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Anaerobic Digestion

Pretreatments of Substrates

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Abstract

The anaerobic degradation process is the biological oxidation of organic matter by the action of specific microorganisms (in the absence of atmospheric oxygen). Organic matter is converted into stable end products at the same time, and the process generates biogas (mainly methane and carbon dioxide) that can be valorized energetically. Recently, there has been a notable increase in the variability of the substrates treated by anaerobic digestion. The main benefits of pretreatment associated with the anaerobic digestion technology include the solids reduction, odor removal, the inactivation and pathogen reduction, less energy use, and increased energy recovery from the methane. The pretreatments methods in anaerobic digestion are physical, chemical, and biological pretreatments. The physical pretreatments include mechanical (ultrasound, mechanical jet, high pressure homogenizer, and mechanical ball mill), thermal, and ultrasonic pretreatments. The chemical pretreatments include alkali, organosolv process, wet oxidation, ozonolysis pretreatment, and acid hydrolysis, and the biological pretreatments (microorganisms) or combined pretreatments (thermochemical or alkali-thermo) are often used.

Keywords: Organic Substrates, Pretreatments techniques, Anaerobic digestion, Waste, Biodegradation

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1.1 Pretreatments in Anaerobic Digestion Process

Anaerobic digestion is an attractive treatment strategy in waste management sector from an environmental point of view and for society benefit by providing a clean fuel from renewable feedstocks [1, 2]. The technical expertise required to maintain industrial scale anaerobic digesters coupled with high capital costs and low process efficiencies had limited the level of its industrial application as a waste treatment technology. However, the anaerobic digestion technology have been recognized as one of the most useful decentralized sources of energy supply [3, 4]. The anaerobic degradation process is suitable for waste treatment; based on the biological oxidation of organic matter by the action of specific microorganisms (in the absence of atmospheric oxygen) as a result of this process, organic matter is converted into stable end products and still, at the same time it generates biogas (mainly methane and carbon dioxide) [5–7] with a considerable energy potential while producing an energy rich biogas (55–70% methane, CH_4) that can be valorised energetically [8]. There are many benefits associated with anaerobic digestion technology, which include mass reduction, odour removal, pathogen reduction, less energy use, and more significantly, the energy recovery in the form of methane [9].

The term technical of biomethanization or bio-hydrogen to refer to anaerobic digestion of the fraction organic of substrates has been used in the past few years. The biometanization of organic wastes is accomplished by a series of biochemical transformation, which can be toughly separated into a first step where hydrolysis, acidification and liquefaction take a place and second step where acetate, hydrogen and carbon dioxide are transformed into methane [10–12]. If the process of anaerobic degradation of municipal solid waste in the landfill reaches the methanogenic phase as soon as possible after the refuse has been deposited, more organic material is transferred into the gaseous phase and less into the liquid phase (leachates). As a result biogas production increases, more portable energy is available and less energy and cost are required for treatment of the leachates [13, 14].

Bio-hydrogen from renewable sources is also known as "green technology" and forms part of the sustainable development and waste minimization issues [15, 16]. Biologically hydrogen can be produced by the photosynthetic and fermentative methods which are more environmental friendly and less energy intensive compared to chemical process [17, 18]. Hydrogen can be produced biologically by biophotolysis (direct and indirect), photo-fermentation and dark-fermentation or by a combination of these processes (such as integration of dark- and photo-fermentation, or biocatalyzed electrolysis) [19]. Biological hydrogen production via dark fermentation of organic wastes is regarded as one of the most promising alternatives for sustainable green energy production [20]. Dark-fermentation among the processes is the most powerful system because of a relatively higher rate of hydrogen production, and many researchers have studied biohydrogen production by fermentative bacteria, such as *Escherichia coli* [21], *Enterobacter* species [22–25] and *Clostridium* species [26].

In the past 25 years there has been a notable increase in the treatment of different substrates by anaerobic digestion. The reliability of this type of treatment has shifted to general acceptance, a fact reflected in the different types of anaerobic digesters operating on an industrial scale in the actuality. Different start-up strategies of anaerobic digestion of substrates (higher organic matter) are detailed in the literature. The main aspects considered of the implementation phase of technology are the type of inoculum used the temperature conditions, the application of pretreatments and the use of different configurations of the process [27]. The benefits of pretreatment associated at anaerobic digestion technology include the solids reduction, odour removal, the inactivation and pathogen reduction, less energy use, and increased in the energy recovery from the methane.

