreatment has been developed [1,9]. With the aid of pretreatment technologies, EPS and recalcitrant cell walls are ruptured and thus, the release of intracellular substances is promoted, increasing their bioavailability. In addition, the rate and degree of organic degradation is enhanced [10]. Hence, as a consequence of these favorable effects, various pretreatment methods have been thoroughly scrutinized, resulting in a lot of advancement in both journal articles and research [11–14]. The pretreatment methods under consideration include physical/mechanical, thermal, chemical, and biological processes, which are applied either as standalone procedures or in proper combinations [9,15,16].

Some of the most commonly applied mechanical processes for sludge pretreatment include, among others, the ultrasonication process, the high-pressure homogenization (HPH) process, the microwave irradiation process, and the electro-kinetic disintegration process [9]. Ultrasonication's mechanism of action involves the cavitation phenomenon. This phenomenon leads to the formation of microbubbles whose implosion brings about the generation of elevated shear forces capable of disrupting sludge structure, increasing the biodegradable matter available for microbial consortium during AD [17]. A pretreatment method where cavitation phenomenon takes place as a typical effect of the high pressure applied is also HPH [15]. Furthermore, another popular mechanical pretreatment option is microwave irradiation, which is reported as advantageous over conventional heating strategies due to its internal heating mechanism that is associated with no heat losses [1]. Moreover, sludge's pretreatment with electro-kinetic disintegration is achieved by employing high-voltage pulsing electric fields. Despite this method's state of the art, it is already being utilized on a large scale [1].

Similar to mechanical pretreatments, thermal and chemical ones are also of high popularity. Thermal sludge disintegration is conducted at temperatures either below 100 °C or between 100 °C and 210 °C, with the temperature range of 160 to 180 °C to be reported as the most effective to apply when high-temperature sludge pretreatment is performed [18]. On the other hand, chemical methods aim to disrupt sludge's structure using alkaline and acidic reagents. Specifically, their mechanisms of action are mainly associated with the pH values. Throughout the literature, sodium hydroxide (NaOH) is the most frequently used base solution during AP while saponification is mentioned as a common effect provoked as a response to high alkalinity values [16]. In case of acid pretreatment, two of the reagents employed to achieve pH levels in the range of 1 to 5.5 are hydrochloric acid (HCl) and sulfuric acid (H_2SO_4) [13]. Ozonation and Fenton's oxidation also lay among chemical methods utilized for sludge pretreatment. These methods are commonly used for sludge disintegration via advanced oxidation through reactions with hydroxyl radicals [19].

Aerobic pretreatment, temperature-phased anaerobic digestion (TPAD), and enzyme-assisted pretreatment are biological processes [20] that appear to be promising alternatives to physical/mechanical, thermal, and chemical ones. Aerobic pretreatment is based on the inherent enzymatic activity of sludge, which is stimulated in the presence of oxygen [21], while TPAD is a pretreatment strategy that is performed via a configuration consisting of two digesters in series; usually, the first digester operates at thermophilic conditions and the second at mesophilic ones [20]. On the other hand, enzyme-assisted pretreatment results in an enhancement in sludge bioconversion with the aid of exogenous enzymes which assist in degrading refractory compounds that resist sludge's inherent enzymatic attack [20]. Combining the aforementioned methods is also a sludge pretreatment option. The most commonly employed integrated systems include mechanical-thermal, mechanical-chemical, and thermo-chemical combinations [9]. Essentially, when applying different methods either simultaneously or sequentially, improved efficiencies in, indicatively, sludge hydrolysis and organic matter solubilization are observed, which seems reasonable as evidence of the synergistic effects provoked [22].

The major goal of this review is to provide better insights into currently applied pretreatment technologies for AD enhancement. In the first place, the basic principles of AD as well as an outline of the structure and composition of the sewage sludge fractions are presented. Secondly, an overview of the relative worth of each pretreatment in terms of their mode of action, potentials, and effectiveness is revealed. Among the methods described, the emerging technology of Supercritical Carbon dioxide Explosion (SCE) as a promising approach for sludge pretreatment that fits within the concept of sustainable biomass processing is reviewed for the first time. Finally, this review aims to provide a brief account of useful guidelines regarding the viability of pretreatment technologies. This discussion arises from the fact that a pretreatment method's feasibility considers many factors and does not only depend on the degree of sludge's biodegradability improvement and subsequent enhancement in methane production. In contrast, since pretreatment requires additional energy input and affects both environment and humans, it is imperative to assess its feasibility from an energetic, environmental, and economic point of view. On these accounts, along with the economic feasibility assessment of each pretreatment method, a first-time attempt in evaluating their viability from an environmental standpoint is provided. Overall, based on the critical outlining of the pretreatment technologies' different aspects, this work aims to assist in coming to a conclusion on a proper method for sludge pretreatment and thus to determine the most feasible route of sludge bioconversion to energy.

2. Anaerobic Digestion Fundamental Steps of Action and Microbial Dynamics

AD is a biological process through which complex organic matter, as that existing e.g., in sewage sludge or other biomasses, is degraded with the aid of sequence of actions of mutually dependent microorganisms and converted into CH_4 and carbon dioxide (CO_2) in the absence of oxygen. AD consists of four major sequential steps namely: (i) hydrolysis, (ii) acidogenesis, (iii) acetogenesis, and (iv) methanogenesis (Figure 1). The stable and efficient performance of these steps is determined by the optimal survival and functionality of different microbes, as well as various parameters, such as pH and carbon, carbon to nitrogen (C/N) ratio, temperature, Hydraulic Retention Time (HRT), Solids Retention Time (SRT), and Organic Loading Rate (OLR) [8,23–25]. Therefore, for an efficient anaerobic bioconversion, the control and monitoring of the operating conditions are of prime importance [26].

Initially, under the assistance of existing extracellular hydrolytic enzymes, high molecular weight organic matter, such as proteins, polysaccharides, and lipids, is converted into oligomers and monomers (e.g., glycose, fatty acids, and amino acids) [23]. Hydrolysis is highly dependent on the substrate's nature. Therefore, when substrate consists of compounds with poor biodegradability, hydrolytic activity has a noteworthy negative impact on AD and hydrolysis can turn into rate-limiting step [3,12]. This way, the availability of hydrolyzed molecules for the ongoing biochemical functions is reduced, resulting in longer retention times, poor level of AD efficiency, and subsequently, insufficient biogas production. Hence, pretreatment seems a useful strategy to overcome these suffering points, which is continuously being explored to serve the degradation of biomass prior to AD. The dominant phyla of the microbial consortium involved in the first phase of AD include Bacteroidetes, Firmicutes and Proteobacteria. Nonetheless, the enrichment of diverse microorganisms can be encouraged in case of changes in anaerobic digester's operational conditions and in the content of the substrate [27]. For instance, in the presence of cellulosic material in the substrate's structure, fungi such as *Neocalimastix*, *Piromyces*, and *Orpinomyces* are involved and play a crucial role during hydrolysis [28]. Additionally, other key genera often detected in hydrolytic phase during sludge digestion, include *Cellulomonas*, *Fibrobacter*, *Succinivibrio*, *Prevotella*, *Ruminococcus*, etc. [1].

Following hydrolysis, the fermentative bacteria involved in acidogenesis step, metabolize the hydrolyzed products to form Volatile Fatty Acids (VFAs) (e.g., acetic acid, propionic acid, butyric acid etc.), alcohols, as well as ammonia and hydrogen. Some of the microorganisms responsible for acidogenesis in a full-scale anaerobic reactor digesting activated sludge have been reported to involve bacteria of Clostridia class and Bacteroidaceae family [29], which is well-known to utilize glucose, propionate, butyrate, and acetate content [30]. However, fermentative bacteria's capability to operate in a wide range of pH values (i.e., between 4.0 and 8.5), allows them to produce different compounds depending on the conditions [25]. Specifically, low pH assists the production of butyric and acetic acids, whereas at higher values, acetic and propionic acids are the major fermentation products [23]. Consequently, based on pH conditions, the predominance of different microorganisms is favored. For instance, *Propionibacteria* spp., *Veillonella gazogenes*, and *C. propionicum* have been found as the most typical residents during propionic acid-type fermentation [31]. However, changes in the microbial community are also observed in case of substrate diversity which can be a consequence of collection areas, people's habits, storage conditions, and treatment [12]. As a result, when reactors are fed with carbohydrates, *Enterobacter* and *Clostridium* dominate fermentation, whereas those using protein-rich substrate as feedstock are commonly dominated by *Aminobacterium* and *Peptostreptococcus*, which can help amino acid decompose into smaller molecules such as short-chain fatty acids [31].

Going a step further, the products generated during acidogenesis are oxidized to acetate and H_2. This oxidation reaction is catalyzed by obligatory hydrogen-producing bacteria, namely acetogens or homoacetogens [29]. Most of the reactions occurring during acetogenesis are thermodynamically favorable only in case of H_2 low partial pressure; otherwise, the overall AD is affected [32]. In this case, syntrophic metabolism plays a key role to avoid accumulation of hydrogen in the digester and thus, digestion failure [33]. The major syntrophic partners found during acetogenesis include the genera of *Syntrophobacter* and *Syntrophomonas* [30,32]; other typical bacteria may include *Pelotomaculum*, *Syntrophothermus*, *Moorella*, and *Desulfovibrio* [1].

During methanogenesis, the final process of AD, the methanogenic archaea of the phylum Euryarchaeota utilize H_2, formate, and acetate as substrate to produce methane. More specifically, the end-product is produced via three main methanogenic pathways with the aid of acetoclastic, hydrogenotrophic, and methylotrophic methanogens. The acetoclastic pathway is majorly constituted of the genera *Methanosaeta* and *Methanosarcina*, which are responsible for acetate's direct cleaving into CH_4 and CO_2. Correspondingly, the hydrogenotrophic methanogens detected during the utilization of H_2 as electron donor for the reduction of CO_2 to CH_4 are mainly members of Methanobacteriales and Methanomicrobiales. In the presence of hydrogenotrophic methanogens, there are also Syntrophic Acetate-Oxidizing Bacteria (SAOB). These bacteria facilitate the formation of H_2 and CO_2 that can be used as substrates through the hydrogenotrophic pathway for CH_4 production [34]. Generally, hydrogenotrophic methanogens have been found to be lower than those involved in the acetoclastic pathway [23]. This may be attributed to the fact that almost 70% of produced methane is a corollary of acetate abundance which is the most important precursor for total methane production [1]. However, an eventual inhibition in acetoclastic pathway due to the co-produced high amounts of ammonia may cause differences in digester microbial populations. This scenario facilitates SAOB and hydrogenotrophic methanogens' predominance over that of acetoclastic methanogens, due to the formers' resistance to large ammonia amounts [35]. In addition, archaea community composition and diversity may also alter in view of changes in temperature and pH [28,36]. Considering that methanogenesis is favored in pH ranges of 6.5 to 8.5, methanogen diversity decreases in case of pH drop due to VFA accumulation, which can be caused due to an increase in hydrolysis rate, mainly when easily biodegradable substrate is used as feedstock. This, in turn, can make methanogenesis the rate-determining step [12]. However, as regards the temperature, it has been reported that the abundance of *Methanosarcina* was lowered from 76.7 to 23.8% and alpha diversity dropped with temperature elevation from 35 to 55 °C. Moreover, Kirkegaard et al. [37] indicated that *Methanosaeta* sp. genus was documented as abundant in mesophilic full-scale reactors. Finally, methylotrophic methanogens are those utilizing methyl compounds (e.g., methanol and methylamines) to synthesize CH_4 and are reported to show an abundance of <1% during anaerobic digestion of sewage sludge in full-scale reactors [38].

The brief outline of AD's main functions presented herein is an attempt to highlight its complexity, bringing up some of the major factors that are commonly involved during process operation and can affect its efficiency, microbial community structure, and activities. Nonetheless, AD is a multifunctional process of such growing importance that requires continuous research to better understand the microbial population within the reactors and microorganisms' responses to changes in digestion conditions, in order to ensure their mutualism, resilience, and concomitant efficient digestion performance [26]. An approach for enhancing AD efficiency deals with substrate's pretreatment. Within this context, understanding the changes in dynamics of microbes that could be promoted by the pretreatment conditions (i.e., pH, temperature, presence of chemicals or catalysts, etc.) and the modifications that pretreatment can cause on the substrate structure and its properties is considered of paramount importance. The fact is that this will allow identification of the mechanisms taking place during pretreatment and revealing key information for its effectiveness and/or improvement necessity [15]. Further knowledge regarding the various parameters affecting the process, the limiting factors, and the microbial population dynamics in AD can be investigated in detail in the recent studies [8,10,23,24,26,34,39].

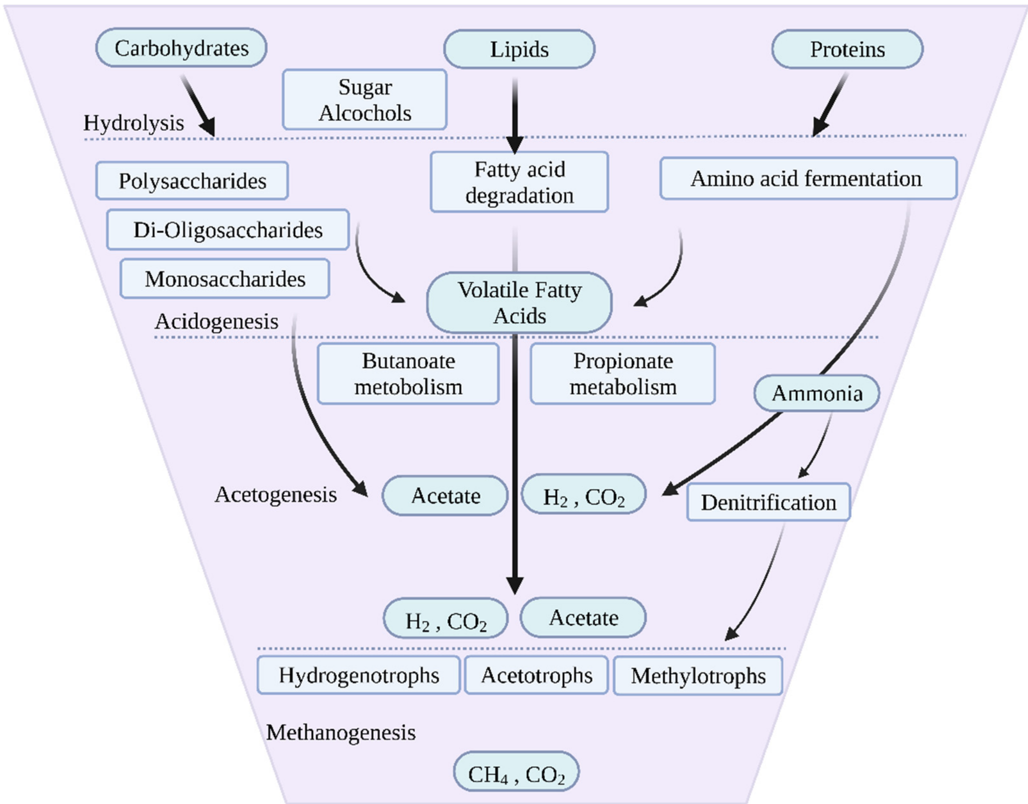

Figure 1. Anaerobic digestion fundamental steps of action, as modified from Campanaro et al. [40].

3. Sewage Sludge's Structural Special Features

As a result of the chemical composition of the influent (raw) wastewater and the changes to which it is subjected throughout the subsequent treatment stages, the resulting sewage sludge fractions vary in terms of composition (i.e., solid, semi-solid, or liquid), characteristics, and quality (Table 1). More specifically, the primary sludge (PS) fraction is produced through primary sedimentation, during which suspended organic and inorganic solids are removed, while secondary sludge (SS) fraction (also called as Waste Activated Sludge—WAS, or simply as sludge) is derived from the biological treatment (aerobic/aeration step) of wastewater to further reduce soluble organic load, suspended solids and in some cases nitrogen and phosphate compounds [41].

It should be noted that SS is less biodegradable than PS. The latter phenomenon is assigned to its complex floc structure and composition, as well as to the poor content of compounds adept at biodegradation, which evidently do not facilitate AD's efficiency [41]. In this light, SS's floc structure has been considerably studied over the last two decades and researchers have concluded that its form and stability are related to the presence of EPS. More particularly, SS's floc structure is mainly compromised by microbial cells and aggregates, organic particles, inorganic substances (i.e., multivalent metal cations, grit, etc.), and water, which are all embedded together in matrixes composed of EPS that act as the bound material [42,43]. Based on the spatial distribution within floc structure, three types of EPS can be distinguished (Figure 2): soluble (S-EPS), loosely bound (LB-EPS), and tightly bound EPS (TB-EPS). S-EPS are distributed in the liquid phase and LB-EPS span from TB-EPS, which in turn surround bacterial biomass. Therefore, sludge's floc structure can be partially characterized as loose suggesting increased porosity, whereas a tight spatial distribution indicates a compact structure [1,10,44].

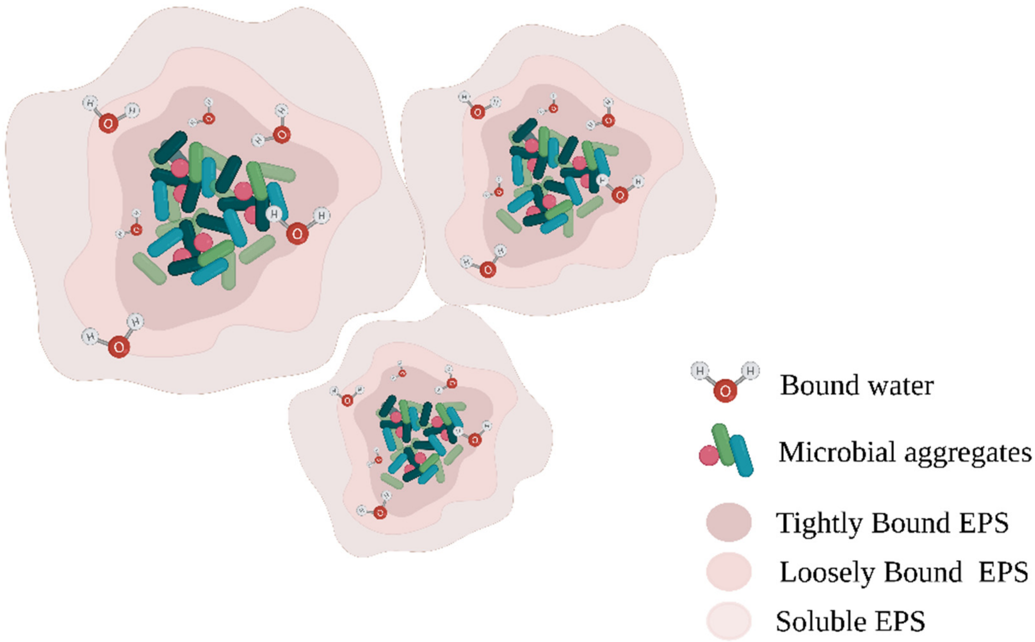

Figure 2. Sewage sludge (bio)floc structure.

Table 1. Typical characteristics of primary and waste activated sludge (WAS), as adapted from literature [1,22,45,46].

