1. Introduction

Lignocellulosic biomass, which is chiefly composed of cellulose, hemicellulose, and lignin, has now been recognized and established as a source of energy, fuels, and chemicals. Amongst the several options for the use of lignocellulosic materials for energy generation, the production of ethanol has attracted particular attention worldwide, becoming the target of intense research and development (R&D) over the past 40 years. However, the technology required for industrial conversion of these materials into ethanol, in an economic manner, has not yet been fully developed. As an example, the necessary biomass pretreatment step has been under thorough investigation, as the production of sugar syrups with high concentrations and yields via enzymatic hydrolysis of biomass requires the pretreatment to be efficient to render the material accessible to the relevant enzyme pool.

A good choice for biomass pretreatment should be made by considering the following parameters: high yields for multiple crops, harvesting times, highly digestible pretreated solid, high biomass concentration, no significant sugar degradation, formation of a minimum level of toxic compounds, high yields of sugars after subsequent hydrolysis, fermentation compatibility, operation in reasonably sized and moderately priced reactors, lignin recovery, and minimum heat and power requirements [1].
Different pretreatment methods produce different effects on the biomass in terms of its structure and composition [2] (Figure 1). For example, the hydrothermal and acidic pretreatments conceptually remove mainly the biomass hemicellulose fraction and alkaline pretreatments remove lignin, whereas the product of a milling-based pretreatment retains its initial biomass composition. As such, the choice of pretreatment as well as its operational conditions determines the composition of the resulting biomass hexose and pentose syrups. Furthermore, cellulose crystallinity is not significantly reduced by pretreatments based on steam, or hydrothermal, or acidic procedures, whereas ionic liquid-based techniques can shift crystalline cellulose into amorphous cellulose, substantially increasing the enzymatic hydrolysis rates and yields. The activity profile of the enzyme blend and the enzyme load for an effective saccharification may also vary according to the pretreatment. Indeed, a low hemicellulase load can be used for a xylan-free biomass and a lower cellulase load will be needed for the hydrolysis of a low crystalline and highly amorphous pretreated biomass material.

![Flow diagram for biomass ethanol production](image)

**Figure 1.** Flow diagram for biomass ethanol production showing different pretreatments options and the composition of the solid pretreated material. SSF: simultaneous saccharification and fermentation

As the pretreatment choice will also be affected by the type of biomass, the envisaged biorefinery model will need to consider the main types of biomass that will be used for the biorefinery operation so as to select an appropriate, and versatile pretreatment method [3]. To date, sugarcane and woody biomass, depending on the geographic location, are strong candidates as the main renewable resources to be fed into a biorefinery. However, due to major differences regarding their physical properties and chemical composition, the relevant pretreatments to be used in each case are expected to be selective and customized. Moreover, a necessary conditioning step for wood size reduction, prior to the pretreatment, may not be necessary for sugarcane bagasse, affecting the pretreatment energy consumption and costs.
Sugarcane is one of the major agricultural crops when considering ethanol production, especially in tropical countries. In Brazil, sugarcane occupies 8.4 million hectares, which corresponds to 2.4% of farmable lands in Brazil. The gross revenue of this sector is about US$ 20 billion (54% as ethanol, 44% as sugar, and 2% as bioelectricity) [4]. In addition, up to 50% of all vehicles in Brazil are flex fuel cars, which corresponds to approximately 15 million cars [5]. Given the above, Brazil is an important player in this scenario, and, consequently, sugarcane bagasse and straw are promising feedstocks for biomass ethanol. Brazil produced, in 2008, 415 million tons of sugar cane residues, 195 million tons of sugarcane bagasse, and 220 million tons of sugarcane straw, whereas the forecasted for the 2012 sugarcane production is 590 million tons, which would correspond to 178 million tons of bagasse, and 200 million tons of straw [6]. Although current R&D has been focused mainly on agricultural residues such as sugarcane residual biomass, woody biomass (hardwoods and softwoods) remains a very important feedstock for cellulosic ethanol production. It is estimated that 370 million dry tons of woody biomass can be sustainably produced annually in the United States. Woody biomass is also sustainably available in large quantities in various other regions of the world such as Scandinavia, New Zealand, Canada, Japan, and South America. Furthermore, short-rotation intensive culture or tree farming offers an almost unlimited opportunity for woody biomass production [7].

This chapter will address an overview of the pretreatments that have been studied for sugarcane and woody biomass aiming at ethanol production using diluted acid, hydrothermal processes, steam explosion, milling, extrusion, and ionic liquids. Advantages and disadvantages of each method will be presented and discussed. The chapter will also discuss the international scenario regarding the existing research and technological choices for the production of biomass ethanol.

2. Diluted acid pretreatment

The use of mineral acids for biomass processing has a historical record dating back to 1819, when concentrated acid was used for wood saccharification aiming at ethanol production [8]. Nevertheless, different technologies using mineral acids have been developed over the last two centuries for converting plant biomass into monosaccharides [9, 10]. The use of acid for biomass pretreatment is conducted with diluted sulfuric or hydrochloric acid (1 to 5%) at 150 °C and pressures up to 10 atm [11]. The efficiency of hemicellulose removal in acid pretreatments is approximately 90%, with sugar losses by degradation at around 1% [12].

The diluted acid pretreatment allows for the deconstruction of the lignocellulosic material structure and the release of sugar monomers, mostly derived from the hemicellulose. If acid pretreatment is carried out under mild conditions of acid concentration and temperature, the hemicellulose fraction can be extracted without significantly affecting the cellulose and lignin biomass content. Unlike cellulose, the hemicellulose is amorphous and branched, being more accessible to hydrolysis agents. This structure allows for the diffusion of acids, which accelerate the hydrolytic process. Therefore, in diluted acid pretreatment, the hemicellulose is preferably removed and hydrolyzed.
The process conditions are crucial in preventing undesirable reactions, which could promote a decrease in monosaccharide yields by the formation of sugar-derived toxic compounds. Temperatures lower than 150 °C reduce sugar degradation, but can result in the decrease of sugar extraction, while temperatures above 160 °C favor the unwanted hydrolysis of the cellulosic fraction, and the formation of toxic compounds [13, 14].

The mechanism of the acid hydrolysis reaction of lignocellulosic materials is described by the following steps (Figure 2) [15]:

1. The diffusion of protons through the wet lignocellulosic matrix;
2. The protonation of the ether–oxygen link between the sugar monomers;
3. The breakage of the ether bond and the generation of a carbocation as an intermediate;
4. The solvation of the carbocation with water;
5. The regeneration of protons and the cogeneration of sugar monomers, oligomers, or polymers, depending on the ether connection that is broken;
6. The distribution of products in the liquid phase (if permitted by their shape and size); and
7. The restart of the process from step 2.

With respect to the material to be treated, some intrinsic characteristics also have an influence during the pretreatment such as the sample phase, the structure and physical accessibility (in the case of heterogeneous hydrolysis), conformation effects, and, finally, the structure and substituents of the sugar ring [16].

The theoretical and fundamental relations among molecular structure, molecular conformation, and the inter-unities bonds of polysaccharides have been evaluated for numerous model experiments. The hydrolytic behavior of glycosidic bonds is also substantially influenced by the conformation of the sugar unit and the inductive effect in these molecules caused by certain substituents in the chain. The half-chair conformation occurring intermittently during the hydrolytic attack is caused by a small rotation of the substituents around the links between carbon atoms 2 and 3, and between carbon atoms 4 and 5, respectively. Generally, the hydrolysis is supported if the axial substituents change to an equatorial position. As the rate of hydrolysis increases with the number of axial groups, the β-anomers are hydrolyzed faster than the corresponding α-forms, with the exception of L-arabinose [16].

Other effects of conformation can accelerate hydrolysis; for example, reducing end bounds are easily hydrolyzed when compared to non-reducing end bounds in polysaccharide chains. C5 substituent’s can also hinder hydrolysis reactions [16].

Furanosidic ring structures are hydrolyzed faster than the pyranosidic rings due to the difference in structural angular tension between furanosidic and pyranosidic rings. For example, in woods, α-D-galactofuranosides are hydrolyzed approximately 100 times faster than α-D-galactopyranosides [16].
The inductive effect describes the fact that different substituents on the ring promote changes in the electron density of the ring oxygen. Electrophilic substituents such as carbonyl and carboxyl groups reduce the protonation and inhibit the C–O fission, thus having a stabilizing effect on the glycosidic bond.

Figure 2. A simplified illustration for the mechanism of hemicellulose acid hydrolysis (adapted from [16]).

Figure 3 shows the inductive effect caused by the presence of glucuronic acid in the glycosidic chain. The carboxyl group induces different electron densities on the oxygen atom of the glycosidic bonds between A–B and B–C. The nucleophilicity is higher in the oxygen between B and C and this reduces the capacity for protonation. Thus, the bond is stabilized, while the glycosidic bond between A and B is activated by the same effect [16].

Figure 3. The inductive effect of the carboxyl group on acid hydrolysis (adapted from [16]).
The main problems associated with acid hydrolysis relate to the formation of toxic compounds from biomass degradation and from equipment corrosion. Such toxic products entail inhibition in cell metabolism when biomass hydrolyzates are used for bioconversion. Steps to remove these inhibitory compounds have been employed to improve the yields in bioconversion processes.

Table 1 presents different conditions of acid pretreatment for different lignocellulosic materials for enzymatic hydrolysis, as well as the cellulose conversion efficiency for hardwood, softwood, and sugarcane bagasse and straw. Historically, acid pretreatment has been the main choice for wood pretreatment [16].