1.1.1 Anaerobic Digestion Pretreatments of Substrates

Anaerobic digesters were originally designed for treatment of sewage sludge and animal waste (manure). However the sewage and manure are not, the biodegradable substrates with the most potential for anaerobic digestion, there are present in very large quantities of substrates, products or waste from agriculture (corn), crop residues, the food industry and market waste, animal waste, sludge sewage and organic matter of municipal solid waste [28] like a typical on-site feedstock, or various organic byproducts, such as slaughterhouse waste, fats oils and grease from restaurants, organic household waste, etc. like a typical off-site feedstock. The substrate composition is a major factor in determining the methane yield and methane production rates from the anaerobic digestion, the more biodegradable substrate the higher the gas yields possible from the system.

Most literature sources report substantial differences in the yields and kinetics of methane on anaerobic digestion depending of type of substrate. Pretreatments by mechanical size reduction, heat treatment and/or chemical treatment could improve its digestibility [29]. The objective of pretreatments stage is to modify the structure of complex materials (usually cellulosic) with decreasing degree of polymerization, the weakening of the bonds of lignin with carbohydrates and increased surface area of particles constitute the remainder. The nature of the apparent association of lignin and carbohydrates is still under discussion [30]. In general substrates for the fermentation process should have a C/N ratio in fermentable mass between 25 and 35. For lower values should be added carbon-rich materials, and otherwise nitrogen-rich materials (manure, sewage sludge, etc.). Table 1.1 shows the C/N ratio set for some substrates. The rapid growth of industrialization and urbanization in the 21st century has resulted in production of unmanageable quantity of sludge from wastewater treatment plants (WWTPs). The sludge from WWTPs one of the most substrates treat from anaerobic digestion [9, 31–34]. The sewage sludge has a C/N between 6:1 and 9:1[12, 35] and digested sludge of 14 to 16 [33, 34, 36] a value well below the C/N ratio recommended (Table 1.1). In this case, pratices of mixing with the municipal solid waste or food waste can increased the C/N ratio favorable to anaerobic digestion [37].

The source separation of municipal solid waste performed in the municipal solid treatment plants is also suitable for anaerobic digestion [38–40]. However, one promising application of anaerobic digestion is the combination of sewage sludge with organic matter from municipal solid waste [11, 41]. To date, many of the anaerobic digesters for both sewage sludge and MSW have no pretreatment practices, and could present some shortcomings such as long retention times, low removal efficiencies of organic matter and instability of the digestion process [42]. In the classical wastewater treatment plant the location for sludge pretreatments could minimise sludge production by either increase sludge age or increased aerobic destruction of organics. Carrere and co-workers [43] suggest cotreatment on activated sludge process or cotreatment on the activated sludge recirculation loop to promote: lower nitrogen limits; final handling costs (especially for final destruction options like incineration), and stabilization performance and pathogen removal. Many agricultural and industrial wastes are examples of food waste and also are ideal candidates for anaerobic digestion because contain high levels of the C/N ratio (superior at 20), like fruit and vegetable [44], Citrus waste [45], of tomato, cucumber, common reed and grass silage [46], sweet potato [47] Sugarcane bagasse [48], Pineapple waste [49], restaurant waste [41, 50–52] and others agricultural wastes particularly (Table 1.1).

Lignocellulosic substrates are considered as the most abundant renewable resource, the major biodegradable fraction in municipal solid waste, with the potential of making a substantial difference in the supply of biofuel. The lignocellulosic biomass contained components such as cellulose (34.0%), hemicellulose (37.5%), lignin (22%) and silicon ash (6.6%) and depending of substrates, for example the corn straw, is necessary to complement with the nitrogen fraction for increase the efficient anaerobic digestion [53]. The biodegradability of cellulose depends on their amorphous or crystalline form and the range lignin content; paper shredded [54], cellulose powered [55] and cardboard [56] (Table 1.1). For biodegradability of lignocellulosic substrate like common reed, grass silage [57], corn silage [41] and corn straw the nitrogen can be added in the form of inorganic form (e.g. ammonia) or organic form (e.g. urea, animal manure or food wastes). Nitrogen fertilizer (e.g. ammonia or urea) is another nitrogen source that can be easily added to the corn straw if nitrogenous wastes are not available [29]. Animal manure and other organic wastes are additional nutrient sources, provided they are readily available for anaerobic digestion; cow manure [58] and swine manure [12].

The pretreatment methods of substrates aim at enhancing anaerobic digestion processes by altering physical or chemical properties and shows the following advantages: increased final handling costs; a trend towards lower nitrogen limits, which is driving up sludge ages and decreasing degradability of activated substrates streams and increased legislative requirements for stabilisation performance and pathogen removal [29]. There is therefore an increased need to review and analyse the different pretreatment options in terms of mechanism, costs, and performance. Here, we review the major classes of physical (mechanical, thermal and ultrasonic), chemical and biological pretreatment methods, and evaluate the likely future of pretreatment prior to substrates stabilisation.

Table 1.1 C/N ratio of substrates in anaerobic digestion.