Characteristics	Primary Sludge (PS)	Waste Activated Sludge (WAS or SS)
Total Solids (TS), %	1–6	0.4–1.2
Volatile Solids (VS) (%TS)	60–85	59–88
Lipids (%TS)	5–8	5–12
Protein (%TS)	20–30	32–41
N (%TS)	1.5–4	2.4–5
P (%TS)	0.8–2.8	2.8–11
K (%TS)	0–1	0.5–0.7
Cellulose (%TS)	8–15	-
Alkalinity (mg/L as $CaCO_3$)	500–1500	580–1100
Organic acids (mg/L as acetate)	200–2000	1100–1700
Iron (%TS)	2–4	2
Al (%TS)	0.2	0.1–13.5
Ca (%TS)	10	0.1–25
Mg (%TS)	0.6	0.6
pH	5–8	6.5–8
Floc size (μm)	53	125 ± 109

The interactions assisting sludge's interior floc structure stability mainly include van der Waals and electrostatic forces, as well as hydrogen bonding, steric effects, and bridging interactions via charged molecules such as metal ions [10]. Bridging and electrostatic interactions mainly contribute to EPS matrix maintenance, which in turn provides sludge's structural form and stability, while determining its integrity and strength towards anaerobic biodegradation [47]. This is mainly assigned to the negative charge of EPS (in circum-neutral pH values), due to the negatively charged functional groups presented in it [48]. As a result, this allows for interaction with the positively charged multivalent metal cations (i.e., Ca^{2+}, Mg^{2+}, etc.), forming quite stable complexes difficult to be hydrolyzed [10,44]. Aside from the protection by the presence of EPS, the direct anaerobic digestion may also be hindered by the microorganism's cell envelope in form of a semi-rigid structure made up of glycan strands crosslinked by peptides [23]. In addition, the semi-rigid structure's further resistance in biodegradation is supported to be a result of an interaction between micro-sized grit particles and the organic matter presented in sludge forming bioinorganic flocs on surface sites [10]. On the above basis, it can be reasonably concluded that the disruption of EPS matrixes and cell walls could be effective for enhancing sludge's biodegradability. Nonetheless, although research has already contributed to some extent to enhancing AD efficiency, it is still imperative to further investigate sludge's physicochemical microstructure and composition. Thus, sludge's disintegration difficulty could be thoroughly understood, fundamentally encouraging enhancement of AD through the modification of the substrate's structure and properties with the aid of proper pretreatment methods.

4. Pretreatment's Beneficial Aspects

The aim of pretreatment is to increase sludge's floc structure accessibility and simplify its composition, in order for compounds amenable to AD to become available for microbial degradation (Figure 3), facilitating biogas and methane generation. Essentially, throughout pretreatment, substrate's floc structure is disrupted, promoting particle size reduction and subsequent increase in surface area. This in turn enhances the enzymatic activity and hydrolysis, fostering the solubilization of organic particulate matter [16]. Furthermore, the enhanced hydrolysis implies the decomposition of high molecular weight organic matter (i.e., polysaccharides and extracellular proteins) into simpler forms susceptible to (anaerobic) digestion. This results from the particle size reduction, which facilitates the displacement of these inner floc components to the outer layers of the sludge. Therefore, their bioavailability and utilization during AD increase [43]. Additionally, sludge dewaterability is also improved, considering that the enhancements mentioned presuppose EPS's looseness or destruction, resulting in the release of bound water [44,49]. Pretreatment can also cause the disruption of refractory cell walls, provoking the release of inner cellular compounds. Their release and subsequent solubility increase due to pretreatment, making these biomolecules available for the microorganism consortium. Thus, given that these organics are commonly hydrophilic, their solubilization and upcoming utilization during AD brings about a subsequent improve in sludge dewaterability [16].

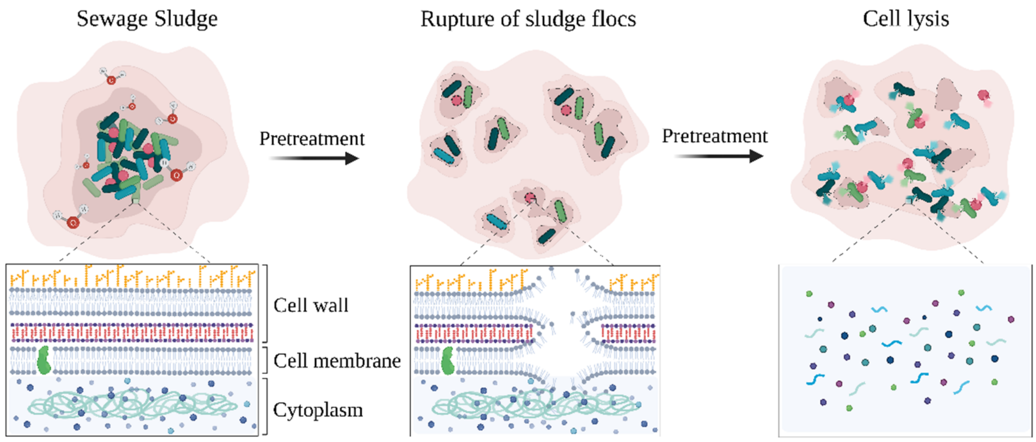

Figure 3. Sewage sludge disintegration, cell lysis, and release of intercellular organic substances due to pretreatment.

Along with the changes in sludge structure, solubilization of organic compounds, and bioavailability, the rate and degree of organic degradation (i.e., hydrolysis) is enhanced and the Hydraulic Retention Times (HRTs) are shortened. A natural consequence of the above is the enhancement of AD efficiency in terms of biogas production, as well as the reduction of requirements in volume and consequently the cost of reactors. In addition, the higher organic degradation can lead to reduced solids content in excess sludge and therefore to reduced handling and disposal costs [12]. Along with these beneficial aspects, pretreatment methods must satisfy some other specific requirements associated with the elimination of the production of inhibitory byproducts and decreased energy input [12]. Meeting these requirements is challenging and is still thoroughly studied as a topic of high scientific interest in the academic community. Within this context, attempts in the simultaneous or sequential appliance of different pretreatment methods have also been made [9] and appreciable results have been reported [15,50–57].

Assessment Criteria of Pretreatment Effectiveness

There are various studies that have been conducted on enhancing sludge's AD through pretreatment. Many of them use Chemical Oxygen Demand (COD) solubilization (also defined as soluble COD–SCOD) as a typical/common parameter to evaluate the effectiveness of a pretreatment and assess the incremental changes it can induce in sludge biodegradability and subsequent biogas and methane production [19,58]. Indeed, there are several studies reporting the direct relationship of COD solubilization and biodegradability without, however, the same proportionality [43,59]. Nevertheless, the same does not apply in all cases, as other researchers found that high COD solubilization does not always imply improved biodegradability or enhancement in AD efficiency [60,61]. Thus, even if a pretreatment method leads to high COD solubilization, it does not ensure enhancement in methane production [62]. More specifically, some research groups reported that although an increase in solubilization indicated hydrolysis acceleration, the improvement in methane production was inadequate [63]. These contradictory outcomes relate to a portion of the COD fraction, which although solubilized during pretreatment, is recalcitrant and cannot be converted by microorganisms to CH_4 [60,64]. An additional explanation for these conflicting behaviors could be attributed to the structural complexity of sludge and especially to the microstructure's physical and biochemical properties, which are influenced by the applied pretreatment. Specifically, it is hypothesized that pretreatment can release some key constituents originally adsorbed into sludge flocs, which are directly involved in hindering bioconversion during AD [65]. Indicatively, the metal ions can disable the activity of some enzymes and thus, inhibit the bacterial growth, negatively influencing biodegradability [66]. In addition, interactions between organic and inorganic constituents may also occur and influence bioconversion [10].

However, the above-mentioned points have not been adequately studied. Therefore, further research is needed regarding the changes in microscopic features and characteristics of soluble content to evaluate pretreatment's influence and effectiveness in relation to the subsequent AD efficiency. This will fill knowledge gaps that could bring about further enhancement in the biodegradability of organic matter and methane generation with the aid of pretreatment methods. Consequently, COD solubilization appears not to be a precise parameter for the evaluation of a pretreatment's effectiveness and the accurate prediction of subsequent biogas production [19]. This view could also be supported considering that (S)COD measurement uses a strong oxidizing reagent to oxidize the total soluble organic matter [67], which a bacterial consortium could be capable of oxidizing partially or not at all. This leads to an overestimation of the organic matter available for biodegradation. In addition, (S)COD determination does not indicate the formation of toxic byproducts (e.g., furfural, hydroxyl-methyl-furfural (HMF) etc.) that may have been produced during pretreatment and could inhibit methanogenic microbial growth and subsequently AD performance and biogas production [35,58].

In contrast, Biochemical Methane Potential (BMP) tests are more accurate to assess pretreatment's effectiveness. BMP tests are performed to determine the individual methane production potential of a given (pretreated) substrate. Therefore, by means of these tests, useful information can also be obtained regarding substrate biodegradability since it is directly associated with methane productivity. Moreover, methane productivity depends on the inoculum activity and microbial characteristics, inoculum to substrate ratio, and rate and degree of hydrolysis [68]. As a result, changes in methane productivity reveal reliable information regarding the pretreatment effects and efficiency, as well as AD performance. The typical outcomes identified from BMP tests (Figure 4) show both the expected economic gains arising from pretreatment and the changes that pretreatment can cause to the biodegradable fraction. More specifically, in case of similar results to those of an un-treated sample (Figure 4a), pretreatment does not provide any additional benefits. However, as illustrated in Figure 4b, an increase in hydrolysis rate can be translated into reduced operational costs because of shorter HRT and need of decreased reactor volume [12]. Even in case of a same reactor size, pretreatment can be still beneficial from an economic standpoint, as it can lead to an increase in the quantity (mass) of feedstock to be processed and thus, in the biogas produced. Furthermore, as shown in Figure 4c, sometimes the time needed for an increment in methane production is longer. This demonstrates that pretreatment has led to the conversion of non-biodegradable organic compounds to forms susceptible to AD and/or to the formation of some organic compounds that microorganisms need longer acclimatization periods to degrade, specifically when the inoculum origin is different from the substrate's [58]. An accelerated AD process, along with increased methane production is another typical outcome of pretreatment and is illustrated in Figure 4d. On the above basis, it can be concluded that BMP testing is a plausible option for assessing the effectiveness of a pretreatment method. In addition, the fact that BMP assay for methane production potential determination is based on a well-established protocol [68,69] makes its use for the evaluation of pretreatment's effectiveness to be strongly suggested.

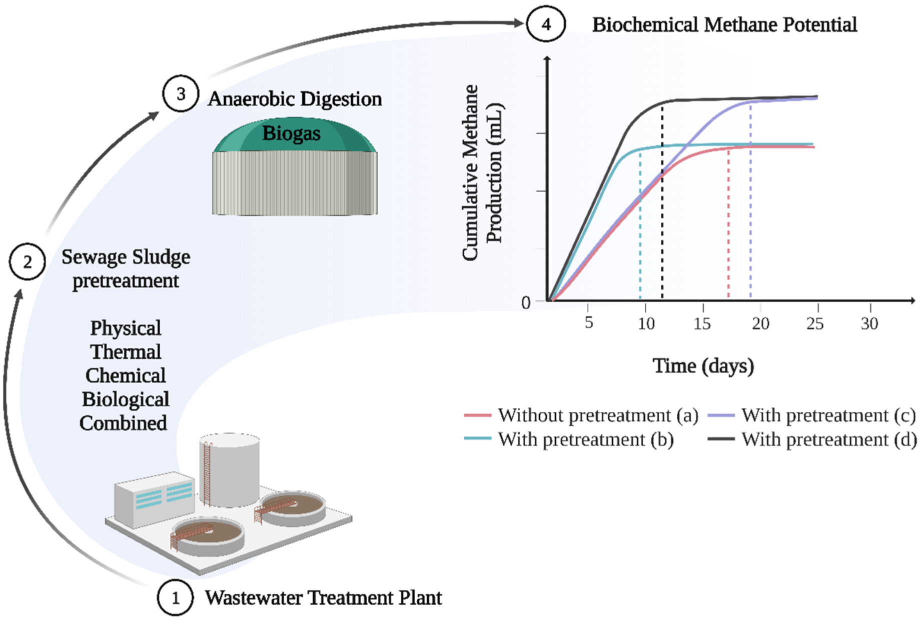

Figure 4. Pretreatment of sewage sludge to enhance anaerobic digestion efficiency and BMP typical outcomes associated with: (a) no additional benefits in case of untreated sludge (without pretreatment), (b) increased hydrolysis rate due to pretreatment, (c) methane production enhancement as consequent of pretreatment, (d) accelerated AD and increased methane production as a result of effective pretreatment. Dotted lines indicate the time point where the available organic matter has been bio-converted to methane.

5. Developed Processes for Sludge Pretreatment Prior to AD

SS (or WAS) is mainly composed of recalcitrant compounds that hinder the smooth hydrolysis performance rendering it as a rate-limiting step, which further influences the overall degree of AD efficiency. Thus, advanced research has been dedicated to developing several pretreatment technologies to be applied prior to AD, aiming to disintegrate sludge in order to overcome the limitations encountered during hydrolysis and enhance sludge's bioconversion efficiency and biogas production. Over the last decades, numerous studies have reported many different pretreatment technologies with a wide range of modes of action and outcomes. In the material to follow, we give an overview of these technologies, which include physical/mechanical, thermal, chemical, and biological processes, as well as combinations, and the improvements they can confer in AD performance. Within this context, the results of studies concerning sludge pretreatment methods prior to AD are discussed, focusing on their effectiveness in terms of COD solubilization, organic matter biodegradability, and the incremental changes they can induce in Volatile Solids (VS) reduction, biogas, and methane production during AD.

5.1. Physical/Mechanical Pretreatment Technologies

Physical pretreatment through ultrasonication, microwave irradiation, high pressure homogenization, and electro-kinetic disintegration (Table 2) is advantageous, considering that no chemical reagents are used, nor are the functional microbes enriched to promote the bioconversion efficiency [12]. The substrate's pretreatment by means of physical/mechanical methods is defined as a reduction in particle size by the application of a physical force. Particularly, this causes the conversion of the organic components into smaller and more soluble fractions, providing increased surface area and thus, higher probability of contact between the substrate and reactor's microbial consortium. Thus, considering that the components become more susceptible to microbial attacks, their biodegradability is extended, resulting in AD process enhancement [70]. In light of this, it has been reported that the larger the particle diameter, the less methane generation there is, implying that the substrate's increased bioavailability and utilization is inversely proportional to particle size [58]. Along with particle size reduction, disruption occurs when physical/mechanical pretreatment mechanisms include the application of intense external pressure that exceeds the internal pressure of cells [42], resulting in the release of intracellular material in the bulk medium. Nonetheless, despite the beneficial aspects of mechanical disintegration, most of the methods belonging in this category are energy intensive [9], which could change in case of excessive energy recovery for pretreatment heat or electricity needs, leading to energetically self-sufficient processes [14,71].

5.1.1. Ultrasonication

Ultrasonication is commonly employed for sludge pretreatment and results in its disintegration through cavitation phenomenon (Figure 5). When cavitation takes place, microbubbles are formed due to pressure alterations by the propagation of ultrasound waves in the medium under frequencies in the range of 20–40 kHz. When the bubbles reach a critical size, they collapse releasing high amounts of energy into the surrounding environment, that lead to extreme temperature and pressure conditions of about 5000 K and 500 bars. Under these conditions, elevated shear forces are generated, which disrupt sludge structure and cause the breakdown of cell walls. Along with these forces, disintegration of sludge can result from the oxidizing effect of reactive radicals (H· and ·OH), whose formation usually presupposes high ultrasonic densities (D_{US}) and frequencies (F_{US}) (i.e., 150–2000 kHz) [17].

In addition to operational parameters such as F_{US}, D_{US}, treatment time, and temperature [8], the content of sludge total solids (TS) and specific energy (E_S) input (kJ/kg TS) are also factors that affect sludge disintegration efficiency and are used to assess the ultrasonication performance [16]. The most important parameter, however, is E_S, an umbrella term equating D_{US}, time, and TS content [19]. Regarding the solids content, Appels et al. [72] reported that when higher, the release of soluble organic matter is enhanced and increases linearly with increasing E_S. Nonetheless, Sahinkaya [56] revealed that for a constant low D_{US} (1.0 W/mL), an increase in TS content may interfere with the propagation of ultrasound waves and thus, reduce their power and effectiveness of pretreatment. Therefore, they concluded that for a positive influence in sludge disintegration, higher ultrasonic energy is required. In light of the above, there is a consensus that the concentration of solids should vary in the optimum range of 2.3–3.2%. This way, adverse effects on overall ultrasonic efficiency are eliminated and increases in energy consumption are avoided [73].

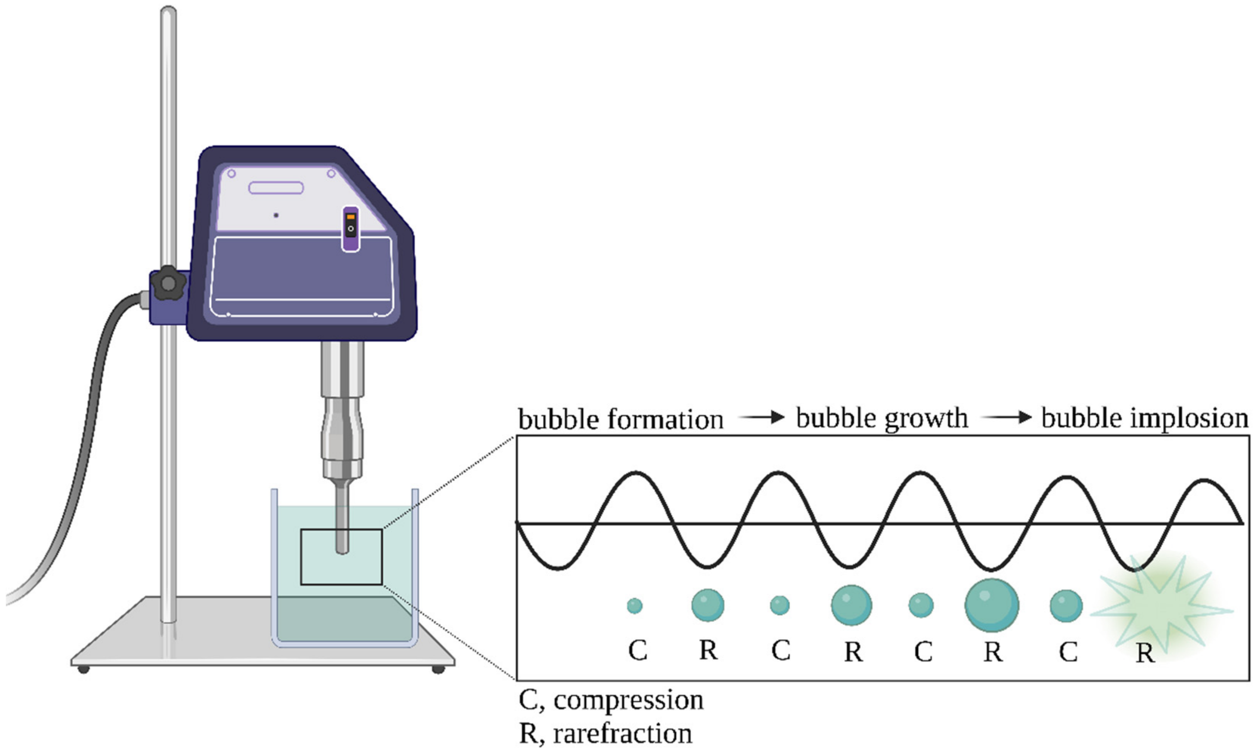

Figure 5. Cavitation phenomenon caused by ultrasonication.

