

Chapter 2

Insight into Various Conventional Physical and Chemical Methods for the Pretreatment of Lignocellulosic Biomass



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Abstract The generation of renewable energy resources as an alternative to fossil fuels is essential to sustain the growing human population. Lignocellulosic biomass is considered an important renewable resource for various value-added compounds and biofuels, as the world is currently poised toward a carbohydrate-based economy. Analogous to petroleum refineries, biorefineries deal with the carbohydrate polymers (cellulose, hemicellulose) and aromatic compounds (lignin), which can be processed into different bioproducts. However, the complex architecture of crystalline cellulose, hemicellulose, and lignin creates high recalcitrance, which requires significant pretreatment steps. Thus, developing cost-effective pretreatment is crucial for the effective separation of the biomass components. In this chapter, first, the basic components of the lignocellulosic biomass have been briefly described followed by the various conventional physical and chemical pretreatment methods. In addition, the efficiency of different biomass-specific pretreatment operations and their combinations has been discussed in detail. Moreover, challenges of the pretreatment processes, like chemical recovery, inhibitory byproducts formation, prolonged and costly methods, and feedstock utilization are also highlighted. Overcoming the challenges has demonstrated the potentiality of the available pretreatment methods in the advanced biological refinery process for the production of biofuels and various value-added compounds.

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Abbreviations

AFEX	Ammonia Fiber Explosion
CrI	Crystallinity index
DA	Dilute acid
DP	Degree of polymerization
GR	Gamma irradiation
ILs	Ionic liquids
LCB	Lignocellulosic biomass
LHW	Liquid hot water
MWR	Microwave radiation
OS	Organosolvent
SE	Steam explosion
WO	Wet oxidation

2.1 Introduction

Environmental pollution and climate changes are two major challenges of the present century, necessitating the use of non-fossil-based carbon-neutral fuel resources. Lignocellulose biomass (LCB) based feedstock presents one of the most suitable alternative resources for biofuels and value-added bioproducts in a sustainable manner. LCB has gained huge attention for promising biorefinery feedstock because of its ample abundance and lower cost than other biomass resources. However, conversion of LCB into useful biofuel or other value-added chemicals takes the integration of a series of chemical as well as biological procedures (Fig. 2.1). Considerable obstacles are involved in the effective utilization of LCB. The major hindrance to the conversion process is the complex organization of the structural components in the lignocellulosic biomass. The primary components of LCB are cellulose ($(C_6H_{10}O_5)_n$), hemicellulose ($(C_5H_8O_4)_m$), and lignin [$C_9H_{10}O_3(OCH_3)_{0.9-1.7}x$] (Akhtar et al. 2016; Jørgensen et al. 2007; Kumar and Verma 2020a). This integral complexity of the plant cell wall leads to major recalcitrance against any kind of deconstruction of the LCB.

Pretreatment is the essential method performed upstream of the LCB to biofuel conversion, in order to overcome the recalcitrance and disrupt the complex organization of cellulose, hemicellulose, and lignin. Pretreatment is a vital component in converting LCB into liquid fuels and chemicals. Pretreatment aims to separate aromatic lignin and polysaccharides (cellulose and hemicellulose) and enhance accessibility to the hydrolytic enzymes for saccharification. The more the LCB is

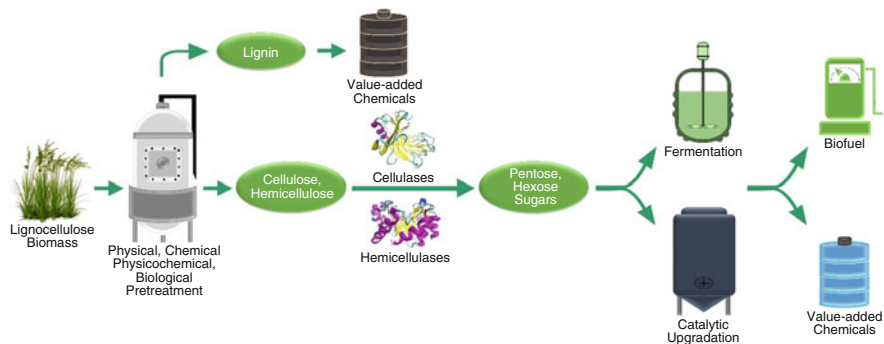


Fig. 2.1 Schematic representation of the general pathway for biofuel and value-added chemical production in lignocellulose biorefinery. The lignocellulosic biomass undergoes steps like pretreatment and enzymatic hydrolysis for removal of lignin and depolymerization of cellulose and hemicellulose, releasing simple sugars for catalytic processing or fermentation for the production of biofuel or value-added chemicals

