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Pretreatment and Lignocellulosic Chemistry

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Abstract Lignocellulosic materials such as wood, grass, and agricultural and forest residues are promising alternative energy resources that can be utilized to produce ethanol. The yield of ethanol production from native lignocellulosic material is relatively low due to its native recalcitrance, which is attributed to, in part, lignin content/structure, hemicelluloses, cellulose crystallinity, and other factors. Pretreatment of lignocellulosic materials is required to overcome this recalcitrance. The goal of pretreatment is to alter the physical features and chemical composition/structure of lignocellulosic materials, thus making cellulose more accessible to enzymatic hydrolysis for sugar conversion. Various pretreatment technologies to reduce recalcitrance and to increase sugar yield have been developed during the past two decades. This review examines the changes in lignocellulosic structure primarily in cellulose and hemicellulose during the most commonly applied pretreatment technologies including dilute acid pretreatment, hydrothermal pretreatment, and alkaline pretreatment.

Keywords Cellulose · Hemicellulose · Lignocellulosics · Pretreatment · Recalcitrance

Abbreviations

DAP	Dilute acid pretreatment
DP	Degree of polymerization
LCC	Lignin–carbohydrate complex
CS	Combined severity
LODP	Leveling-off degree of polymerization
CP	Cross-polarization
MAS	Magnetic angle spin
NMR	Nuclear magnetic resonance
HMF	5-Hydroxymethylfurfural
SEM	Scanning electron microscope
LHW	Liquid hot water
AFEX	Ammonia fiber explosion
ARP	Ammonia recycled percolation

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Introduction

Increasing global energy demand, unstable and expensive petroleum resources, and concern over global climate changes have led to the development of renewable energy sources that can supplement fossil fuels [1]. As a result, future reductions in fossil fuel consumptions will reside in a multifaceted approach including nuclear, solar, hydrogen, wind, and particularly biofuels, which many countries have already initiated by advancing research and development programs [2]. Bioethanol has been a popular choice as a renewable energy source because of its high octane number, heat of vaporization, and compatibility with modern motor vehicles. At present, bioethanol production largely represents the first-generation biofuel which is produced from readily processable bioresources such as starch from corn and simple sugars from sugar cane [3–5]. However, as the demand for food resources increases, the search for renewable nonfood resources to displace substantial amounts of nonrenewable fossil fuels rests largely on low-cost lignocellulosics [6]. Lignocellulosics, such as wood, grass, and agricultural and forest residues, are the most abundant

renewable feedstocks on the planet, with approximately 200 billion tons produced annually in the world [7]. Compared to grain, oil seed, or sugar crops, lignocellulosics have a higher productivity per hectare and require less input per unit of biomass produced [8]. More importantly, lignocellulosics grown on secondary land do not compete with food crops and can be acquired from agricultural and forest residues which complement current farm and forest products practices.

For the conversion of lignocellulosics to ethanol, their polysaccharides (cellulose and hemicellulose) need to be broken down into the corresponding monosaccharides, which subsequently are fermented to ethanol by microorganisms. Although concentrated and dilute acid hydrolysis of lignocellulosics, respectively, have been used to produce sugars suitable for ethanol production, utilization of enzymes is viewed as the most viable strategy since enzymatic hydrolysis of lignocellulosics offers several advantages such as higher yield, lower by-product formation and energy requirement, mild operation condition, and environmental benign processing compared to chemical hydrolysis. However, native lignocellulosics are recalcitrant to decomposition from microbes and enzymes due to their physical features and chemical composition/structure [1]. Pretreatment is thus an essential step for overcoming this recalcitrance and increasing fermentable sugar yields from biological deconstruction step. It utilizes various technologies such as chemical treatment to alter the physicochemical, structural, and compositional properties of lignocellulosic biomass, thereby making cellulose more accessible to enzymes during hydrolysis step [9, 10]. Although pretreatment has the potential to improve the efficiency and reduce the cost for bioethanol production, it is still one of the most expensive processing steps. Among various pretreatment technologies developed during the past two decades, dilute acid pretreatment (DAP), hydrothermal pretreatment, and alkaline pretreatment are the major chemical techniques being developed. Understanding the carbohydrate chemistry during these leading pretreatment technologies is therefore essential since pretreatment chemistry is important owing to its impacts on lignocellulosic processing and bioethanol conversion. The purpose of this work is to review the structural changes, primarily in cellulose and hemicellulose, for these leading pretreatment technologies. This will be done by explaining the composition of lignocellulosics, giving an overview of factors contributing to lignocellulosic recalcitrance, and summarizing the hemicellulose and cellulose behaviors during DAP, hydrothermal, and alkaline pretreatments following the acid–neutral–alkaline order. This fundamental insight is a key feature needed to develop more efficient/cost-effective pretreatments in the future.

Lignocellulosic Composition

Most terrestrial plants are primarily composed of three major components: cellulose (38–50 %), hemicellulose (23–32 %), and lignin (10–25 %) [11]. Table 1 summarizes the average contents of these three major components from several common biofuel crops.

Cellulose is a linear polymer made up of β-D-glucopyranosyl units linked with 1→4 glycosidic bonds with cellobiose as the repeating unit. Cellulose fibers are bundles of microfibrils stabilized laterally by hydrogen bonds between hydroxyl groups on linear cellulose chains. These hydrogen bonds stiffen cellulose chains and promote aggregation into a crystalline structure [11]. The most common crystalline form of native cellulose is cellulose I that has parallel glucan chains and strong intramolecular hydrogen bonds. In nature, cellulose I exists as two crystalline suballomorphs, cellulose I_α and I_β. Cellulose I_α has a one-chain triclinic unit cell whereas cellulose I_β has a monoclinic two-chain unit cell [18]. The relative amounts of cellulose I_α and I_β have been found to vary between samples from different origins. Whereas cellulose I_α has been found rich in the cell wall of primitive microorganisms such as some algae and in bacterial cellulose, cellulose I_β has been found rich in higher plants such as cotton, wood, and ramie fibers [18]. In addition to the crystalline and amorphous regions, some researchers have suggested that cellulose also contains a para-crystalline portion, which has more order and less mobility than amorphous cellulose structure [19, 20]. The degree of polymerization (DP) of cellulose in plants is typically in the range of 1,510 to 5,500 [21].

Hemicelluloses are the second most common polysaccharides in nature [12]. Unlike cellulose, hemicellulose is composed of combinations of pentoses [xylose (Xyl) and arabinose (Ara)] and/or hexoses [mannose (Man), galactose (Gal), and glucose (Glc)], and it is frequently acetylated and has side chain groups such as uronic acid and the 4-*O*-methyl

Table 1 The average contents of major components from common biofuel crops

	Composition (%, dry basis)			Reference
	Cellulose	Hemicellulose	Lignin	
Switchgrass	45	30	12	[12]
Poplar	45	21	24	[13]
Miscanthus	48	30	12	[14]
Corn stover	40	25	17	[12]
Wheat straw	38	27	20	[15]
Rice straw	37	34	12	[16]
Sugarcane bagasse	40	24	25	[12]
Cotton stalk	31	11	28	[17]

ester. The chemical nature of hemicellulose is dependent on the source. In general, the dominant component of hemicellulose from hardwoods and agricultural plants, such as grasses and straws, is xylan, while glucomannan is prevalent for softwoods [11]. Xylan is a heteropolysaccharide with a homopolymeric backbone chain of 1,4-linked β-D-xylopyranosyl units [12]. The branches of xylan vary from species to species, which may contain arabinose, glucuronic acid, or the 4-*O*-methyl ether, acetic, ferulic, and *p*-coumaric acids [22]. Hemicellulose is amorphous and hydrophilic in the fiber wall and acts as an interfiber bonding agent serving as support for cellulose microfibrils. The DP of hemicellulose is typically in the range of 50 to 300 [11], which is much lower than that of cellulose.

Lignin is an amorphous, cross-linked, and three-dimensional phenolic polymer. It consists of three phenylpropane units called guaiacyl (G), syringyl (S), and *p*-hydroxyphenyl (H) units, and their respective precursors are three aromatic alcohols (monolignols), namely, coniferyl, sinapyl, and *p*-coumaryl alcohols (Fig. 1) [11]. In general, softwood lignin is almost exclusively composed of guaiacyl units (G lignin), together with a small quantity of *p*-hydrophenyl units (H lignin), whereas hardwood lignin contains both guaiacyl and syringyl units (G and S lignin) together with a small proportion of *p*-hydrophenyl units as well. Additionally, lignin derived from grass and herbaceous crop contains all the three units (G, S, and H lignin) along with *p*-hydroxycinnamic acids (*p*-coumaric acid, ferulic acid, and sinapic acid) [11, 23].

Lignin is relatively hydrophobic and covalently linked to hemicelluloses, and it fills the spaces in the cell wall between cellulose and hemicelluloses. This cellular arrangement gives strength to the plant tissue and prevents the collapse of the water-conducting elements.

Factors Contributing to Lignocellulosic Recalcitrance

Several factors are believed to contribute to the recalcitrance of lignocellulosics to biological and chemical deconstruction. They include the structure and content of lignin, acetylated hemicelluloses, lignin–carbohydrate complexes (LCCs), cellulose crystallinity and DP, pore volume, and specific surface area of cellulose [8].

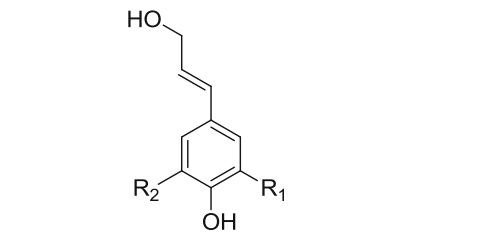

Fig. 1 Three building blocks of lignin

Lignin acts as a physical barrier to prevent enzyme access to the carbohydrate fraction of lignocellulosics. Although the detailed mechanism that explains the protective effect of lignin against enzymatic hydrolysis is still unclear, the cross-linkages between lignin and carbohydrate, the structure and distribution of lignin in lignocellulosics are believed to be significant [24]. In addition, during enzymatic hydrolysis, enzymes tend to irreversibly bind to lignin through hydrophobic interactions that cause a loss in their activities [25]. Such nonproductive binding of enzymes to lignin has been suggested to be responsible for the requirement of high enzyme loadings [26–30].