E_S usually varies in the range 1000–40,000 kJ/kg TS, with that of 5000 kJ/kg TS reported to be as the threshold, ensuring improved disintegration and COD solubilization [1,74]. For an E_S value between 10,868 and 23,226 kJ/kg TS, the obtained results from the study by Tytla et al. [75] demonstrated the positive impact of ultrasound pretreatment, resulting in an increment in biogas production. Specifically, it was reported that with the aid of two different experimental ultrasonic devices, the incremental changes achieved when compared to the untreated sample, corresponded to percentages of 13.0% and 19.7%. Similarly, within the range of 15,000 to 35,000 kJ/kg TS using different OLRs, Lizama et al. [65] also reported ultrasonication as a suitable technology for AD enhancement. In this study, it was revealed that with increasing E_S, a corresponding increase in biogas yield was observed for all the OLRs tested. However, the positive trend changed when exceeding 25,000 kJ/kg TS. This observation was attributed to a hypothesis dealing with the formation of insoluble and/or inhibitory compounds because of high temperatures and energy input [65].

Furthermore, studies using low F_{US} during ultrasonic pretreatment have also been conducted. Indicatively, Li et al. [76] examined ultrasonic pretreatment of WAS at 20 kHz for D_{US} of 0.5 W/mL and evaluated its efficiency for treatment times between 0 and 100 min. For pretreatment duration of 80 min, the results presented an increment in biogas yield with methane content of 53.8%, which was related to the SCOD significant release observed at this sonication treatment time. Although most low-frequency ultrasonic applications vary between 20 and 40 kHz, Delmas et al. [77] examined the audible frequency of 12 kHz. Given that at lower frequencies, the microbubbles' critical size for implosion is increased [78] leading to more violent cavitation effects, promising results regarding sludge disintegration were expected. Indeed, it was concluded that the degree of sludge disintegration (DD_{COD}%) (calculated as defined by Kim et al. [51]) was enhanced compared to when a frequency of 20 kHz was applied and was gradually increased by increasing input power from 50 to 360 W.

The effect of temperature has also been evaluated, especially in case of temperature-uncontrolled sonication in order to differentiate whether biodegradation is a consequence of temperature or ultrasonic effects. Several studies have demonstrated that in case of temperature control during ultrasonic pretreatment, the methane yield achieved was lower compared to uncontrolled conditions [60,79]. For instance, Le et al. [80] revealed that better performance in terms of DD_{COD} was observed under the coupled effect of ultrasound waves and temperature, probably due to the additional mechanisms occurred during low-thermal pretreatment (see Section 5.2.1.) that are associated with biodegradation improvement. Similarly, Sahinkaya and Sevimli [81] studied ultrasound pretreatment at 80 °C and revealed that its effectiveness in terms of disintegration was better than that achieved with the aid of standalone sonication and an increment of 13.6% in methane production was achieved. Nevertheless, extreme temperatures should be avoided. This is attributed to the fact that high temperatures increase the vapor content in the cavitation bubbles, leading to a less efficient collapse and concomitant decrease in the effects of shear forces [77].

Furthermore, in addition to the use of ultrasonication for enhancing AD, its impact regarding sludge dewaterability has also been examined [78,82]. Sonication's capability to disintegrate sludge, destruct EPS structure, reduce particle size, and thus, release bound water is also expected to improve dewaterability [82]. However, excessive pretreatment may lead to the formation of fine particles and larger surface areas available to re-absorb water, resulting in the deterioration of dewaterability [83]. As a result, when increasing the energy input levels, dewaterability is not favored, given that the higher the energy the more the disintegration of the particles [84]. Thus, it has been reported that dewaterability increases only when DD_{COD} varies between 2% and 5% [85]. Moreover, when DD_{COD} is below 2%, the sludge flocs are not adequately destructed, while above 5% the formation of fine particles is favored and thus, deteriorate dewaterability. Hence, the addition of conditioning chemical agents (e.g., solutions of polyelectrolytes) can cause re-flocculation of the smallest fragments and improve dewaterability. Taking into consideration the latter aspects, Fe et al. [86] examined the relation between DD_{COD} value and dewaterability. They demonstrated that for a specific energy input ranging 0–2200 kJ/kg TS dewaterability is increased, with the better results observed at 800 kJ/kg TS, where EPS concentration varied from 400 to 500 mg/L and particle size did not exceed 90 µm.

5.1.2. Microwave Pretreatment

Microwaves are a form of electromagnetic radiation, which occur at a frequency range of 300 MHz to 300 GHz. However, the frequencies usually applied in industry are close to 0.915 GHz with the most common reported in the literature to be that of 2.45 GHz, most likely due to practical constraints regarding equipment availability [87]. What makes microwave pretreatment (MP) preferable compared to conventional heating strategies is associated with its internal heat mechanism, which in contrast to thermal methods, is translated into minimal or no heat losses [13,88]. Generally, through MP, sludge's floc and EPS structure is disrupted, bound organic matter is released, and the cell walls which are no longer protected within the EPS matrix are destroyed. MP's principle of action relies on thermal and non-thermal effects. Thermal effects are related to the presence of dipolar molecules (e.g., water) in sludge. Under the oscillating electromagnetic field generated by microwaves, the alignment and realignment of polar molecules' dipoles is provoked, which results in friction effects, liberating heat [19]. High temperature brings on heating of intracellular liquor to boiling point. Consequently, such a change in physical state within the cell causes the rupture of the cell walls/membranes, due to stress triggered by the increase of internal pressure (Figure 6) [89]. Along with the thermal effects, non-thermal ones are consequent of a change in the dipole orientation of polar molecules in an effort to be adjusted to the intruding electromagnetic radiation. Thus, whether the orientation of macromolecules compromising the cell membranes takes place, the hydrogen bonds assisting their structure break. Inevitably, intracellular substances are released, while changes in the secondary and tertiary structures of the microorganisms' proteins occur by virtue of denaturation [90].

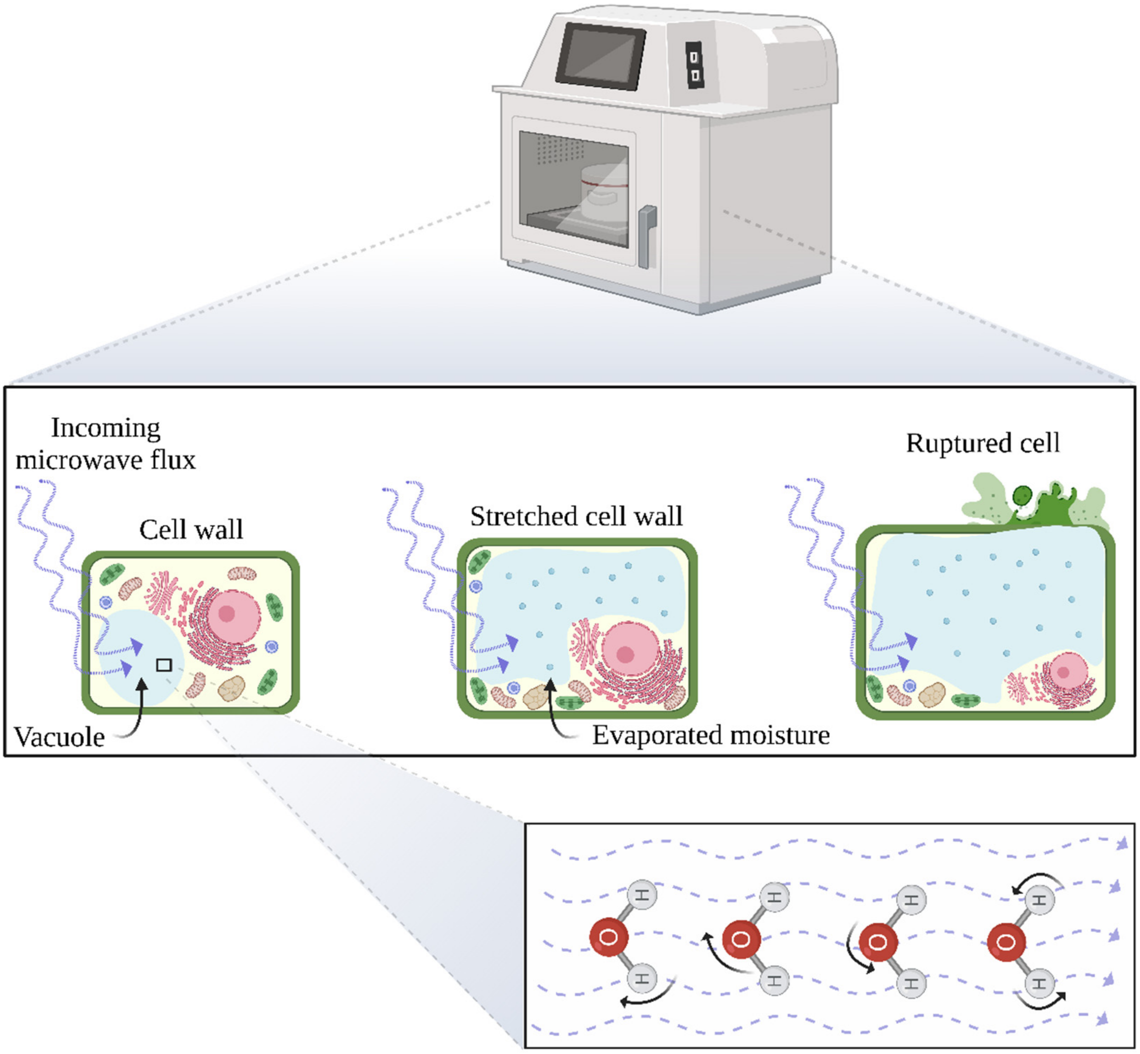

Figure 6. Microwave pretreatment's principle of action.

A common approach to assess a pretreatment method's effectiveness is associated with the incremental changes it can induce in terms of sludge solubilization [91,92]. Sludge solubilization is affected by solids concentration, which seems reasonable considering that sludge solids concentration influences microwave energy absorption and consequently the quantity of energy transferred to the sample [93]. Fang et al. [94] applied high temperature MP (at 170 °C) on sludge concentrated at 7%, 9%, and 13% and concluded that SCOD was gradually increased when increasing solids concentration. More specifically, using sludge of 13% solids concentration, an increment of 32% was observed in SCOD concentration with increasing pretreatment duration. In respect to sludge solubilization, Appels et al. [90] demonstrated that treatment of thickened sludge under 336 kJ/kg TS of specific energy (E_S) and 800 W of power induced an increment of 214%, as well as an augmentation in biogas production equal to 50% over the untreated sample. Similar to this study, using thickened sludge (43.6 g TS/kg), Houtmeyers et al. [95] reported a significant solubilization improvement (117%) and surplus increment of 20% in biogas production applying E_S of 96 kJ/kg TS and 800 W MP power. Along with the changes in solubilization, high solids concentrated sludge can contribute to reducing the energy input requirements. The latter is associated with the absence of free water; otherwise, in case of microwave heating, it absorbs a substantial part of the irradiated energy, negatively influencing MP efficiency [96].

With respect to the microwave E_S, its positive association with the increase in solubilization has also been reported [87]. Serrano et al. [97] conducted MP assays at E_S inputs varying from 0 to 30,000 kJ/kg TS, while the powers applied were 400 W and 700 W. In both cases, an increasing trend in solubilization was observed with increasing E_S. When 700 W of power was applied, a significant increase of 212% in SCOD was revealed at around 20,000 kJ/kg TS of energy input, while a surplus increment of 3% was observed for increasing E_S at 30,000 kJ/kg. In contrast, the results under 30,000 kJ/kg at 400 W, presented only a 30% increase. In addition, SCOD was higher at 700 W than this at 400 W and 30,000 kJ/kg, even with specific energy input of 10,000 kJ/kg. On those grounds, it was concluded that higher-power pretreatment is more effective and may be capable of reducing the amount of E_S required for efficient MP performance. This indicated that the amount of energy supplied to the substrate is generally controlled by microwave power [87]. MP's effectiveness regarding the increase in surface area and subsequent increase in methane yield has also been documented. Martinez et al. [98] demonstrated that among the irradiation energy input values examined (488–2700 kJ/L) for the pretreatment of mixed sludge, the highest increase in surface area was observed at 975 kJ/L, while insignificant improvements were detected up to 2700 kJ/L. The same trend also applied for methane yield (based on batch tests), indicating its direct relationship to the surface area (R^2 = 0.967). The observed increment, compared to the untreated sludge at 975 kJ/L, reached 46%.

Even though non-thermal microwave breakdown mechanisms have been speculated in the literature [87,99–101], evidence of their occurrence is still mostly equivocal. In order to scrutinize whether non-thermal effects occur, most researchers have compared Conventional Heating (CH) and MP under alike heating conditions. Some of them observed no additional improvements in solubilization and biodegradability due to non-thermal effects [100,101], while others reported increments in both SCOD to total COD ratio (SCOD/TCOD) and biogas production, compared to CH [102]. These inconsistent conclusions along with the fact that the monitoring of internal temperatures in molecular level is difficult, indicate that the creation of a pattern regarding the non-thermal effect on the substrate is challenging and needs more effort to be clarified. Finally, it is important to note that MP has also appeared to be effective against pathogens [91] and dewaterability improvement [19].

5.1.3. High-Pressure Homogenization

High-Pressure Homogenization (HPH) pretreatment's working principle to disintegrate sludge involves the use of high pressures and relies on abrupt pressure alterations, as well as on typical effects of fluid/dispersion dynamics such as the formation of mixing eddies due to high turbulence, cavitation phenomenon, and shear forces [16]. For the process to begin, external high pressure is provided using a pump and sludge is obligated to flow through narrow gaps between the homogenizer valve and the valve seat (Figure 7). Once the substrate passes this flow restriction paths, the energy applied is converted into kinetic force, bringing on turbulence in it and shearing of microorganism cells due to generation of eddies. Effective cell disruption also results in a high velocity jet of suspended cells that collides on the stationary surface of an impact ring, positioned near the exit of the narrow gaps [103]. Along with these effects, as consequence of velocity acceleration, an abrupt drop of the liquid's pressure below the vapor pressure occurs. As a result, the formation of vapor bubbles is enhanced. As the liquid exits the valve, pressure recovers because of the reduction in velocity, leading to cavitation and bubbles' implosion [70]. Through the abovementioned destructive processes, the disruption of sludge flocs is facilitated, cell membranes rupture and organic substances amenable to AD are released. Moreover, the number of large particles decreases dramatically, increasing the surface area available for enzymatic attack accelerating hydrolysis [1].

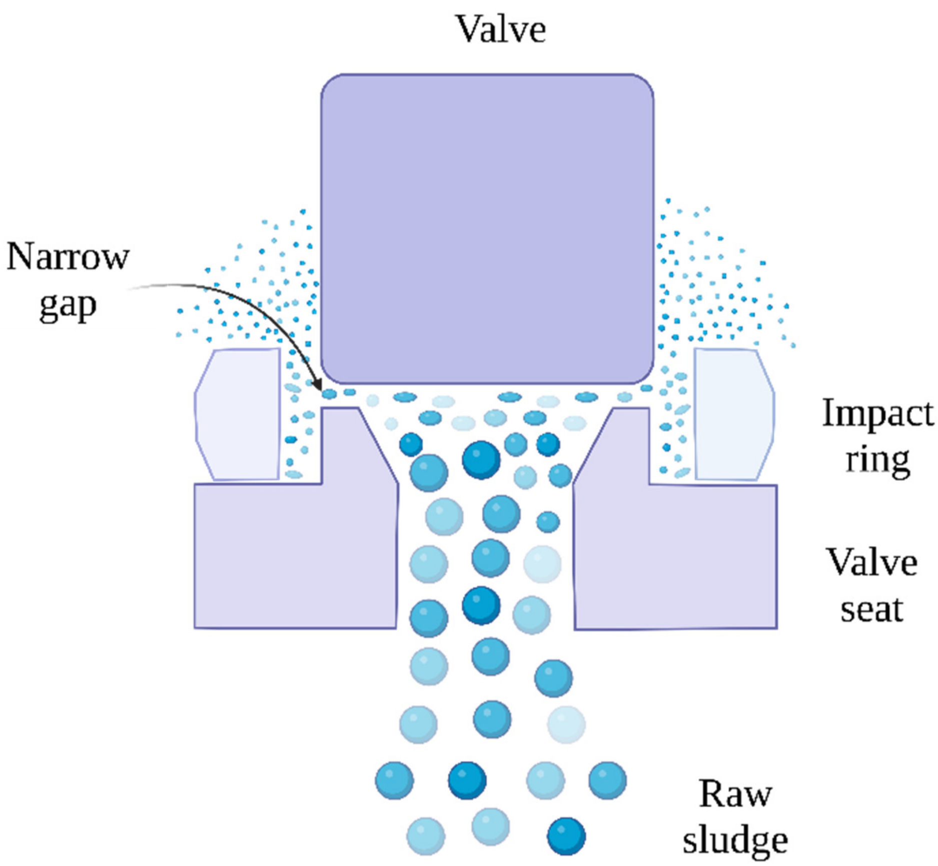

Figure 7. Typical high-pressure homogenization schematic configuration.

Homogenization cycle number and pressure are parameters that influence HPH pretreatment performance in terms of both sludge disintegration and solubilization, as well as biogas production effectiveness. Zang et al. [104] applied homogenization pressures between 20 MPa and 80 MPa and observed an increasing trend in DD_{COD} with a corresponding increase in pressure. However, this trend did not apply for increasing the TS content and the results indicated that DD_{COD} decrement was consistent for all the conditions tested. As with increasing the homogenization pressure, HPH's effectiveness also improved under multiple treatment cycles. Specifically, it was reported that the DD_{COD} increase was intense during the first two cycles, whereas the subsequent increases over the next two homogenization cycles were less than 10%. In a follow-up study and under corresponding pressure range and number of cycles, the same research team scrutinized the changes in solubilization and solids reduction when sludge was subjected to HPH pretreatment [105]. Concretely, organic matter solubilization was found to increase with both homogenization pressure and number of cycles. In addition, given that a reduction in Total Suspended Slides (TSS) and Volatile Suspended Solids (VSS) by 31% and 37%, respectively, was gained during AD, HPH pretreatment was identified as effective in enhancing the release/dissolution of organic matter from solid matrix in the bulk medium. The results also indicated a linear increase in solids reduction with homogenization pressure, whereas significant reduction was only detected at the first two homogenization cycles. Finally, a linear correlation of approximately 99% between SCOD solubilization and VSS reduction was also reported [105].

Improvements in biogas production when AD is coupled with HPH pretreatment have also been documented. Particularly, Zhang et al. [106] reported that the maximum cumulative biogas production was achieved after a 7-day AD, fed with sludge pretreated under pressure of 50 MPa and two homogenization cycles. The increment observed corresponded to a percentage of 115% compared to raw substrate. Along with this, sufficient methane content of 64% was achieved, which was also higher than that of the untreated sample. Positive results for biogas production have also been reported by Onyeche [107]. Specifically, for pretreated concentrated sludge at 150 bars with flow rate of 2.7 m^3/h, a sludge reduction of 23% was achieved, while more than a 30% increment in biogas production was demonstrated. Finally, it is important to note that there are several full-scale processes based on HPH technology which are commercially available. For instance, the patented MicroSludge™ process was first applied by Rabinowitz and Stephenson [108]. According to this process, sludge is first treated with the aid of proper chemicals aiming to decrease the strength of cell walls. Cell rupture is then provided as the cells pass through a high-pressure homogenizer at 800 kPa [109]. Other relevant full-scale processes developed are Crown® and Cellruptor. The former is based on the cavitation phenomenon [110], while the second one uses pressures lower than 10 bars to compress CO_2 into sludge, which upon rapid depressurization causes excessively high shear forces and irreversible rupture of the cell walls [11].