Substrate		C/N ratio	Reference
Sludge	Sewage sludge Digested sludge	6:1 9:1 16:1	[12] [35] [59] [36] [33] [34]
Municipal solid wastes	Organic fraction	9–12:1	[38] [39] [40]
Food waste	Fruit Citrus Sweet Potato Sugarcane bagasse Pineapple waste Restaurant waste	35–50:1 24:1 25:1 100:1 21:1 14–38:1	[44] [45] [47] [48] [49] [50] [51] [41] [52]
Lignocellulose	Corn straw Corn silage Grass silage	60–120:1 10:1 9–25:1	[29] [41] [46]
Cellulose	Paper shredded Cellulose powered Cardboard	175:1 175:1 350:1	[54] [55] [56]
Animal Waste	Cow manure Pig manure	20–35:1 10–20:1	[58] [60]

1.2 Physical Pretreatment

Problems such as low degradation of substrate, low methane yield and problems with instability of process are the limiting steps in the anaerobic degradation. The physical include mechanical (mechanical jet, high pressure homogenizer, and mechanical ball mill), thermal [61, 62] and ultrasonic pretreatments. The application of physical pretreatment can increase the rate of hydrolysis and increase the anaerobic biodegradability of substrates, especially municipal solid waste [63].

1.2.1 Mechanical Pretreatment

The mechanical pretreatment size reduction improves of the solids of substrate by rupturing cell walls and making the biodegradable components more accessible to microorganisms. The size reduction technique increases the accessibility of microorganisms to the residue particles due to breaking of large structures in shorter chains, improving the speed and efficiency of hydrolysis. The mechanical pretreatment methods most commonly used are mechanical jet, high pressure homogenizer, mechanical ball mill, etc. [64–66]. According to Tchobanoglous [67], the basic operations used in the separation and processing of materials, mainly municipal solid waste, are as follows:

1. Reduction by size: This operation is used to process materials for direct use as the case of compost or as part of a recovery of recoverable fractions. There are several types of size reduction units as hammer mills, crushers and pipes cutting shredders.
2. Separation by size: It involves the separation of a mixture of materials in two or more lots by using one or more screening surfaces and, generally, on a dry. The most commonly used types of sieve in the separation of municipal solid waste are vibrating screens, trommel screens and the screens on disk.
3. Separation by density: The separation of crushed MSW is based on two main components: light fraction (paper, plastic and organic) and heavy fraction (metals, wood, etc.). Technologies used are pneumatic classification, stoners, flotation and dense media separation.
4. Separation by electromagnetic field: direct application in the recovery and separation of ferrous and non ferrous metals. There are units of magnetic separation, electrostatic separation and eddy current separation.
5. Compaction: The main objective of this procedure is to increase the efficiency of storage and transportation of materials. For example, stationary compactors, equipment, packaging and pelletizing.

Hwang [62] report an increase in the solubility of the waste (measured on the basis of chemical oxygen demand, COD), which led to an improvement in the efficiency of the process stages of anaerobic digestion. Although different mechanical pretreatments have shown significant impact on biogas production enhancement, and the major challenge of using mechanical pretreatment is high energy requirement [68].

1.2.2 Thermal Pretreatment

The thermal pretreatment is a stage that improves the efficiency of anaerobic digestion process because de the thermal hydrolysis leads to partial solubilisation of substrates, the organic and inorganic compounds are efficiently solubilised during thermal treatment [69], reduces digester volume and enhances biogas production [70, 71].

In anaerobic digestion process the thermal hydrolysis could be applied in the pre-treatment stage with the objective of improve the substrate biodegradation, and also could be used for treatment of thickened sludge before dewatering, to reduce solids to be disposed of and increase solid content in dewatered cake. The thermal energy result can be recovered for heating temperature anaerobic process. There are two conventional operational temperature levels for anaerobic digesters, which are determined by the species of methanogens in the digesters: mesophilic or thermophilic. The mesophilic temperature which takes place optimally around 20–35°C where mesophiles are the primary microorganism present [72], and the thermophilic which takes place optimally around 45–55°C at elevated temperatures up to 70°C where thermophiles are the primary microorganisms present [73].

In Table 1.2, an overview of some thermal pretreatments of substrates in anaerobic digestion studies are showed. In general the most frequently alkali thermal pretreatment studies reported in literature for values of temperature, pressure and hydraulic retention time (HRT) are: optimal temperature in the range of 160–180°C and HRT from 30 to 60 min [37]. Substrates like food waste (TS of 10%) showed the best results with 175°C, 4 MPa, 30 min [63] and 175°C, 2 MPa, 30 min [69]. For municipal solid waste with thermochemical pretreatment with NaOH the best conditions for carrying out are: high temperature (180°C), intermediate dose of NaOH (3 g/L) and intermediate pressures (3 bar) [27].