Hemicelluloses sheath cellulose microfibrils and the acetyl groups of hemicelluloses are believed to sterically hinder enzyme attack [8]. Unbranched hemicelluloses (xyloglucan, homoxylan, and mannan) form hydrogen bonds with the surface of cellulose fibrils, whereas hemicelluloses and the side chains of branched hemicelluloses (uronic acid and arabinose) may be covalently bonded to lignin to create enzyme-impenetrable cross-links [31]. These cross-links are also called LCCs, which are believed to include phenyl glycoside bonds, esters, and benzyl ethers (Fig. 2) [32]. LCCs are thought to be the major impediments to enzyme access to cellulose [31, 33]. Therefore, hydrolysis of hemicellulose and cleavage of LCCs can also open the plant cell wall structure. Sierra et al. [8] suggested that moderate hemicellulose removal (>50 %) is required to significantly increase the enzymatic digestibility of cellulose [9].

Furthermore, removal of hemicelluloses increases the mean pore size and the specific surface area of cellulose [34] that are influential structural features related to noncomplexed cellulase adsorption on the cellulose surface and subsequent enzymatic deconstruction [30] since cellulases must bind to the surface of cellulose before hydrolysis can take place. One of the impacts of pretreatment is to enlarge pore sizes to create more surface area and enhance cellulase penetration into biomass. Zeng and coworkers [35] have demonstrated that an increased surface area results in more exposed cellulose, thereby increasing the initial enzymatic hydrolysis rate of cellulose.

The effect of cellulose crystallinity on the overall cellulose-to-glucose conversion has been an issue for extensive studies. The degree of cellulose crystallinity is expressed in terms of the crystallinity index (CrI) according to the data from X-ray diffraction (XRD) technique, which is defined by Segal et al. [36] as follows:

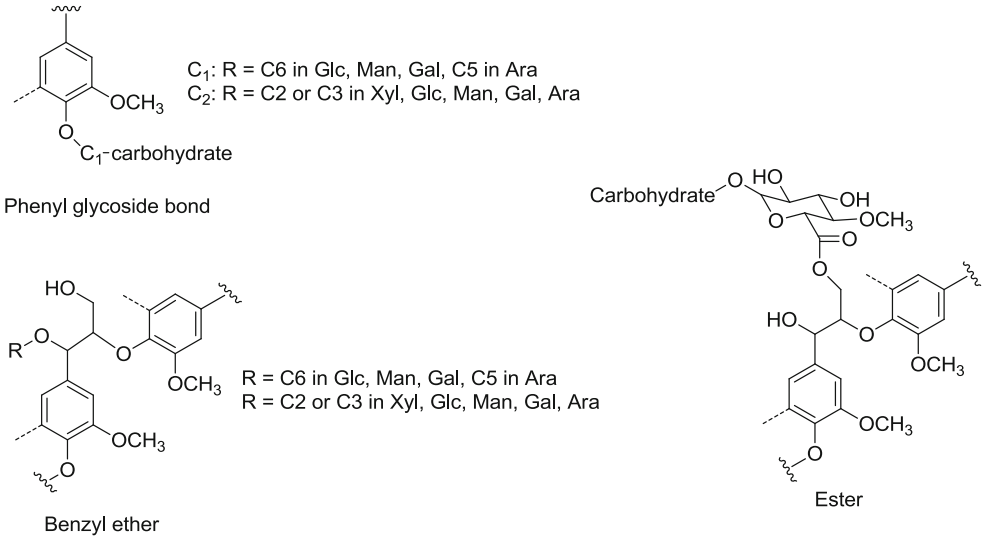

Fig. 2 Lignin–carbohydrate complex linkages

$$\mathrm{CrI} = 100 \times \left[\left(I_{002} - I_{\mathrm{amorphous}}\right)/I_{002}\right]$$

- I_{002} is the intensity for the crystalline portion of cellulose at about 2θ=22.5°.
- $I_{\mathrm{amorphous}}$ is the minimum intensity corresponding to the amorphous portion at about 2θ=18°.

In addition to the XRD peak height method that has been used in about 70 to 85 % of the studies [37], the CrI can also be determined from the areas of the crystalline and amorphous C4 signals using the following formula, based on solid-state NMR analysis [38]:

$$\mathrm{CrI} = 100 \times \left[A_{86-92\mathrm{ppm}}/\left(A_{79-86\mathrm{ppm}} + A_{86-92\mathrm{ppm}}\right)\right]$$

- $A_{86\text{-}92\ \mathrm{ppm}}$ is the area of the crystalline C4 signal.
- $A_{79\text{-}86\ \mathrm{ppm}}$ is the area of the amorphous C4 signal.

Furthermore, it has been shown that different measurement techniques give different CrI values, but the order of crystallinity is relatively consistent within each measurement technique [37].

From recent literatures, Zhang and Lynd [39], Sannigrahi et al. [40], Zhu et al. [41], Yoshida [42], Mittal et al. [43], and Ioelovich and Morag [44] have suggested that the degree or rate of enzymatic hydrolysis of cellulose declines with increasing cellulose crystallinity (Table 2), while Puri [45], Grethlein [46], and Thompson et al. [47] did not observe this strong correlation. It has been reported that the initial enzymatic hydrolysis rate of different cellulose allomorphs decreases in the following order: amorphous>III_I>II>I, which has been attributed to the enhanced specific surface areas compared to cellulose I [48]. The slower hydrolysis rate of native crystalline cellulose (cellulose I) has been attributed to the presence of strong interchain hydrogen bonding between adjacent chains in a cellulose sheet and weaker hydrophobic interactions between cellulose sheets, resulting in the stability of crystalline cellulose nanofibers that strongly resist chemically or biologically catalyzed degradation [18, 49, 50]. In addition, Weimer et al. [48] stated that the increased enzymatic hydrolysis rate of cellulose III_I was due to its lower crystallinity, lower packing density, and higher distances between hydrophobic surfaces compared to cellulose I.

Recently, Hall et al. [51] have demonstrated that the initial enzymatic hydrolysis rate of cellulose decreases linearly as crystallinity increases. Additionally, differences in the adsorption properties of cellulases on crystalline and amorphous cellulose are also believed to be related to the reactivity difference between crystalline and amorphous cellulose [52, 53]. Hall et al. [51] have observed that the amount of adsorbed enzymes appeared to decrease linearly with crystallinity only at CrI above 45 %, whereas a constant amount of adsorbed enzymes leading to higher hydrolysis rate was observed at lower degrees of crystallinity (i.e., CrI < 45 %). They inferred that the adsorbed enzymes on the cellulose with low degrees of crystallinity are more active at the same overall concentration, which is owing to a more open cellulose structure that hinders enzymes from residing on neighboring chains from hindering one another [54]. Exo-cellulases may also locate a chain end faster with an open structure so that hydrolysis may occur quicker. However, the hydrolysis rate is limited to the high degrees of crystallinity because the internal surface of highly crystalline cellulose is poorly accessible to enzymes, leading to low enzyme adsorption. Based on the conflicting results from the literature, further studies are needed to determine whether

Table 2 Cellulose conversion yield versus crystallinity for different substrates [41, 42]

Sample	Crystallinity index (%)	Extent of enzymatic hydrolysis (%)	Enzyme loadings	Reference
Ground high lignin poplar	60.2	3.5 in 1 h	30 FPU/g for cellulase from Spezyme CP	[41]
Ground high lignin poplar	25.9	25.0 in 1 h	10 FPU/g for cellulase from Spezyme CP	[41]
Ground high lignin poplar	16.4	29.8 in 1 h	10 FPU/g for cellulase from Spezyme CP	[41]
Ground high lignin poplar	8.2	31.9 in 1 h	10 FPU/g for cellulase from Spezyme CP	[41]
Ground low lignin poplar	66.1	14.8 in 1 h	10 FPU/g for cellulase from Spezyme CP	[41]
Ground low lignin poplar	32.0	42.1 in 1 h	10 FPU/g for cellulase from Spezyme CP	[41]
Ground low lignin poplar	17.5	53.3 in 1 h	10 FPU/g for cellulase from Spezyme CP	[41]
Untreated *Miscanthus*	54.2	12.0 in 72 h	328 U/g for cellulase from Celluclast 1.5 L and 268 U/g for β-glucosidase from Novozyme 188	[42]
Untreated *Miscanthus*	41.9	16.2 in 72 h	328 U/g for cellulase from Celluclast 1.5 L and 268 U/g for β-glucosidase from Novozyme 188	[42]
Untreated *Miscanthus*	24.8	27.9 in 72 h	328 U/g for cellulase from Celluclast 1.5 L and 268 U/g for β-glucosidase from Novozyme 188	[42]
Ground delignified *Miscanthus*	55.9	65.8 in 72 h	328 U/g for cellulase from Celluclast 1.5 L and 268 U/g for β-glucosidase from Novozyme 188	[42]
Ground delignified *Miscanthus*	53.0	79.8 in 72 h	328 U/g for cellulase from Celluclast 1.5 L and 268 U/g for β-glucosidase from Novozyme 188	[42]
Ground delignified *Miscanthus*	45.7	82.3 in 72 h	328 U/g for cellulase from Celluclast 1.5 L and 268 U/g for β-glucosidase from Novozyme 188	[42]

All Samples are Natural Substrates

cellulose crystallinity provides a clear indication of enzymatic digestibility of cellulose.

The DP of cellulose has also been postulated to play a role in its susceptibility to enzymatic deconstruction of cellulose. During enzymatic hydrolysis, endo-cellulases are involved in cleaving internal β(1→4) linkages of cellulose chains, decreasing the DP of cellulose and exposing reactive ends that can be attacked by exo-cellulases [55, 56]. Exo-cellulases are known to have a marked preference for substrates with lower DP [39, 57], thus it is expected that reduction in the DP of cellulose would produce more chain ends with higher enzyme accessibility to cellulose. Furthermore, it has been shown that cellulose with shorter chains is more amenable to enzymatic deconstruction due to the absence of strong hydrogen bonding [58–60]. One of the earliest studies focusing on the effect of the DP of cellulose on enzymatic saccharification (Table 3) [45] suggested that the DP of cellulose may play an important role in the enzymatic degradation of cellulose, especially in the initial rate of hydrolysis. However, Sinistyn and co-workers [61] showed that reduction in the DP of cotton linters while keeping the crystallinity index constant had negligible impact on the enzymatic hydrolysis rate. Zhang and Lynd [62] observed that a decrease in cellulose DP was less effective in accelerating enzymatic hydrolysis than an increase in the accessibility of β-glycosidic bonds. Such results suggest that the understanding of the impact of cellulose chain length on enzymatic hydrolysis is still developing.