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>T (°C)</th>
<th>Time (min)</th>
<th>H₂SO₄ (wt %)</th>
<th>Enzyme loading (FPU/BGU)</th>
<th>Enzymatic digestibility (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Athel pine¹</td>
<td>165</td>
<td>8</td>
<td>1.4</td>
<td>15FPU/52.5BGU</td>
<td>60 (160 h)</td>
<td>[17]</td>
</tr>
<tr>
<td>Spruce¹</td>
<td>180</td>
<td>30</td>
<td>5.0</td>
<td>15FPU/g solid</td>
<td>55 (24 h)</td>
<td>[18]</td>
</tr>
<tr>
<td><em>Eucalyptus camaldulensis</em>²</td>
<td>165</td>
<td>8</td>
<td>1.4</td>
<td>15FPU/52.5BGU</td>
<td>38 (160 h)</td>
<td>[17]</td>
</tr>
<tr>
<td>Mixed wood (10% birch and 90% maple)²</td>
<td>230</td>
<td>0.12</td>
<td>1.17</td>
<td>---</td>
<td>95</td>
<td>[19]</td>
</tr>
<tr>
<td>Sugarcane bagasse</td>
<td>130</td>
<td>15</td>
<td>0.5</td>
<td>7FPU/3.5BGU</td>
<td>41.5 (72 h)</td>
<td>[20]</td>
</tr>
<tr>
<td>Sugarcane straw</td>
<td>195</td>
<td>10</td>
<td>1.0</td>
<td>15FPU/10BGU</td>
<td>72.4</td>
<td>[21]</td>
</tr>
</tbody>
</table>

¹Softwood; ²Hardwood

Table 1. Examples of sugarcane and woody biomass pretreated with diluted acid.

2.1. Advantages and disadvantages of acid pretreatment

Pretreatment with diluted sulfuric acid has been reported as one of the most widely used processes due to its high efficiency [14]. This pretreatment removes and hydrolyzes up to 90% of the hemicellulose fraction, rendering the cellulose fraction more accessible to hydrolytic enzymes. However, it presents important drawbacks related to the need for a neutralization step that generates salt and biomass sugar degradation with the formation of inhibitors for the subsequent fermentation step such as furfural from xylose degradation. The removal of inhibitors from the biomass sugar syrups adds cost to the process and generates a waste stream. Additionally, mineral acids are corrosive to the equipment, calling for the use of more sturdy materials alongside higher maintenance costs. Acid recovery is also costly. The availability of the biomass acid pretreatment and the knowledge that has been built up on this subject highlights its important and costly drawbacks. In addition, the environmental problems caused by its waste streams have called for the need for other options for the pretreatment of lignocellulosic materials.
3. Hydrothermal pretreatments

3.1. Liquid hot water (LHW) pretreatments

The liquid hot water (LHW) treatments are also called hot compressed water treatments, hydrothermolysis [22, 23], aqueous or steam/aqueous fractionation [24], uncatalyzed solvolysis [25, 26], and aquasolv [27]. LHW is based on the use of pressure to keep water in the liquid state at elevated temperatures (160–240 °C). This process changes the biomass native structure by the removal of its hemicellulose content alongside transformations of the lignin structure, which make the cellulose more accessible to the further enzymatic hydrolysis step [1, 28]. Differently from steam-explosion treatment, LHW does not use rapid decompression and does not employ catalysts or chemicals. Nevertheless, as with the acid treatment, LHW depolymerizes hemicelluloses to the liquid fraction. In this case, sugars are removed mostly as oligosaccharides, and the formation of the inhibitors furfural and 5-hydroxymethylfurfural (HMF) is at a slightly lower level [28], depending on the process conditions. To avoid the formation of inhibitors, the pH should be kept at between 4 and 7 during the pretreatment, because at this pH, hemicellulosic sugars are retained in oligomeric form, and monomer formation is minimized. The removal of hemicellulose also results in the formation of acetic acid in the liquid fraction.

LHW pretreatment, whose most important parameters are the biomass moisture content, the operation temperature, and the residence time [29], is usually done in a pressure tank reactor where two streams can be obtained after filtration of the biomass slurry: a solid, cellulose-enriched fraction and a liquid fraction rich in hemicellulose-derived sugars. The solid phase is therefore constituted by cellulose and lignin along with residual hemicellulose. There are three types of reactor design for LHW pretreatment. For co-current reactors, the biomass liquid slurry passes through heat exchangers where it is heated to the appropriate temperature (140–180 °C) and kept for 10–15 minutes as the slurry passes through an insulated plug-flow snake-coil, followed by the slurry-cooling concomitant to heat recovery via the countercurrent heat exchange with the incoming slurry. Flow-through technologies pass hot water at 180–220 °C and approximately 350–400 psig. The resulting pretreated biomass has enhanced digestibility and a significant portion of the lignin is also removed. In countercurrent pretreatment, the biomass slurry is passed in one direction while water is passed in another in a jacketed pretreatment reactor. Temperatures, back pressures, and residence times are similar. In the flow-through pretreatment reactor, water or acid is passed over a stationary bed, and removes some of the biomass components including lignin. Although LHW can result in the partial depolymerization and solubilization of lignin, the re-condensation of lignin-derived, soluble compounds is also observed. Flow-through systems have been reported to be more efficient in terms of hemicellulose and lignin removal in comparison to batch systems for some types of biomass via the addition of external acid during the flow-through process [30].

There have been many studies on the use of LHW for the pretreatment of corn fiber [28, 30-33], wheat straw [34, 35], and sugarcane bagasse [36, 37]. Studies on woody biomass from Eucalyptus [38-40], and olive tree biomass [41] have also been reported.
Several works have reported about the optimal LHW pretreatment conditions in terms of temperature and residence time. For the pretreatment of corn stover the best conditions were reported to be 190 °C and 15 min, resulting in a 90% of cellulose conversion after enzymatic hydrolysis [33], while for wheat straw the optimum treatment temperature was found to be 188 °C during 40 min, which resulted in 79.8% of cellulose conversion and releasing of 43.6% of hemicellulose derived sugars to the liquid fraction. Nonetheless, when response variables were analyzed separately, the best conditions for the recovery of hemicellulose-derived sugars from wheat straw, at up to 71.2%, were found at 184 °C during 24 min, whereas the optimal conditions for a cellulose conversion of 90.6% were found to be 214 °C during 2.7 min [35].

For sugarcane bagasse, top-performing LHW runs are favored by high temperatures (≥ 220 °C) and a short residence time (≤ 2 min) associated with low solids concentrations (≤ 5%), reaching 87% of simultaneous saccharification and fermentation (SSF) conversion, and 81% of xylan recovery. However, it is reported that the use of LHW using a solid concentration of more than 1% can significantly decrease the ethanol fermentation rate due to inhibition [36].

The LHW pretreatment of *Eucalyptus* biomass was studied in two steps as follows: in the first step the pretreatment in which a temperature range from 180 to 200 °C was studied gave the highest total xylose recovery yield of 86.4% at 180 °C for 20 min. In the second step of the pretreatment, a temperature range from 180 to 240 °C was studied for intervals of time up to 60 min. The authors concluded that the efficiency of LHW for the cellulose conversion rate was more sensitive to temperature than residence time. The optimum reaction conditions for the second step of the pretreatment with minimal degradation of sugars were found to be 200 °C for 20 minutes, where the total sugar recovery from *Eucalyptus grandis* after 72 h of enzymatic saccharification reached 96.63%, which is superior to the yield from a single-step pretreatment with hot water or diluted acid [40].

LHW pretreatment of olive tree biomass resulted in a 72% glucose yield from cellulose hydrolysis after 72 h of saccharification using 2% of solids concentration during pretreatment, while for higher solids content the glucose yields were strongly affected reaching 70%, 60%, 57%, and 43% when using 5%, 10%, 20%, and 30% of solids, respectively [41]. A two-stage process which combines the LHW for hemicellulose removal and a treatment for delignification (e.g. ammonia pretreatment) has also been suggested for further improvement of enzymatic hydrolysis [42].

### 3.2. Steam pretreatment

The steam treatment is quite similar to LHW, with the major difference between the processes being related to the contact of the liquid phase with the biomass. For LHW, the biomass is in direct contact with the liquid phase at the bottom of the reactor, which prevents the use of high solids content, while in the steam pretreatment, the biomass is at the top of the reactor and not in direct contact with the liquid phase, in a similar manner to that of an autoclaving process. Using steam pretreatment means that it is possible to use a higher solids content of 50% or more, whereas for the LHW, in most cases, the solids content is lower than 10%. Sim-
Similarly to what is observed for LHW, the hemicellulose fraction is extracted by direct contact with water-saturated steam and due to the high temperatures and high solids concentration (around 50%), lignin and biomass polysaccharides can be extracted and degraded, releasing derived products such as furfural, HMF, and derived acids at high concentrations [36].

Sugarcane bagasse pretreatment by steam using high solids content (≥ 50%) at 200 °C for 10 min allowed a poor xylan recovery of 12%; nevertheless, an SSF yield of 79% was observed and the dissolved xylan content was found to be 89%. When the steam treatment was carried out at 220 °C for 2 min, the xylan recovery was increased to 48%, and the SSF yield and the dissolved xylan content were 85% and 88%, respectively, indicating the efficiency of high temperatures coupled with very short pretreatment times for high solids concentrations [36].

Aiming to improve the recovery of xylan sugars, most of the steam treatment studies report the use of SO$_2$ as the catalyst. When this procedure was used for sugarcane bagasse, it allowed for the recovery of 57% of hemicellulose-derived sugars and minimal amounts of sugar-degradation compounds were formed. The overall highest sugar yield achieved from the bagasse cellulose enzymatic hydrolysis was 87% [43]. There are several reports on the use of steam pretreatment associated with rapid decompression as a pretreatment for several biomass types. In this chapter, this type of treatment will be addressed as a steam-explosion treatment.