The sludge from wastewater treatment is one of the most important substrates of anaerobic digestion and combination of thermal pre-treatments and anaerobic digestions is most investigated in literature in order to improve their dewaterability (in order to lyse sludge cells) and improve the commercialized of this stage in some industrial plants. In general, an optimal temperature in the range from 160 to 180°C and treatment times from 30 to 60 min for activated sludge (TS of 96–99%) [71], but there are best results with temperature from 120 to 180°C and hydraulic retention time 0–30 min [31, 74, 75]. Pressure associated to these temperatures may vary from no set pressure to 4000 kPa.

Bougrier and co-workers [71] suggest a thermal treatments classification acoording the impact on biogas production: 1) treatments at temperatures of 70°C or 121°C which led to a 20–30% biogas production increase and, 2) treatments at 160–180°C which led to a 40–100% biogas production increase. In conclusion, the 160–180°C pre-treatments are thus most efficient to enhance sludge anaerobic digestion but theses results dont are favorable to increase in biogas production (from 40 to 100%). According Mottet and *et al.* [76] the methane production kinetics are strongly linked to the acetate and propionate kinetics, a limiting effect on the methane production. In studies with thermal treatment of thermophilic anaerobic digestion of sludge, the acetate degradation was associated to the first methane production phase and the propionate degradation was associated to the second methane production phase.

Table 1.2 Thermal pretreatments of substrate in anaerobic digestion.

Substrate	Thermal Pretreatment	Reference
Food waste	175°C, 4 MPa, 30 min	[63]
	175°C, 2 MPa, 30 min	[77]
	180°C, 3 bar, 30 min	[27]
Sludge	70°C, 9–72 hours	[72]
	120°C, 30 min	[73]
	121°C, 1,5 Mpa, 30 min	[31]
	121°C, 60 min	[77]
	170°C, 3 bar, 30 min	[78]
	170°C, 30 min	[79, 80]
	170°C, 60 min	[81]
	175°C, 40 min	[82]
	175°C, 90 min	[74]

The application of temperatures above 200°C could promote an inhibitory effect on the digestion process [83], due to hydrolysis of microbial cell components (DNA and RNA involving the polymerization of sugars and amino acids) increasing the concentration of nitrogen and phosphorus in the solution. Also, temperatures higher than 180°C lead to the production of recalcitrant soluble organics or toxic/inhibitory intermediates, hence reducing the biodegradability [84]. The optimum temperature and duration of pretreatment conditions are largely depending on the nature of the substrate and the most significant of high-temperature in the thermal pretreatment is its high energy requirement.

In the first studies some authors refer to problems associated with this type of pretreatment, associated with excessive energy requirements for heating and cooling of the residue. However, actually there are some industrial processes been commercialized, and numerous studies dealing with the performances and effects of thermal pretreatment on physico-chemical properties of sludge and its implementation at full scale plants are described in literature, for example: cell lysis, solubilization, molecular structure, filterability variations, etc. [85–87].

1.2.3 Ultrasound-assisted Pretreatment

The application of high intensity ultrasound to a liquid system may lead to physical and chemical reactions that can significantly modify the structure the material present in the liquid. Sound propagates in the form of waves (sound waves) with a given height (intensity) and distance between waves (frequency). Ultrasound is the term used to any wave above 20 kHz, which is the higher frequency that humans can hear. However, the extremely high frequencies cause a phenomenon known as cavitation. When ultrasonic waves passes trough a medium it generates gas bubbles, which are subsequently compressed and expanded by the passing sound waves until reaching a critical point where they eventually implode producing local extreme conditions of temperature and pressure [88–92].

The local high temperature and pressure of the "shock waves" can seriously affect the structure of organic matrixes, releasing intercellular material that can be more easily accessed and increasing the contact between the reaction medium and substrate improving downstream processing. Depending of the frequency and intensity of the ultrasonic waves, bubbles can oscillate more before imploding, generating bigger bubbles (although fewer bubbles are generated) with higher accumulated energy. In general, bigger bubbles are formed with lower frequencies (e.g. 20 kHz) while smaller bubbles are generated by the use of higher frequencies (e.g. 40 kHz). In this context, lower frequencies are usually used when the objective is the destruction of the material being submitted to ultrasound pretreatment (such as in the pretreatment of substrates) while higher frequencies are used to process more delicate materials, such as in the cleaning of electronics and jewels [88–92].