Dilute Acid Pretreatment

Dilute acid pretreatment can significantly reduce lignocellulosic recalcitrance and it has been successfully applied to a wide range of feedstocks, including softwoods, hardwoods, herbaceous crops, and agricultural residues [63–68]. It is usually performed over a temperature range of 120 to 210° C, with acid concentration typically less than 4 wt.% and residence time from a few seconds to an hour in different types of reactors such as batch [69], plug flow [70], percolation [71], countercurrent [72], and shrinking bed reactors [72–74]. Although a variety of acids such as hydrochloric acid, nitric acid, phosphoric acid, and peracetic acid have been employed, sulfuric acid has been most widely used since it is inexpensive and effective [75, 76]. Table 4 summarizes recent DAP results for different substrates.

The combined severity (CS) factor is used for an easy comparison of pretreatment conditions and for facilitation of process control, which relates the experimental effects of temperature, residence time, and acid concentration [77].

$$\mathrm{CS} = \log\{t \exp[(T - T_{\mathrm{ref}})/14.7]\} - \mathrm{pH}$$

- t is the pretreatment time (min).
- T is the pretreatment temperature (°C).
- T_{ref} is 100°C.

DAP is one of the most important chemical pretreatment technologies because of its high hemicellulose solubilization and recovery and its high yields in subsequent enzymatic

Table 3 DP versus extent of enzymatic hydrolysis for different substrates [45]

Substrate	Treatment	DP	Extent of enzymatic hydrolysis (%)
Bagasse	Untreated	925	28
	CO_2 explosion, 5 min at 200°C and 3.45 MPa	572	78
	Alkali explosion, 5 min at 200°C and 3.45 MPa with 8 % NaOH	550	85
Wheat straw	Untreated	1,045	29
	CO_2 explosion, 5 min at 200°C and 3.45 MPa	698	81
	Alkali explosion, 5 min at 200°C and 3.45 MPa with 6 % NaOH	662	85
E. regnans	Untreated	1,510	9
	Ozone treatment, 15 % ozone charge and 50 % solids in water	1,065	86
P. radiata	Untreated	3,063	3
	Ozone treatment, 15 % ozone charge and 50 % solids in water	2,900	87
Cotton linters	Untreated	3,170	38
	Ball-milling for 15 min	2,214	57

deconstruction of cellulose. However, DAP is still among the most expensive steps in biomass conversion to fuels [78], primarily owing to the additional costs for acid, special reactor material, and acid neutralization step.

Hemicellulose Behavior During Pretreatment

A primary effect of DAP is to hydrolyze hemicelluloses and to disrupt the lignin structure so that the treated biomass has increased enzyme access to cellulose fraction. Hemicellulose, mainly xylan, is hydrolyzed to fermentable sugars during DAP as glucomannan is relatively stable in acid [34]. In general, less xylan remains in the pretreated solid residues at higher-severity pretreatment conditions [79–85] as shown in Fig. 3. At lower CS, most of the released xylan is accumulated in the liquors in the form of xylose, whereas at higher CS, the released xylan in the liquors is partially converted to furfural [80, 81]. Kabel et al. [81] demonstrated that the amount of furfural and the xylan loss increased as both CS increased and the percentage of residual xylan decreased.

It is well known that two fractions of xylan with different reactivities towards hydronium-catalyzed hydrolysis, such as DAP, exist in feedstocks (two-fraction theory) [86, 87]. This is attributed to the difference in accessibility and in chemical structure between different zones of xylan [86, 88, 89], resulting in fast- and slow-reacting xylans. Several researchers have applied this two-fraction model to investigate the kinetics and mechanism of xylan hydrolysis [83, 90, 91]. However, the fast- and slow-reacting xylans have not been distinctly defined despite that this concept has been applied for several decades. Recently, Shen and Wyman

Table 4 Summary of DAP conditions for different substrates

Substrate	Pretreatment conditions	CS	Cellulose conversion yield (%)	Enzymes loadings	Reference
Corn stover	121°C, 2.0 % H_2SO_4, 120 min	2.01	75.6 in 72 h	40 FPU/g for cellulase from Celluclast 1.5 L, and 8 CBU/g for β-glucosidase from Novozym 188	[64]
	121°C, 2.0 % H_3PO_4, 120 min	1.28	56.0 in 72 h	40 FPU/g for cellulase from Celluclast 1.5 L, and 8 CBU/g for β-glucosidase from Novozym 188	[64]
Cotton stalk	121°C, 2.0 % H_2SO_4, 60 min	1.71	23.9 in 72 h	40 FPU/g for cellulase from Celluclast 1.5 L, and 70 CBU/g for β-glucosidase from Novozym 188	[17]
Aspen	175°C, 0.25 % H_2SO_4, 30 min	2.10	42.3 in 72 h	60 FPU/g for cellulase from Spezyme CP and 120 CBU/g for β-glucosidase from Novozyme 188	[65]
	170°C, 1.10 % H_2SO_4, 30 min	2.60	88.0 in 72 h	7.5 FPU/g for cellulase from Celluclast 1.5 L and 11.25 CBU/g for β-glucosidase from Novozyme 188	[66]
Switchgrass	140°C, 1.0 % H_2SO_4, 40 min	1.79	75.0 in 72 h	15 FPU/g for cellulase from Spezyme CP and 30 CBU/g for β-glucosidase from Novozyme 188	[67]
Sweet sorghum bagasse	180°C, 1.0 % H_2SO_4, 20 min	2.67	53.9 in 60 h	20 FPU/g for cellulase	[68]

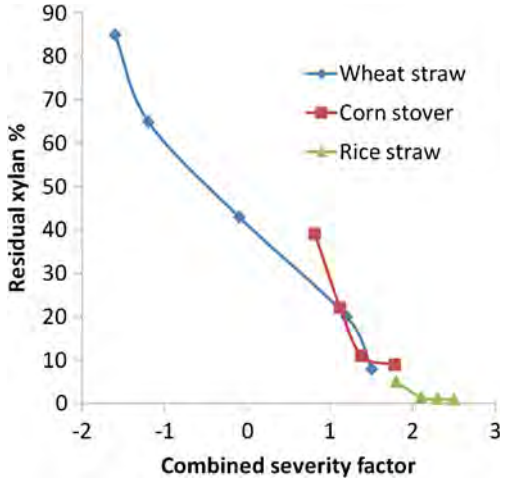

Fig. 3 Residual xylan content versus pretreatment severity for different feedstocks [81, 83, 84]

[92] defined that the fast-reacting xylans were the portion that directly forms monomeric xyloses through an autohydrolysis mechanism, whereas the slow-reacting xylans were the portion that forms oligosaccharides by an autohydrolysis mechanism (or further forms monomeric xyloses by acid catalysis in DAP) plus the portion that remains unreacted in the solid xylan in biomass. According to these definitions, Shen and Wyman [92] calculated that the percent of fast- and slow-reacting xylans in corn stover during DAP were 9.0 and 91.1 %, respectively. A total of 10.6 % of the slow-reacting xylan (or 9.7 % of total xylans) belongs to the unreacted fraction. In addition, it has been postulated that the two-fraction theory considered in xylan degradation could also be applied to deacetylation [93] since the accessibility problems cause differences in the reactivity of both xylan and acetyl groups bound to xylan chains. Kabel et al. [81] showed that the hydrolyzed acetyl groups became an in situ source of acetic acids that further catalyzes xylan depolymerization, whereas another fraction of the acetyl esters remained covalently linked to the xylan backbone and were released from the residue together with the xylan as esterified xylo-oligosaccharides. This latter fraction is believed to be a stronger inhibitor to cellulases than pure xylo-oligosaccharide owing, in part, to the steric hindrances of the acetyl groups.

Furthermore, it has been hypothesized that xylan is dissolved in the reaction media first as high M_w (DP> 25) material followed by cleavage of more and more bonds between xylose residues upon higher severity pretreatment conditions [80]. This hypothesis was confirmed by Kabel et al. [81] in 2007. Their work showed that the more severe the pretreatment the more low M_w (DP<9) xylans and the less high M_w xylans were detected in the liquor. Additionally, the proportions of medium M_w (DP 9–25) xylans first increased slightly upon medium severity (CS −0.4 to 1.2) but then decreased rapidly at higher severity in favor of low M_w xylans formation.

It was originally hypothesized that a gradual hydrolysis of xylan during DAP would begin at the solvent-exposed cell walls, the apoplast (the free diffusional space outside the plasma membrane), and then work its way inward [94]. This hypothesis assumed that the center of the cell wall would be the most protected from acid hydrolysis. Contrary to this hypothesis, Brunecky et al. [94] and Jung et al. [95] have observed the migration of xylan from the center cell wall to the lumen and middle lamella prior to being hydrolyzed into soluble oligomers during DAP especially at high temperature. Brunecky et al. [94] have provided two possible explanations for the above observation. One possible explanation is that the solubility of the large xylo-oligosaccharides drops sufficiently for them to precipitate from the solution when the hydrolyzate cools at the end of pretreatment. The large xylo-oligosaccharides could then redeposit on the outer surfaces of the cell walls. Another possibility is that the association of a fraction of the xylan with lignin causes xylan redistribution since it is well known that lignin migrates and forms spherical droplets on the outer surfaces of cell walls, particularly on the lumen and in the middle lamella during DAP especially at high temperature [96, 97]. The hydrophobic nature of lignin could hinder the access of the acid in the hydrolyzate to the ether linkages of the xylo-oligosaccharides, which explains why this fraction of xylan appears to be more difficult to hydrolyze during DAP [98].

Although DAP achieves high xylan-to-xylose conversion yields, undesired by-products such as furfural, formic acid, acetic acid, and uronic acid are formed from xylan and side chain groups of hemicellulose especially under high-severity pretreatment conditions. This not only lowers the sugar yields of hemicelluloses, but several of the by-products also severely inhibit the formation of ethanol during the fermentation process. Figure 4 shows the reaction pathways of the formation of inhibitory by-products (furfural and formic acid).

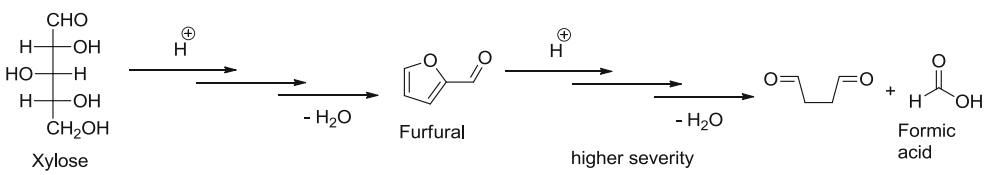

Fig. 4 Mechanisms of the formation of furfural and formic acid from xylose

Cellulose Behavior During Pretreatment

The majority of the hemicelluloses (xylose, mannose, arabinose, and galactose) from substrate including hardwood (such as poplar), agricultural residue (such as corn stover), and grass (such as switchgrass) are removed during DAP. The hydrolyzation of cellulose and subsequent solubilization of glucose would take place if the pretreatment conditions are too severe [82–84, 99–102]. Foston and Ragauskas [99] stated that the degradation of cellulose is an acid-catalyzed and thermally accelerated chain scission mechanism. The reaction takes place within the fibril structure from within either a crystalline or amorphous region of cellulose. This process consists of two major stages: an initial rapid hydrolysis of the more solvent accessible amorphous region and a latter much slower hydrolytic attack of the crystalline portion [20, 103].