3.3. Advantages and disadvantages of LHW and steam pretreatments

LHW and steam pretreatments are attractive from a cost-savings perspective, as they do not require the addition of chemicals such as sulfuric acid, lime, ammonia, or other catalysts. Moreover, the reactors do not require high cost materials and maintenance due to their low-corrosion potential. Additionally, these treatments do not alter the biomass glucan content, as a glucose recovery rate of 97% was observed for sugarcane bagasse that was pretreated by both methods [36]. The main differences between the features of the two treatments relates to hemicellulose extraction, which is higher for the LHW, and the biomass load, which is higher for the steam pretreatment, with the obvious corresponding advantages and disadvantages. In contrast to steam pretreatment, LHW allows for a higher xylan recovery associated with the lower formation rate of inhibitors.

4. Steam-explosion pretreatment

Steam explosion is one of the most used methods for lignocellulosic biomass pretreatment. This process was initially developed in 1926 by the Masonite Corporation, Canada, for the production of fiberboard from wood [44]. From 1970–1980, the process was adapted to treat wood and agriculture residues aiming at improving the cellulose enzymatic hydrolysis and cattle feed production from lignocellulosic materials. A batch-type device was available from Iotech Corporation at pilot-plant scale in 1983 and a continuous device was available from Stake Technology, both from Canada, in the ‘80s.
In this process, the biomass is subjected to pressurized steam at temperatures ranging from 160 to 260 °C (corresponding to 0.69–4.83 MPa) for a few seconds to 20 minutes, followed by a rapid decompression of the reactor, by opening up an outlet, sharply reducing the temperature and interrupting the reactions. This approach combines chemical and mechanical forces in order to solubilize the hemicellulose fraction and render the cellulose more accessible to the enzymatic hydrolysis [1, 28, 45-47].

In the first step, called autohydrolysis, the water at high temperature causes the release of organic acids (mainly acetic acid) from the hemicellulose biomass moiety, followed by a chain reaction where the hemicellulose is partially solubilized and hydrolyzed [46]. The mechanical effects are caused by the rapid decompression, which results in the disruption of the cell wall fibers and, by extension, particle size reduction, and increased porosity [48]. In association with the partial hemicellulose hydrolysis, lignin is degraded to some extent, and a small portion is removed from the material [49].

At the end of the process, an insoluble solid fraction and a liquid fraction are obtained. The solid fraction contains primarily cellulose and the partially modified lignin. The liquid fraction—whose pH is in the range of 3.5 to 4.0 depending on how many of the acid chains are released [47]—contains soluble carbohydrates derived from hemicellulose in the form of oligomers and monomers in a proportion that is subject to the process conditions [11, 50]. A balance between the temperature and time of the pretreatment should be achieved in order to minimize the formation of phenolic compounds, furfural, and hydroxymethylfurfural from the degradation of lignin, five-carbon sugars (C5), and six-carbon sugars (C6), respectively. The formation of such compounds, as they may be inhibitory to the subsequent enzymatic hydrolysis and ethanol fermentation-production steps, should be minimized as much as possible.

Another approach for the steam-explosion process has been the use of impregnating agents such as SO₂, CO₂, and H₂SO₄, which can improve the effectiveness of the pretreatment, increasing the efficacy, and decreasing the residence time. In some materials, such as softwood, the intrinsic level of organic acids is not enough to promote the degradation of the hemicellulose backbone. Thus, the addition of a mineral acid can reduce the initial pH to below 2 and promote the hydrolysis. On the other hand, the use of acids as impregnating agents may require an additional step for pH adjustment. Moreover, such an approach may enhance partial carbohydrate and lignin degradation, resulting in an increase in the formation of toxic compounds, which will affect enzymatic hydrolysis, and fermentation [50]. Steam-explosion technology without additives has been successfully performed for ethanol production from hardwoods and for a wide range of agricultural residues [1].

The use of CO₂ was tested as an impregnating agent in the steam-explosion pretreatment of sugarcane bagasse and straw, and the pretreatment was assessed in terms of glucose yields after enzymatic hydrolysis [50]. For sugarcane bagasse, the highest glucose yield (86.6% of the theoretical level) was obtained after pretreatment at 205 °C for 15 min. For straw, the highest glucose yield (97.2% of the theoretical level) was obtained after pretreatment at 220 °C for 5 min. The reference pretreatment, using impregnation with SO₂ and performed at 190 °C for 5 min, resulted in an overall glucose yield of 79.7% and 91.9% for bagasse and
leaves, respectively. The production of toxic compounds from the dehydration of sugars (mainly furfural and hydroxymethylfurfural) was less than 1%.

Many patents [51-53] have been granted for steam-explosion, and these processes have been widely tested in pilot- and demonstration-scale ethanol plants, and are considered to be close to commercialization [47]. Iogen Corporation (Ottawa, Canada) was the first company to commercialize cellulosic ethanol; at full capacity, the demonstration plant was designed to process approximately 20–30 tons per day of wheat, barley, and oat straw, and to produce approximately 5000–6000 liters of cellulosic ethanol per day [54].

In Brazil, the pretreatment of lignocellulosic materials by steam explosion was initially developed at the Foundation of Industrial Technology in 1981. Studies which were performed on bench scale (a 1.6-L reactor) with sugarcane bagasse, Eucalyptus, and elephant grass, produced a wealth of data regarding the degree of hydrolysis and solubilization of the hemicellulose fraction, and the susceptibility of the treated material to enzymatic saccharification in the presence or absence of impregnating agents (SO2 and H2SO4). These studies [55, 56] showed that the enzymatic saccharification enhancement due to steam explosion was more prominent for Eucalyptus, though steam exploded bagasse, and steam exploded grass were more accessible to attack by enzymes in comparison to treated Eucalyptus. In contrast to Eucalyptus, treatment conditions for achieving higher saccharification yields for bagasse and elephant grass were similar to those for optimal hemicellulose recovery in the process liquid stream. The use of the optimum time and temperature conditions for sugarcane bagasse (200 °C for 5–7 min) resulted in a 63% C-5 sugar recovery as soluble oligomers and an increase in glucose yield after enzymatic hydrolysis at standard conditions from 13% (untreated bagasse) to 56%. The use of 0.25% sulfuric acid as the impregnant agent promoted a sharp decrease in the optimal pretreatment time (1 min) and allowed a higher recovery of the hemicellulose fraction (90%) as monomers. Saccharification yields were at the same levels as those observed for the treatment without acid addition. For Eucalyptus, the best pretreatment conditions for C-5 sugar recovery (61%) were 200 °C for 3.5–5 min, while those for achieving higher enzymatic hydrolysis yields were 200 °C for 9 min, in which saccharification yields increased from almost zero (untreated wood) to 29.9%. Moreover, a study evaluated the effects of the explosion step in the steam-explosion process by comparing sugarcane bagasse pretreatment by steam explosion at 200 °C with that at the same temperature but without the explosion process [56]. Although both pretreatment processes showed almost identical chemical effects (hemicellulose autohydrolysis and cellulose and hemicellulose recovery), steam-exploded bagasse was 14.4% more accessible to attack by cellulases. Engineering studies were also carried out in order to expand the reactor scale to 0.2 and 2 m3, both of which were tested and operated successfully [57]. These results allowed for the development of a joint project with the Sugar and Alcohol Plant, Iracema (Iracemápolis, SP), for the installation and operation of an unit with the capacity to produce treated bagasse enough to feed 150 cows/day. These studies have contributed to the implementation of the steam-explosion process of sugarcane bagasse for the production of cattle feed, currently operating in Brazil.
4.1. Advantages and disadvantages of steam-explosion pretreatment

The main advantages of steam explosion relate to the possibility of using coarse particles, thus avoiding a biomass-size conditioning step, the non-requirement for exogenous acid addition (except for softwoods, which have a low acetyl group content in the hemicellulosic portion), a high recovery of sugars, and the feasibility for industrial implementation. Moreover, the soluble stream rich in carbohydrates derived from hemicellulose in the form of oligomers and monomers may be easily removed and used as feedstock for the production of higher added-value products such as enzymes and xylitol [58]. Other attractive features include less hazardous process chemicals and conditions, the potential for significantly lower environmental impact, and lower capital investment [59]. The fact that the steam-explosion process does not require previous grinding of the raw biomass is an important feature, considering that the energy required to reduce the particle size before the pretreatment (pre-grinding) can represent up to one-third of the total energy required in the process [60].

The main drawbacks related to steam-explosion pretreatment are the enzyme and yeast inhibitors generated during the pretreatment, which include furfural and hydroxymethyl furfural; the formation of weak acids, mostly acetic, formic, and levulinic acids, the two latter acids being derived from furfural’s and hydroxymethyl furfural’s further degradation; and the wide range of phenolic compounds produced due to lignin breakdown. Several detoxification methods have been developed in order to reduce the inhibitory effect, which represent additional costs in the overall process. Other limitations of this method include the incomplete disruption of the lignin–carbohydrate matrix [11].

5. Mechanical pretreatments

Mechanical pretreatments of biomass aim primarily to increase the surface area by reducing the feedstock particle size, combined with defibrilization or reduction in the crystallinity degree. This approach facilitates the accessibility of enzymes to the substrate, increasing saccharification rates and yields. The most studied biomass mechanical pretreatment for biomass is the milling process, mainly the ball-milling and disk-milling pretreatments. Another mechanical treatment to be considered is extrusion, even though this process involves additional thermal and/or chemical pretreatments.