Another important factor affecting cavitation is the ultrasonic wave intensity (power output). If enough energy is not provided to the medium, bubbles will only oscillate and will not implode and therefore will not damage the residue structure. Although the bubbles will not implode, it still may be able to increase contact between the medium and the substrate by mixing caused by the oscillation of the bubbles. On the other hand, the necessary intensity of ultrasound to promote cavitation will depend of several factors, such as the medium, volume, temperature, pressure and the substrates being processed, among others. Most ultrasonic devices available for the pretreatment of substrates use frequencies between 20 and 40 kHz, with power intensities ranging from 50–80 Watts to more than 20.000 Watts. Volume is an important aspect since it will dilute overall ultrasound power and ultrasound intensity is given in watts per cm^2. Although complete disintegration of the substrates is possible, a proper balance between cavitation effect and power consumption is necessary since it can be a serious drawback considering overall processing costs [88, 89, 93, 94].

On the other hand, other processes may take place by the application of ultrasound, such as dissociation of water, where hydroxyl radiacal are formed and similar reactions to those observed in advanced oxidation processes (ozone,H_2O_2,UV) can take place which that can explored to pretreat any residue using the same principles of these processes. These sonochemical reactions may be able to affect volatile pollutants by pyrolytic processes in the cavitating bubbles and non-volatile pollutants by the previously mentioned hydroxyl radical oxidation [93, 94].

Therefore, the application of ultrasound may have a great potential to be used before actual treatment of residues. Several reviews about the role of ultrasound in the pretreatment of residues before anaerobic digestion have been recently published and they provide a deeper view of the mechanisms by which ultrasound may be useful as a pretreatment technique [93–96].

There are also several recent applications of ultrasound for the pretreatment of wide range of residues. It has been reported, for example, that ultrasonic pretreatment increased daily biogas production, methane production and significantly reduced volatile solids during the anaerobic digestion of waste activated sludge. A short pretreatment of 15 minutes of sonication increased initial soluble COD concentration from 50 mg/L up to a value of 2500 mg/L. Increased performance of anaerobic digestion was attributed to an increased soluble substrate which increased anaerobic biodegradability concurrently. Also, an economical analysis of the use of ultrasound as pretreatment revealed that it is economically feasible [97].

Ultrasound was used as pretreatment of raw sewage sludge before being fed to the mesophilic and the thermophilic anaerobic digestion reactors and significantly improved COD removal efficiency and biogas production, especially under mesophilic conditions. Also, it was observed a reduction in the pretreated sludge micropollutants content. However, removal of contaminants depended of the compound characteristics. Ultrasound pretreatment assisted was removal of naphthalene, particularly in the mesophilic conditions, while pyrene removal remained at same efficiency level with and without ultrasonic pretreatment [98].

Muller *et al.* [99] studied the use of ultrasound in a pretreatment mode and in an internal recycle line and observed that using a relatively low-energy sonication system, improved anaerobic digestion efficiency for waste-activated sludge. There was a 13 to 21% increase in biogas yield and an increase in total and volatile solids destruction of 3 to 10.3%. Additionally, ultrasonic pretreament generated a more stable biosolids product, with a 2 to 58% reduction in organo-sulfur gas production from dewatered biosolids cakes.

Appels *et al.* [8] observed that ultrasound pretreatment increased soluble COD-fraction accompanied by an increasing presence of BOD, increased release of volatile fatty acids, and increased biogas production by more than 40% at low specific energy-inputs. Pérez-Elvira *et al.* [100] used ultrasound as pre-treatment to improve anaerobic digestion of secondary sludge. Theyr observed that sonication pretreatment increased biogas yield from 248 (control reactor) to 349 mL CH_4/g VS (41% increase in full-stream sonication). However, digestion of the soluble compounds generated during incubation of sonicated sludge appeared to be less degradable compared to those solubilised by ultrasound or incubation alone, which showed no benefit in combining both treatments.

Shao *et al.* [101] studied the effect of ultrasonic pretreatment on sludge and reported that ultrasonic pretreatment greatly improved sludge dewaterability. Nickel and Neis [102] evaluated the disintegration of biosolids for improved biodegradation using ultrasound and observed that the quantity of dissolved organic substrate increased as well as the degradation rate and the biodegradability of organic biosolids mass.

Bougrier *et al.* [80] compared the effects of ultrasounds, ozonation and thermal pre-treatment before anaerobic digestion of waste activated sludge. In terms of batch anaerobic biodegradability, best results were obtained with ultrasounds with an energy of 6250 or 9350 kJ/kg TS and a thermal treatment at 170 or 190°C. Ultrasound pretreatment (as well as other pretreatments) also had effects on physico-chemical characteristics of sludge, reducing apparent viscosity. Mean diameter of sludge flocs were reduced only by ultrasound pretreatment, which also increased capillary suction time. Pérez-Elvira *et al.* [103] compared thermal hydrolysis (170°C for 30 min), and the ultrasounds pre-treatment (30 kJ/kg TS) under thermophilic and two stage temperature phased anaerobic digestion. A general increase on biogas production was observed when compared to conventional methods, but thermal treatment of the sludge resulted on the highest increased biogas production and ultrasonic pretreatment resulted in a degradation of the dewaterability properties of the digestate.