In general, the crystallinity of cellulose increases throughout the process of DAP as shown in Table 5. Foston and Ragauskas [99] have observed that the para-crystalline content of cellulose from poplar and switchgrass appears to increase with pretreatment temperature based on solid-state ^{13}C CP/MAS NMR studies. They suggested that the majority of the increase in crystallinity and para-crystalline percentage is primarily due to localized hydrolyzation and removal of cellulose in the amorphous regions. The more solvent accessible amorphous regions are more prone to degradation during pretreatment at a higher temperature because cellulose hydrolysis is thermally accelerated.

In addition, the relative proportion of both the crystalline and para-crystalline forms can also be affected by ultrastructural transformation mechanisms and/or hydrolyzation at crystalline surfaces. For example, it has been observed that the relative intensity of the cellulose I_α form decreases while the relative intensity for the other crystalline allomorphs increases with residence time for both poplar and switchgrass during DAP [99]. This data suggest that the cellulose I_α form is susceptible to either selective degradation by acidic hydrolyzation and/or transformation to other crystal allomorphs during pretreatment. In fact, conditions in DAP could potentially promote cellulose annealing [105–107] of cellulose I_α into cellulose I_β crystal. This transformation is attributed to the metastable properties of the triclinic one-chain crystal structure of cellulose I_α [103, 108–110]. It has been suggested that, during DAP, cellulose I_α is primarily converted to pare-crystalline cellulose, followed by conversion to cellulose I_β while simultaneously a small fraction of para-crystalline cellulose slowly transforms into crystalline cellulose [99].

Interestingly, Sannigrahi et al. [107] have observed a large increase in the relative proportion of cellulose I_β accompanied by a decrease in the relative proportions of both cellulose I_α and para-crystalline region from dilute acid pretreated pine. This suggests that the types of lignocellulosic materials and exact pretreatment conditions influence cellulose crystalline allomorphs and para-crystalline contents during DAP.

DAP leads to the reduction in the DP of cellulose especially at high-severity pretreatment conditions, which increases the enzymatic digestibility of cellulose. The DP of cellulose from different substrates decreases gradually until reaching a nominal value, namely, the leveling-off degree of polymerization (LODP) throughout the course of DAP [79, 111]. The initial faster DP reduction phase is believed to represent the hydrolysis of the reactive amorphous region of cellulose, whereas the slower plateau rate phase corresponds to the hydrolysis of the slowly reacting crystalline fraction of cellulose [21, 111].

Undesired by-products such as 5-hydroxymethylfurfural (HMF), levulinic acid, and formic acid are formed from glucose during DAP especially under high-severity conditions. Similar to the by-products formed from xylose, these by-products not only lower the sugar yields of cellulose but also severely inhibit the formation of ethanol during the fermentation process. Weak acids (formic acid, acetic acid, uronic acid, and levulinic acid) have been proposed to inhibit microorganism cell growth by uncoupling and intracellular anion accumulation mechanisms [112]. Furfural and HMF have been shown to be able to damage microorganism cell growth by reducing enzymatic and biological activities, breaking down DNA, and inhibiting protein and RNA synthesis [113]. In addition, it was found that some inhibitors such as acetic acid and furfural can interact antagonistically on cell growth, resulting in a greater drop in the specific growth rate than the sum of reductions caused by the individual inhibitors. Palmqvist and Hahn-Hagerdal [114] have

Table 5 Crystallinity index (CrI) before and after DAP for different substrate [99, 100, 104, 107]

Substrate	Pretreatment conditions	CrI (%) before pretreatment	CrI (%) after pretreatment	Reference
Corn stover	160°C, 0.5 % H_2SO_4, 20 min	50.3	52.5	[100]
Poplar	160°C, 1.0 % H_2SO_4, 5 min	49.9	70.5	[99]
	190°C, 2.0 % H_2SO_4, 70 s	49.9	50.6	[100]
Sugarcane bagasse	80°C, 50 % peracetic acid, 2 h	42.6	63.0	[104]
Loblolly pine	180°C, 0.5 % H_2SO_4, 10 min	62.5	69.9	[107]

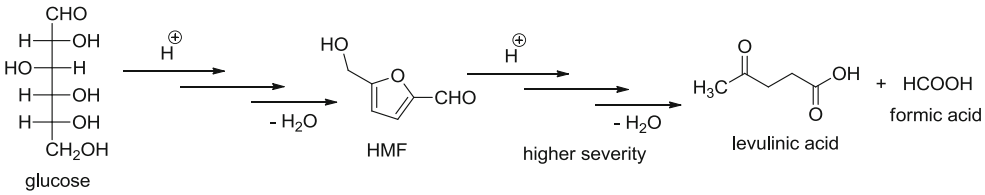

Fig. 5 Mechanisms of the formations of HMF, formic acid, and levulinic acid

written an excellent review about the detailed mechanism of inhibition by these inhibitors. Figure 5 shows the reaction pathways of the formations of HMF, levulinic acid, and formic acid. It has been suggested that DAP should be performed at high temperatures and short residence times since these pretreatment conditions would promote hemicellulose solubilization while minimizing the degradation of hemicellulose and cellulose to undesired inhibitors.

DAP does not lead to significant delignification. Several studies have instead found that the acid-insoluble (Klason) lignin content of dilute acid-pretreated material is often higher than the starting material as shown in Table 6.

Although this might be caused by the removal of significant amounts of hemicelluloses and certain amounts of cellulose while retaining most of lignin, some researchers have hypothesized that this is due to the repolymerization of polysaccharide degradation products (such as HMF and furfural) and/or polymerization with lignin (Fig. 6) to form a lignin-like material termed pseudo-lignin [118, 119].

Recently, Sannigrahi et al. [76] have demonstrated that pseudo-lignin can be generated from carbohydrates without a significant contribution from lignin during DAP especially under high-severity pretreatment conditions. Scanning electron microscope studies on dilute acid-pretreated holocellulose revealed the presence of pseudo-lignin spherical droplets on the surface of dilute acid-pretreated holocellulose. ^{13}C CP/MAS analysis of pretreated holocellulose indicated significant peaks from carbonyl, aromatic, methoxy, and aliphatic structure. These peaks were attributed to the structure of pseudo-lignin, which account for the additional lignin detected by wet chemical analysis. Furthermore, the intensities of these peaks increased as pretreatment severity increased, suggesting an acid-catalyzed disproportionation mechanism of formation for pseudo-lignin.

In summary, DAP typically results in a decrease in the DP and an increase in the degree of crystallinity of cellulose together with an increase in the relative proportion of the more stable cellulose I_β form. However, DAP especially carried out under high-severity pretreatment conditions will lead to the formation of inhibitory sugar degradation products and to the formation of pseudo-

Table 6 Klason lignin content before and after pretreatment for different substrates [67, 95, 107, 115–117]

Substrate	Pretreatment conditions	Klason lignin before pretreatment (%)	Klason lignin after pretreatment (%)	Reference
Corn stover	160°C, 0.5 % H_2SO_4, 20 min	17.2	26.8	[115]
Sweet sorghum bagasse	180°C, 1.0 % H_2SO_4, 10 min	15.2	26.5	[67]
Loblolly pine	200°C, 1.0 % H_2SO_4, 2 min	29.4	46.2	[107]
Raw bagasse	111°C, 2.0 % H_2SO_4, 10 min	14.0	20.6	[116]
	111°C, 2.0 % H_2SO_4, 12.5 min	14.0	23.0	[116]
	111°C, 2.0 % H_2SO_4, 15 min	14.0	27.2	[116]
	111°C, 2.0 % H_2SO_4, 17.5 min	14.0	26.6	[116]
Poplar	160°C, 1.0 % H_2SO_4, 10 min	23.2	24.2	[95]
	175°C, 2.0 % H_2SO_4, 10 min	23.2	38.5	[95]
Switchgrass	160°C, 1 % H_2SO_4, 2 min	31.2	48.5	[117]
	160°C, 1 % H_2SO_4, 5 min	31.2	60.8	[117]
	160°C, 1 % H_2SO_4, 10 min	31.2	44.3	[117]

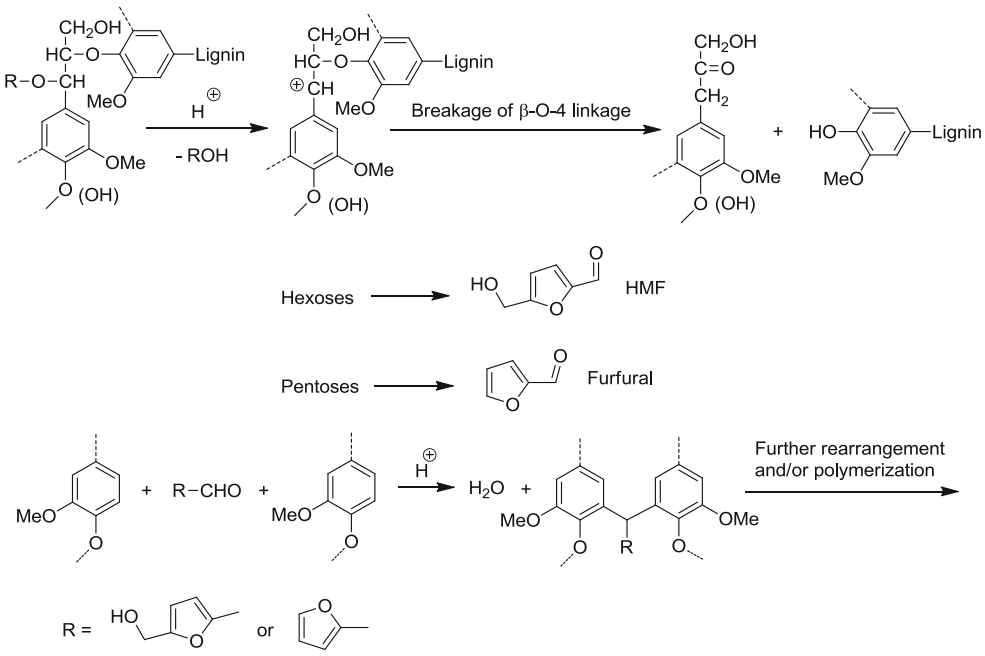

Fig. 6 Lignin fragmentation and repolymerization of HMF and/or furfural with lignin during DAP

lignin. The increase in the degree of crystallinity of cellulose and the formation of pseudo-lignin may lead to the inhibition of cellulase enzymatic hydrolysis. This suggests that further development in tailoring the chemistry of DAP is needed.