5.1. Milling

Different types of milling processes can be used to improve the enzymatic hydrolysis of lignocellulosic materials [61]. The main objective of milling pretreatment is to reduce particle size in order to increase the biomass-specific surface during biomass fibrillation and to reduce cellulose fiber organization, which is measured by a decrease in crystallinity. These effects can be produced by a combination of chipping, for final particle sizes of 10–30 mm, or grinding or milling, for final particle sizes of 0.2–2 mm [11]. It is important to emphasize that macroscopic particle size reduction does not lead to significant improvements of bio-
mass enzymatic saccharification, which is solely achieved by using a milling process that alters the biomass structure at a nanoscopic level. Ball milling and wet disk milling (WDM) are the most common biomass milling pretreatments.

5.1.1. Ball milling

The ball milling process uses mechanical shear stress and impaction to produce powdered material [62]. This process can be done in the wet or dry state. A combination of chemicals, such as acids, bases and organic solvents, can also be applied depending on the main treatment purpose. In general, the process uses a rotary drum and balls of different sizes made from different materials (tungsten, ceramic or stainless steel). The effect of ball milling on the biomass particle size, structure and crystalline degree depends on the rotation speed, operation time and ball size. Ball milling treatment can be considered a kind of ultra-fine grinding and fibrous materials can present between 10-20 µm in terms of particle size [63]. Highly crystalline cellulose has a strong interchain hydrogen-bonding network that confers a high resistance to enzymatic hydrolysis, whereas amorphous cellulose is readily digestible [64].

For biomass pretreatment after the material is fed into the rotary drum equipped with balls, drum rotation around a horizontal axis causes a reduction on the material particle size [65]. The ball milling process can drastically alter the complex heterogeneous network structure of wood cell walls, and with a long pretreatment time, cellulose crystallinity can be significantly reduced, which increases the ratio of amorphous cellulose, thus improving the saccharification yields [65]. However, it was found that nano-fibrillation of woody biomass by ball milling in the wet state can improve the saccharification yield without a significant decrease in cellulose crystallinity [65]. They found the crystallinity index on the ball-milled biomass to be ca. 41% in comparison to 68% for the raw material. Thus, the decrease in particle size to a powder-like material and the increase of surface area seemed to be the main factors that promoted the hydrolysis of treated wood.

Results obtained with the pretreatment of sugarcane biomass have shown that ball milling treatment of bagasse for 60 min and sugarcane straw for 90 min results in glucose and xylose yields of 78.7% and 72.1% for bagasse and 77.6% and 56.8% for straw, respectively. In both cases, the enhancement in cellulose digestibility was related to the reduction of cellulose crystallinity to nearly an amorphous level [66]. In another study, the 20 min pretreatment of Eucalyptus using a planetary ball milling process was insufficient for improving enzymatic digestibility, even though the crystallinity index decreased from 59.7% to 7.6%. When a prolonged milling time of 120 min was used, the enzymatic digestibility of both glucan and xylan increased, while the degree of crystallinity of the material was almost the same as that milled for 20 min. Additionally, the digestibility of glucan and xylan and their total yield were 76.7%, 63.9% and 74%, respectively, even at a substrate concentration of 20% and an enzyme dosage of 4 FPU/g of substrate, indicating that ball milling is extremely efficient to enhance biomass reactivity to enzymes [39].
5.1.2. Advantages and disadvantages of ball milling

Mechanical disruption of cellulose by ball milling is a candidate method for a significant increase of cellulose-accessible surface area without the loss of low-molar mass components. After ball milling treatment, without the use of additive chemicals, the treated material keeps the same chemical composition of the untreated material and there is no generation of liquid fractions, gas or inhibitors. As such, ball milling is an environmentally friendly pretreatment method for lignocellulosic biomass. However, milling processes are known to be very energy intensive, depending on the material characteristics and the target particle size [36]. Taking into account the high energy requirements of milling and the continuous rise of energy prices, it is likely that this process is not economically feasible [67]. Moreover at an industrial scale, ball milling equipment requires high dimensions; nevertheless, in specific cases the balls can be replaced by bars for efficient milling depending on the amount of biomass to be used. Recently, a new milling pretreatment method for lignocellulosic biomass was described by using disk milling in a wet state, as described below [68].

5.1.3. Wet disk milling (WDM)

WDM is a recently introduced biomass pretreatment process able to produce milled biomass with low levels of inhibitors; it is considered to be feasible for industrial implementation. This technique has been shown to increase the degree of biomass fibrillation and the nano space between the microfibrils, thus promoting the accessibility of the cellulolytic enzyme pool to cellulose [69]. The disk mill is a type of crusher that can be used to grind, cut, shear, fiberize, pulverize, granulate or blend. In general, the suspended material is fed between opposing disks or plates that can be grooved, serrated or spiked. The force applied in the material will depend on the type of disks, the distance between the disks and its rotation speed. For biomass processing using WDM, a water suspension (1–5% of solids) of the lignocellulosic material is passed between two ceramic non-porous disks that are separated by a distance of 20–100 µm and that have a rotational speed of around 1800 rpm. This process can be repeated according to the required number of WDM cycles; very small particle sizes with high specific surface areas have been observed for a minimum of five cycles [66, 68].

A study on WDM pretreatment of sugarcane bagasse and straw showed that enzymatic hydrolysis yields increased with the number of WDM cycles; maximum sugar yields were obtained with 20 cycles, leading to glucose and xylose yields of 49.3% and 36.7% for sugarcane bagasse and 68.0% and 44.9% for sugarcane straw, respectively [66]. Hydrolysis yield data for 10 WDM cycles showed a glucose yield for bagasse of 31.5%, while a glucose yield of 56.1% was observed for straw, confirming that WDM is more efficient for straw.

As WDM is a recent procedure for biomass pretreatment, there is limited information on the pretreatment of several different types of biomass. However, reported data for the pretreatment of rice straw showed that after 10 cycles of WDM it was possible to achieve glucose and xylose yields of 78.5% and 41.5% respectively, with an energy consumption of 5.4 MJ/kg of rice straw. The authors evaluated energy consumption using 60 min ball milling for the pretreatment of rice straw. The process used 108 MJ/kg of rice straw, a value 20-fold higher than that for 10 cycles of WDM [69].
5.1.4. Advantages and disadvantages of WDM

Although WDM pretreatment presents lower energy consumption than that for ball milling, it requires large amounts of water due to low solids loading (1–5%), which is a drawback that may hinder its industrial application. WDM of rice straw has been reported to require almost the same energy (5.4 MJ/kg of biomass) as hydrothermal pretreatment, exemplifying the possibility of using milling for biomass pretreatment [69].

5.2. Extrusion process

Screw extruders were originally designed to extrude polymers and were also developed for food and feed processing [70-72]. An extruder can provide many functions, such as cooking, forming, kneading, degassing, dehydration, expansion, homogenization, mixing, sterilization, shaping, densification and shearing [73]. These functions can be performed in the same process, depending on the size of the extruder and the screw design. Since the 1990s, there has been an increase in the number of studies that use extrusion for biomass processing, such as for the extraction of compounds [74-76], densification [73, 77] and biomass pretreatment for enzymatic saccharification [78-87]. For lignocellulosic biomass pretreatment, extrusion processing can provide a unique continuous reactor working at higher throughput and solid levels. The extrusion equipment provides temperature control and efficient pulverization by applying a high shearing force. This process also allows the advantageous simultaneous combination of thermomechanical and chemical pretreatment.

The extruder consists of a barrel with a rotating screw (or screws) that squeezes and conveys the material continuously from the input to the output. The barrel is normally segmented, which allows temperature control (heating or cooling) along its length and feeding ports for additive injection. Different types of screw elements can be installed onto the shafts for screw configuration in accordance with the process requirements. The possibility to design the screw configuration using many combinations of elements renders the process very flexible. There are conveying, backward-conveying and kneading elements that can be threaded in different ways to provide mixing, shearing, elongation flow, and pressure build up. The use of kneading disks, which can be staggered at diverse angles (typically 30°, 45°, 60° and 90° of stagger) in forward or reverse directions, can impart a high shearing stress by forcing the material to pass through the small clearances between the disks and between the disks and the barrel surfaces [88, 89]. It is also possible to configure sealed regions where the pressure can be significantly higher than other zones.

There are several types of extruders that can be classified according to the number of shafts: single-screw, twin-screw or multiple-screw extruders. The single-screw extruder presents a unique screw rotating in a stationary barrel and is more applicable for distributive mixing without changes in physical properties of the material [89]. On the other hand, some types of twin-screw extruders can provide distributive and dispersive mixing; twin-screw extruders are normally applied to obtain changes in physical properties of materials, such as the reduction in particle size by high shearing forces [89]. The twin-screw extruder can be classified according to the rotation directions of the two screws: counter rotating (opposite directions) [90] or co-rotating (same directions) [88]. The counter-rotating design is used for a
very high shear force; nevertheless, it can generate excessive wear and tear. The co-rotating twin screw can be operated at high screw speeds, resulting in high outputs, while maintaining the required shear force, mixing and conveying properties. Figure 4 shows an example of screw configuration and temperature control versatility of a twin-screw extruder used for biomass pretreatment [82].

Figure 4. Example of a twin-screw configuration. The elements can be exchanged to be adapted to the type of biomass and the pretreatment conditions, such as retention time and shearing force.

Thus, the co-rotating twin-screw extruder is one of the most promising configurations for biomass processing due to its flexibility to carry out biomass deconstruction under relatively high stress, temperature and pressure. The twin-screw extruder is also easy to operate and economically suitable for large-scale production [80]. However, the use of kneading elements and sealed regions, with reverse elements, for biomass pretreatment at high rates and high solid concentration depends on the flow properties of the biomass. The extrusion of lignocellulosic biomass is difficult due to poor flow properties inside the extrusion barrel, leading to accumulation, burning and blocking of the die during the process [82]. This material can be mixed with water or additives to increase the viscosity and its flow capability, reducing the operational torque and transposing the aforementioned problems. The combination of chemical pretreatment inside the extruder, i.e., alkaline [91, 92], acid [93, 94] and ionic liquids [95], can also increase flow and reduce torque, allowing the use of extrusion as a continuous mixing reactor for biomass processing.