5.1.4. Electro-Kinetic Disintegration for Sludge Pretreatment

Electro-kinetic Disintegration (ED) technology, also known as "pulsed electric field", uses high-voltage pulsing electric fields ranging between 20 kV and 30 kV [1], aiming to disrupt the sludge flocs and cell membranes, and solubilize the complex organic matter. Considering the cell membrane's dielectric properties and structure built of polar molecules, when subjected to external electric field with the aid of immersed electrodes, the charges created can cause membrane disruption. Essentially, the applied field induces at each side of the membrane the accumulation of negative and positive charges, respectively (based on the facing electrode), creating increased trans-membrane potential (Figure 8). That in turn, causes membrane compression owing to the intense attraction of opposing charges. Once the normal trans-membrane potential is overcome, membrane's structural stability weakens and bursts, releasing the intracellular organic material into the bulk solution [111].

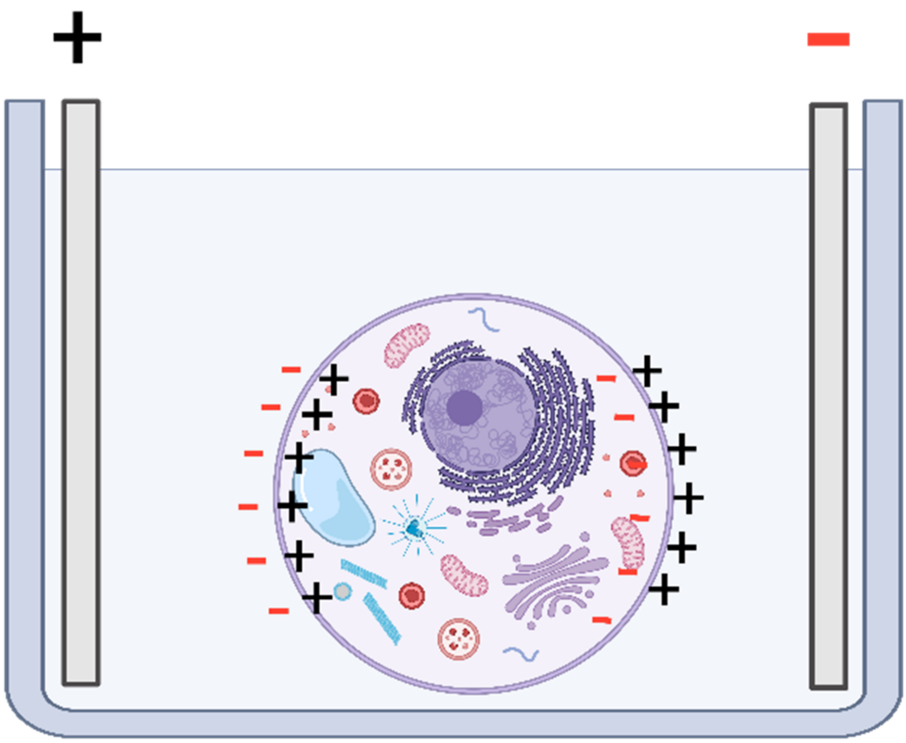

Figure 8. Increased trans-membrane potential due to the application of external electric field.

Among the published studies that investigated the effect of ED technology on sludge disintegration, Lee and Rittmann [112] reported a percentage of 220% increment in SCOD compared to raw substrate, as well as an increase in methane production rate and TCOD removal. Accordingly, Choi et al. [113] applied ED on WAS and demonstrated that SCOD/TCOD ratio was augmented by 4.5 times. Moreover, the researchers revealed that this method provoked a 2.5-fold increase in biogas production over the untreated sludge when applying a pulsing electric field of 19 kV, 110 Hz and a very short pulse period that corresponded to 1.5 s. Despite ED being an innovative sludge pretreatment method, it has already been widely utilized in the industry. A full-scale implementation of appreciable results, namely OpenCel™, has been described by Rittmann et al. [114]. When 63% of the sludge influent was ED pretreated, an increment of 40% in biogas production and a reduction in biosolids equal to 30% were reported. These percentages were estimated to further increase by 20% and 10%, respectively, in case of full ED pretreatment. In this light, the researchers noted that for a sludge inlet flow rate of about 380 m^3/day, the annual economic benefit generated by full-ED pretreatment was estimated to be nearly $540,000 net of maintenance and operating expenses. Corresponding economic benefits due to incremental changes in SCOD, biogas production and reduction of biosolids, have also been reported in another full-scale installation of OpenCel™ [115]. A commercial electro-kinetic disintegration device with multiple full-scale installations in both Europe and US is BioCrack. During a BioCrack pilot test, thickened WAS was used as influent. The fact that the disintegration of both TSS and VSS was high, indicated that sludge flocs were broken. However, BioCrack's effectiveness regarding COD solubilization was inadequate (only 0.30%). Based on these results, it was concluded that BioCrack seemed to be more appropriate in accelerating the rate of anaerobic digestion rather than extent the digestion efficiency [115]. Nonetheless, German Vogelsang, a well-known manufacturer specializing in electro-kinetic disintegration devices, claims a 20% increment in biogas production, a reduction in the consumption of power, and significant downstream energy savings of approximately 30% by using BioCrack technology for sludge pretreatment [1].

5.1.5. Physical/Mechanical Pretreatments—Concluding Remarks

Among the physical/mechanical pretreatment methods reviewed in this section, microwave pretreatment is the only one that has not been yet commercialized, despite its relative effectiveness. Specifically, microwave pretreatment results in significant increase in SCOD, improved biodegradability, as well as enhancements in both biogas and methane production. Additionally, specific energy and microwave power have been reported to be the operating parameters that mainly affect SCOD. In respect to this, SCOD is reported to be pronounced with increasing the power applied for the pretreatment; a gradual increase in this parameter has also been documented with increasing sludge's TS content. Likewise, ultrasonication is found to be an effective method that can provoke sludge disintegration and improve solubilization. However, to ensure these positive effects, the specific energy input should exceed 5000 kJ/kg TS. Furthermore, as long as the specific energy input ranges between 0 and 2200 kJ/kg TS, ultrasonication is reported to improve sludge dewaterability. Similar to the other physical/mechanical pretreatment methods, high-pressure homogenization's effectiveness regarding sludge disintegration, solubilization and biogas production has also been demonstrated. The main parameters reported to influence high-pressure homogenization pretreatment performance include the homogenization cycle number and pressure. Finally, among the physical/mechanical pretreatment methods described, electro-kinetic disintegration is the most innovative. However, this technology is already widely implemented in the industry, which seems reasonable considering the incremental changes it can induce in sludge solubilization, biogas and methane production, and in the reduction of the digestate solids. Two more reasons that justify the extensive utilization of this method at industrial scale are associated with its ease of operation and the short pretreatment durations.

Table 2. Effects of physical/mechanical and thermal pretreatment methods on anaerobic performance.

Pretreatment Technology	Pretreatment Conditions	Effects of Pretreatment	Anaerobic Digestion Conditions	Anaerobic Digestion Performance	References
Ultrasonication	- Type of sludge: WAS, Mixed Sludge, TWAS * - Specific Energy: 52–108,000 kJ/g TS - Power: 50–750 W - Frequency: 20–200 kHz - Time: 0.5–80 min - Temperature control ≤ 80 °C	- DD_{COD} increase by 9–21% - Up to 1.5-fold increase in SCOD - SCOD/TCOD ratio increase from 0.02 to 0.10 - Increase in biodegradability of TWAS up to 15.5%	- AD: Batch, (semi) continuous, TPAD–Batch assays - Scale: Laboratory, Pilot, Full - Temperature: Thermophilic, Mesophilic	- VS reduction: 6–180% - Biogas production: 4–83% - Methane production: 31–74% - Methane yield up to 95%	[12,16,19,65,75,95,116–121]
Microwave	- Type of sludge: WAS, Mixed, TWAS - Specific Energy: 96–20,000 kJ/g TS - Power: 400–1250 W - Frequency: 300 MHz–300 GHz - Time: 0.63 s–3.5 min	- Up to 3.6-fold increase in SCOD - 3.0-fold to 9.5-fold increase in SCOD/TCOD ratio - Increase in biodegradability by 20–35%	- AD: Batch, (semi) continuous, - semi continuous TPAD - Scale: Laboratory - Temperature: Thermophilic, - Mesophilic	- VS reduction: 23–53.1% - Biogas production: 16–570.7% - Methane production up to 20% - Methane yield up to 102%	[10,16,90,95,98,122–124]
High-pressure Homogenization	- Type of sludge: WAS, Mixed Sludge, Concentrated Sludge - Specific Energy: 300–3380 kJ/g TS - Pressure: 150 to ~827 bars - Cycles: 1–2	- DD_{COD} increase up to 43% - Up to 6.2-fold increase in SCOD	- AD: Batch, semi–continuous, 2TPAD - Scale: Laboratory, Pilot, Full - Temperature: Thermophilic, - Mesophilic	- Increase in VS reduction: 7–138% - Sludge reduction up to 23% - Increase in biogas production: 17–115% - Increase in methane content - Increase in methane yield up to 30%	[13,15,16,106,107]
Electro-kinetic Disintegration	- Type of sludge: WAS, Mixed Sludge - Specific Energy: 10–34 kWh/m^3 - Short pulse periods (sec)	- 2.6-fold to 4.4-fold increase in SCOD - Up to 4.5-fold increase in - SCOD/TCOD ratio	- AD: Batch, (semi) continuous - Scale: Laboratory, Full - Temperature: Mesophilic	- VS reduction up to 9% - Biosolids reduction by 30% - 2.5 times higher biogas production - Methane production: 33–100%	[13,16,112–114]
Low temperature pretreatment	- Type of sludge: WAS, Mixed Sludge, Dewatered Sludge - Temperature: 50–95 °C - Time: 15 min to 7 days	- DD_{COD} increase by 15–30% - 8-fold to 32-fold increase in SCOD - Increase in sludge disintegration rate	- AD: Batch, (semi) continuous - Scale: Laboratory, Pilot - Temperature: Thermophilic, Mesophilic	- VS reduction:10–45% - Biogas production: 10–984% - Methane production: 5–124% - Methane yield: 12–48%	[10,12,16,19,125,126]
High temperature pretreatment	- Type of sludge: WAS, Mixed Sludge, High Solids and Dewatered Sludge - Temperature: 100–210 °C - Pressure: 3–21 bars - Time: 20–90 min	- DD_{COD} increase by 34.7–42.5% - Up to 63-fold increase in SCOD - Up to 4.3-fold increase in SCOD/TCOD ratio	- AD: Batch, (semi) continuous - Scale: Laboratory, Pilot, Full - Temperature: Thermophilic, Mesophilic	- VS reduction: 7–105% - Biogas production: 25–150% - +40.2% in methane production - Biogas yield: 25–79% - Reduction of the digestate solids	[10,13,16,19,127–129]

* Thickened waste activated sludge.

5.2. Thermal Pretreatments

Thermal Hydrolysis (TH) is an effective pretreatment method of significant commercial interest that has been extensively studied in order to improve AD of sewage sludge [130]. By means of heat, the cells presented in sludge are ruptured and cellular components are released as an inevitable corollary of cell walls and cell membranes' chemical bonds breakage [42]. A positive influence of TH on the solubilization of organic compounds has also been reported [63]. TH of sludge is typically performed at temperatures ranging between 50 °C and 210 °C, where it is usually retained for a predefined time period of a few minutes to several hours [15]. For a final desired temperature below 100 °C, the process is characterized as low-temperature pretreatment (Table 2). Correspondingly, a high-temperature pretreatment occurs when sludge's temperature is increased from the ambient temperature to the range of 100 to 210 °C [13].

Furthermore, heating is provided with the aid of steam passing through heat exchangers or its direct injection to the sludge [16,18]; temperature rising with autoclaving and radiant heating with the aid of electric heat is also conducted [70]. It should be underlined that a successful and effective thermal sludge disintegration is highly dependent on the applied temperatures and time [13,126,131]. In addition, besides the beneficial contribution of TH in solubilization of organic compounds, this technology attains in increasing solids reduction during AD and thus, in the reduction of the digestate solids produced, easing handling and transportation costs [41]. TH also lowers sludge viscosity, which enables higher solids concentration (10–12%) to be fed to the anaerobic digester, without creating obstacles during digestion stirring [132]. Other advantages of TH pretreatment include enhanced dewaterability and sludge sanitation (pasteurization) as a result of (at least partial, but significant) pathogen removal [130,132–134]. Inactivation of pathogens allows for the production of Class A biosolids, that are defined by United States Environmental Protection Agency (USEPA) (Part 503 Regulation) as safe for agricultural reuse [135]. Additional TH-related observations include increased sludge biodegradability and biogas production. [18,127,131]. Nonetheless, the high energy required for sludge heating (i.e., steam) at high temperatures makes thermal pretreatment prohibitively expensive to implement [70]. However, this can be offset by producing high-quality biogas and incorporating a Combined Heat and Power (CHP) system. As a result, the heat recovered from the CHP units cooling systems and exhaust gases, cover all the heating needs, and thus, reducing the overall cost of thermal pretreatment application [14,136].

5.2.1. Low-Temperature Sludge Pretreatment

Although most TH research has been conducted at high temperatures with increased pressure and treatment duration less than 60 min, low-temperature hydrolysis (LTH) in the range of 50 °C to 95 °C has also been studied. In view of the high energy consumption entailed by high-temperature treatment and the possibility of recalcitrant compounds and inhibitory intermediates formation, pretreatment at low temperatures has been scrutinized, aiming to overcome these drawbacks [18,125,126,131,137,138].

In Liao et al. [131] the thermal pretreatment of high solids sludge (15% TS) at temperatures ranging from 50 to 80 °C for a duration up to 90 min was examined. Liao et al. concluded that SCOD proportionally increased with increasing temperature from 50 °C to 60 °C, 70 °C and 80 °C for 30 min of pretreatment duration. In this temperature range, the COD solubilization increase was found to be positively correlated to biodegradability, considering that the organic matter became more accessible for anaerobic microorganisms. Thus, the observed increase in biogas production was also expected. More specifically, the biogas production increment reported after 30 min of pretreatment, was 7.3%, 15.6%, and 24.4% for 60 °C, 70 °C, and 80 °C, respectively. In addition, they concluded that low-temperature pretreatment can effectively accelerate hydrolysis rate, given that the kinetic constant at 70 °C and 80 °C was doubled. Furthermore, Prorot et al. [139] reported the increase in SCOD as primarily responsible for the acceleration of biogas production rate in the temperature range of 65 to 95 °C and treatment duration of 20 min. However, despite the organic matter solubilization and improved substrate availability, the impact of heat on sludge biodegradability was limited, considering that AD of both raw and pretreated sludge led to almost similar biogas production. This observation was assigned to the fact that thermal pretreatment promoted only a de-flocculation of macro-flocs and therefore not a sufficient floc and EPS breakage/disintegration.

In the study of Ruffino et al. [125] increased treatment durations (1–15 h) at temperature values of 70 °C, 80 °C, and 90 °C were tested and the increase in both temperature and time were revealed to positively affect the portion of SCOD. The changes in SCOD were expressed by means of DD_{COD} parameter. Specifically, after 1 h of pretreatment, the DD_{COD} increased by 15% for both 70 °C and 80 °C, while for 90 °C, the increment observed was equal to 19%. However, when Ruffino et al. increased the tested treatment duration at 15 h, surplus increments of 10%, 13%, and 11% were observed for each examined temperature value. In addition, the obtained results from batch tests, indicated that when the sludge was treated at 70 °C, 80 °C, and 90 °C for 3 h, corresponding incremental changes of 18.9%, 22.7%, and 26.1% in biogas production and 21%, 29.2%, and 31.4% in methane production were observed. Accordingly, Liu et al. [140] also examined the effect of LTH on AD performance and achieved a 3-fold and 5-fold increase in methane productivity when sludge was pretreated at 90 °C for 1 h and 36 h, respectively. A proportional increase with prolonging the pretreatment duration was also noted for SCOD concentration. In both studies, SCOD rose dramatically only in the first 1 to 3 h, experiencing a slight increase from thereafter. Therefore, it could be assumed that an enhancement in biogas production is not only explained by an increase in solubilization but also by prolonged treatment durations, increasing this way sludge's biodegradability [18,19].

Furthermore, TH appears as responsible for changes in the rheological properties of sludge [141,142]. Specifically, recent studies reported the decrease of apparent viscosity with LTH [125,143] and mentioned that this can lead to reduced stirring power requirements and thus, to reduced energy consumption [131].

5.2.2. High-Temperature Sludge Pretreatment

Among the numerous studies that applied high-temperature hydrolysis (HTH) for sludge pretreatment to improve its solubilization and biodegradability during AD, the majority of them reported significant effectiveness for temperatures ranging from 160 to 180 °C and treatment duration in the range of 20 to 40 min. The concomitant developed pressure as a result of these temperatures ranges between 600 and 2500 kPa [144].

HTH's impacts documented throughout the scientific literature [130] include improved sludge biodegradability and organic matter solubilization (i.e., primarily of carbohydrates and proteins), as well as particle size reduction due to sludge de-flocculation. Nevertheless, adverse effects mainly associated with the formation of refractory compounds that promote biodegradation's inhibition instead of enhancement, have also been reported. Indicatively, Carrere et al. [145], conducted batch experiments using pretreated sludge at the temperature range of 60–210 °C and demonstrated that once the temperature did not exceed 190 °C, sludge solubilization and biodegradability were positively influenced. The increase in solubilization and biodegradability has been linked to an increase in methane production. The latter finding was consistent with other studies, which reported an increase in AD as a result of increased particulate matter transfer to the bulk medium caused by HTH [18,19,42,145,146], which in turn accelerated digestion rate, reducing the HRT [127]. However, when Carrere et al. pretreated sludge at 210 °C, the Maillard reactions that occurred weakened biodegradability due to the production of non-biodegradable high molecular weight polymers such as melanoidins. Melanoidins are products of carbohydrate and amino acids polymerization, which can even inhibit the degradation of other organics and produce color changes [147,148]. An incremental trend regarding solubilization was also revealed by Lu et al. [129], when both temperature (130 °C to 170 °C) and treatment duration (10 to 60 min) were increased. Furthermore, as evidence of temperature's effect, an incremental difference of approximately 25% in biogas yield was noticed from the bottom temperature limit to the upper one examined, under a constant reaction time of 30 min. In contrast to the study of Carrere et al. [145], Maillard reactions did not occur in such an extent that could provoke inhibition, since the temperature range studied was kept below 190 °C. However, there are studies reporting that although further increase in biodegradability and methane production is prevented with rising the temperature up to 220 °C, sludge solubilization continues to grow, as a result of the production of soluble but recalcitrant compounds that do not contribute in bioconversion to methane [19,149,150]. Finally, it is also worth noting that Maillard reaction products develop at either the ideal temperature range (160–180 °C) or lower temperatures. As demonstrated by a few recent studies [133,151], the refractory soluble COD (rsCOD) tends to increase as temperature rises within the range of 160 °C to 180 °C [135]. Particularly, Toutian et al. [151] determined a 3.9 to 9.4% increase in rsCOD from 130 °C to 170 °C. Accordingly, treatment durations from 1 to 7 days and temperatures ranging between 55 °C and 70 °C, may also lead to similar and sometimes lower biodegradability in comparison to the untreated sludge [19].