Hydrothermal Pretreatment

Hydrothermal pretreatment refers to the use of water in the liquid or vapor phase to pretreat lignocellulosic materials. Hydrothermal pretreatment is particularly promising because it provides several potential advantages including no requirement for catalysts or special reactor materials or preliminary feedstock size reduction [120]. Liquid hot water (LHW) and uncatalyzed steam explosion are the two major hydrothermal pretreatment technologies.

Liquid Hot Water Pretreatment

Liquid hot water is one of the old technologies applied for pretreatment of lignocellulosics, where lignocellulosics undergo high-temperature cooking in water with high pressure. Pressure is utilized to maintain water in the liquid state at elevated temperatures of 160–240°C [121]. LHW has been performed in co-current, countercurrent, or flow-through reactors. In the co-current process, the water–lignocellulosics slurry is heated to pretreatment conditions and held for the required residence time. In the countercurrent process, the water and lignocellulosics flow in the opposite directions. In the flow-through process, hot water flows through a stationary bed of lignocellulosics [8]. Table 7 summarizes recent LHW pretreatment results for different substrates. Similar to DAP, the severity factor is used to measure the treatment intensity in LHW, which is defined as

Table 7 LHW pretreatment results for different substrates [35, 123–125]

Substrate	Pretreatment conditions	Cellulose conversion yield (%)	Enzymes loadings	Reference
Wheat straw	200°C, 40 min	95.8 in 72 h	15 FPU/g for cellulase from Celluclast 1.5 L and 15 IU/g for β-glucosidase from Novozyme 188	[123]
	195°C, 20 min	87.5 in 72 h	15 FPU/g for cellulase from Celluclast 1.5 L and 15 IU/g for β-glucosidase from Novozyme 188	[124]
	195°C, 40 min	81.2 in 72 h	15 FPU/g for cellulase from Celluclast 1.5 L and 15 IU/g for β-glucosidase from Novozyme 188	[124]
Corn stover	190°C, 15 min	69.6 in 168 h	15 FPU/g for cellulase from Spezyme CP and 65 IU/g for β-glucosidase from Novozyme 188	[35]
Switchgrass	200°C, 10 min	77.4 in 48 h	49 FPU/g for cellulase from Celluclast 1.5 L and 40 IU/g for β-glucosidase from Novozyme 188	[125]

follows [122]:

$$R_0 = t \exp[(T - 100)/14.75]$$

- t is the pretreatment time (min).
- T is the pretreatment temperature (°C).

LHW pretreatment without adding any catalyst is attractive due to its simplicity and significant reduction in the chemical and materials of construction costs compared to DAP. Although LHW pretreatment suffers from lower hemicellulose sugar yields than DAP, it has much lower cellulose degradation.

Hemicellulose Behavior During Pretreatment

The goal of LHW pretreatment is to solubilize hemicelluloses and to increase cellulose digestibility. During LHW, water acts as a weak acid and releases the hydronium ion, which causes depolymerization of hemicellulose by selective hydrolysis of glycosidic linkages, liberating *O*-acetyl group and other acid moieties from hemicellulose to form acetic and uronic acids. The release of these acids has been postulated to catalyze the hydrolysis of hemicelluloses and oligosaccharides from hemicelluloses [126–130].

However, this postulate was challenged since it cannot explain the large difference in the performance of a flow-through reactor that removes much more hemicellulose especially at high flow rate (Fig. 7). This is because the large amount of water used in a flow-through reactor quickly dilutes and removes organic acids, which lowers the organic acid concentrations and minimizes the time for them to act on the solid hemicellulose.

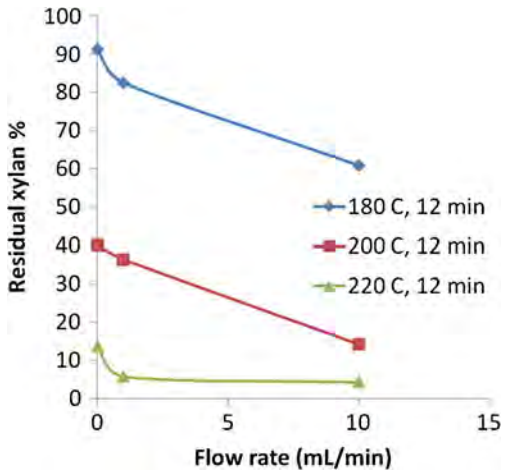

Fig. 7 Flow rate versus xylan removal for LHW pretreatment of corn stover [131]

Liu and Wyman [131] postulated that the long-chain hemicellulose oligomers and unreacted hemicellulose could form hydrogen bonds with water molecules to form an "ice-like" layer that slows the access of water to hemicellulose during LHW pretreatment. Additionally, it was hypothesized that the flow of liquid would enhance the removal of less soluble oligomers and disturb the "ice-like" layer, leading to reduce the thickness of the stagnant fluid layer surrounding the solid particles and to lower the resistance to penetration of water into the solids for hydrolysis and diffusion of oligomers into solution [131]. Therefore, hemicellulose hydrolysis is controlled by both chemical reaction (i.e., temperature and acid concentration) and mass transfer effect [132]. At relatively high acid concentration condition, mass transfer is insignificant since the hydronium ions would rapidly hydrolyze long-chain oligomers to more rapidly dissolving short-chain species and then to monomers. This is why hemicellulose solubilization is mainly controlled by temperature and acid concentration in DAP, while mass transfer is believed to play a more important role in LHW pretreatment [131, 132], where most of hemicellulose sugars are released as oligomers [80, 131–135]. However, the detailed mechanism accounting for the enhanced hemicellulose removal with flow rate is still not fully understood [136]. Furthermore, it was found that lignin–hemicellulose–oligomers and their solubility might significantly affect the rates and yields of hemicellulose solubilization [136].

It has been observed that hemicellulose is easily dissolved in water together with a considerable part of lignin at approximately 180°C [90, 131, 137, 138], and solubilization of hemicellulose increases significantly with pretreatment severity (i.e., temperature and time). Garrote et al. [129] and Vegas et al. [130] showed that the medium molecular weight (DP 9–25) xylo-oligosaccharides were predominant in LHW pretreatment, and their proportions decreased slightly with severity due to the increased decomposition. The amount of low molecular weight (DP<9) fraction increased with severity while the proportion of high molecular weight (DP>25) fraction declined with severity. These authors concluded that the majority of xylan-derived products in LHW pretreatment corresponded to xylo-oligosaccharides with DP less than 25 [129, 130].

Similar to DAP, the two-fraction model can also be applied to xylan hydrolysis in LHW pretreatment [92, 139, 140]. Based on the definitions of the fast- and slow-reacting xylans, the percent of fast- and slow-reacting xylans in corn stover during LHW pretreatment were determined to be 9.0 and 91.8 %, respectively. A total of 31.0 % of the slow-reacting xylan (or 28.5 % of total xylans) belongs to the unreacted fraction [92]. In addition, it has been shown that the kinetics of deacetylation follow a similar trend to xylan

solubilization [93, 139–141], and the two-fraction model can also be applied to deacetylation during LHW pretreatment [80, 93, 139, 140, 142].

Furfural and HMF will be formed from the degradation of pentoses and hexoses, respectively, during LHW pretreatment. However, the quantities of sugar degradation products generated are lower than DAP (Table 13) [143, 144] and would not significantly inhibit the fermentation process if LHW pretreatment is performed under 220°C [128]. In fact, LHW maximizes the solubilization of the hemicellulose fraction while minimizing the formation of monosaccharides and thus also the formation of sugar degradation products when pH is maintained between 4 and 7 [133, 145–147].

Cellulose Behavior During Pretreatment

LHW pretreatment results in preserving most of the cellulose in solid form, and the amount of glucan retained is greater than in DAP [123]. Recently, Yu and Wu [148] have investigated the hydrolysis behavior of amorphous and crystalline cellulose in LHW pretreatment. According to them, the minimal temperature required to rupture the glycosidic bonds in the chain segments within the amorphous portion of cellulose appears to be approximately 150°C, whereas for the crystalline portion of cellulose it is 180°C. Furthermore, low-DP glucose oligomers are produced at 180°C, whereas large-DP glucose oligomers are released at temperatures above 200°C. Clearly, this difference in the hydrolysis behavior between amorphous and crystalline cellulose is due to the significant ultrastructural differences in the amorphous and crystalline portions of cellulose. Therefore, amorphous cellulose is more reactive than crystalline cellulose and several researchers have reported that the crystallinity index of cellulose increased after LHW pretreatment (Table 8), although no significant change in cellulose crystallinity has also been observed when the LHW pretreatment severity is low [149]. The DP of cellulose decreases progressively until reaching LODP during LHW pretreatment [79, 111], which is similar to those reviewed for DAP.

There has been no pseudo-lignin generation reported during LHW pretreatment to date. However, the possibility of pseudo-lignin generation could not be ruled out especially under high-severity pretreatment conditions. It is reported that acid-insoluble (Klason) lignin content significantly increases after LHW pretreatment (Table 8) [125, 149, 150]. Although this can be explained by the removal of components such as extractives and hemicelluloses, it is believed that the increase in lignin content is too significant to be only due to this reason.