The extrusion process was reported for starch conversion [96] and for wood pulping [97]. A twin-screw extruder was used to fibrillate wood chips to produce individual fibers, which have higher aspect ratios than the wood flour particles usually used for wood–polymer composites [98]. Twin-screw extruders were also used as extractors of lipids [74] and essential oils [75]. The extraction of hemicelluloses via alkaline solubilization using a twin-screw extruder for pentose production from the hardwood Populus tremuloides was also reported [76]. The major advantage of using twin-screw for extraction of biomass compounds is that kneading and reverse screw elements can cause severe compression of the material and allow the simultaneous extraction and liquid/solid separation in a very efficient manner.

Significant improvements in sugar yield after enzymatic hydrolysis have been reported for biomass pretreatment based on extrusion; examples include corn stover [92], Miscanthus
big bluestem, prairie cord grass, switchgrass, Indian grass. Nevertheless, there are no reports for the use of extrusion for pretreatment of sugarcane bagasse and straw aimed at cellulosic ethanol production.

The use of single-screw extrusion for the pretreatment of corn stover and soybean hulls at high solid loadings (75–80%) resulted in 54–61% sugar recovery. The pretreatment of Douglas fir wood (coniferous, Pseudotsuga) was performed by a counter twin-screw extruder using cellulose affinity additives (ethylene glycol, glycerol and dimethyl sulfoxide) to effectively fibrillate the wood cell wall and lower the extrusion torque. However, it has been suggested that torque is more effective for fibrillation than temperature and the swelling effects of additives. The enzymatic conversion of extruded products into glucose was three to six times higher than that of untreated material. Ethylene glycol was found to be the most effective additive for fibrillation, achieving a glucose yield of 62.4%. In another study, sawdust and wood chips were pretreated using a twin-screw extruder; this resulted in the recovery of 65% of glucose upon enzymatic hydrolysis, which was over 10-fold higher than that of untreated material.

Extrusion was also used in combination with hydrothermal and chemical pretreatment. A single-screw extruder was reported for the pretreatment of wheat straw in conjunction with NaOH, Na2S and H2O2; nevertheless, the mass flow rates and concentration were limited to low values of 10–30 g wheat straw (dry basis)/min and 12–33% solids concentration. The use of a twin-screw extruder in combination with the ammonia fiber explosion (AFEX) process for the pretreatment of milled dry corn stover showed that the extrusion process with ammonia compares well to batch AFEX pretreatment. The pretreatment of milled (under 3 mm) Douglas fir (softwood) and Eucalyptus (hardwood) by LHW followed by a co-rotating twin-screw extruder was also reported. The glucose yields obtained by extrusion after LHW were higher than those for the individual use of LHW for both types of biomass. Results for Douglas fir were fivefold higher, compensating for the limitations of LHW for this material as LHW is known to be less effective for softwood than for hardwood. The Douglas fir wood was also treated using a batch-type kneader with twin-screw elements. The biomass was pulverized by ball milling for 20 min followed by kneading for 20 min. The maximum glucose yield was 54.20% (25.40 wt% based on initial wood weight). However, glucose yield was improved by heating the extruded biomass with water under pressure (135 °C and 0.25 MPa), revealing that only mechanical kneading with water showed limitation for enhancing the accessibility of cellulose to enzymes.

The thermomechanical extrusion of wheat bran and soybean hulls led to reduced sugars yields of 65–73% and 25–36%, respectively. The combination of lower temperature and high residence time (low screw speed) or higher temperature and low residence time (high screw speed) led to higher sugar yields; these authors also tested the combination of chemicals (NaOH, urea and thiourea) with extrusion, with no significant improvements.

The combination of twin-screw extruder and diluted acid pretreatments was recently reported for rice straw and rape straw. When rice straw was pretreated with 3% sulfuric acid at 120 °C a low (32.9%) glucose yield was observed. However, the use of extrusion/acid pretreatment followed by a hot water extraction step enhanced the enzymatic hydrolysis.
yield from 32.9% to 60.9%. The hot water hemicellulose extraction step allowed the conversion of 83.7% of xylan to xylose and favored cellulose hydrolysis [93]. Rape straw extrusion pretreatment with 3.5% sulfuric acid at 165 °C obtained a glucose yield of 70.9% [94].

The high sugar recovery due to extrusion pretreatment is related to fibrillation, the increase in surface area [79-81, 86, 87] and pore size [103], which facilitate the access of enzymes to cellulose. Some authors have reported that the crystallinity, which confers resistance to enzymatic hydrolysis, was not significantly reduced in extruded biomass [79, 102] and therefore was not related to the increase in biomass digestibility. Moreover, an increase of 82% in the crystallinity of soybean hulls by thermomechanical pretreatment, using a twin-screw extruder was reported [82]; as there was no change in material composition, crystallization of the amorphous structure during thermomechanical extrusion was suggested. Some researchers have also noted the crystallization of cellulose in the presence of moisture and heat, as has been observed for wood pretreated by steam explosion [104], cotton linter and wood treated in aqueous media after ball milling [105] and hemp cellulose treated by wet ball milling [106]. In accordance with the aforementioned, some researchers suggested that the opening of the cell wall structure at a microscopic scale is sufficient for enzymatic saccharification, regardless of the cellulose crystallinity index [79]. Furthermore, the combination of thermomechanical and/or chemical pretreatments can deconstruct the hemicelluloses chains and/or remove part of the hemicelluloses and lignin, facilitating biomass digestibility [82, 102, 107].

5.2.1. Advantages and disadvantages of extrusion pretreatment

The twin-screw extruder is highly efficiency for pulverization by applying high shearing forces and shows adaptability to different processes, such as chemical, high-pressure applications and explosion pretreatments (steam or other solvents) [79-81, 92, 108, 109]. The process is easy to operate and the extrusion process allows the continuous pretreatment of large amounts of biomass with high throughputs, which is advantageous in comparison to batch procedures for the industrial setting. Extrusion compares well to pretreatment technologies that have as drawbacks the batch processing mode, low solids loading or the use of large amounts of water, as already mentioned. Extrusion allows temperature control and does not require washing and conditioning steps, as required with diluted acid, alkali or ionic liquid pretreatments and does not produce effluent; thus there is no effluent disposal cost, no solids loss and no significant safety issues [86]. In comparison to other mechanical pretreatments, the extrusion process is normally less energy intensive than the milling pretreatment options. If extrusion is combined with chemical pretreatment, due to its effective ability of kneading and mixing, the process requires less chemical loadings and thus less residual effluents are formed; the combination of extrusion with chemical pretreatment can further reduce energy consumption as it is economically suitable for large-scale operation. Furthermore, extrusion does not produce fermentation inhibitors, such as furfural and hydroxyl methylfurural; nevertheless, low concentrations of acetic acid have been reported [84-87]. However, the extrusion pretreatment of lignocellulosic biomass requires the use of additives to increase the flow ability inside the barrel and avoid the accumulation, burning
and blocking of the die during the process. Another disadvantage in comparison to other biomass pretreatments is the low yields of enzymatic hydrolysis achieved.

6. Ionic liquid pretreatment

Ionic liquids (ILs) can be defined as salts that melt below 100 °C and are composed exclusively of ions. The first report of a room temperature IL dates back to 1914 [110] and did not prompt any significant interest at that time. It was in the 1980s that these chemicals have come under intense worldwide attention due to the implications for their use as solvents [111, 112]. The fact that many ILs can be liquid at room temperature and, in general, present a negligible vapor pressure has justified the attention that this group of chemicals has received. They have also been suggested as candidates to substitute for low-boiling-point solvents, such as toluene, diethyl ether and methanol. In addition, ILs are versatile materials and often called designer solvents because their physical and chemical properties can be tuned to meet a specific purpose by preparing new ILs with different combinations of ions [113].

ILs have become increasingly trendy over the past few years in the biomass field due to the ability of some members of this class of chemicals to dissolve a wide variety of biomass types. ILs have been reported for the pretreatment of cellulose [114] and lignocellulosic materials, such as rice straw [115], sugarcane bagasse [116, 117], wheat straw [118], switchgrass [119], Miscanthus [120] and wood [121, 122, 123], among others. However, this concept is not new since in 1934 a patent claimed that certain organic salts were capable of dissolving cellulose and alter its reactivity [124]; nevertheless, at that time this publication did not generate any important reaction in the scientific community. In 2002, a research group from the University of Alabama investigated new compounds, now known as ILs, based on the concept of cellulose dissolution by a molten salt described by Graenacher in 1934. As result, they found that the IL 1-methyl-3-butyl imidazolium chloride ([Bmim][Cl]) could dissolve up to a 10% solution of cellulose by stirring cellulose with the IL while heating (100 °C). When heating was performed in a microwave oven, the dissolution achieved was up to 25% (wt%) [125]. Their pioneer work has now been cited over 1000 times and is considered a breakthrough that has set the basis for a novel concept for lignocellulosic biomass pretreatment.