susceptible to hydrolysis, the more yield will be achieved for the fermentation process. Different types of pretreatment processes are employed to overcome the recalcitrance and breakdown of crystalline cellulose, opening the hemicellulose-cellulose surface with improved accessibility for further chemical conversion (Bhutto et al. 2017; Chen et al. 2017; Agrawal and Verma 2020; Bhardwaj et al. 2021). Notably, each pretreatment method has its specific consequence on the structure of the cellulose, hemicellulose, and lignin of the LCB. The physical and chemical changes of the LCB fractions are directly linked to the overall operation and effectiveness of the downstream process in terms of hydrolysis, substrate solubilization, fermentation rate, mixing, ethanol yield, etc. Thus, the choice of proper pretreatment method based on the LCB feedstock type and its effects on downstream steps is essential for the overall production of biofuels and chemicals.

In this chapter, first, the components of the LCB have been summarized followed by a discussion of the various conventional physical and chemical pretreatment methods. The challenges of different pretreatment methods are also highlighted along with the production of biofuels and various value-added compounds. Understanding the overall process helps in selecting the proper pretreatment method with subsequent operations based upon the type of feedstock, hydrolysis, and fermentation steps.

2.2 Components of Lignocellulosic Biomass

Plant cell wall mainly comprises polysaccharides such as cellulose, hemicellulose, aromatic polymer lignin, and a trace amount of pectin (Fig. 2.2) (Ververis et al. 2004). These complex composite materials provide the basic structural support to the plants. Cellulose remains in the core, surrounded by hemicellulose, whereas lignin

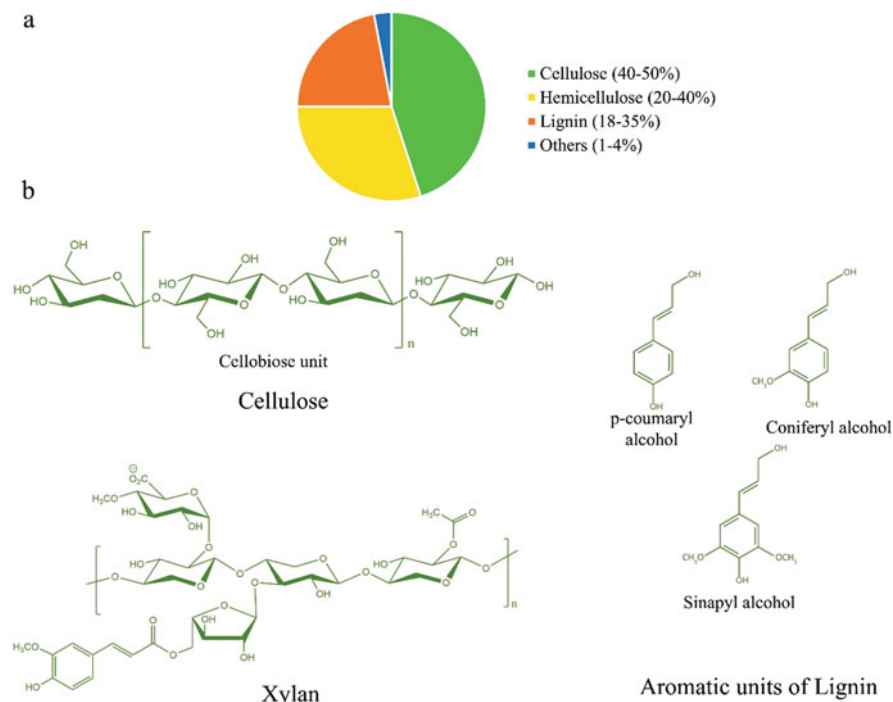


Fig. 2.2 Composition of the lignocellulosic biomass: (a) Cellulose (40–50%), hemicellulose (20–40%), and lignin (18–35%) along with a trace amount of pectin, protein, extractives, and ash make up the lignocellulosic biomass. (b) Chemical structures of the major constituents of lignocellulosic biomass. Cellulose is a homopolymer of β -1,4-glycosidic linked glucan chains, whereas xylan is hemicellulose composed of β -1,4-linked xylose residues. Lignin is an aromatic compound consisting of mainly p-coumaryl, coniferyl, and sinapyl units

appears at the outermost part of the plant material. Cellulose is a linear homopolymer of β -1, 4 linked glucose, whereas hemicellulose is a branched heterogeneous polysaccharide consisting of monomers like xylan, arabinoxylan, and others. Pectin is made up of complex polysaccharides such as α -linked galacturonic acid and rhamnose monomers. Lignin is also heterogeneous in nature and mainly contains alkyl-aromatic units forming a branched polymer. The composition of some popular lignocellulosic plant biomass is given in Table 2.1.