The type of sludge is another factor that influences pretreatment effectiveness and subsequent AD performance. The inherent nature of primary sludge which contains easily biodegradable components, renders its disintegration with HTH of little use/interest, compared to WAS. Indeed, it has been reported that AD performance is fainted when pretreated mixed sludge is used, while the improvement over the untreated sludge is more pronounced for solely pretreated WAS [41]. This fact was also supported in the comprehensive review of Devos et al. [135], where VS reduction increase (in %) was used as performance indicator to assess the impact of sludge type on AD efficiency, based on the temperature range at which sludge was pretreated. The conclusions reached were result of a compilation of literature data that were statistically analyzed through Kruskal–Wallis and Wilcoxon tests. For WAS, the authors reported that in the temperature range of 160 °C to 200 °C, the increase in VS reduction values was significantly greater than that reported at 100–140 °C, while no significant difference was observed between the ranges of 140–160 °C and 160–200 °C. Particularly, in ascending order of temperature ranges, an escalating increase (in percentage points) of 5 ± 3 pt.%, 14 ± 5 pt.% and 19 ± 7 pt.% was documented in VS reduction after AD due to HTH. However, such an intense increase in VS reduction was not observed when HTH was applied for mixed sludge. That was indicated by the slightly VS reduction incremental changes, which, in the temperature ranges tested, were 2.7 ± 0 pt.%, 9 ± 2 pt.%, and 8 ± 4 pt.% compared to the untreated mixed sludge [135].

Apart from sludge disintegration and biodegradability enhancements, another major benefit of HTH deals with digestate dewaterability improvement. Throughout the available literature, there is a consensus regarding the positive influence of HTH on sludge dewaterability [134,146], with more appreciable results reported above 150 °C [135,152]. Among the different authors, it is unanimously agreed that dewaterability is strongly related to EPS matrix degradation, considering its strong water binding capacity that makes it responsible for capturing water inside the sludge flocs [153]. Thus, once the temperature increases and the molecules collide with each other, the EPS matrix is broken down, releasing bound water [12]. When HTH pretreatment is performed above 150 °C, enhancement in stimulation of flocculation has also been reported as a consequence of chemical bonds creation, reducing the number of fine floc particles [154].

Owing to the aforementioned beneficial aspects and effectiveness in enhancing AD performance, HTH has been in high popularity in full-scale applications around the world. The most common commercially available TH processes include Cambi™ Thermal Hydrolysis Process (CambiTHP™) (Cambi Group AS, Asker, Norway) and Exelys/BioThelys™ (Veolia Water Technologies, St-Maurice, France). CambiTHP™ process consists of three steps, according to which sludge is first preheated in a tank to eliminate problems associated with energy consumption during pumping under pressure and heat exchanger corrosion, followed by hydrolysis with the aid of direct steam injection in a reactor, and a final depressurization in the flash tank. Hydrolysis process operates in the temperature range of 150 to 165 °C for a retention time of 20–30 min and pressure around 6 to 9 bars per batch. What enables cells to rupture relates to the shear forces created during rapid release of pressure when discharged into the flash tank [18,155].

Correspondingly, Exelys™, a continuous mode operating version of BioThelys™ batch TH system, does not employ either a preheating tank or a flash system but operates to similar temperature (~165 °C) and pressure ranges (~9 bars) with CambiTHP™ system [41,135]. Like Exelys™, BioThelys™ is capable of solubilizing organic matter, as well as producing 25–35% less solids that need to be disposed. The additional advantages when applying BioThelys™, include increased biodegradability and biogas production (30–50%) and improved dewaterability with sludge cake TS content increased by more than 7 pt.%. The energy recovery due to parallel operation of batch mode hydrolysis reactors and use of one's flash steam for preheating the other has also been underlined [18]. Several other commercialized methods operating nowadays are Lysotherm® [156], Haarslev's Hydrolysis System (HCHS), and Turbotec® [110,157,158].

5.2.3. Thermal Pretreatment—Concluding Remarks

Pretreatment of sewage sludge using thermal methods can result in significant increments in DD_{COD}, COD solubilization, as well as in biodegradability. In case of low-temperature hydrolysis, COD solubilization, biogas production and biogas production rate have been reported to increase with increasing temperature at constant pretreatment duration. However, when prolonging the pretreatment duration, a proportional increase in COD solubilization can also be induced. Thus, considering the influence of temperature and time, the selection of their values seems to be fundamental. Additionally, low-temperature hydrolysis is considered as less costly and presents lower energy requirements compared to high-temperature hydrolysis. Regarding high-temperature pretreatment, the optimum performance range is considered to be in the range of 160 °C to 180 °C and pretreatment duration between 20 and 40 min. Otherwise, when temperature exceeds 190 °C Maillard reactions may occur that can lead to the formation of recalcitrant compounds, which are soluble but non-biodegradable, deteriorating the production of biogas. It is important to note that although both low and high-temperature hydrolysis contribute to the overall enhancement of AD performance, only the latter one has already been transferred to industrial scale. One reasonable explanation deals with the extensive treatment durations applied for sludge pretreatment at low temperatures, which, on an industrial scale, may turn out to be unprofitable. Conclusively, the main effects documented when applying commercialized HTH processes, include the increase of organic matter solubilization, biodegradability and biogas production, the improvement of dewaterability and digestate quality, as well as the enhancement of VS reduction.

5.3. Chemical Pretreatments

The basic aim of Chemical Pretreatment technologies (CP) is to disintegrate sludge's floc structure with the aid of strong reagents, such as alkali and acids, as well as oxidation methods during which hydrogen peroxide, ozone, and Fenton's reagent are most commonly used (Table 3). As a result of the most opened structure induced by CP, the interaction between microbial cells and chemicals is facilitated, promoting the solubilization of cell walls and membranes, and favoring the enzymatic attacks to the intracellular content [16]. To that end, the rate-limiting step of hydrolysis is accelerated, resulting in shortened HRTs, enhanced biogas production, while dry sludge production is reduced, considering the improved bioavailability of more soluble compounds [70]. Although these positive effects and advantages are associated with ease of CP operation and simple equipment, these methods are not preferred in such extent as physical ones do, due to the purchase of chemicals that increase the operation and maintenance cost and their inability to be recovered [9].

5.3.1. Alkaline and Acid Pretreatment

Among the most common base solutions used during Alkaline Pretreatment (AP), including potassium hydroxide (KOH), magnesium hydroxide ($Mg(OH)_2$), and calcium hydroxide ($Ca(OH)_2$), that of sodium hydroxide (NaOH) is the most frequently employed, owing to its effectiveness in terms of solubilization even by using low dosages [43,159]. The positive effects of AP are mainly associated with the pH values, which in turn are responsible for modifications in the sludge structure and surface properties, as well as in the EPS electrostatic charge. A reasonable explanation of such modifications is associated with the substrate's characteristics and EPS physicochemical properties. For high concentrations of Na^+, sludge flocs' negative charge tends to increase. That is a consequence of a multivalent cations (e.g., Ca^{2+}) replacement by Na^+, resulting in a bridging breakdown and EPS matrix disruption [48]. In addition, under high pH values the deprotonation of functional acidic groups (i.e., carboxylic groups and amino groups) presented in EPS, can result in electrostatic repulsions between the negatively charge EPS and thus, enhanced disintegration [19].

Other effects provoked by high pH values include cell membrane's lipid bilayer saponification, inducing the release of intracellular components. Along with this, saponification provides swelling of solids and a consequent increased specific area, enabling anaerobic microorganisms' accessibility to substrate's biodegradable matter. Additionally, liquid phase richness in released intracellular organic molecules is related to substrate cells' inability to sustain their turgor pressure as a result of deficient protection by the EPS matrix [160]. Throughout the literature, it has been stated that AP performance in terms of DD_{COD} tends to increase with increasing the NaOH dosage [16,161]. However, when high concentrations of NaOH are used, residual amounts may cause inhibition of AD as a result of microorganisms' inactivation; thus, pH adjustment to the range of methanogenesis is necessary, leading to an inevitable increase in pretreatment cost [16]. Nevertheless, a small amount of residual alkali could be beneficial in stabilizing pH during acidogenesis process, improving the buffering capacity of the system [57].

AP's effectiveness and influence on AD performance has been investigated by Shao et al. [162] and the results revealed this method as promising to implement. Particularly, among the different pH values examined (8, 9, 10, 11, and 12), it was demonstrated that TSS and VSS reduction was pronounced by increasing NaOH dosage for a corresponding pH that did not exceed 11. With respect to biogas production, an increment of 15.4% was reported compared to raw sludge at pH 10, whereas a reduction of 18.1% was observed with increasing alkalinity at pH 12. Biogas production deterioration has been attributed to the formation of soluble but with poor biodegradability substances and the production of refractory compounds during Maillard reactions, without, however, affecting the increase of SCOD, as similarly observed during HTH [19,162]. Encouraging results regarding AP's effectiveness in terms of solubilization and biogas production during AD were also reported by Xu et al. [54]. They concluded that the solubilization achieved during pH value of 10 using 5 N NaOH, was the maximum observed compared to thermo-alkaline, electrochemical, and thermal pretreatments. Regarding biogas production, an increment of 41.41% was revealed compared to raw (untreated) substrate. Along with these improvements, the authors demonstrated that AP presented the highest daily biogas production rate.

Accompanying the changes in SCOD due to pretreatment, the influence of AP on solubilization of carbohydrates and proteins, which are the main components of SCOD, has also been scrutinized throughout the literature. More specifically, recent studies reported that the content of soluble carbohydrates and proteins is increased with increasing pH using NaOH, while values ranging below 10 adversely affect their solubility [43,160]. Among the reagents used during AP is also $Ca(OH)_2$. Nonetheless, results obtained when using $Ca(OH)_2$ during AP have revealed lower SCOD than this with the same NaOH dosage. That was attributed to the Ca^{2+} bivalent cations and their interactions with the hydroxyl and carboxylic functional groups of sludge flocs [163,164]. It was supported that these interactions led to dissolved organic polymers' re-flocculation, decreased COD solubilization but better dewaterability of the digestate because of calcium bridging [165].

As far as Acid Pretreatment (AcP) is concerned, the most commonly employed chemical reagents include HCl, H_2SO_4, H_3PO_4, and HNO_2 and pH ranges between 1 and 5.5 [13]. As in case of AP, during acidification, polymers are degraded into oligomers and monomers, facilitating hydrolysis and increasing hydrolysis rate [15]. Additionally, AcP has been identified as capable of solubilizing sludge and increasing proteins and carbohydrate's bioavailability as a consequence of their soluble concentration increment. Indeed, Sousa et al. [43] observed that proteins and carbohydrate concentrations increased at pH 2 up to 3.2 and 3.7 times, respectively; this observation was comparable to other studies [56,166]. In the study by Sahinkaya et al. [56], solubilization of both carbohydrates and proteins contained in WAS was increased with decreasing pH in the range of 1 to 2 using H_3PO_4. Accordingly, Devlin et al. [166] reported AcP with HCl as effective for COD solubilization and highlighted that this coincided with an increase in biogas production. During semi-continuous experiments of 21-day digestion period, an increment of 17% and 32% was presented under pH 2 and 1, respectively. Interestingly, a difference in biogas production rate was observed between the two acidic conditions; the days required for pH 2 to achieve the biogas yield observed for pH 1 by day 7, were nearly doubled. However, strong AcP at pH levels lower than 2, presuppose the use of high acid amounts, increasing the cost of pretreatment process. To avoid the inhibition of methanogenic microorganisms, an additional increase in cost is inevitable, considering the mandatory pH adjustment to alkalinity values. Amongst others, acids are associated with corrosion of equipment [8] and the need for reactors withstanding acidic conditions, increasing the capital investment cost [19]. In addition, there are studies supporting the formation of inhibitory compounds (e.g., furfural and hydroxyl-methyl-furfural-HMF) under strong acidic pretreatment. To avoid extreme pH values but meanwhile maintaining the efficiency of acid pretreatment, coupling of AcP and thermal pretreatment has been scrutinized [167,168].

5.3.2. Ozone Pretreatment

Ozonation is a well-known Advanced Oxidation Process (AOP) which utilizes the unstable molecule of ozone (O_3) as a strong oxidant agent in the field of water processing to serve its disinfection. Lately, its use in WAS pretreatment has received significant interest, considering ozone's capability to disrupt cells membranes, promote the release of intracellular material into the medium, and oxidize both particulate and soluble organic matter, contributing to the overall improvement of sludge biodegradability. Ozone's mechanism of reaction with organic substrates can be direct or indirect with the aid of highly reactive hydroxyl radicals. Hydroxyl radicals are not characterized by any selective mechanism, exhibiting the potential to react, almost without exception, with every organic compound [19]. In contrast, ozone's direct reaction depends on the structure of reactants. Specifically, ozone favors the oxidation of molecules holding double bonds between the carbon atoms, as well as of aromatics [169]. A parameter that influences AD performance and thus, needs to be controlled during ozonation pretreatment, is oxidizing reagent's dose. Increased doses usually result in increased sludge biodegradation. Nevertheless, surpassing specific dose limits might result in a drop in COD solubilization and methane yield [1,170]. Goel et al. [171] reported that an ozone dose of 0.015 gO_3/g TS in WAS resulted to a lower COD solubilization than that obtained for 0.05 gO_3/g TS; when increasing the ozone dose, improved results regarding solids reduction efficiencies (from 35 to 90%) and methane production were also obtained. However, Silvestre et al. [172] noted that an increasing ozone dose above 0.063 gO_3/g TSS negatively affected biogas production as a result of mineralization, a process that is pronounced at higher doses [154]. More specifically, this observation was attributed to mineralization's detrimental contribution in converting organic matter to CO_2 and thus, decreasing the utilizable amount of carbon being available for biogas production. Among studies, there is no consensus about the most appropriate ozone dose for maximizing biodegradation, most likely due to variations in the type and composition of sludge used [19,172]. Nevertheless, a range of O_3 doses that had revealed appreciable results in terms of methane production during the performance of batch tests under similar experimental conditions (i.e., temperature and HRT), varies from 0.0035 to 0.15 g O_3/g TS [170].

Despite the benefits of ozone pretreatment regarding the AD performance, this method is energy-intensive, due to the high requirements in energy to produce ozone (12.5 kWh/kg O_3) and transfer it to the sludge (2.5 kWh/kg O_3) [16]. In view of these high energy demands, the possibility of reducing the costs through the generation of micro/nano-bubbles has also been examined in order to facilitate ozone mass transfer as a result of the increased contact area between bubbles and liquid [15]. This way, both acceleration in the formation of hydroxyl radicals and subsequent speed up in sludge solubilization were observed. In the framework of eliminating the footprint of ozonation process, the use of in-line injectors has also been proposed. This could reduce the need for contact chambers [70]. Available commercialized ozonation systems include Aspal SLUDGE™ (Air Liquide, London, UK) and Praxair® *Lyso*™ (Praxair Technology, Inc., Danbury, CT, USA), which present improvements in terms of sludge reduction, dewaterability and energy consumption [1,173].

5.3.3. Fenton Oxidation

The Fenton process also lays among the commonly used technologies for sludge pretreatment via advanced oxidation. As partly in the case of ozonation, its mechanism of action is based on the oxidation abilities of hydroxyl radicals, which are generated through the decomposition of hydrogen peroxide (H_2O_2) by ferrous ions (Fe^{2+}) under acidic conditions [174,175]. Owing to their high oxidation potential (+2.80V), these strong oxidizers can react, non-selectively, with several co-existing recalcitrant compounds in order to disintegrate them into forms degradable by the microorganisms, inducing improvements in biogas production. Hydroxyl radicals are also effective in breaking the cell walls and thus, in releasing inner cellular materials; enhancements in sludge dewatering have also been reported [1]. In addition to these beneficial aspects, the non-toxic nature, abundance and low cost of iron element, explains Fenton process' widely acceptance and studying [2].

The main operational parameters affecting the performance of Fenton oxidation include the concentrations of the reagents and pH. Given that under extremely low pH values protons become more abundant, a subsequent radical scavenging provokes, deteriorating the oxidation capacity of Fenton process. Accordingly, when pH exceeds the value of 4, the catalytic capacity of ferric ions (Fe^{3+}) is decreased, as a result of precipitation (in form of insoluble hydrous oxides). Therefore, the optimal range is proposed to vary from 2 to 4 [176]. Both researcher groups of Dewil et. al. [177] and Pilli et al. [178], applied Fenton process for the pretreatment of WAS at pH 3 with 0.7 g Fe^{2+}/g H_2O_2, using 50 g H_2O_2/kg TS and 60 gH_2O_2/kg TS, respectively. Although the former group aimed to enhance the specific biogas production, only a slight increase of 6.8% was demonstrated with increasing H_2O_2 dosage. However, this result indicated the effectiveness of Fenton pretreatment in disintegrating recalcitrant compounds to more degradable forms. Pilli et al. [178] noticed an enhancement of 15% in methane yield compared to raw (untreated) substrate, which corresponded to 496 mL CH_4/g VS_{added}. A corresponding increment of 19.4% was also revealed in the study of Erden and Filibeli [179] for 4 g Fe^{2+}/kg TS and 60 g H_2O_2/kg TS. Usually, the higher the H_2O_2 concentration, the more pronounced the sludge disintegration efficiency is [174]. However, the reagent's excessive use may lead to adverse effects due to the scavenging of the hydroxyl radicals, lowering their concentration. The same applies for the Fe^{2+} when used without limitation [176]. In addition, the fact that this type of oxidation presupposes acidic conditions, obligates the neutralization of sludge before digestion, leading to unavoidable increase in pretreatment cost [2,16,19].

Another negative aspect associated with Fenton process is related to the formation of Fenton sludge (also called as iron sludge), which contains significant amounts of ferric hydroxide complexes. These complexes pose adverse environmental issues and restrict the digested sludge's usage options in the disposal stage [175]. To minimize to some extent the production of Fenton sludge, the use of heterogeneous Fenton process (HFP) has been proposed. During HFP, the decomposition of H_2O_2 occurs on the surface of the solid catalyst. Owing to the catalyst's structure, the electron transfer to ferric ions is facilitated, leading to regeneration of Fe^{2+} and thus, to the decrease of the ferric-hydroxide complexes. Other advantages associated with HFP include the minimization of iron ions leaching and the extension of the working pH range, preventing precipitation [176]. In view of the above, Sahinkaya et al. [174] conducted a study to compare conventional Fenton process and HFP. More precisely, both processes were implemented at pH 3, for an oxidation period of 1 h, under 4 g $Fe^{2+/0}$/kg TS and 40 g H_2O_2/kg TS. In case of HFP, zero valent iron (Fe^0) was used in form of powder. The results showed that the conventional catalysis presented better performance in terms of methane production (38%), compared to the heterogeneous one (26.8%). That was ascribed to the dissolved form of ferrous ions (i.e., Fenton's reagent) used in the former case, which facilitated the rapid oxidation of Fe^{2+} to Fe^{3+} and the simultaneous generation of radicals. In contrast, in case of HFP, the concentration of ferrous ions in the bulk solution was lower in the beginning and Fenton reactions required first the dissolution of Fe^0 to proceed [174].