Zhang *et al.* [104] studied the effect of ultrasound-assisted pretreatment on the solid content of sludge, its biological activity, in the soluble chemical oxygen demand and on proteins and nucleic acids concentrations. They observed that ultrasonic pretreatment effectively degraded and inactivated the sludge and that soluble chemical oxygen demand, supernatant proteins and nucleic acids concentrations, and sludge mass reduction and inactivation degrees increased with the increase of sonication treatment and intensity. They also concluded that higher energy ultrasound was more efficient than lower energy ultrasound for the sludge treatment. Salsabil *et al.* [105] observed an important improvement of anaerobic biodegradability of activated sludge by ultrasonic pretreatment (108,000 kJ kg TS^{-1}), which was attributed to the increase of the instantaneous specific soluble chemical oxygen demand uptake rate. Ultrasound also increased biogas production but depended on the disintegration degree of the sludge.

The combination of high disintegration degree of sonicated sludge prior to an anaerobic digestion led to very good results in term of sludge reduction (80%). Braguglia *et al.* [106] used ultrasound pretreatment to maximize the digestibility of biological sludge for agricultural use. They observed that despite low sonication energy, sludge digestion performance improved in terms of solids degradation and biogas production depending on the soluble organic load. Biogas production from pretreated sludge increased up to 30%. They concluded that ultrasound-assisted pretreatment can be used to guarantee self-sustainability of mesophilic digestion of sludges. However, they also recommended thickening to be used to achieve a positive energy balance.

Research about the use of ultrasound-assisted pretreatment for increasing biogas production is extensive, with several different applications. In general, it is seen as an effective technique and that low-frequency ultrasound treatment is technical and economic feasible to be used at large scale plants. Although ultrasound is increasingly being used it is important that optimum operating conditions are used, but this is an issue still open to debate. On the other hand, current research is also indicating that the best approach may be a combination of different techniques, which have different mechanisms of action. Zhang *et al.* [107] for instance, combined pretreatment with ultrasound (40 KHz, 50 W) and lime (560 mg/L) to improve the performance of mesophilic two-phase anaerobic digestion of waste activated sludge with low-volatile solid content, which was achieved. However, the settlement performance and dewatering property of pretreated sludge was reported to be negatively affected after anaerobic digestion.

Seng and co-workers [108] compared ultrasonic, chemical, and combined chemical–ultrasonic pretreatments (NaOH dose of 10 mg g^{-1} TS (total solids) and specific energy input of 3.8 kJ g^{-1}TS) on waste activated sludge disintegration and its subsequent digestion at different solids retention times. The combined chemical–ultrasonic pretreatment, resulted in significantly improved release of soluble chemical oxygen demand. Additionally, methane production with combined pretreatment was approximately 31% higher than control, while when using only ultrasound, an increase of approximately 23% was observed. On the other hand, the highest total solids removal was achieved with the ultrasonic pretreatment (16.6%), whereas the highest volatile solids removal was achieved with combined chemical–ultrasonic pretreatment (24.8%).

1.3 Chemical Pretreatment

Pretreatment of the agricultural residues by mechanical size reduction, heat treatment and/or chemical treatment usually improves its digestibility. Chemical pretreatment methods include alkali pretreatments (alkaline hydrolysis and alkaline peroxide); organosolv process; wet oxidation (treatment with water and air or oxygen at temperatures above 120°C for a period of e.g. 30 min); ozonolysis pretreatment (treatment with ozone); acid hydrolysis pretreatment (sulfuric acid is the most applied acid). In the case of anaerobic pretreatment of the substrates, the methods of alkali are the mostly used for increase at performer in terms of solubilization of chemical oxygen demand (COD) and biogas production.

In the literature are studies that show different types of alkaline agents such as NaOH, KOH, $Mg(OH)_2$ and $Ca(OH)_2$. Sodium hydroxide (NaOH), also known as lye and caustic soda, is a caustic metallic base. The procedure of alkaline pretreatment of substrates in anaerobic digestion consists of adding the dose of NaOH on the substrate at room temperature (25°C) with a contact time of 24 hours, finally the samples are filtered through a nylon mesh of 20 micron pore size [109][63] or centrifugal [110] obtaining a liquid fraction (supernatant) and a solid (precipitate). The alkaline environment causes swelling of the organic particles, making them more susceptible to enzymatic attack by improving the biodegradability in the solid phase in contrast to acid hydrolysis, characterized first for a mechanism of solubilization of organic material [111]. So, the additional buffer capacity, resulting from the addition of alkali, could help the neutralization of organic acids (VFA) during the digestion stage, attenuating the inhibitory effect.