Uncatalyzed Steam Explosion Pretreatment

Steam explosion is one of the most common pretreatments applied to the fractionation of biomass components to weaken the lignocellulosic structure and increase its chemical and biological reactivity. In this method, lignocellulosic particles are treated with high-pressure saturated steam for a period of time and then the pressure is swiftly released, which makes the lignocellulosic material undergo an explosive decompression. Steam explosion is typically carried out at a temperature of 160–260°C (corresponding pressure 0.69–4.83 MPa) for several seconds to a few minutes before the material is exposed to atmospheric pressure [10]. Addition of an acid catalyst such as SO_2 or preferably H_2SO_4 because it is inexpensive [153] to steam explosion can significantly increase its hemicellulose sugar yields [154]. Therefore, the sugar yields are highly dependent on the pretreatment conditions. Since the hemicellulose and cellulose behaviors in the acid-catalyzed steam explosion pretreatment are similar to those reviewed in DAP, only uncatalyzed steam explosion pretreatment will be discussed here. This technology combines both chemical and physical pretreatments into one step. The mechanical effects are caused by a sudden decompression so that fibers are separated. The severity factor (S_0) can also be applied here to compare the different degrees of uncatalyzed

Table 8 Cellulose crystallinity and Klason lignin content before and after LHW pretreatment for different substrates [100, 125, 149–151]

Substrate	Pretreatment conditions	CrI (%) before pretreatment	CrI (%) after pretreatment	Klason lignin (%) before pretreatment	Klason lignin (%) after pretreatment	Reference
Poplar	200°C, 10 min	49.9	54.0	NA	NA	[100]
Tamarix	160°C, 8 min	41.0	50.6	15.1	24.0	[149]
	180°C, 9 min	41.0	51.4	15.1	29.3	[149]
	200°C, 10 min	41.0	55.5	15.1	40.1	[149]
Wheat straw	195°C, 6 min	NA	NA	22.6	25.5	[150]
Coastal bermuda grass	170°C, 60 min	50.2	69.4	17.6	21.9	[151]
Switchgrass (leaves)	200°C, 10 min	NA	NA	19.5	39.2	[125, 152]

steam explosion pretreatment used, which is defined as follows [155]:

$$S_0 = \log\{t \exp[(T - T_{\text{ref}})/14.75]\}$$

- t is the pretreatment time (min).
- T is the pretreatment temperature (°C).
- T_{ref} is 100°C.

Hemicellulose Behavior During Pretreatment

In steam explosion, steam penetrates the plant cell wall, causing hemicellulose hydrolysis and lignin transformations owing to high temperature, thereby increasing cellulose digestibility [73]. If there is no external acid added to the system, acetic acid released from acetylated hemicellulose has been considered as the main acid, which catalyzes further hydrolysis of hemicellulose. In addition, water itself also possesses certain acid properties at high temperature [156], thereby further catalyzing hemicellulose hydrolysis during steam explosion pretreatment. In general, the hemicelluloses are dissolved as oligosaccharides after acid hydrolysis, which are partially further hydrolyzed to monosaccharides.

Chen et al. [157] have investigated the kinetics and mechanism of hemicellulose removal during uncatalyzed steam pretreatment of hardwoods. They observed that both xylan removal and deacetylation rates increased initially and then decreased gradually throughout the course of pretreatment. This behavior is similar to those reviewed for LHW pretreatment. The initially dissolved xylan had a high DP with a high degree of acetylation since the acetyl groups increase xylan solubility [158], whereas lower DP xylo-oligosaccharides with a lower degree of acetylation were dissolved in the later reaction stage as the majority of xylan were removed from the material. Additionally, Chen et al. [157] have also observed that lignin-free xylan would not dissolved at 160°C until the DP is lower than 25, which explains why the initial xylan dissolution rate increased with time, owing to random acid hydrolysis process. After the lignin-free xylan is removed, the remaining xylan removed is believed to be in the form of LCCs and its DP decreases with time.

As steam explosion pretreatment conditions get more severe, there are more condensation and repolymerization reactions taking place between the decomposition products of hemicelluloses such as furfural and lignin [159–161], leading to an increase in acid-insoluble (Klason) lignin content after pretreatment (Table 9). It has been hypothesized that two types of reactions occur during steam explosion pretreatment: initially faster depolymerization of native lignin and hemicellulose by acid hydrolysis followed by condensation and repolymerization reactions, leading to the formation of pseudo-lignin that is responsible for increased acid-insoluble (Klason) lignin content [159, 162].

Cellulose Behavior During Pretreatment

In general, cellulose is the least altered component during uncatalyzed steam explosion pretreatment, owing to its high degree of crystallinity and the presence of inaccessible microfibrils that decreases accessibility [118]. Asada et al. [163] have examined the chemical characterization of the cellulose component in steam explosion-pretreated bagasse. They observed that the DP of cellulose decreased significantly as the steaming time increased until reaching a minimum value of about 700 and then increased slightly. Asada et al. [163] suggested that cellulose was depolymerized at a relatively short steaming time but repolymerized at a relatively long steaming time. The slight increase in cellulose DP during steam explosion pretreatment has also been reported by Wang and coworkers [161], which was accompanied by the transformation of some parts of amorphous cellulose into crystalline cellulose [161]. The steam explosion pretreatment also affects cellulose crystallinity. Asada et al. [163] observed that the degree of crystallinity increased as the steaming time increased until reaching a maximum value of 45 % and then decreased slowly. They hypothesized that steam explosion crystallized some parts of the amorphous portion of cellulose but extended steaming times could convert the crystalline portion back to noncrystalline portion again. This hypothesis is somewhat different from the general concept that steam explosion can selectively hydrolyze amorphous portions of cellulose [161, 168] and transfer some amorphous portions of cellulose to crystalline portions [108, 164–166].

Table 9 Klason lignin content before and after uncatalyzed steam explosion pretreatment for different substrates [67, 159]

Substrate	Pretreatment conditions	Klason lignin content (%) before pretreatment	Klason lignin content (%) after pretreatment	Reference
Sweet sorghum bagasse	160°C, 5 min	15.2	23.4	[67]
Pinus pinaster	190°C, 4 min	36.2	55.1	[159]
	190°C, 8 min	36.2	54.3	[159]
	210°C, 4 min	36.2	59.7	[159]
	210°C, 4 min	36.2	58.2	[159]

The increase in cellulose crystallinity (by approximately 10–20 % depending on the pretreatment severity) after steam explosion pretreatment has also been reported by other researchers in the literature [159–161, 167–169].

HMF is generated from degradation of glucose from acid hydrolysis of cellulose during uncatalyzed steam explosion pretreatment. This is undesired because HMF will inhibit the formation of ethanol in the fermentation process. The mechanism of HMF formation in steam explosion pretreatment is similar to that in DAP, which has been shown previously (see Fig. 5).

LHW Versus Uncatalyzed Steam Explosion

In general, both LHW and uncatalyzed steam explosion pretreatments have the potential to reduce lignocellulosic recalcitrance and increase sugar yields from enzymatic hydrolysis of cellulose. It is reported that LHW pretreatment is comparable to uncatalyzed steam explosion pretreatment with respect to the cellulose conversion yields at equivalent pretreatment severity [141]. However, steam explosion pretreatment can be performed at higher solid concentrations which often generate more inhibitors. It also suffers from lower hemicellulose recovery than LHW pretreatment [128, 146], whereas LHW pretreatment produces large amounts of hemicellulose oligosaccharides that must be further hydrolyzed to fermentable monosaccharides [133, 134]. In addition, the concentration of solubilized products is lower in LHW pretreatment compared to steam explosion pretreatment [137]. This is probably caused by higher water input in LHW pretreatment than in steam explosion pretreatment [34, 121]. However, higher water input in LHW pretreatment demands more energetic requirements, which is not favorable for developing at a commercial scale.

Alkaline Pretreatment

Alkaline pretreatments have received numerous studies as another major chemical pretreatment technology besides DAP. Alkaline pretreatments can be divided into two groups based on the chemical used: (a) pretreatments that use sodium or calcium hydroxide and (b) pretreatments that use ammonia [170]. Alkaline pretreatment efficiencies are mainly affected by reaction temperature, pretreatment time, and alkali loading. This process is basically a delignification process and is more effective on hardwoods, herbaceous crops, and agricultural residues with lignin contents lower than softwoods [63]. Compared with DAP, alkaline pretreatments have some practical operational advantages including lower reaction temperatures and pressures, no need for complicated reactors, and allow for the reuse of residual alkali [63, 171]. However, one limitation of alkaline pretreatments is that some alkali are converted to salts or incorporated as salts into lignocellulosics during the pretreatment process so that the treatment of a large amount of salts becomes a challenging issue for alkaline pretreatments [9, 63]. Another limitation is the capital cost of recycling alkali.

Sodium Hydroxide and Lime Pretreatment

Sodium hydroxide and lime are the major chemicals applied to alkaline pretreatment of lignocellulosics. Although sodium hydroxide has been shown to effectively enhance cellulose digestibility (Table 10) [172–174], it has several disadvantages, such as cost [175], safety concerns, and difficult to recover. Therefore, lime has received much more attention since it is inexpensive (about 6 % cost of sodium hydroxide) [175], has improved handling, and can be recovered easily by using carbonated wash water [9, 176]. In addition, lime has been proven to successfully reduce lignocellulosic recalcitrance (Table 10).

Hemicellulose Behavior During Pretreatment

A significant amount of hemicellulose is solubilized during alkaline pretreatment although generally less than DAP. It is expected that the solubilization of hemicellulose in conjunction with substantial lignin reduction can improve enzymatic hydrolysis of cellulose [17]. The mechanism of pretreatment is believed to involve saponification of intermolecular ester bonds that crosslink hemicellulose and lignin (Fig. 8) [10, 63]. The presence of these LCC linkages is believed to prevent the selective solubilization and removal of wood components such as hemicelluloses and lignin in biorefining processes [32]. Therefore, saponification leading to the cleavage of these linkages and the exposure of cellulose microfibrils can increase the enzymatic digestibility of cellulose. Acetyl groups and various uronic acid substitutes are also removed by alkali, thereby increasing the accessibility of hemicellulose and cellulose to enzymes [174]. In addition, it is found that more hemicellulose is solubilized as compared to lignin and cellulose during alkaline pretreatment [180].

He et al. [180] have characterized hemicelluloses from untreated and dilute NaOH-treated rice straws by FT-IR spectroscopy. According to these data, dilute NaOH pretreatment did not change the hemicellulose structure significantly, but it altered certain functional groups and linkages. For instance, the reduction in the carbonyl stretching region attributed to the loss of hemicellulose acetyl and uronic ester groups, was observed by different researchers [100, 161, 180, 181]. In addition, a decrease

Table 10 NaOH and lime pretreatment results for different substrates [67, 173, 177, 178]

Substrate	Pretreatment conditions	Cellulose conversion yield (%)	Enzyme loadings	Reference
Corn stover	55°C, 0.073 g $Ca(OH)_2$/g raw biomass, 4 weeks	98 in 96 h	15 FPU/g for cellulase from Spezyme CP and 40 CBU/g for β-glucosidase from Novozyme 188	[177]
Switchgrass	120°C, 0.1 g $Ca(OH)_2$/g dry biomass, 2 h	60 in 72 h	5 FPU/g for cellulase from Cytolase CL and 28.4 CBU/g for β-glucosidase from Novozyme 188	[172]
Sweet sorghum bagasse	25°C, 0.5 M NaOH, 90 min	87 in 24 h	20 FPU/g for cellulase from Celluclast 1.5 L and 50 CBU/g for for β-glucosidase from Novozyme 188	[173]
	25°C, 1.0 M NaOH, 60 min	86 in 24 h	20 FPU/g for cellulase from Celluclast 1.5 L and 50 CBU/g for for β-glucosidase from Novozyme 188	[173]
	25°C, 2.5 M NaOH, 90 min	92 in 24 h	20 FPU/g for cellulase from Celluclast 1.5 L and 50 CBU/g for for β-glucosidase from Novozyme 188	[173]
Jatropha curcas	100°C, 0.1 g $Ca(OH)_2$/g raw biomass, 3 h	68.9 in 48 h	0.24 ml/g for cellulase from ACCELLERASE 1500	[178]
Corn stover	120°C, 0.075 g $Ca(OH)_2$/g dry biomass, 4 h	88.0 in 7 days	25 FPU/g for cellulase from Spezyme CP and 28.4 IU/g for β-glucosidase from Novozyme 188	[179]

in the contents of β-glycosidic linkages between hemicellulose sugar units was reported in the literature [180].