Based on the concept of cellulose dissolution described by Swatloski and co-workers [124] and lately by another work that has shown that [Bmim][Cl] was also able to partially dissolve wood [126], many research groups have described processes of biomass pretreatment with ILs; most of these studies document the complete or partial dissolution of lignocellulose under heating conditions followed by precipitation with water as an antisolvent. The aim of this procedure is to recover a pretreated part of the biomass that is highly susceptible to enzymatic attack. After IL pretreatment, the biomass native structure is altered in the recovered material in such a manner that the reconstructed cellulose is essentially amorphous compared to highly crystalline untreated cellulose [127].
The mechanism for IL cellulose dissolution has been investigated by applying different analytical methods. In one study, nuclear magnetic resonance (NMR) relaxation measurements on [Bmim][Cl] confirmed that chloride ions form hydrogen bonds with the cellulose hydroxyl group in a stoichiometric 1:1 ratio [128]. This interaction causes the break of intermolecular and intramolecular hydrogen bonding between cellulose fibrils, which ultimately leads to cellulose dissolution. Additionally, depending on the type of IL, an efficient extraction of lignin can be facilitated by the cellulose dissolution process, as more lignin can be exposed to the solvent [121, 126].

Different combinations of anion and cation compositions have been examined for biomass pretreatment, as the dissolution of biomass components is highly affected by the nature of the IL. In general, in order to dissolve cellulose, the anion of the IL must be a good hydrogen bond acceptor [123, 129]. The most promising anions have been shown to be chlorides, acetates, formates and phosphates. It has also been demonstrated that cations play a role in cellulose solubility as the imidazolium cation, whose electron-rich aromatic π system interacts with cellulose hydroxyl oxygen atoms via nonbonding and π electrons, prevents the cross-linking of cellulose molecules. In general, the most appropriate cations for cellulose dissolution are based on methylimidazolium and methylpyridinium cores, with allyl-, ethyl-, or butyl-side chains [130].

Considering the IL pretreatment of sugarcane bagasse, the IL 1-ethyl-3-methylimidazolium acetate ([Emim][Ac]) has been selected among six ILs studied as the best choice for pretreatment (120 min, 120 °C and 5% solid loading) as it was possible to reach a glucose yield of 98.2% after 48 h of enzymatic hydrolysis of 2.5% pretreated bagasse loading using commercial enzymes at a dosage of 15 FPU/g bagasse [116]. The authors suggested that the resulting pretreated biomass was highly digestible due to its amorphous-like structure, the high ability of [Emim][Ac] to extract lignin and the increased specific surface area (SSA) of 131.8 m²/g compared to an SSA of 1.4 m²/g measured for untreated bagasse. In another study, yields of 69.7% of reducing sugars were obtained for the enzymatic hydrolysis (30 FPU/g substrate) of 2% bagasse loading pretreated with [Emim][Ac] for 15 min at 145 °C, using a 14% solid loading during pretreatment [131]. Since high-solid loading during pretreatment was applied, [Emim][Ac] was ineffective in bagasse delignification, even though it was able to reduce the biomass crystallinity.

Some studies have combined other pretreatment strategies to IL pretreatment of sugarcane bagasse to reduce the pretreatment time and increase the efficiency. In an HCl-catalyzed pretreatment process in IL aqueous solutions, optimum conditions for the sugarcane bagasse pretreatment was obtained at 130 °C, 30 min, using a water:[Bmim][Cl]:HCl solution (%) of 20:78.8:1.2. Cellulose digestibility yields corresponding to 94.5% were obtained after 24 h saccharification of 2% glucan loading, using commercial enzymes at a dosage of 20 FPU/g glucan; the pretreatment for 120 min using solely [Bmim][Cl] resulted in 29.5% cellulose conversion [132]. Other reports of sugarcane bagasse pretreatment using [Bmim][Cl] have also reported low glucose yields of 38.6% (120 °C, 120 min) [116] and 62% (140 °C, 90 min) [117].
The reduction of cellulose crystallinity is usually reported as a main effect of IL pretreatment. However, in pretreatment of sugarcane bagasse catalyzed by acid, using HCl-[Bmim][Cl] [132] or H$_2$SO$_4$-1-butyl-3-methylimidazolium methylsulfate ([Bmim][MeSO$_4$]) systems [133], cellulose crystallinity remained unaltered even though a significant increase of cellulose digestibility was achieved. The authors suggested that digestibility increase was due to the highly effective and simultaneous removal of xylan and lignin that facilitated cellulose enzymatic saccharification efficiency. Table 2 presents the experimental conditions for the pretreatment of sugarcane bagasse with ILs.

<table>
<thead>
<tr>
<th>IL</th>
<th>Pretreatment association</th>
<th>Temperature (°C)</th>
<th>Time (min)</th>
<th>Solid loading (%)</th>
<th>Enzyme dosage (FPU/g)</th>
<th>Substrate loading (%)</th>
<th>Glucose yield (%)</th>
<th>Reducing sugars yield (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Emim][Ac]</td>
<td>-</td>
<td>120</td>
<td>30/120</td>
<td>5</td>
<td>15</td>
<td>2.5</td>
<td>95.3/98.2</td>
<td>-</td>
<td>[116]</td>
</tr>
<tr>
<td>[Emim][Ac]</td>
<td>-</td>
<td>120</td>
<td>5</td>
<td>15</td>
<td>1.0</td>
<td>69.7</td>
<td>-</td>
<td>-</td>
<td>[134]</td>
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<tr>
<td>[Emim][Ac]</td>
<td>-</td>
<td>145</td>
<td>15</td>
<td>14</td>
<td>30</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
<td>[131]</td>
</tr>
<tr>
<td>[Emim][Ac]</td>
<td>-</td>
<td>140</td>
<td>continuous</td>
<td>25</td>
<td>15</td>
<td>2.5</td>
<td>90.3</td>
<td>-</td>
<td>[95]</td>
</tr>
<tr>
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<td>-</td>
<td>140/150</td>
<td>90</td>
<td>5</td>
<td>15*</td>
<td>1.0</td>
<td>62.0/100</td>
<td>-</td>
<td>[117]</td>
</tr>
<tr>
<td>[Bmim][Cl]</td>
<td>-</td>
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<td>120</td>
<td>5</td>
<td>15</td>
<td>2.5</td>
<td>38.6</td>
<td>-</td>
<td>[116]</td>
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<td>130</td>
<td>30</td>
<td>10</td>
<td>20*</td>
<td>2.0</td>
<td>93.5/94.5</td>
<td>-</td>
<td>[132]</td>
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<tr>
<td>[Bmim][Cl] NH$_4$OH-H$_2$O$_2$</td>
<td></td>
<td>100</td>
<td>60</td>
<td>3</td>
<td>20</td>
<td>2.0</td>
<td>-</td>
<td>90.0</td>
<td>[135]</td>
</tr>
<tr>
<td>[Amim][Cl]</td>
<td>-</td>
<td>120</td>
<td>120</td>
<td>5</td>
<td>15</td>
<td>2.5</td>
<td>43.3</td>
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<td>[Amim][Cl] NH$_4$OH-H$_2$O$_2$</td>
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<td>100</td>
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<td>[Bmim][MeSO$_4$]</td>
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<td>60</td>
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<td>74.0</td>
<td>-</td>
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<tr>
<td>[Mmim][DEP]</td>
<td>-</td>
<td>120</td>
<td>120</td>
<td>5</td>
<td>15</td>
<td>2.5</td>
<td>61.9</td>
<td>-</td>
<td>[116]</td>
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</tbody>
</table>

* Enzyme dosage per gram of cellulose

Table 2. Sugarcane bagasse IL pretreatment parameters and corresponding data for enzymatic saccharification and sugar yields

Many reports can be found for the pretreatment of wood biomass with ILs. Initial studies have focused on the use of ILs to dissolve lignocellulosic biomass aiming its fractionation [126, 136]. Moreover, the possibility to perform the derivatization of wood components in situ using the biomass IL solution was considered an interesting approach to reduce the number of steps to produce derivatives, such as acylated cellulose from raw materials [137]. More recently there have been reports on the enzymatic digestibility of recovered wood biomass after IL dissolution. Over 90% cellulose hydrolysis was obtained after Pinus radiata pretreatment with [Emim][Ac] at 120 °C for 180 min, using a 5% solid loading during pretreatment [138]. The authors demonstrated that the IL pretreatment induced compositional and structural changes in the wood, including extraction and deacetylation of the
hemicellulose fraction and loss of lignin ether linkages. The cellulose crystallinity was altered, prompting the suggestion that cellulose I was transformed, to some extent, to cellulose II. However, in contrast to an earlier report for the pretreatment of maple wood flour with [Emim][Ac] in which up to 80% of delignification was achieved [121], no significant delignification of *P. radiata* was observed. The glucose saccharification yields obtained for the maple wood flour pretreated at 130 °C for 90 min reached 95%. In contrast to the high yields obtained after wood pretreatment with [Emim][Ac], the use of [Emim][Cl] was shown to be ineffective, as only 30% of total sugars were obtained after saccharification of pretreated eucalyptus at 150 °C for 60 min [139].

A comparison of the effects of newly synthesized ILs has also been performed for hardwood (barked mixed willow) and softwood (pine sapwood). The ILs 1-butyl-3-methylimidazolium hydrogen sulfate [Bmim][HSO₄] and 1-ethyl-3-methylimidazolium methyl sulfate [EMIM][MeCO₂] were mixed to 20% water and used for the pretreatment of both materials [120]. The pretreatment of the softwood sample with those ILs was ineffective as the maximum cellulose-to-glucose conversion achieved was 30%, while the pretreatments of hardwood samples with [Bmim][HSO₄] and [EMIM][MeCO₂] resulted in glucose yields of over 80% and 60%, respectively. Table 3 presents the experimental conditions for the pretreatment of woody biomass with ILs.