The NaOH concentration is very variable from one author to another:

–20 meq/ L = 0.8 g/L [109]

–20–80 meq/ L = 2.4 g/L [74]

–175 meq/ L = 7 g/L [31]

Jerger and collaborates [112] reported an improvement in the rate of methane gas production using three different species of forest residues, previously treated with a solution of NaOH to 17%. According Pavlostathis [113] the alkaline pretreatment at room temperature wheat straw substrate, with NaOH at 50 meq/L showed an increase of biodegradability over 100%. The sludge pretreatments indicate an increase in solubility above 46% [114]. Some authors suggest that the greater the proportion of total solids of sludge greater the removal of organic matter to the same dose of NaOH and the same retention time, in this case the total solid concentration of 2% of sludge resulted at chemical oxygen demand (COD) reduction of 47%, compared with 39% of other sludge with a concentration of 1% ST, the HRT was 7.5 days and the dose NaOH used, 20 meq/ L [114]. On the other hand, according to Ray [115], the alkaline pretreatment of sludge reactors allowed biofilm reduces Hydraulic Retention Time (HRT) from 15 to 2 days. After, the alkaline pretreatment was studied in cellulosic waste, the application of alkali as NaOH promotes the breakdown of lignin, so the area increases cellulose surface and decreases the crystallinity [116].

The ammonia pretreatment show several advantages as nitrogen source for biodegradation of substrate, promote biogas production, and is a method easy to operate in the shorter pretreatment time recommended for large-scale biogasification of corn straw [29]. In general, the carbon-to-nitrogen ratio (C/N) for anaerobic digestion should be within the range of 25–35%, some organic substrates needs to be supplemented to increase the nitrogen fraction for more efficient anaerobic digestion and nitrogen can be added in the form of inorganic form (e.g. ammonia) or organic form (e.g. urea, animal manure or food wastes) since once nitrogen is released from the organic matter, it becomes ammonium (NH_4^+) which is water-soluble so recycling nitrogen in the digested broth reduces the amount of nitrogen needed [53]. Then the ammonia treatment has several advantages over the other ones, since ammonia itself is a nitrogen source for biodegradation and that no separate wastewater streams are generated from the pretreatment process. Zhong and collaborates [29] studied the effects of corn straw pretreatments using Fungus *Pleurotus florida* and chemicals such as ammonia on the biogasification performance by anaerobic digestion. The ammonia was added at 500 g of the corn straw to moisture contents of 22% and the prepared flasks were covered with plastic films and closed with a plastic ring, then placed in a chamber at ambient temperature

(152°C) for 20 days. At the end of the experiment, the pretreated corn straw was dried in an electronic oven at 80°C for 48 h and then kept in a refrigerator for chemical analyses and finally our anaerobic digestion experiments showed that the biogas productivity was increased by all the pretreatments [29].

1.4 Biological Pretreatment

The aim of biological pretreatment is to prepare the substrates for the enzymatic degradation and the best method and conditions of pretreatment depend greatly on the type of substrate. The microorganisms can used to degrade organic substrates, several fungi, e.g. brown-, white- and soft-rot fungi, and bacteria have been used for this purpose [117]. Bacteria can be used for biological pretreatment of substrates in anaerobic digestion. Kurakake *et al.* [118] studied the biological pretreatment of office paper with two bacterial strains (*Sphingomonas paucimobilis and Bacillus circulans*) and obtained improve of the enzymatic hydrolysis; the sugar recovery was enhanced up to 94% for office paper.

Depending on the type substrates (household waste, industrial waste water timber distillery wastewater, etc., the enzymatic attack could be made with different types of fungi or combinations of them (*Aspergillus niger, Aspergillus awamori, Aspergillus oryzae, Aspergillus terreus, etc.*) [119]. Taniguchi *et al.* [12] evaluated biological pretreatment of rice straw using four white-rot fungi (*Phanerochaete chrysosporium, Trametes versicolor, Ceriporiopsis subvermispora, and Pleurotus ostreatus*) and the pretreatment with *Pleurotus ostreatus* resulted in selective degradation of the lignin, and increased the susceptibility of rice straw to enzymatic hydrolysis. Also, solid-state fermentation of orange peels by fungal strains of *Sporotrichum, Aspergillus, Fusarium and Penicillum* enhanced the availability of feed constituents and reduced the level of the antimicrobial substances [121]. In a similar work, cultivation of white-rot fungi was used to detoxify olive mill wastewater and improve its digestion [122].

Low energy requirement, no chemical requirement, and mild environmental conditions are the main advantages of biological pretreatment. However, the treatment efficiency is very low in most biological pretreatment processes [123]. At the end, the corn straw must be dried in an electronic oven at 80°C for 48 h and then kept in a refrigerator for chemical analyses for composition determination and anaerobic digestion experiments [29]. Therefore, the biological pretreatments need extra stage to ensure the attack after enzyme, and this alternative may be done after the pretreatments previously discussed (physical or chemicals).