5.3.4. Chemical Pretreatments—Concluding Remarks

Alkaline and acid pretreatment, ozonation and Fenton oxidation are all methods reported to enhance AD efficiency. However, their performance depends on several factors that need to be considered/controlled in order to ensure their effectiveness. The parameter reported to influence ozonation's effectiveness the most is O_3 dose, which once it is in the range of 0.0035 to 0.15 g O_3/g TS, appreciable results in terms of sludge solubilization, biodegradability and solids reduction can be obtained. Otherwise, adverse effects as a result of mineralization may occur, leading to insufficient biogas production. Regarding Fenton oxidation, the operating parameters that affect its performance are the concentration of the reagents and pH. If pH exceeds the value of 4, precipitation of Fe^{3+} occurs, deteriorating the oxidation capacity. Moreover, Fenton process is hindered in case of excessive reagent use, which can lead to low pH values and thus, to hydroxyl radicals' scavenging due to the abundance of protons. Alkaline pretreatment's effects also depend on pH values, and reagent dosage is considered as a key parameter of this method's effectiveness. Specifically, it has been stated that better efficiencies are achieved with increasing alkalinity; however, high alkali doses may also hinder the activity of microorganisms and/or lead to Maillard reactions. The inactivation of microorganisms can also happen in case of acid pretreatment due to extreme low pH values. For this reason, once the pretreatment is completed, pH adjustment to alkalinity values is mandatory. Finally, it is worth noting that among chemical pretreatments, ozonation is the only one available for full-scale applications, given that the rest methods present drawbacks associated with the corrosion of the equipment, the formation of inhibitory compounds, and high costs due to the purchase of chemicals.

Table 3. Effects of chemical pretreatment methods on anaerobic performance.

Pretreatment Technology	Pretreatment Conditions	Effects of Pretreatment	Anaerobic Digestion Conditions	Anaerobic Digestion Performances	References
Alkaline pretreatment	- Type of sludge: WAS, Mixed Sludge - Reagents: NaOH, KOH, $Mg(OH)_2$, $Ca(OH)_2$ - Doses reported: 0.1 mol NaOH/L, 157 g NaOH/kg TS, 4 mol NaOH/L, 8 g NaOH/m^3 wet sludge - pH: 8–12	- DD_{COD} increase by 22.3–26.9% - 2-fold to 8.7-fold increase in SCOD - Increase in SCOD/TCOD ratio - Reduced sludge viscosity	- AD: Batch, (semi) continuous - Scale: Laboratory - Temperature: Thermophilic, Mesophilic	- VS reduction: 11.5–133% - TSS and VSS removal up to ~13% - Increased biodegradability - Decreased biodegradability in high alkali doses - Biogas production increase up to 41.41% - Biogas yield: 1.5–15.4% - Methane production: 13–120% - Methane yield increase up to 83.3%	[10,13,15,16,19,54,162]
Acid pretreatment	- Type of sludge: WAS - Reagents: HCl, H_3PO_4, HNO_2 - Doses reported: 0.011 mmol HCl/g TS, 8.75 mL HCl/kg wet sludge - pH: 1–2	- 3.2-fold to 4-fold increase in soluble carbohydrates - 3.7-fold to 6-fold increase in soluble proteins - Up to 3-fold increase in SCOD	- AD: Batch, (semi) continuous - Scale: Laboratory - Temperature: Mesophilic	- VS reduction up to 5% - Biogas production: 12–32% - Methane yield increase up to 14.3%	[16,19,43,56,166]
Ozonation (O_3)	- Type of sludge: WAS, Mixed Sludge, Sewage Sludge - Doses reported: 10 mg O_3/g TSS, 0.09g O_3/g MLSS, 0.1 gO_3/g COD, 15–180 mgO_3/g TS, 0.0035–0.15 gO_3/g TS - pH: 11 - Duration: 1–3 h	- DD_{COD} increase up to 18% - 2.7-fold to 28-fold increase in SCOD	- AD: Batch, Batch F/I0.8, lab–scale AS–MBR, (semi) continuous - Scale: Laboratory, Pilot, Full - Temperature: Mesophilic, Thermophilic	- VS reduction up to 9% - 1.6-fold VSS reduction - Biogas production: 8–200% - Methane production: 5–80% - Up to 1.8-fold increase in methane yield - Up to 2.2-fold increase in methane production rate	[10,13,15,16,19]
Fenton oxidation	- Type of sludge: WAS - Methods: Conventional and Heterogenous Fenton process (HFP) - Doses reported: 46–114 H_2O_2/Fe^{2+} molar Ratio, 60 g H_2O_2/kg TS and 0.07 g Fe^{2+}/g H_2O_2, 4.2 g Fe^{2+}/kg TS, 60 g H_2O_2/kg TS, 40 g H_2O_2/kg TS and 4 g $Fe^{2+/0}$/kg TS, 50 mg H_2O_2/g TS, 7 mgFe^{2+}/g TS - pH: 2–3 - Duration: 30–60 min	- DD_{COD} increase up to 23.6% for Fe^{2+} - DD_{COD} increase up to 16.7% for Fe^0 - 11.9-fold to 44-fold increase in SCOD	- AD: Batch, (semi) continuous - Scale: Laboratory - Temperature: Mesophilic and Thermophilic	- VS reduction: 31–72% - Biogas production increase up to 30.2% for Fe^{2+} - Methane production increase up to 38.0% for Fe^2 - Biogas production increase up to 24.4% for Fe^0 - Methane production increase up to 26.8% for Fe^0 - Biogas yield: 1.5–15.4% - Methane production: 10–38 % - Methane yield increase up to 15%	[10,12,13,15,16,19,178]

5.4. Biological Pretreatments

Pretreatments progressively gaining the scientific/research interest over the last decade are those utilizing aerobic, anaerobic, and enzymatic methods (Table 4) to speed up sludge hydrolysis and enhance AD. The increasing attention is attributed to their promising results and environment-friendly nature [180], as well as the fact that these methods produce non-toxic wastes comparing to chemical pretreatment methods. However, biological pretreatments' capability to solubilize the organic particulate matter, improve methane production, and operate under mild operational conditions does not negate the fact that enzymes' high prices and the need for additional equipment (e.g., pretreatment aeration devices or a second digester) may limit those processes' cost-effectiveness [20].

5.4.1. Aerobic Pretreatment

Aeration pretreatment (also called pre-aeration) is one of the biological pretreatments capable of converting sludge's particulate organic matter into biodegradable forms, improving the rate-limiting step of hydrolysis and enhancing AD start up [21]. This technology involves substrate's treatment prior to AD in the presence of oxygen, with the aid of facultative anaerobic and aerobic microorganisms already presented in sludge. Essentially, pre-aeration facilitates the decomposition capacities of the endogenous hydrolytic microbial communities, improving their hydrolytic activities through the excretion of extracellular enzymes such as proteases [20]. Aeration has been reported to be performed using either air stones [21], compressed air [181], or open flasks [182]; air or oxygen injection has also been reported [183]. Oxygen requirements are ascribed to its use as a final electron acceptor so as facultative microorganisms would carry out the metabolic processes needed to degrade the organic matter and ensure their growth [184].

Studies that examined WAS or mixed sludge pretreatment by means of pre-aeration deal with the stimulation of sludge's inherent enzymatic activity under the combination of oxygen supply and temperatures that do not exceed 70 °C. This way, the hydrolytic enzymes produced by enzyme-excreting microorganisms degrade organic matter that otherwise would remain recalcitrant and could not be utilized under anaerobic conditions. In view of the above, this process is also referred as "autohydrolysis" owing to the utilization of sludge's inherent hydrolysis potential [15,144]. A proposed autohydrolysis process set-up includes trials that apply aerobic conditions in thermophilic reactors before AD performance in mesophilic ones. This process is commonly reported as thermophilic aerobic digestion combined with mesophilic anaerobic digestion, with the abbreviation of TAD-MAD [20].

Within this context, Carvajal et al. [182] investigated the effect of such treatment on WAS at lab scale to stimulate enzymes that catalyze organic matter hydrolysis and enhance AD. Indeed, after a 12 h of autohydrolysis the COD solubilization was improved by 39%. The effect of pretreatment was also reflected as methane productivity enhancement up to 23%. Similarly, Jang et al. [181] studied at lab scale the effect of aeration on mixed sludge, aiming to improve the subsequent MAD. Aeration was carried out at 55 °C in 2-L reactors with a 1-day HRT and an air flow rate of 2.5 $L_{air}/L_{reactor}$/min. These conditions resulted in higher microbial community diversity, enhanced hydrolytic activities and sludge biodegradability. In addition, an increment of 13% in methane yield was reported, while the methane production rate achieved after 19 days was 42% higher than that displayed by the control during a 20-day HRT operating MAD. Another proposed set-up for pre-aeration performance, includes the injection of air directly into the anaerobic digester for a predefined period, which is then cut off for AD to begin [185,186]. Both research groups of Montalvo et al. [185] and Barati Rashvanlou et al. [186] who performed this kind of reactor set-up obtained appreciable results, revealing increments in methane production of over 200% compared to the untreated sludge.

Another type of aeration pretreatment is defined as "micro-aeration" and involves the infusion of restricted quantities of air or oxygen into the bioreactor throughout the entire anaerobic digestion process [187]. Therefore, both aerobic and anaerobic activities are enabled and take place within the same bioreactor [188]. Although the conventional consideration is that oxygen inhibits methanogens that are strict anaerobes, there are studies reporting that limited quantities can be beneficial [21,183]. For instance, Morello et al. [189] evaluated at lab-scale (using 500 mL batch reactors) the effects of micro-aeration on degradability of sewage sludge and subsequent methane production, applying Air Loading Rates (ALRs) in the range of 0–16.83 L_{air}/kg VS_{added} throughout the 30-day AD process. To avoid overpressure, micro-aeration was performed after biogas sampling, 3 to 5 times per week. The results indicated that ALRs over 1.37 L_{air}/kg VS_{added} were inhibitory. The ALR of 1.37 L_{air}/kg VS_{added} was also the rate that was revealed as the threshold at which the highest increment in methane production was observed, equating to a percentage of 19%. This observation was ascribed to the contribution of facultative aerobic consortia to the degradation of organic matter, enhancing hydrolysis. Regarding VFA concentrations, it was reported that at low ALRs, VFA accumulation was controlled, which was consistent with the higher methane production obtained at these oxygen doses.

5.4.2. Two-Stage Digestion

AD is typically a single-stage biological process during which all the reaction steps (i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis) and anaerobic activities occur within the same bioreactor, without being physically separated. However, in an attempt to intensify and enhance AD efficiency by improving hydrolysis step, temperature phased anaerobic digestion (TPAD) is commonly implemented [144]. According to TPAD, digestion process is compromised by a digester that operates at thermophilic or hyper-thermophilic temperatures and is in series with a mesophilic AD reactor. Essentially, the first digester, which is considered as the pretreatment step, is fed with the raw substrate and the effluent is subsequently transferred to the mesophilic one [16,20]. The first digester is designed to provide the conditions needed to primarily promote the steps of hydrolysis and acidogenesis, while the second mainly supports AD's steps of acetogenesis and methanogenesis [19,190]. The thermophilic temperature range commonly varies between 45 °C and 70 °C and a typical design uses HRTs of 9 h to 48 h. These HRTs are long enough to favor hydrolytic and acidogenic microorganisms rather than acetogens and methanogens, owing to the formers' faster reaction kinetics. Accordingly, retention times between 14 and 30 days are applied for mesophilic digestion [19].

This pretreatment strategy provides better pH control and thus, increased stability during mesophilic digestion, ensuring that methanogenic activity is not suppressed. Meanwhile, TPAD allows for higher OLRs as a consequence of accelerated hydrolysis due to elevated temperatures and enhanced organic solids destruction through thermophilic stage. Along with these benefits, TPAD possesses advantages such as shortened HRT, higher methane production and inactivation of pathogens, rendering the digestate utilizable for soil conditioner [9,190]. Despite these benefits, this kind of pretreatment configuration has not been extensively applied in pilot and full scale due to its infancy and concomitant complexity. That draws the attention for further examination in terms of parameter optimization and energy balance [1].

Up to now, several studies have been carried out aiming to investigate the benefits resulting from the utilization of a TPAD configuration. In the study of Hameed et al. [30], two TPAD systems of two semi-continuous reactors were examined individually. The first reactor in one system was operated at 45 °C (TPAD-I), while the other was operated at 55 °C (TPAD-II). The second reactor of each system was operated at mesophilic conditions (i.e., 35 °C). The obtained results demonstrated that both TPAD-I and TPAD-II were efficient and presented almost the same levels of specific methane yield. However, TPAD-I exhibited higher methane production rate, equal to 3.55 ± 0.47 L $CH_4 \cdot L^{-1} \cdot day^{-1}$. Additionally, TPAD-II appeared instabilities because of increased VFA and NH_3 levels.

The detected differences between the two system performances were attributed to the initial temperatures, which dictated the microbial community composition changes and diversity in each system. Therefore, based on the dominating members of core microbial population, the adaptation speed under operational conditions and susceptibility to instabilities differed [30]. According to another study, the first phase in the TPAD system examined was operated at the hyper-thermophilic temperature of 70 °C. It was concluded that the pretreated sludge presented increased COD solubilization, up to 40% of the influent particulate matter. Additionally, increments between 20% and 50% were achieved in biogas production compared to those revealed from the conventional single-stage mesophilic and thermophilic AD systems studied [191]. The TPAD system was also examined by Ge et al. [192] who conducted a systematic analysis to find the first digester's optimum operating conditions. They concluded that the highest performance in terms of biodegradability and methane yield during the subsequent mesophilic AD, can be obtained after anaerobic pretreatment at 65 °C for 1 to 2 days and pH 6–7. In the framework of TPAD processes, other set-up options proposed include two-stage thermophilic AD, as well as inverted phase fermentation (IPF). Both configurations have shown interesting results regarding biogas production increment, effluent sanitary quality, and HRT reduction [20].

5.4.3. Enzyme-Assisted Pretreatment to Enhance AD Performance

Given their primary capability to degrade complex organic matter and compounds of high molecular weight into simpler forms and function as catalysts for several biochemical reactions, hydrolytic enzymes are of vital importance during AD [193]. However, several refractory compounds presented in sludge resist in enzyme attack, rendering hydrolysis step as rate-limiting, which in turn deteriorates the overall AD performance. Considering this, research regarding the addition of exogenous enzymes has been conducted to address constraints associated with the rate-limiting step of hydrolysis. Indeed, several studies have obtained promising results and revealed that enzyme-assisted pretreatment strategy appeared to improve biomass conversion, COD solubilization and enhance both biogas and methane production [194,195].

As listed in the comprehensive review of Bremond et al. [20], there are four ways to perform enzyme addition (Figure 9). Specifically, substrate's pretreatment can be conducted either in a separate vessel or with the direct addition of enzymes in the bioreactor of a single-stage AD or in the first digester of a TPAD system. Sludge pretreatment through the addition of enzymes in the leachate recirculation system, has also been reported as an option. Compared to other pretreatment methods, enzyme-assisted pretreatment offers advantages associated with lower energy demands and no chemical requirements, while eliminating the formation of toxic by-products. Nonetheless, the cost of this method remains quite high. This is attributed to the fact that the enzymes utilized (i.e., proteases, amylases, lysozymes, or enzyme blends) are commonly commercially prepared, rendering this method unfeasible to implement in full-scale applications. A promising alternative that can be concerned as a proper way to control the cost of this pretreatment method, is bioaugmentation [16]. This technique can be defined as the enrichment of endogenous specific enzymes or concentration increase of specific indigenous microorganisms that can secrete such enzymes [196]. In this regard, Yu et al. [197] fermented *Bacillus subtilis* and *Aeromonas hydrophila*, which were isolated from WAS, to produce two enzyme mixtures rich in amylase and protease, respectively. When these mixtures were used in combination to pretreat sludge for 28 h of incubation time at 37 °C, the subsequent mesophilic AD resulted incremental changes in both SCOD and biogas production. Specifically, after 11 days of AD an increase of 23% in biogas production was revealed compared to the untreated substrate. Similarly, Yin et al. [198] investigated the effect of in-situ produced fungal mash on WAS hydrolysis and subsequent AD performance. A reduction of 19.1% in VS and an increment of 53% in methane yield were achieved.

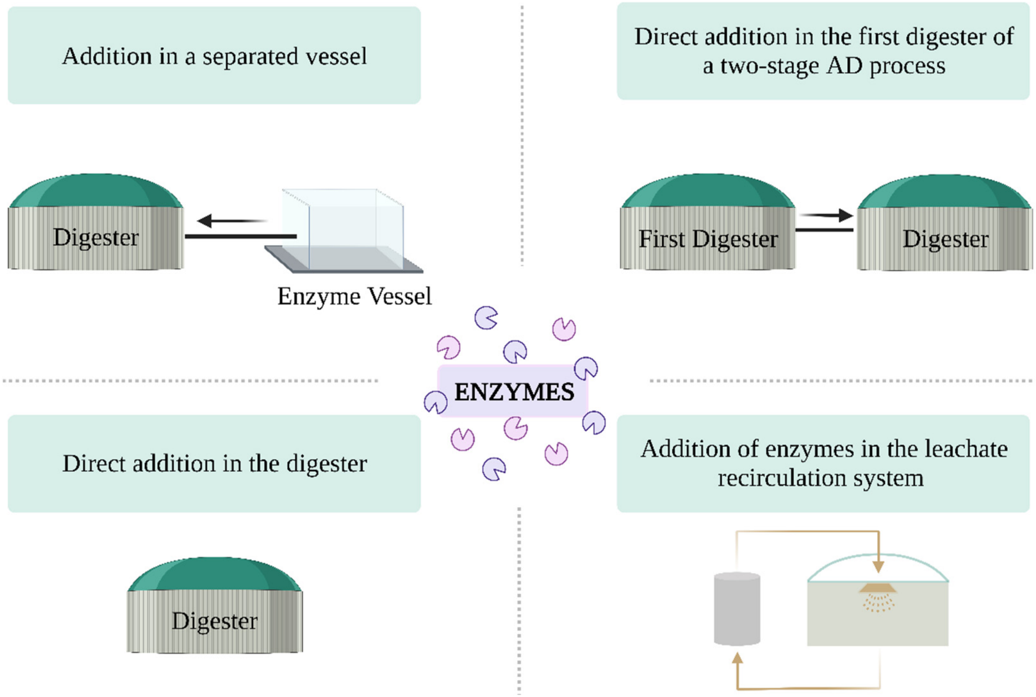

Figure 9. Different ways to perform enzyme addition to enhance AD performance.

Since not all the molecules can serve as proper substrates for an enzyme due to the latter's specificity, the addition of appropriate enzymes is of high importance for an effective pretreatment. In light of this and the fact that sludge mainly consists of carbohydrates, proteins, and lipids, the enzymes examined for the respective degradation of these constituents were mainly amylases, proteases, and lipases [15]. However, owing to the complex composition of WAS, the use of individual enzymes does not always result in significant hydrolytic activity and the addition of enzyme mixtures seems more promising for efficient pretreatment. Referring to the results of Yang et al. [199], the synergy of amylase and protease in a mixture ratio of 1:3, revealed better results in terms of both COD solubilization and VSS reduction, than those obtained when these enzymes were separately used as (bio)catalysts. During sole addition, however, the authors concluded that enzymatic pretreatment efficiency with amylase outperformed that of protease. Additionally, although enzymes can function in wide pH and temperature ranges, effective hydrolysis presupposes the environmental conditions where the different enzymes act optimally; otherwise, their inactivation or even denaturation occurs. Enzyme dosage also presents a factor affecting pretreatment, as the more it increases, the more the active sites for hydrolysis to take place exist [195]. However, a reasonable addition of enzymes should be determined since further increases have been observed to induce insignificant higher efficiencies regarding biodegradability, solids reduction and methane production. Thus, from an economic standpoint, the lowest possible dosages should be determined and preferred [199]. In addition, enzyme dosage is highly dependent on the operational conditions occurring. Specifically, hydrolysis activity tends to diminish when enzymes operate under suboptimal levels of pH and temperature. Therefore, higher enzyme dosing is needed to achieve comparable hydrolysis efficiencies to those accomplished under optimum conditions; otherwise, a rise in the overall pretreatment cost is inevitable [195].