<table>
<thead>
<tr>
<th>IL</th>
<th>Biomass</th>
<th>Temperature (°C)</th>
<th>Time (min)</th>
<th>Solid loading (%)</th>
<th>Enzyme dosage (FPU/g)</th>
<th>Substrate loading (%)</th>
<th>Glucose yield (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Emim][Ac]</td>
<td><em>Pinus radiata</em></td>
<td>120/150</td>
<td>30</td>
<td>5</td>
<td>20</td>
<td>1.5</td>
<td>93/81</td>
<td>[138]</td>
</tr>
<tr>
<td>[Emim][Ac]</td>
<td>Maple wood flour</td>
<td>125</td>
<td>120</td>
<td>33</td>
<td>4.9</td>
<td>1.0</td>
<td>72</td>
<td>[140]</td>
</tr>
<tr>
<td>[Emim][Ac]</td>
<td>poplar</td>
<td>125</td>
<td>120</td>
<td>33</td>
<td>4.9</td>
<td>1.0</td>
<td>65</td>
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<tr>
<td>[Emim][Ac]</td>
<td>Maple wood flour</td>
<td>130</td>
<td>90</td>
<td>5</td>
<td>NI</td>
<td>NI</td>
<td>95</td>
<td>[121]</td>
</tr>
<tr>
<td>[Emim][Cl]</td>
<td><em>Eucalyptus globulus</em></td>
<td>150</td>
<td>60</td>
<td>5</td>
<td>180</td>
<td>5</td>
<td>30</td>
<td>[139]</td>
</tr>
<tr>
<td>[Emim][Cl]</td>
<td><em>Nathofagus pumilo</em></td>
<td>150</td>
<td>30</td>
<td>5</td>
<td>180</td>
<td>5</td>
<td>40</td>
<td>[139]</td>
</tr>
<tr>
<td>[Bmim][HSO₄]</td>
<td>Mixed willow</td>
<td>120</td>
<td>120</td>
<td>10</td>
<td>60*</td>
<td>2</td>
<td>80</td>
<td>[120]</td>
</tr>
<tr>
<td>[Bmim][HSO₄]</td>
<td>Pine sapwood</td>
<td>120</td>
<td>120</td>
<td>10</td>
<td>60*</td>
<td>2</td>
<td>30</td>
<td>[120]</td>
</tr>
<tr>
<td>[EMIM][MeCO₂]</td>
<td>Mixed willow</td>
<td>120</td>
<td>120</td>
<td>10</td>
<td>60*</td>
<td>2</td>
<td>60</td>
<td>[120]</td>
</tr>
<tr>
<td>[EMIM][MeCO₂]</td>
<td>Pine sapwood</td>
<td>120</td>
<td>120</td>
<td>10</td>
<td>60*</td>
<td>2</td>
<td>25</td>
<td>[120]</td>
</tr>
</tbody>
</table>

* Enzyme dosage per gram of cellulose; NI – Not informed

Table 3. Woody biomass IL pretreatment parameters and corresponding data for enzymatic saccharification and sugars yields
6.1. Advantages and disadvantages of IL pretreatment

ILs are able to disrupt the plant cell wall structure by the solubilization of its main components. This class of salts is also able to alter cellulose crystallinity and structure, rendering the amorphous cellulose prone to high rates and yields from enzymatic saccharification. Indeed, this combination of effects generates a pretreated material that can be easily hydrolyzed into monomeric sugars when compared to other pretreatment technologies, also rendering the enzymatic attack faster as the initial hydrolysis rate is greatly increased [116, 119]. In order to achieve high cellulose conversion yields (>80%) using other pretreatment processes, enzymatic saccharification times of 48–72 h are generally reported. However, in the case of IL pretreatment of bagasse and also some woody types, those yields can be obtained in less than 24 h with enzymatic hydrolysis. Nevertheless ILs are still too expensive to be used for biomass pretreatment at the industrial scale; however, the possibility of recovering the extracted lignin opens up the possibility for producing high-value products in addition to ethanol, which would favor the economics of a biorefinery based on IL biomass pretreatment. Indeed, modeling studies have shown that selling lignin can effectively lower the minimum selling price of ethanol to the point where lignin becomes the main revenue source of an IL-based biorefinery [141].

There are many challenges to be addressed before ILs can be considered as a real option for biomass pretreatment, including their high cost and the consequent requirement for ionic liquid recovery and recycling, and the high IL loading required for most IL pretreatment processes reported. It has been shown that the reduction in IL loading is more important than increasing the rate of IL recycling [141]. Aiming to tackle IL cost, two recent works have addressed this issue and were successful in reducing the IL requirement, demonstrating that it is possible to increasing biomass loading up to 33% [95, 140]. Moreover, a continuous pretreatment process using ILs by applying a twin-screw extruder as a mixing reactor has been developed [95]. Many works have also reported the use of recycled ILs up to 10 cycles without significant loss in pretreatment efficiency [121, 132, 140]; nevertheless the development of energy-efficient recycling methods for ILs for large-scale applications is still an open issue. It is also noteworthy that most studies have performed enzymatic saccharification of IL-treated biomass at low-biomass loadings (<5%). Data on saccharification yields obtained on high-biomass consistency hydrolysis assays (>15%) are also needed to truly evaluate the effectiveness of IL pretreatment on enhancing the enzymatic hydrolysis rate. ILs toxicity to enzymes and fermentative microorganisms must also be addressed as ILs trace residues may negatively affect the performance of enzymes [142] and inhibit fermentation [143]. Some research groups are now looking for new enzymes that are stable in ILs [144, 145].

Despite the current restrictions and the clear need for research and development to pave the way for the industrial use of ILs, ILs have great potential use within the engineering perspective of a biorefinery due to their uncommon and specific chemical features and their selectivity toward biomass processing.
7. Other pretreatment processes

According to the foregoing, this chapter has covered the most relevant pretreatment techniques for sugarcane and woody biomass as well as the new trends in this field. Below, we present other pretreatments, such as alkaline, ammonia fiber expansion and biological, which are also of relevance. Other important methods such as organosolv, ammonia percolation, and oxidative reactions using hydrogen peroxide or ozone will be dealt with elsewhere.

7.1. Alkaline pretreatment

This pretreatment is similar to the Kraft pulping process used in the pulp and paper industries. Nevertheless, sodium, potassium, calcium, and ammonium hydroxides have been employed for the pretreatment of lignocellulosic biomass, sodium hydroxide has been the most studied reagent [146-148]. However, calcium hydroxide is advantageous due to its low cost, higher safety besides its recovery as insoluble calcium carbonate through reaction with carbon dioxide [149]. Lime pretreatment has been used in studies carried out with several lignocellulosic materials, such as sugarcane bagasse [150], switchgrass [151], rice straw [152] and poplar wood [153].

The main effect of alkaline pretreatments is the biomass lignin removal thereby reducing the steric hindrance of hydrolytic enzymes and improving the reactivity of polysaccharides. It is believed that the mechanism involves saponification of intermolecular ester bonds between xylans and lignin, increasing the material porosity. The addition of air/oxygen to the reaction mixture dramatically improves delignification, especially in the case of materials with high lignin content. The removal of acetyl groups from hemicellulose by the alkalis also exposes the cellulose and enhanced its enzymatic hydrolysis [2]. The alkali pretreatment also causes partial hemicellulose removal, cellulose swelling and cellulose partial decrystallization [149].

In the alkaline process the biomass is soaked in the alkaline solution and mixed at a mild controlled temperature in a reaction time frame from hours to days. It causes less sugar degradation than the acidic pretreatments. The necessary neutralizing step, prior to the enzymatic hydrolysis, generates salts that can be partially incorporated to the biomass. Besides removing lignin the pretreated material washing also removes inhibitors, salts, furfural and phenolic acids.

7.2. Ammonia fiber expansion (AFEX) pretreatment

Another pretreatment that deserves attention is the ammonia fiber expansion (AFEX), which is a physicochemical process very similar to steam explosion, in which lignocellulosic biomass is exposed to liquid ammonia at high temperature and pressure for a period of time, with a subsequent quick reduction of the pressure [154]. In a typical AFEX process, the dosage of liquid ammonia is 1-2 kg of ammonia/kg of dry biomass and the temperature and residence time are around 170 °C and 30 min, respectively [2].

The AFEX technology has been used for the pretreatment of several lignocellulosic materials including wood, switchgrass, sugarcane bagasse and corn stover [154-158]. Over 90% hy-
drolysis of cellulose and hemicellulose was obtained after AFEX pretreatment of bermuda-grass (approximately 5% lignin) and bagasse (15% lignin) [157]. Although hardwood pretreatment, like poplar, requires harsher AFEX conditions to obtain equivalent sugar yields upon enzymatic hydrolysis, poplar (Populus nigra x Populus maximowiczii hybrid) AFEX-pretreated at 180 °C, 2:1 ammonia to biomass loading, 30 minutes residence time by using various combinations of enzymes (commercial cellulases and xylanases) achieved high glucan and xylan conversion (93 and 65%, respectively) [159].

This process presents some disadvantages, such as the use of ammonia solvent itself, that should be recycled and handled with caution to make the process environmentally feasible, and also from an economic point of view the ammonia consumption needs to be minimized [47]. However, there are some advantages in this pretreatment, like the feasibly solvent recover and the hydrolysate from AFEX is compatible with fermentation microorganisms without the need for conditioning [160].

7.3. Biological pretreatment

Biological pretreatment employs various types of rot fungi, being the white-rot fungi the most effective for biological pretreatment of lignocellulosic biomass. The aim of biological pretreatment processes are the lignin degradation by microorganisms, through the action of lignin degrading enzymes such as peroxidases and laccases [2]. The most investigated fungus for lignin degradation is Phanerochete chrysosporium [161].