It is reported that the amount of furfural or HMF in the hydrolysates obtained with alkaline pretreatment is much lower than that with DAP (Table 13) [182]. He et al. [180] observed that the content of cold and hot water extractives increased significantly after NaOH pretreatment. These extractives were generated mainly from the degradation of hemicellulose and partially from the degradation of lignin and cellulose.

Cellulose Behavior During Pretreatment

Sodium hydroxide or lime pretreatment of lignocellulosic materials decreases the DP of cellulose and causes swelling of cellulose, leading to an increase in its internal surface area [183]. This makes cellulose more accessible for enzymes and bacteria. The changes in cellulose structure after NaOH treatment were represented by the reduction in the intensity of hydroxyl stretching (3424 cm^{-1}), indicating that the hydrogen bonds of cellulose were disrupted [100, 161, 180]. This leads to an increase in the accessibility of cellulose to enzymes. A decrease in the intensities of C–H stretching and C–O–C stretching peaks revealed that the $–CH_2$ and C–O–C portions of cellulose were also ruptured after pretreatment [100, 180]. In addition, the breakage of β-1,4-glycosidic linkages between cellulose sugar units was observed [180]. All the above changes to cellulose structure after NaOH pretreatment were reported to enhance cellulose digestibility by enzymes.

In terms of cellulose crystallinity, many researchers [100, 173, 180] observed that the crystallinity of cellulose increased after alkaline pretreatment (Table 11), which is probably owing to the removal of the amorphous components by alkali. In addition, Wu et al. [173] observed that the crystallinity of cellulose declined after 5 M NaOH pretreatment, although it increased after dilute NaOH pretreatment (Table 11). This is probably attributed to the changes in cellulose crystal structure, cellulose amorphization, and/or the microscopic structures of lignocellulosics caused by

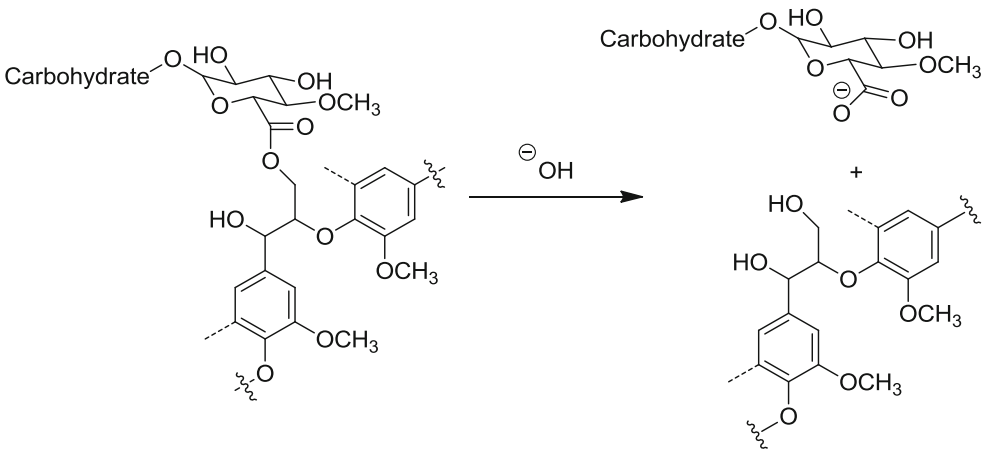

Fig. 8 Saponification of lignin–carbohydrate complex with alkali

Table 11 Cellulose crystallinity before and after alkaline pretreatment [100, 173, 180]

Substrate	Pretreatment conditions	CrI (%) before pretreatment	CrI (%) after pretreatment	Reference
Rice straw	20°C, 6 % NaOH, 3 weeks	60.3	64.3	[180]
Bagasse	25°C, 1 M NaOH, 1 h	51.8	61.3	[173]
	25°C, 2.5 M NaOH, 1 h	51.8	54.3	[173]
	25°C, 5 M NaOH, 1 h	51.8	42.8	[173]
Poplar	65°C, 0.5:1 $Ca(OH)_2$ to biomass, 4 weeks	49.9	54.5	[100]
Corn stover	55°C, 0.5:1 $Ca(OH)_2$ to biomass, 4 weeks	50.3	56.2	[100]

concentrated NaOH. In general, dilute alkali results in greater hydrolyzation of the amorphous regions than crystalline regions, and the occurrence of the peeling reaction in the amorphous regions of cellulose leads to an increase in the crystallinity of cellulose after dilute alkaline pretreatment.

Ammonia Fiber Explosion Pretreatment

Ammonia is another desirable pretreatment reagent because (a) it is an effective swelling reagent for lignocellulosic material, (b) it has high reaction selectivity with lignin over carbohydrates and it cleaves ester linkages in the LCCs, (c) it is a noncorrosive chemical, and (d) it is easy to recover and recycle because of its high volatility [184]. Ammonia pretreatment can be carried out in either a batch or a flow-through reactor. In the batch mode, known as ammonia fiber explosion or ammonia fiber expansion (AFEX) pretreatment, the lignocellulosic material is treated with liquid anhydrous ammonia at temperatures between 60 and 100°C and high pressure for a variable period of time (such as 30 min), and then the pressure is released. Due to the design of a commercial-scale AFEX process requiring that adequate ammonia contacts with moist biomass followed by removing adequate ammonia with minimal costs, the conventional approach to perform AFEX is to treat moist biomass (0.1–2 g H_2O/g dry biomass) with liquid ammonia (0.3–2 g NH_3/g dry biomass) while heating (40–180°C) the biomass–water–ammonia mixture for a period of time (5–60 min) before rapidly releasing the pressure [185]. This swift pressure release leads to a rapid expansion of the ammonia gas that causes swelling and physical disruption of biomass fibers. In addition, about 95 % of the ammonia can be recovered in the gas phase and recycled, with a small amount of ammonia that remains in the lignocellulosics which might serve as a nitrogen source for the microbes in the fermentation process [9, 186, 187]. AFEX is another physicochemical pretreatment and its effects can be presented as a combination of steam explosion and alkaline pretreatment [170]. Unlike steam explosion pretreatment where biomass slurry is produced, AFEX generates dry pretreated solids without any liquid stream. AFEX has been applied to various lignocellulosic materials including rice straw, corn stover, and switchgrass. However, AFEX is not effective on lignocellulosics with high lignin content such as softwood [182, 188]. Table 12 summarizes recent AFEX pretreatment results for different substrates.

Table 12 AFEX pretreatment results for different substrates [25, 151, 189, 190]

Substrate	Pretreatment conditions	Cellulose conversion yield (%)	Enzyme loadings	Reference
Switchgrass	100°C, 1:1 ammonia to biomass loading, 80 % moisture content, 5 min	93 in 168 h	15 FPU/g for cellulase from Spezyme CP and 40 IU/g for β-glucosidase from Novozyme 188	[189]
Coastal Bermuda grass	90°C, 1:1 ammonia to biomass loading, 60 % moisture content, 5 min	87.1 in 48 h	30 FPU/g for cellulase from *Trichoderma reesei*	[151]
	100°C, 1:1 ammonia to biomass loading, 60 % moisture content, 5 min	89.0 in 48 h	30 FPU/g for cellulase from *Trichoderma reesei*	[151]
	80°C, 1:1 ammonia to biomass loading, 60 % moisture content, 30 min	97.3 in 48 h	30 FPU/g for cellulase from *Trichoderma reesei*	[151]
Corn stover	90°C, 1:1 ammonia to biomass loading, 60 % moisture content, 5 min	93 in 168 h	60 FPU/g for cellulase from Spezyme CP	[190]
	90°C, 1:1 ammonia to biomass loading, 60 % moisture content, 5 min	93 in 168 h	31 mg/g for cellulase protein from Spezyme CP and 33 mg/g for β-glucosidase protein from Novozyme 188	[25]
Poplar	180°C, 2:1 ammonia to biomass loading, 233 % moisture content, 30 min	93 in 168 h	125 mg/g for cellulase protein from Spezyme CP and 33 mg/g for β-glucosidase protein from Novozyme 188	[25]

Hemicellulose Behavior During Pretreatment

In AFEX, the combination of chemical and physical effects leads to partial solubilization of hemicelluloses and the disruption of the cell wall complex structure, thereby increasing the accessibility of cellulose and hemicellulose to enzymes [9, 25, 187, 190]. Removal of acetyl groups from hemicellulose results in the formation of acetamide and acetic acid, but it is reported that AFEX removes the least amount of acetyl groups from lignocellulosic material compared to other leading pretreatment technologies [100]. AFEX can be considered as a 'dry to dry' process since dry mass recovery following AFEX treatment is almost 100 % [190]. Although results from the literature have suggested that the physical removal of hemicellulose and lignin from plant cell walls into separate liquid phases is necessary to enhance the enzymatic digestibility of cellulose, AFEX can still achieve more than 90 % conversion of cellulose and hemicellulose to fermentable sugars at optimal conditions (Table 12). Furthermore, the enzymatic digestibility of AFEX-treated material at low enzyme loadings is very high compared with other pretreatment technologies [191]. These all suggest that ammonia may affect hemicellulose differently from other chemicals.