On account of the abovementioned aspects and WAS composition complexity, it is reasonably acceptable that for an effective enzymatic pretreatment, a combination of appropriate choices in terms of enzyme mixture type, ratio, dosage, as well as pH and temperature ranges, is needed. For this to happen, further research is still required to define specific enzymes for specific substances contained in WAS [3]. This way, it could be feasible to draw enough information to customize a proper blend of enzymes for WAS pretreatment. To obtain a more comprehensive insight research in assessing each operating parameter's individual effect on different enzymes is also necessary. This could encourage the establishment of an optimum operational range for each parameter, that would be a compromise and tolerate by the enzymes involved, while also favoring the activity of their major proportion, in an effort to maximize hydrolysis efficiency and overall AD performance.

5.4.4. Biological Pretreatments—Concluding Remarks

All the biological pretreatment methods reviewed in this section appear to be efficient in enhancing methane production. However, aeration is reported to exhibit the most appreciable results, revealing increments that can even reach 200%. This significant efficiency can be attributed to the activity of facultative anaerobic microorganisms, which in the absence of oxygen would be negligible. On the other hand, the enhanced AD performance provoked by temperature phased anaerobic digestion can be assumed that associates to this process's configuration, which allows for better pH control, preventing the formation of inhibitory compounds. Along with this benefit, two-stage digestion possesses advantages associated with low energy demands, while it is reported to sterilize sludge providing biosolids free of pathogens. However, this process is considered slow and requires the use of additional equipment, which in turn limits its cost-effectiveness. Similar to temperature phased anaerobic digestion, the high price of the enzymes utilized for enzymatic pretreatment remains a principal drawback, which negatively affects its cost. Nonetheless, this method appears promising in improving COD solubilization, biodegradability, biogas, and methane production. To ensure, such improvements, however, the enzyme mixture type, ratio, and dosage, as well as pH and temperature ranges applied, should be selected with special consideration.

Table 4. Effects of biological pretreatment methods on anaerobic performance.

Pretreatment Technology	Mechanism Involved and Pretreatment Conditions	Effects of Pretreatment	Anaerobic Digestion Conditions	Anaerobic Digestion Performances	References
Temperature-Phased Anaerobic Digestion (TPAD)	- Type of sludge: WAS, Mixed Sludge - Two–stage anaerobic process - First reactor: sludge pretreatment under thermophilic AD conditions (hydrolysis and acidogenesis) - Temperature: 45–70 °C - Time: 9h to 6 days - Second reactor: mesophilic AD (acetogenesis and methanogenesis) - Temperature: 37 °C - Time: 14–45 days - Other TPAD systems reported: two–stage mesophilic or thermophilic digestion	- 4.5-fold to 8.3-fold increase in SCOD - Low energy demand and low-quality thermal energy requirements - Sterilization of pathogens	- AD: Batch, (semi) continuous - Scale: Laboratory, Pilot - Temperature: Thermophilic, Mesophilic	- VS reduction: 10–70% - Biogas production: 11–50% - Methane production increase up to 85% - Methane yield: 11–54%	[1,3,12,16,19]
Aerobic pretreatment	- Type of sludge: WAS, Mixed Sludge - Mechanisms: - Pre–aeration (autohydrolysis) - Performances: in separate vessel/reactor, in the same reactor for predefined period prior to AD - Air flow: 72–300 L/h - Duration: 12 h to 2 days - Temperature up to 70 °C - Means of aeration: air stones, compressed air, open flasks	- Increase in SCOD - Increased microbial diversity	- AD: Batch, (semi) continuous - Scale: Laboratory, Pilot - Temperature: Mesophilic	- VS reduction: 11–67% - Methane production: 19–211% - Methane yield: 13–122%	[3,10,15,20,21,181–183,185,186]

Table 4. *Cont.*

Pretreatment Technology	Mechanism Involved and Pretreatment Conditions	Effects of Pretreatment	Anaerobic Digestion Conditions	Anaerobic Digestion Performances	References
Aerobic pretreatment	- Micro–aeration - Performance: throughout the entire AD process - Air loading rates: 0.68–1.37 Lair/kg VS_{added} - Temperature: 35 °C - Duration: 30–day AD - Means of micro–aeration: air or oxygen injection				[3,10,15,20,21,181–183,185,186]
Enzyme-assisted pretreatment	- Type of sludge: WAS, Mixed Sludge - Source of enzymes: - Commercialized enzymes (proteases, amylases, lysozymes or enzyme blends) or bioaugmentation - Enzyme addition: direct, in separated vessel, in the leachate recirculation system - Temperature: 35–60 °C - Duration: 3–36 h	- Up to 4-fold increase in SCOD/TCOD ratio - 1.3-fold to 47-fold increase in SCOD - Increased SCOD release with incubation time	- AD: Batch, (semi) continuous - Scale: Laboratory, Pilot - Temperature: Mesophilic, Thermophilic	- VS reduction up to 58% - VSS reduction: 10–68.43% - TSS reduction up to 17.14% - Biogas production: 12–20% - Biogas yield: 23.1–40% - Methane yield increase up to 53%	[3,10,16,19,20,197–199]

5.5. Combined Pretreatment Methods

Although the pretreatment methods are commonly applied individually as standalone procedures, the sequential or simultaneous application of some different methods has also been scrutinized [9]. The combined pretreatment has been of high popularity owing to better efficiencies in terms of sludge hydrolysis, solubilization of organic constituents, biogas and methane yield, compared to when these methods are applied alone [54,161,200–202]. Additionally, coupling of single pretreatments in some cases, enables the improvement of some limitations that occur during the implementation of individual methods, which are usually associated with increased energy consumption, high capital, investment and operation costs [12]. For example, when alkaline pretreatment is combined with the thermal one, it provokes a synergistic effect that allows reducing both the energy requirements and alkali reagent quantity, without adversely affecting the pretreatment efficiency [22]. Therefore, considering the beneficial aspects arising from combined pretreatment, several studies have been conducted to evaluate these integrate systems, with the most common (Table 5) to include the mechanical-thermal [200,202,203], mechanical-chemical [123,202,204], and thermo-chemical combinations [51,205].

Mechanical and thermal coupled pretreatment takes advantage of the shear forces created by mechanical methods to disintegrate sludge structure and disrupt the cell walls, as well as of heating to further soften the sludge components, promoting an even increased solubilization before AD. For instance, Kim and Youn [202], studied the performance of thermal pretreatment on WAS for increasing temperature from 30 °C to 90 °C and treatment duration of 0, 1, 2, and 3 h, in a sequential combination with ultrasonication at power of 0.4 kW/L for 20 min. The results revealed an increment of 30% in sludge hydrolysis, compared to the individual ultrasonic pretreatment. In addition, the authors highlighted that irrespectively to thermal duration, SCOD presented an increasing trend from 30 °C (22–24%) to 50 °C (28–30%), whereas a further increase up to 90 °C did not offer any additional incremental changes. Finally, they concluded that heating plays a crucial role in solubilization; meanwhile, it was mentioned that the higher the temperature and the longer the treatment time was, the more the ultrasonic cavitation effect was deteriorated, due to the increase of vapor content in the cavitation bubbles.

The order the pretreatment methods are applied also affects the overall effectiveness. In the study of Mostafa et al. [201], it was demonstrated that by altering the sequence of thermal-ultrasonic pretreatment set, solubilization was positively affected, presenting a surplus increment of 4%. That was assigned to ultrasonication, which led to a bulkier solids content than that resulted when it was not applied prior to thermal pretreatment, thereby increasing the availability of compounds for heating.

The concomitant performance of mechanical and chemical pretreatment methods has also been evaluated as alternative for enhancing the disintegration of sludge. Specifically, accompanying the mechanical forces that assist the breakdown of sludge flocs, the use of chemical reagent(s) presents the additional benefit of accelerating the rate-limiting step of hydrolysis during the anaerobic bioconversion, rupturing the cell walls and increasing the solubility of inner cellular components. Along with the improvements in solubilization, several studies that examined such integrated/combined systems have demonstrated improved biogas quality and thus, increment in the methane content [70]. The combination of ultrasonication with ozonation (UO) [55], Fenton's oxidation (UF) [206], or alkaline pretreatment (UA) [200,207] and the application of microwaves (MP) or high pressure homogenization (HPH) with concomitant base dosing [123,161,204,208] are all examples of mechanical/chemical mechanisms integration that have presented appreciable results of effectiveness.

Due to the pivotal role of alkali addition in COD solubilization [43], there are several studies that have focused on coupling mechanical with alkaline pretreatment (AP). In the study of Babu et al. [200], it was demonstrated that ultrasonication at pH 12 with NaOH addition presented an improved SCOD, compared to that obtained by the individual ultrasonication. Specifically, it was mentioned that ultrasonic pretreatment aided in the flocs breakage, which led to a more opened structure and promoted the OH^- attacks, resulting in a better disintegration. Similarly, Jang and Ahn [208] reported the synergistic effect of MP and AP, which resulted in an increase in SCOD/TCOD ratio (up to 18 times), compared to the untreated sludge. The former's thermal and non-thermal effects caused the rupture of cell walls and membranes, while AP further pronounced the weakening of cell walls, the solubilization of their compositional materials and the release of intracellular components, mainly due to the lipid bi-layer saponification. A significant increase in methane yield was also revealed.

Accordingly, Dognan and Sanin [204] conducted batch experiments and demonstrated that MW in combination with AP led to a surplus ~10% increase in both SCOD and methane yield compared to when MW was applied individually. It is also worth noting that coupling of MW and AP provides an alternative option for sludge disintegration with shorter treatment time periods, less energy consumption and chemical quantities than those required at individual chemical and conventional heating pretreatment methods [123]. Finally, another studied combined pretreatment includes the coupling of HPH and AP's mechanisms. In the research study by Fang et al. [161], HPH beneficial integration with AP was quantified as an increase in the methane content, compared to that obtained when HPH was applied as a standalone procedure, indicating an improved biogas quality.

Thermo-chemical pretreatment also exhibits improved organic matter solubilization in comparison to the corresponding single pretreatments, as evidence of attaining the advantages of both methods [209,210]. Commonly, chemical pretreatment is performed with the addition of alkali agents [51,211,212], ozone, or hydrogen peroxide [70], and is combined with either low (50 to 90 °C), or high (115 to 170 °C) temperature for pretreatment [15,205]. Although high temperature chemical-assisted pretreatment (HTCP) has been reported to reveal appreciable results regrading COD solubilization and AD performance enhancement [213], it is energy-intensive and capable of offsetting the bioenergy produced by pretreatment heat and electricity needs [212]. This fact limits its implementation, drawing attention to a more viable alternative, which is the low-temperature chemical-assisted pretreatment (LTCP). This process is most frequently performed with alkali addition owing its effectiveness to the organic matter solubilization, even when using low dosages [159,212].

Among the single pretreatment methods (i.e., ultrasonication, alkaline, and low-temperature pretreatment) examined in the study of Babu et al. [200], AP was revealed as the most efficient, with the best performance in terms of SCOD. This value was significantly increased by 43% when AP was combined with thermal pretreatment. The fact that AP was applied (for 1 h) prior to heating facilitated the overall performance. A reasonable explanation of this behavior was associated with the AP-induced sludge floc disintegration, saponification, and subsequent release of intracellular components. This way, thermal pretreatment's solubilization performance was enhanced due to processing sludge rich in already defragmented constituents [201].

Recently, the positive influence of DD_{COD} when merging the positive effects of AP and high-temperature pretreatment (i.e., high-temperature alkaline pretreatment–HTAP) has also been reported. Specifically, Liu et al. [214] reported a surplus increase of 13,4% to the DD_{COD} achieved via individual thermal pretreatment. Regarding AD performance, it was revealed that when HTAP pretreated sludge was used (at 134 °C, under 3.4 bars for 30 min in an autoclave at pH 12), the methane yield obtained at HRT of 25 days was 234.9 ± 13.0 mL CH_4/g VS_{added}. In contrast, the methane yield observed after 25-day AD of the thermal pretreated sludge (at 134 °C) was 192.6 ± 13.7 mL CH_4/g VS_{added}. Although these values were decreased with reducing the HRT to 20 and 15 days, HTAP continued to present a higher efficiency than the individual thermal one. Among the several studies, Gorzna et al. [211] also highlighted that solely heating at 60 °C for 60 min revealed almost a 2-fold lower SCOD than that obtained when combined with NaOH dosing of 16 g NaOH/kg TS. That indicated the high organic matter solubilization obtained under these conditions. When this pretreatment combination was tested on semi-continuous AD trials with HRT of 15 days, an increment of 8% and 27% was detected in methane content and methane yield, respectively, compared to raw sludge. Finally, the authors concluded that a low dose of NaOH in combination with low-temperature pretreatment could be profitable for full-scale applications. That was assigned to the additional energy produced, increasing the possibility for energy independency, and the high VS reduction achieved in AD, due to pretreatment, which resulted in a digestate of a lower TS content for dewatering and disposal, hence reducing the operational costs.

Combined Pretreatments—Concluding Remarks

Upon comparing individual pretreatments to combined ones, the latter present better efficiencies. Nonetheless, taking advantage of the benefits arising from combined pretreatment presupposes the selection of methods that can provoke synergistic effects. To maximize the benefits of combined pretreatment, the synergistic mechanisms should be first thoroughly understood before coupling two different methods. Along with this, the optimum operational conditions should be determined. Finally, the sequence of the combination should also be considered, given that altering the order of two proven feasible pretreatment methods can deteriorate their efficiency.

Table 5. Effects of combined pretreatment methods on anaerobic performance.

Pretreatment Combinations	Effects	Disadvantages	References
Thermo-Chemical			
- Type of sludge: WAS, Mixed Sludge - Low temperature: 50–90 °C - High temperature: 115–170 °C - Chemicals used: NaOH, KOH, O_3, HCl, H_2O_2	- Increase in DD_{COD} - Increase in SCOD - Improved solids reduction - Significant increase in biogas production up to 630% - Increased methane production up to 154%	- Formation of non-biodegradable compounds - Cost of chemicals - High energy requirements	[10,15,51,70,205,209–212,214]
Thermo-Mechanical			
- Type of sludge: WAS, Thickened Mixed Sludge - Low temperature-assisted ultrasonication (30–90 °C) - High temperature-assisted sludge pretreatment (160–180 °C) under pressure	- Altering the sequence of pretreatments affects solubilization - Increase in SCOD - Improved solids reduction - Up to 50% increase in methane yield - Significant increases in soluble concentrations of proteins and carbohydrates	- Cavitation deterioration in high temperatures - High energy requirements	[200–203,215]
Mechanical-Chemical			
Type of sludge: WAS, Mixed Sludge Ultrasonication—acid addition	- Increase in sludge disintegration up to 40%	Need for chemicals	[10,13,15,55,123,161,200,204,206–208]
Ultrasonication—alkaline addition	- DD_{COD} increase by 10–90% - Significant increase in SCOD - Biogas production: 38–55%		
Ultrasonication—ozonation	- Increase in SCOD - Decrease in particle size and VS reduction 18–21% - Biogas production: 26–36%		
Mechanical-Chemical			
Microwave—alkaline addition	- Up to 18-fold increase in SCOD/TCOD ratio - Up to 52.5% increase in sludge solubilization - Biogas production: 44–228% - VS reduction: 28–262%	Need for chemicals	[10,13,15,55,123,161,200,204,206–208]
HPH—alkaline	- Increase in SCOD with increasing NaOH dose - Biogas production up to 47% - VS reduction: 16–41%		
HPH—ozonation	- Increase in SCOD - Biogas production: 6 % for semi-continuous tests and up to 800% for batch tests - VSS reduction: 60% for batch tests		

6. Other Emerging Technologies

The growing interest in green chemistry and green technologies has prompted the development of alternative and environmentally friendly pretreatment methods that use green solvents and fit within the concept of sustainable biomass processing. Supercritical fluids lay among the most attractive solvents fit for purpose, with CO_2 the most examined [216,217]. A method that uses CO_2 as solvent is Supercritical Carbon dioxide Explosion (SCE). SCE involves the compression of biomass with CO_2 in the reaction chamber for a predefined period, above solvent's critical pressure and temperature. These conditions allow the penetration of $scCO_2$ in the micropores of substrate's matrix and cell walls. Then, the mixture is abruptly decompressed. Once the decompression occurs, the supercritical fluid rapidly leaves the substrate matrix, provoking its quick and severe expansion. This way, the complex sludge structure and cell walls are disrupted and the release of intracellular substances is enabled, increasing their availability for microbial biodegradation [218,219].

Recently, Mitraka et al. [219] were the first to introduce the use of SCE as an efficient approach for sludge pretreatment. Particularly, this research group conducted SCE's optimization using Response Surface Methodology (RSM), in order to establish an optimum set of pretreatment conditions that would ensure an increase in the substrate's biodegradability and subsequent methane generation. It was concluded that the optimum set corresponded to temperature of 115 °C and short duration of 13 min. Under these conditions, the obtained results revealed a significant increment of 8.7% in methane yield compared to raw sludge, implying the efficient use of $scCO_2$ as an alternative green reactant for sludge pretreatment. In addition, the combination of SCE with thermal, alkaline, and acidic hydrolysis was examined. The inference reached was that all the combinations, except from that with acidic hydrolysis, contributed to a statistically significant increase in methane yield compared to that observed for untreated sludge.

The results obtained from this study clearly justified the feasibility of SCE in sludge pretreatment to make it more available for microorganism consortium during AD, as well as its potential as an attractive green alternative over classical pretreatment methods. The main advantage attributed to this method's beneficial aspect relies on reducing or even eliminating the use of organic solvents, as well as reducing downstream purification processes mainly because CO_2 can be easily separated by simple expansion to atmospheric conditions. In addition, this method benefits from the fact that CO_2 is often a waste product from many industrial processes and, as such, can be obtained with low cost. Based on that, this CO_2-based technology has the potential to contribute to a greener solution concerning the deal with the excess of CO_2 produced. In view of the above, SCE seems to be a promising approach considering the overarching goals of green chemistry, envisioning positive environmental impacts. Even though this technology demonstrates some advantages and can offer solutions for problems such as product purity, process efficiency, and environmental impact that traditional technologies [220] for biomass pretreatment fail or are unable to solve, current efforts for biomass pretreatment with SCE do not yet guarantee its economic viability. This is attributed to the large investment costs requirements, mostly due to the high-pressure equipment. Therefore, a techno-economic assessment is needed to access its potential economic feasibility and find the most adequate application of SCE in biorefineries so as to decide for a further development in commercialized level. For the widespread utilization of SCE in large scale, the implementation of process intensification concepts together with mass and energy integration should also be evaluated. Such techniques can potentially result in reduced costs due to a better utilization of installations and equipment, and to an improved process performance in general. Therefore, for a further direction of future research, it would be essential to combine these concepts with techno-economic analyses in order to obtain evaluations in terms of cost-effectiveness and environmental impact, considering the proposed technology's state of the art.