The biological pretreatment of sugarcane straw was evaluated by screening eight microorganisms, including bacteria and fungi, for an incubation time of 30 days. The fungus Aspergillus terreus was found as the most effective strain, resulting in 92% reduction in the lignin content [162]. The pretreatment of sugarcane straw was also evaluated using the fungus Cariporiopsis subvermispora with the objective to reduce cooking times and chemicals load for the organosolv pulping. The pretreatment was effective regarding the decomposition of lignin, however high cellulose losses were pointed as negative side effects [163]. Another study evaluated the pretreatment of sugarcane bagasse with the white-rot fungus Pleurotus sajor-caju PS 2001 using a 45 days incubation time, in order to modify its lignin content. However, in this case, the aim of the study was to provide a more digestible substrate for the production of cellulases by the fungus Penicillium echinulatum [164].

The pretreatment of the Japanese red pine Pinus densiflora was studied using three white-rot fungi. The fungus Stereum hirsutum was able to selectively degrade lignin resulting in a less recalcitrant biomass after eight weeks of pretreatment. As consequence, the sugar yields obtained after the hydrolysis of the pretreated red pine with commercial enzymes was 21% higher when compared to non pretreated control samples [165].

The main advantages of such processes are the low capital cost, low energy, no requirement for chemicals, fewer hydrolysis and fermentation inhibitors produced during pretreatment and mild environmental conditions [166]. However, the biological processes require a very long residence time, when compared to other pretreatment techniques and result in very low reaction rates. Additionally, most microorganisms consume part of the substrate as a
nutrient for its growth during the pretreatment, which affects negatively the sugar yield at
the end of the process [3]. In addition, the consumption of lignin also reduces the biomass
energy utilization. At present, the use of biological pretreatments may represent a competi-
tive option only if associated with other pretreatment techniques, in order to reduce the en-
ergy requirement of the total pretreatment process [167]. In future, if less recalcitrant
genetically modified plant materials are available, biological pretreatments may represent
an important alternative.

8. Conclusion

Sugarcane and woody biomass, which are abundant and readily available, are frontrunner
materials as lignocellulosic feedstock for the production of biomass ethanol despite its dif-
ferences in regard to structure and chemical composition, which relates to different respons-
des for the same type of pretreatment.

In general, the biomass lignin content, which is an important parameter for enzymatic sac-
charification, is higher in woody biomass than in agricultural residues, such as sugarcane bi-
omass. This is particularly true for softwood, which responds poorly to several pretreatment
techniques, as shown throughout this chapter. This fact corroborates the need for the devel-
opment of tailor-made pretreatments based on the biomass type, so that a suitable choice
can benefit the subsequent bio-based conversion steps for enzymatic hydrolysis and ethanol
fermentation.

The choice of pretreatment should also take into account the foreseen utilization of the main
biomass molecular components (cellulose, hemicelluloses and lignin) for the ethanol pro-
duction process or within the framework of the biorefinery concept. Considering the use of
an ethanologenic microorganism able to ferment C6 and C5 sugars, it would be desirable to
apply pretreatments such as milling or extrusion, avoiding the formation of a separated
hemicelluloses stream, as observed for acidic pretreatment. However, even for the case of
hydrothermal or steam pretreatments, the operational conditions can be fitted to minimize
the removal of hemicellulose. Considering now a biorefinery concept which broadens the
biomass derived products, the C6 sugars could still be fermented into ethanol, while the C5
stream could be used for the production, via biotechnological routes, of a wide range of
chemicals with higher added value. In that cause, the best suited pretreatments would be
the acid pretreatment, which releases mostly C5 sugars, steam-based and LHW processes,
which separates an oligosaccharides-rich stream. In both cases, lignin can be used as a val-
uable solid fuel or as a source of aromatic structures for the chemical industry.

Regarding innovative and promising biomass pretreatment technologies, the use of ILs
stands out. These versatile class of chemicals can be tailored to suit the selective extraction
and recovery of the biomass components, such as the recovery of a cellulose-hemicellulose
rich material in an amorphous form which is prone to enzymatic hydrolysis with high yields
and rates. Additionally, the possibility of recovering the extracted lignin broadens and in-
creases the efficiency for the use of biomass.
Table 4 lists the pretreatment options presented in this chapter and its general effects in the biomass composition and structure. All pretreatments cause an increase in the surface area, which responds for the increased enzymatic digestibility of the treated materials. However, the substantial decrease in cellulose crystallinity is only observed for the treatments using ball milling and IL. This effect is of paramount importance for the increased rates and yields of cellulose enzymatic hydrolysis. The acid, LHW and steam explosion pretreatments are more effective on hemicelluloses and on the modification of the lignin structure, which also cause a higher formation of inhibitors in comparison to milling, extrusion and IL pretreatments.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Increase of SSA</th>
<th>Reduction of CrI</th>
<th>Removal of hemicellulose</th>
<th>Removal of lignin</th>
<th>Modification of lignin</th>
<th>Formation of toxic compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Alkali</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>LHW</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Steam explosion</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Ball milling</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WDM</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Extrusion</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ionic liquid</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>nd</td>
</tr>
</tbody>
</table>

+++ expressive effect; ++ moderate effect; + low effect; - no effect; nd: not determined
SSA: Specific surface area
CrI: Crystallinity index
LHW: Liquid hot water
WDM: Wet-disk milling

Table 4. General effects of different pretreatments on the composition and structure of the biomass.

Table 5 presents sixteen biomass ethanol plants (pilot, demonstration and commercial scale) which are operating or under construction. It is also presented, for each case, the feedstock and the biomass pretreatment that is used in these facilities. At the current scenario the majority of the units have implemented processes that generate a hemicelluloses rich stream: three units use diluted acid, three units use LHW and three units use steam-explosion pretreatment. Two units describe its process as a thermal-mechanical pretreatment which could also generate of a hemicelluloses rich stream. One unit applies a mild alkaline pretreatment that precludes lignin separation and the remaining four units have not disclosed the choice of pretreatment. A variety of feedstocks, such as pine wood chips, wood wastes, forest residues, garden waste, wheat, barley and oat straw, corn cob, corn stover, corn straw as well as perennial energy grasses, are used with different pretreatment types.

As the pretreatment step accounts for a substantial part of the biomass ethanol production cost, it is expected that the research in this field will continue to seek for improvements of existing methods or for the development of new and more advanced options.
| Pretreatment                        | Company               | Location               | Biomass                                                             | Scale | Capacity m$^3$y$^{-1}$ | Status   | Ref.   |
|------------------------------------|-----------------------|------------------------|                                                                    |       |                       |          |       |
| Dilute acid hydrolysis$^1$         | SEKAB                 | Örnsköldsvik, Sweden   | pine wood chips                                                    | D     | 5715                  | OP       | [168] |
| Diluted acid                       | Abengoa Bioenergy     | Salamanca, Spain       | corn cob, corn stover and wheat straw                             | D     | 5080                  | OP       | [169] |
| Diluted acid                       | Abengoa Bioenergy     | Hugoton, USA           | corn stover, wheat straw, and switchgrass                        | C     | 95000                 | UC       | [169] |
| Hydrothermal                       | Inbicon               | Fredericia, Denmark    | wheat straw                                                        | P     | 1397                  | OP       | 170   |
| Hydrothermal                       | Inbicon               | Kalundborg, Denmark    | wheat straw                                                        | D     | 5400                  | OP       | 170   |
| Hydrothermal                       | Chemtex/Proesa        | Crescentino, Italy     | perennial grass ($Arundo donax$ - giant red) and wheat straw      | C     | 50000                 | UC, Start by end 2012 | 171   |
| Steam-explosion                    | IOGEN                 | Ottawa, Canada         | wheat, barley and oat straw                                        | D     | 1800                  | OP       | [54]  |
| Steam-explosion and wet oxidation$^2$ | BioGasol             | Aakirkeby, Denmark     | straw, garden waste, energy crops and grass                       | D     | 5080                  | UC       | [172] |
| Steam-explosion and wet oxidation$^2$ | BioGasol             | Boardman, USA          | wheat straw, wood chips and corn stover                           | D     | 10233                 | UC       | [172] |
| Low acid impregnation and thermal-mechanical | Blue Sugars Corp | Upton, USA             | wood wastes                                                        | D     | 3600                  | OP       | [173] |
| Thermal-mechanical                 | Süd-Chemie AG - Clariant | Straubing, Germany    | wheat straw                                                        | D     | 1270                  | UC       | [174] |
| Mild alkaline process              | DuPont                | Vonore, USA            | corn cobs, corn stover and switchgrass                            | D     | 947.5                 | OP       | [175] |
| Not informed                       | BP Biofuels           | Highlands County, USA  | perennial grass                                                    | C     | 136440                | UC       | [176] |
| Not informed                       | POET-DSM              | Emmetsburg, USA        | corn cobs, leaves, husk, and stalk                                 | C     | 95000                 | UC, Start by end 2013 | [177] |
| Not informed                       | DuPont                | Nevada, USA            | corn stover                                                        | C     | 102330                | UC       | [175] |
| Not informed                       | Proctanol 2G, Futurool | Pomacle, France        | wood wastes, agricultural and forest residue, garden waste and perennial grass | P     | 180                   | -        | [178] |

All pretreatments were followed by separated enzymatic hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF).

The data presented in this table was based on the official information provided in each company website.

Scale was defined as follow: Pilot – P; D – Demonstration; Commercial – C.

Operational status was defined as follow: Under construction – UC; Operational - O

$^1$ Two reactors in series, the hemicellulose is hydrolyzed in the first reactor and the cellulose is decomposed in the second reactor at >200 °C.

$^2$ Combination of steam-explosion and wet oxidation, applying both the addition of oxygen and a pressure release at high temperature (170-200°C)

Table 5. Pilot, demonstration and commercial scale biomass ethanol plants.
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