Recently, several researchers have reported that subtle changes in the inner cell wall localization of the lignin and hemicelluloses during AFEX pretreatment account for the increase in cellulose accessibility [96, 150, 192]. During AFEX pretreatment, ammonia causes a series of ammonolysis (amide formation) and hydrolysis reactions (acid formation) in the presence of water, which cleave LCC ester linkages such as diferulates cross-linking arabinose side chains of xylan [192]. Cleavage of these lignin–hemicellulose ester linkages facilitates the solubilization and removal of lignin and hemicellulose oligomers, thereby exposing the embedded cellulose microfibrils [192]. A recent study of AFEX-pretreated corn stover by Vismeh et al. (manuscript in preparation) revealed that more than 90 % of diferulate isomers were released as diferuloyl amides whereas only about 5 % were released as diferulic acids, reflecting the relative contributions of ammonolytic and hydrolytic reactions during AFEX pretreatment. Furthermore, the rapid pressure release leading to the expansive decompression of ammonia at the end of pretreatment would lead to the formation of large pores at the middle lamella. Chundawat et al. [192] stressed that these large pores would greatly facilitate the accessibility of cellulases because the radius of gyration of exo-cellulase Cel7A from *Trichoderma reesei* is smaller than the pore size. In addition, increasing cell wall porosity without extensively extracting lignin and hemicellulose could prevent the collapse and aggregation of cellulose microfibrils, thereby preserving pretreatment effectiveness [96, 117]. The mechanism discussed above partially explains how AFEX pretreatment reduces lignocellulosic recalcitrance.

One of the major advantages of AFEX pretreatment is the minimal formation of inhibitors such as furfural and HMF (Table 13) for the fermentation process [193]. Also, it is reported that acetamide could stimulate rather than inhibit microbial metabolism [187, 192, 194]. Since some phenolic fragments of lignin, aliphatic organic acids, and other cell wall extractives including arabinoxylan oligomers may remain on the surface of cellulose, water washing of AFEX-pretreated solid residue does greatly improve sugar yields [195].

Cellulose Behavior During Pretreatment

AFEX pretreatment has a much lower impact on the DP of cellulose than other leading pretreatment technologies such as DAP [100]. Another major chemical effect of AFEX is cellulose decrystallization (Table 14) [24, 100, 196] that allows cellulose to be more easily degraded in the enzymatic hydrolysis process. In general, lower moisture content in AFEX pretreatment tends to produce less crystalline samples (Table 14) [24]. This is probably owing to the high moisture in the biomass which

Table 13 Concentrations of furfural and HMF from different pretreatments for different substrates [144]

Pretreatment	Conditions	Concentration (mg/L) of furfural from			Concentration (mg/L) of HMF from		
		Corn stover	Polar	Pine	Corn stover	Poplar	Pine
DAP	180°C, 0.7 % H_2SO_4, 8 min, 10 g/L solid concentration	220	220	190	44	64	170
LHW	180°C, 8 min, 10 g/L solid concentration	8.0	2.6	2.5	2.3	0.45	1.3
Ammonia	180°C, 0.1 % (*w*/*w*) aqueous NH_4OH, 8 min, 10 g/L solid concentration	0.40	0.50	0.65	0.89	0.079	0.16
Lime	180°C, water containing 0.1 g $Ca(OH)_2$/g biomass, 8 min, 10 g/L solid concentration	1.5	1.8	5.4	2.3	0.36	0.63

Table 14 Cellulose crystallinity before and after AFEX pretreatment for different substrates [24, 100]

Substrate	Pretreatment conditions	CrI (%) before pretreatment	CrI (%) after pretreatment	Reference
Poplar	180°C, 2:1 ammonia to biomass loading, 233 % moisture content, 30 min	49.9	47.9	[100]
Corn stover	90°C, 1:1 ammonia to biomass loading, 5 min	50.3	36.3	[100]
	90°C, 1:1 ammonia to biomass loading, 60 % moisture content, 5 min	50.3	36.3	[24]
	90°C, 0.7:1 ammonia to biomass loading, 60 % moisture content, 5 min	50.3	31.2	[24]
	90°C, 1:1 ammonia to biomass loading, 40 % moisture content, 5 min	50.3	23.5	[24]
	90°C, 0.7:1 ammonia to biomass loading, 20 % moisture content, 5 min	50.3	16.8	[24]

promotes the formation of ammonia hydroxide [189] that hydrolyzes more hemicellulose and lignin, thereby increasing the relative crystallinity of cellulose. The reduction in cellulose crystallinity after AFEX pretreatment may be attributed to the generation of more amorphous cellulose [100].

It is well known that both native cellulose allomorphs cellulose I_α and I_β can be converted irreversibly into cellulose III_I by treatment with anhydrous liquid ammonia [193, 197]. Chundawat et al. [50] suggested that, upon going from cellulose I_β to cellulose III_I, the interaction between anhydrous liquid ammonia and cellulose I_β leads to a decrease in the number of intrasheet (intra- and interchain) hydrogen bonds and to the formation of intersheet hydrogen bonds. Such subtle structure alternation within the cellulose III_I hydrogen bonding network was found to significantly increase the enzymatic hydrolysis yield of cellulose III_I compared to native cellulose I_β [50]. This was attributed to, in part, the larger structural fluctuations along the intra- and intersheet directions of both the crystalline cores and the surface chains of the cellulose III_I fibrils, thereby making individual glucan chains for cellulose III_I more readily accessible by cellulases than cellulose I_β [50]. Furthermore, the surface chains of cellulose III_I have a higher tendency to form hydrogen bonds with water molecules and have a lower percentage of the *tg* (trans-gauche) conformation state for the cellulose C6 hydroxymethyl group. These structural properties typically associated with amorphous cellulose suggest the 'amorphous-like' nature of the surface chains of cellulose III_I [50], contributing as another possible reason to the enhanced enzymatic digestibility of cellulose III_I compared to cellulose I_β.

There is no significant transformation of the native cellulose I to cellulose III_I during conventional AFEX pretreatment [192], which has been attributed to the amount of water present. This is expected since water would compete with ammonia's ability to intercalate and disrupt the elementary microfibril hydrogen bonding network, thereby preventing the transition of cellulose I to III_I [198]. However, the enhanced enzymatic digestibility of cellulose III_I suggests that optimizing AFEX

Table 15 ARP pretreatment results for different substrates [184, 200–203]

Substrate	Pretreatment conditions	Klason lignin before pretreatment (%)	Klason lignin after pretreatment (%)	Cellulose conversion yield (%)	Enzymes loadings	Reference
Waste oak wood	130°C, 15 wt.% ammonia, 5.0 ml/min, 20 min	17.5	6.6	86.1 in 72 h	60 FPU/g for cellulase	[200]
Oak wood	170°C, 15 wt.% ammonia, 5.0 ml/min, 10 min	19.2	7.9	87.4 in 72 h	60 FPU/g for cellulase	[200]
Poplar	175°C, 10 wt.% ammonia, 1.0 ml/min, 60 min	26.0	15.3	87.0 in 72 h	30 FPU/g for cellulase from Cytolase CL	[201]
Corn stover	170°C, 15 wt.% ammonia, 5.0 ml/min, 90 min	17.2	2.6	92.5 in 72 h	10 FPU/g for cellulase from Spezyme CP	[184, 202]
	170°C, 15 wt.% ammonia, 5.0 ml/min, 10 min	17.2	5.1	90.1 in 72 h	15 FPU/g for cellulase from Spezyme CP, and 30 CBU/g for β-glucosidase from Novozyme 188	[203]
	170°C, 10 wt.% ammonia, 5.0 ml/min, 10 min	17.2	5.1	87.7 in 72 h	15 FPU/g for cellulase from Spezyme CP, and 30 CBU/g for β-glucosidase from Novozyme 188	[203]

pretreatment conditions to maximize the transformation of cellulose I to cellulose III_I may further increase cellulose accessibility to cellulases.

Ammonia Recycled Percolation Pretreatment

Ammonia pretreatment can also be performed in a flow-through reactor known as ammonia recycled percolation (ARP) pretreatment, where aqueous ammonia passes through a column reactor containing lignocellulosic materials. The primary factors influencing ARP pretreatment efficiency are reaction time, temperature, ammonia concentrations, and the amount of liquid throughput. Normally, the reactor temperature is fixed at 140–210°C, the reaction time can vary from 5 to 90 min, and the percolation rate is approximately 5 ml/min with ammonia concentration of 5–15 wt.% [9, 10, 199, 200]. This pretreatment technology has been successfully applied to hardwoods [200, 201], agricultural residues (Table 15) [184, 202–204], and with slightly less efficiency to softwoods [205].

Hemicellulose Behavior During Pretreatment

The primary effects of ARP pretreatment are delignification (Table 15) and hemicellulose solubilization. The solubilized hemicelluloses are mainly in their oligomeric forms. The extent of hemicellulose deacetylation is much more significant than AFEX pretreatment; for instance, Kumar et al. [100] observed 88.1 % of deacetylation of corn stover for ARP while it is 32.5 % for AFEX. Furthermore, decomposition of carbohydrates during ARP pretreatment is insignificant; thus, less inhibitors are produced, which is a very important benefit in this pretreatment process.

Cellulose Behavior During Pretreatment

Cellulose remains intact during ARP pretreatment (more than 92 % of glucan is retained) [184, 202, 203]. Similar to AFEX pretreatment, ARP pretreatment does not significantly reduce the DP of cellulose [100]. It removes a significant portion of amorphous hemicellulose and lignin; thus, the relative crystallinity index of cellulose increases after ARP pretreatment [184, 202, 203]. This leads to an increase in the enzymatic digestibility of cellulose because the reduction in hemicellulose and lignin contents improves enzyme access to cellulose and reduces nonspecific binding of enzymes with lignin despite an increase in the relative crystallinity index.

Conclusions and Perspectives

Most of the leading pretreatment technologies that have been described herein are effective on one or more factors that contribute to lignocellulosic recalcitrance. Despite much research that have been dedicated to understanding the chemistry and the plant cell wall structure changes during various pretreatment technologies, the insufficient knowledge on cell wall structure, ultrastructure, and pretreatment effects still limits the economics and effectiveness of pretreatment. For instance, the biological and chemical properties of plants are very complex, which are composed of at least 35 different cell types that are distinct in composition, structure, and ultrastructure [206]. Additionally, the actual mechanism of LCCs preventing chemical and biological deconstruction of cell walls is poorly understood [31]. Although researchers have put a lot of effort into optimizing the pretreatment effectiveness, the fundamental science behind these optimizations is still not fully understood. Furthermore, there has been a lack of mechanistic understanding of the ultrastructural and physicochemical changes occurring within the cell wall at the molecular level and the cellular/tissue scale during various pretreatment technologies [31]. It is thus essential to understand the effects of pretreatment on plant cell walls at a more fundamental level in order to develop a cost-effective pretreatment technology with maximum fermentable sugar recovery, minimum inhibitor production and energy input, low demand of post-pretreatment processes, and low capital costs for reactors, water, and chemicals.

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