7. Economic Feasibility of Sludge Pretreatment Methods

The selection of an optimal pretreatment method depends not only on the degree of sludge biodegradation and methane conversion efficiency, but also on economics and energetic benefits. Technical performance is the first step toward assessing a pretreatment technology. However, to be economically viable, it must offer enough economic benefit to balance installation costs, operation, and maintenance costs, as well as its energy requirements [13,19,70,221]. In general, the pretreatment technologies that result in a considerable improvement of methane yields and an increase in biogas production, lead also to high operating and maintenance (O&M) costs [16]. Indicatively, pretreatment technologies with high O&M costs include ultrasonication, high temperature thermal method, high-pressure homogenization, and ozonation. In addition to that, high energy requirements remain another crucial barrier, especially for thermal, microwave, and physical/mechanical treatments, with high capital costs being an additional drawback for thermal and microwave technologies [222,223]. Chemical and biological pretreatments are low energy requirement methods, thus considered to be self-efficient and cost-effective despite their disadvantages [12,22]. However, in case of chemical technologies (acid/alkalis and oxidation with Fenton), the high cost of chemicals used results in negative profits and remains a challenge [12,16,19,223].

Table 6 provides a brief comparison of costs (investment/capital and O&M) and energy requirements between the various pretreatment methods analyzed in this paper, taking into account economic analyses conducted in previous studies. To this end, techno-economic analysis based on laboratory performance data showed that microwave, enzyme-assisted, and mechanical-chemical pretreatments showed the best economic performance [70].

Ultrasonication appears to be one of the most widely used methods, among the physical/mechanical pretreatment technologies previously described, despite the high energy requirements [3,16]. However, when the enhanced biogas generation balances the increased cost, its application can be viable. Microwave pretreatment is also characterized from high energy consumption [19]. Nevertheless, previous research has demonstrated that capital investments as well as energy requirements are lower compared to ultrasonication, thus, O&M costs appeared also reduced in small-scale systems [16,70]. Another popular physical pretreatment option is the high-pressure homogenization (HPH) process. The main characteristics of this method are the easy operation, the great energy efficiency characteristics and the lower investment and operation costs compared to the previous options [1,16,70]. Concerning thermal pretreatments, high energy demand appeared to be one of their main disadvantages, since it makes the application of this option costly. Nonetheless, the enhanced biogas recovery together with the additional high-quality biogas produced can be effectively exploited generating electricity on-site, and consequently reduce the pretreatment cost [12,16,70,222].

It should be noted that the purchase of chemical reagents increases the overall O&M cost of chemical pretreatments. However, the literature indicates that the production of high-quality biogas along with the reduction of the disposed sludge amounts can balance these additional costs [70]. More specifically, acid/alkali and Fenton oxidation pretreatments are easy to use methods, with high methane conversion efficiency, low energy requirements, and low investment cost [1,12,16,19,70], even though the costs of added chemicals remain a principal drawback. In contrast, ozonation is a more expensive option compared to other chemical processes, due to the high energy demand for ozone production [1,16,70]. The high energy requirements result not only in high operational cost, but also in increased capital costs.

Biological pretreatments are in general self-efficient methods [22] with low energy supply requirements and lower operational costs since no additional chemicals or other processing equipment are required [3,16,70]. However, previous studies indicated high installation cost, which makes their application less attractive [3,22]. Furthermore, as regards the enzyme-assisted method, it has been reported that the amount of enzymes needed increases the operational cost [12,19,70] similar to chemical pretreatment technologies.

Therefore, it is suggested that its integration with other available pretreatment technologies may result in an economically feasible option [12]. Finally, the combination of the above-mentioned pretreatment methods may provide an efficient and feasible alternative. More specifically, these integrated systems (i.e., thermo-chemical, thermo-mechanical, and mechanical-chemical) often reduce the energy demand of each method, while requiring lower capital and operational costs [12,70]. For instance, when chemical technologies are integrated with either mechanical or thermal techniques, methane production is increased significantly, while at the same time the energy demand is reduced [22]. Nevertheless, in some cases, the added costs of both processes prevent their full-scale application.

Table 6. Qualitative assessment of the energy requirements and economic feasibility of different sludge pretreatments technologies.

Pretreatment Technology	Investment Cost	Operational Cost	Energy Requirements	References
Physical/Mechanical Pretreatment				
Ultrasonication	Medium to High	Medium to High	High	[1,3,12,16,19,22,70,221–223]
Microwave pretreatment	Medium to High	Medium to High	High	[1,12,16,19,22,70,97,221–223]
High-pressure homogenization	Medium	High	High	[1,3,16,70,222]
Electro-kinetic disintegration	High	High	High	[1,16,222]
Thermal Pretreatment				
Low temperature pretreatment	Low to Medium	Low to Medium	High	[12,16,19,22,70,222]
High temperature pretreatment	High	High	High	[1,12,16,19,22,97,222,223]
Chemical Pretreatment				
Alkaline pretreatment	Low	Medium	n/a	[1,12,16,19,70,222,223]
Acid pretreatment	Low	Medium	n/a	[1,3,12,16,19,70,223]
Ozonation	High	High	High	[1,12,16,70,222,223]
Fenton oxidation	Low to Medium	High	Low	[1,12,16,19,70]
Biological Pretreatment				
Temperature-Phased Anaerobic Digestion (TPAD)	Low	Low to Medium	Low	[1,12,19,70,222]
Aerobic pretreatment	n/a	Low to Medium	Low	[3,12,70,222]
Enzyme-assisted pretreatment	Low	Medium to High	Low	[12,16,19,70,221,222]
Combined Pretreatments				
Thermo-Chemical	High *	n/a	High	[12,70]
Thermo-Mechanical	High *	n/a	High	[12,70]
Mechanical-Chemical	n/a	Medium	Medium	[12,70]

* Depending on the selected system(s).

8. Environmental Assessment of Sludge Pretreatment Methods

Apart from the high energy demand and the economic burdens of the various pretreatment technologies, their environmental impact is another important obstacle that in most cases make them unsustainable. However, to the best of our knowledge, limited information can be found available in the literature that address the environmental evaluation of sludge pretreatment alternatives [1]. Previous research has documented that the impacts related with the application of any of the above-mentioned pretreatment methods cannot be excluded when the environmental burden of a sludge management system is studied [224]. More specifically, any of the pretreatment options analyzed in this paper uses energy and/or chemical substances, thus having diverse effects both on the environment and humans.

Chemical pretreatments such as acid and alkaline techniques are recommended due to their good net environmental performance, while ozonation and thermal technologies need to be optimized in terms of energy efficiency in order to reduce their environmental impact [224]. More specifically, Carballa, Duran, and Hospido [224] demonstrated that, among chemical methods, the acid technology performs better in terms of climate change (GWP) and abiotic depletion (ADP) categories, while the alkaline option presents better results in eutrophication (EU) and toxicity potentials. Especially for the GWP category, low impacts are expected since chemical methods do not require much energy [225]. However, the ozonation option would cause greater environmental harm, since improving the AD process does not compensate for the environmental burdens associated with this pretreatment [224]. Furthermore, concerning the thermal pretreatment alternatives, the results offered by Mainardis in [226] suggest that thermal scenarios present the worst environmental performance in the categories that are influenced by the high energy supply, such as climate change (GWP) and fossil depletion (ADP). At the same time, they show a better profile for impact categories such as human toxicity (HTT), freshwater ecotoxicity (FET), and eutrophication (EP), due to the lower requirement in reagents, as well as the effects of the thermal energy generation during the process [226].

The environmental impacts of some physical/mechanical pretreatments are also presented in the literature. In particular, ultrasonication at laboratory scale showed poor results mainly for climate change and fossil depletion categories due to the high energy demands [226]. At the same time, when applied in a full-scale, ultrasonication resulted in the lowest impacts in climate change and green-house-gases emissions, fossil depletion and stratospheric ozone depletion, due to its optimal input to output energy ratio [226]. Another physical/mechanical pretreatment technology that has been studied is the microwave method. Bozkurt and Apul [87] reported the primary environmental benefits of this method, which include among others the reduced digested solid loading in landfills. On the other hand, it was observed that the increased methane production and methane emissions into the atmosphere affects significantly the climate change impact category [87]. Similarly, based on the results presented in the same study, the release of heavy metals and dissolved solids during the pretreatment could have also important impacts that need to be considered.

Figure 10 illustrates the LCIA results concerning the pretreatment technologies studied in this paper as they have been presented in previous studies. It should be highlighted that the results consider the impacts associated with the operational phase, while the system boundaries include not only the different pretreatments, but also the AD units and the final disposal/use of the sludge (details are provided in Appendix A, Table A1). To this end, the main contributor to the most of impacts categories is the AD unit, while the negative effect is related to the indirect air emissions. Thus, the impact of all pretreatments can be considered negligible compared to the other units of the studied systems since pretreatment units have lower impact than the others. However, if the impact categories are studied only for the various pretreatment methods, the main contributor to the impact is indirect emissions, whereas electricity also has a small negative effect. In addition to that, the impact of pretreatments is linked to their impacts on energy recovery, transportation requirements, and nutritional loadings, emphasizing the necessity to evaluate its performance over its whole life cycle [227].

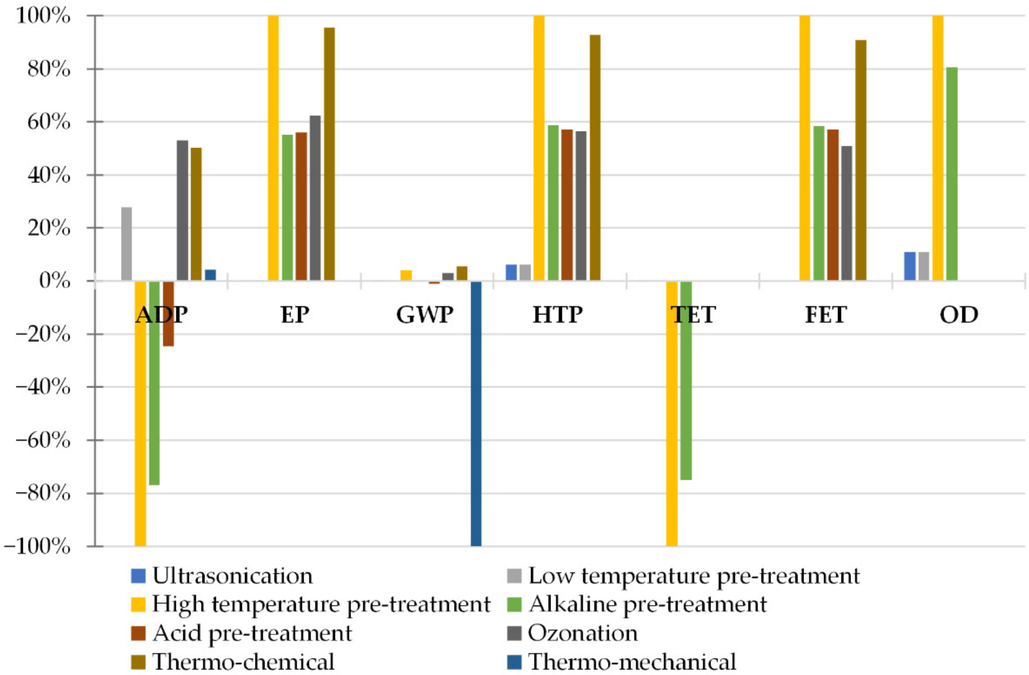

Figure 10. Relative impact of most studied pretreatment technologies, taking the highest absolute value for every category as baseline. ADP: Abiotic Depletion, EP: Eutrophication, GWP: Climate Change, HTTP: Human Toxicity, TET: Terrestrial Ecotoxicity, FET: Freshwater ecotoxicity, OD: Ozone Layer Depletion.

9. Conclusions and Future Perspectives

Rising concerns regarding energy safety, along with the environmental impacts and increased treatment and disposal costs of the vast sewage sludge amounts produced worldwide from the wastewater treatment plants, have drawn attention to developing technologies that offer solutions to these problems. A way-out technology to these concerns is anaerobic digestion. Through this technology, the increasing amount of sewage sludge produced is utilized as a second-generation biofuel feedstock and issues concerning its handling before disposal are addressed. Essentially, anaerobic digestion has a great potential in reducing the overall solids mass, lessening the overall operating cost of a wastewater treatment plant, while it advocates for environmental protection and sustainability owing to its contribution in the renewable energy field. Nonetheless, sludge's composition complexity and poor biodegradable content obstruct anaerobic digestion performance. On these accounts, several studies have been conducted to develop methods that will serve sludge pretreatment prior to anaerobic digestion, aiming to improve its efficiency.

This paper critically discusses the recent advances in sludge pretreatment methods and reviews their effectiveness in enhancing anaerobic digestion. In general, all the methods presented herein have the potential to solubilize particulate and complex organic matter contained in sludge, thus facilitating the biodegradation of molecules that otherwise would remain recalcitrant. This in turn accelerates the hydrolysis process and renders these methods as promising in boosting biogas or methane generation and reducing the sludge solids that need to be disposed after anaerobic digestion. Specifically, the methods that reveal the best results in terms of sludge solubilization include the thermal and enzymatic pretreatments, as well as ozonation and Fenton oxidation, followed by mechanical and biological ones (i.e., two-phased anaerobic digestion and aeration). Whatever the positive contribution of the pretreatment technologies in improving anaerobic digestion performance is, each one of them has its demerits. The drawbacks may range from high operational costs and energy requirements to the formation of refractory compounds or inhibitory byproducts when high-temperature thermal methods are applied, or high concentration of chemicals are used during alkaline pretreatment. These undesired compounds can be part of the soluble fraction. Nevertheless, they are not convertible to CH_4, indicating the potential that increased solubilization does not always conduce to enhanced anaerobic digestion efficiency. Therefore, more detailed analysis and research is needed to clarify the changes promoted by pretreatment in the complex sludge components. This way, a more thorough grasp of a method's effectiveness and mechanism of action can also be attained.

Among the numerous methods available for sludge pretreatment, physical/mechanical (except for microwave), ozonation, and especially thermal processes have already been implemented at industrial scale. However, solid techno-economic assessment analyses of novel technologies that have been assessed only in lab and pilot scale are still needed. By this, better evaluations can be obtained, which will assist the selection of an optimal pretreatment method and estimate its feasibility before seriously considering up-scaling and applying it at a wastewater treatment plant. Based on the results reported throughout the literature, the combination of pretreatment methods seems to be a more efficient and feasible option to implement than individual methods. Nonetheless, more studies should be conducted to fully understand their synergistic effects and fill knowledge gaps that will aid in improving anaerobic digestion process. Moreover, further research is required to assess the impact of combining processes for sludge pretreatment upon overall cost-effectiveness. In addition, the fact that most applications have been examined at laboratory scale does not allow estimating the real cost at a larger scale. Therefore, the assessment of scaling implications is of primary importance in order to obtain a more realistic estimation of the energy and economic costs. Finally, this review incorporates an environmental assessment of pretreatment methods to provide useful guidelines that will assist in evaluating their viability. Nevertheless, there is a need to further study, analyze, and compare the environmental impacts of the available pretreatment technologies based on experimental data, integrating both laboratory and full-scale conditions. Overall, it is concluded that balancing operational, economic, and environmental concerns for selecting an efficient and sustainable pretreatment application is difficult. Thus, future work should examine the incorporation of full-scale experience and a more integrated and energy-efficient waste management strategy.

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Appendix A

Table A1. Relative impact of most studied pretreatment technologies.

Pretreatment Technology	Abiotic Depletion	Eutrophication	Climate Change	Human Toxicity	Ecotoxicity		Ozone Layer Depletion	References
	ADP (kg Sb-eq)	EP (kg PO43-eq)	GWP (kg CO_2-eq)	HTP (kg 1,4-PDB-eq)	TET (kg 1,4-PDB-eq)	FET (kg 1,4-DB-eq)	CFC-11 eq	
Physical pretreatment								
Ultrasonication	1.4×10^{-6} **	6.6×10^{-7} **	4.7×10^{-3} **	9.1×10^{-5} ** 6.8×10^{-2} **	1.3×10^{-4} **		7.0×10^{-10} **	[226]
Microwave pretreatment								
High-pressure homogenization								
Electro-kinetic disintegration								
Thermal Pretreatment								
Low temperature pretreatment	2.3×10^{-3} **	6.1×10^{-7} **	7.1×10^{-3} **	8.6×10^{-5} ** 6.8×10^{-2} **	1.2×10^{-4} **		7.1×10^{-10} **	[226]
High temperature pretreatment	2.66×10^{-3} * 1.75×10^{-3} -8.27×10^{-3} ***	3.70×10^{-3} * 1.13×10^{-5} -5.68×10^{-5} ***	5.21×10^{-1}* 6.85×10^{-3} 2.03×10^{-2} ***	1.11 * 2.36×10^{-4} -1.21×10^{-3} ***	9.68×10^{-3} -4.60×10^{-2} ***	3.89×10^{-2} * 1.84×10^{-4} 1.82×10^{-2} ***	4.04×10^{-9} 6.45×10^{-9} ***	[224,225]
Chemical Pretreatment								
Alkaline pretreatment	-6.60×10^{-4} * 1.66×10^{-4} -6.36×10^{-3} ***	2.04×10^{-3} * 1.20×10^{-6} -4.47×10^{-5} ***	2.18×10^{-2} * 6.16×10^{-4} 1.48×10^{-2} ***	6.52×10^{-1} * 2.65×10^{-5} -9.44×10^{-4} ***	1.89×10^{-3} -3.45×10^{-2} ***	2.27×10^{-2} * 2.20×10^{-5} -7.31×10^{-4} ***	2.66×10^{-10} 5.20×10^{-9} ***	[224,225]
Acid pretreatment	-2.03×10^{-3} *	2.07×10^{-3} *	-1.28×10^{-1} *	6.33×10^{-1}		2.22×10^{-2} *		[224]
Ozonation	2.20×10^{-3} *	2.31×10^{-3} *	3.89×10^{-1} *	6.27×10^{-1} *		1.98×10^{-2} *		[224]
Fenton oxidation								

Table A1. *Cont.*

Pretreatment Technology	Abiotic Depletion	Eutrophication	Climate Change	Human Toxicity	Ecotoxicity		Ozone Layer Depletion	References
	ADP (kg Sb-eq)	EP (kg PO43-eq)	GWP (kg CO_2-eq)	HTP (kg 1,4-PDB-eq)	TET (kg 1,4-PDB-eq)	FET (kg 1,4-DB-eq)	CFC-11 eq	
Biological Pretreatment								
Temperature-Phased Anaerobic Digestion (TPAD)								
Aerobic pretreatment								
Enzyme-assisted pretreatment								
Combined Pretreatments								
Thermo-chemical	4.15×10^{-3} *	3.53×10^{-3} *	6.98×10^{-1} *	1.03 *		3.53×10^{-2} *		[224]
Thermo-mechanical	1.03×10^{-3} 3.56×10^{-4} ****		182.62 -12.65 ****					[227]
Mechanical-chemical								

* The system included the different pretreatments applied, the anaerobic digestion process with energy recovery and the disposal of the digestate in agricultural land [224]. ** The studied system included all the processes involved in sludge treatment line from pretreatment step to digestate agricultural reuse [226]. *** Regarding the system boundaries, the sludge line comprises the different pretreatments, the AD unit and final disposal, considering only the impacts associated with the operational phase [225]. **** The sub-systems include the in-plant stabilization operations using AD and the transport and application of sludge to soil as replacements of commercial fertilizers. Pretreatment prior to digestion was also included [227].

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