1.5 Combined Pretreatment

Pretreatment by physical, chemical or biological means is a well investigated in the anaerobic digestion technology. There are many some efforts in the pretreatment of waste materials: both to enhance the bio-digestibility of the wastes and to increase the biogas production. According to Taherzadeh and Karimi [117] an effective pretreatment should meet the following requirements: (a) avoiding formation of possible inhibitors for hydrolytic enzymes and fermenting microorganisms, (b) minimizing the energy demand, (c) reducing the cost of size reduction for feedstocks, (d) reducing the cost of material for construction of pretreatment reactors, (g) producing less residues and (e) consumption of little or no chemical and using a cheap chemical. When the pretreatment by physical, chemical or biological does not have efficient results, combined pretreatment can be used: physical-chemical, chemical-biological, etc.

The thermochemical pretreatment, is a physical-chemical pretreatment and was studied for municipal solid waste anaerobic digestion by Owen [124]. The results showed a slight increase of COD (chemical oxygen demand) soluble of municipal solid waste when applied at temperatures between 150–225°C, markedly decreasing the hydrolysis above 225°C. Also three chemicals pretreatment (NaOH, ammonia, and urea) were reported by Zhong and collaborates [29]. NaOH, ammonia, and urea were added into the solution of corn straw in the flasks (1 litre) at concentrations 8%, 5%, and 4% (wt%), respectively. And the resulting moisture contents were 48%, 22%, and 38% for the three groups, respectively. Finally, all the prepared flasks were covered with plastic films and closed with a plastic ring, then placed in a chamber at ambient temperature (152°C) for 20 days. At the end of the experiment, the pretreated corn straw was dried in an electronic oven at 80°C for 48 h and then kept in a refrigerator for chemical analyses for composition determination and anaerobic digestion experiments to investigate the effect of different chemical treatments on biogas production.

According to the literature, there are interesting application alternatives to the pretreatment in the organic fraction of municipal solid waste, which would bring economic benefits by reducing the volume of the digester and therefore the costs of treatment. Fdez-Güelfo and collaborates [42] studied alkali-thermo pretreatment (sodium, NaOH) with different temperatures, pressures, sodium concentrations, type of atmosphere and percentages by volume of inoculums and concluded that: i) the pressure and temperature are parameters that influences significantly at the yield of solubilization of organic matter expressed in terms of COD and total acidity, minimum pressure (1 bar) and maxima (10 bar); and ii) the alkali concentration and temperature are factors that influences significantly at the efficiency of solubilization of organic matter expressed in terms of COD, maxima concentration (5 g/L).

Another type of pretreatment studied was simultaneously combining pretreatment by ultrasonic vibration (120 W/h and 20 KHz) and alkaline (40 meq/ L) by Ying-Chih [61]. The combination of the pretreatment was more effective than alkaline pretreatment alone being equal, with which it reached a speed of hydrolysis of 97.8 mg/L.min.

1.6 Concluding Note

The anaerobic digestion is one of the most effective biological processes to treatment a wide variety of substrates, and the prime advantages of this technology are: organic wastes with a low nutrient content can be degraded by co-digesting with different substrates in the anaerobic bioreactors and the process simultaneously leads to low cost production of biogas. The technical expertise required to maintain industrial scale anaerobic digesters coupled with high capital costs and low process efficiencies had limited the level of its industrial application as a waste treatment technology. However, different factors such as substrate and co-substrate composition and quality, environmental factors (temperature, pH, organic loading rate), and microbial dynamics must be optimized to achieve maximum benefit from this technology in terms of both energy production and organic waste management.

The benefits of the pretreatment of substrates (altering physical or chemical properties) associated at anaerobic digestion technology include: the solids reduction, odour removal, the inactivation and pathogen reduction, energy reduction, and increased in the energy recovery from the methane. Considering that the pretreatments of substrates aim at enhancing anaerobic digestion processes, increased final handling costs, deceasing degradability of organic matter and increased legislative requirements for stabilisation performance and pathogen removal.

The pretreatments methods in anaerobic digestion are physical, chemical and biological pretreatments. The physical include mechanical (ultrasound, mechanical jet, high pressure homogenizer, mechanical ball mill), thermal and ultrasonic pretreatments. The chemical pretreatment includes alkali, organosolv process, wet oxidation, ozonolysis pretreatment and acid hydrolysis), and the biological (microorganisms) or combined pretreatments (thermochemical or alkali-thermo) are very used.

When the pretreatment by physical, chemical or biological does not show efficient results, the combined pretreatment could be recommended (physical-chemical, chemical-biological, etc.). Some authors has found pretreatments efficient to enhance the biodegradability of substrates but with results which are not entirely favorable to increase in biogas production and others have found pretreatments to be efficient in increasing the methane levels in the biogas.

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