2.2.1 Cellulose

Cellulose is the most abundant organic matter available on earth, which represents the primary constituent of the plant cell wall. Out of all the components of the lignocellulosic biomass, cellulose represents a vast amount of fermentable sugar

Table 2.1 Composition of some industrially important lignocellulosic biomass including agricultural wastes which can serve as potential lignocellulosic biomass sources in biorefineries

Plant material	Major components of lignocellulosic biomass			References
	Cellulose (wt %)	Hemicellulose (wt %)	Lignin (wt %)	
Alfa Alfa	21.8	12.4	9.7	Dijkerman et al. (1997)
Bermuda grass	47.8	13.3	19.4	Li et al. (2009)
Corn cobs	35–39	38–42	4.5–6.6	Okeke and Obi (1994)
Cotton seed hairs	80–95	5–20	0	Howard et al. (2003)
Hard Woods	45 ± 2	30 ± 5	20 ± 4	Kuhad et al. (1997)
Maize	35	16.8	7	Kasthuraiah and Sai Kishore (2017)
Miscanthus	45	27	12	Kasthuraiah and Sai Kishore (2017)
Reed canary straw	42.6	29.7	7.6	Bridgeman et al. (2008)
Rice straw	41	21.5	9.9	Lee (1997)
Soft woods	42 ± 2	27 ± 2	28 ± 3	Kuhad et al. (1997)
Sugar cane	40	27	10	Kuhad et al. (1997)
Sweet sorghum bagasse	34–45	18–28	14–22	Saini et al. (2015)
Switchgrass	45	31	12	Howard et al. (2003)
Wheat straw	44	29.6	10.4	Bridgeman et al. (2008)

feedstock and a major focus of biofuel and value-added chemical generation on an industrial scale. However, it is more recalcitrant to the catalytic deconstruction process than other carbohydrate polymers. Cellulose is a linear chain of homopolysaccharides with anhydrous glucose units connected by β -1,4-glycosidic bonds.

Dimerization of glucose monomers through β -1,4-glycosidic bond forms cellobiose which is the basic structural unit of cellulose. The spatial organization of the β -1,4-glycosidic bonds inside the glucan chains gives rise to specific inter and intramolecular hydrogen bonding interactions imparting high crystallinity, making it insoluble in water or other common solvents (Bishop 2015; Kumar and Verma 2020b). Also, each cellulose chain comprises a reducing end at one end and a non-reducing end at the other (Fig. 2.3). During chain elongation, new glucose monomers are added to the non-reducing end. Proper knowledge of cellulose crystallinity is crucial for understanding the depolymerization process. The β -1,4 linked glucan chains form a highly ordered crystalline structure. It is reported from the X-ray diffraction data that the crystalline microfibrils contain 24–36 linear chains (Endler and Persson 2011; Fernandes et al. 2011). In some cases, loss of crystallinity results in disordered regions, which are known as amorphous cellulose. The highly ordered crystalline cellulose is separated by such amorphous regions. Interchain as well as intrachain hydrogen bondings due to the spatial organization of the hydroxyl groups of the glucose along with the different pyranose ring arrangements in the

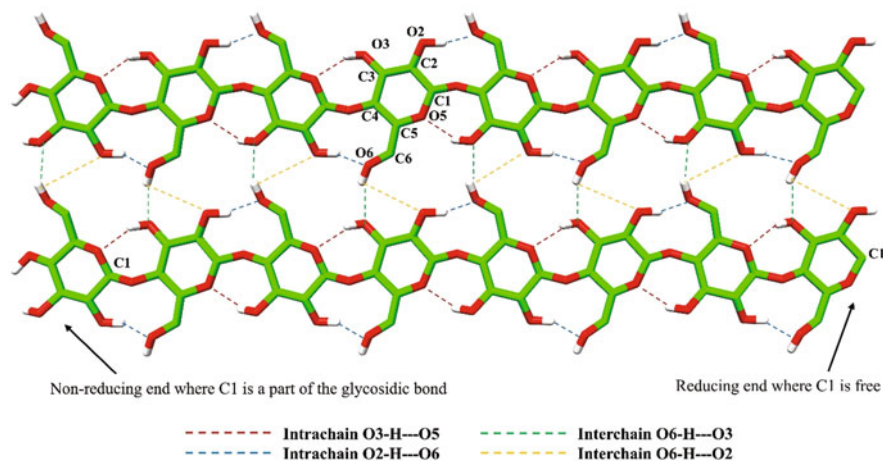


Fig. 2.3 Inter and Intra-hydrogen bonding in cellulose. Intrachain hydrogen bonding forms between $O2H2 \cdots O6$ and $O3H3 \cdots O5$, whereas interchain interaction occurs via $O6H6 \cdots O3$ hydrogen bonding between two cellulose chains. Carbon, oxygen, and hydrogen are shown in green, red, and white, respectively

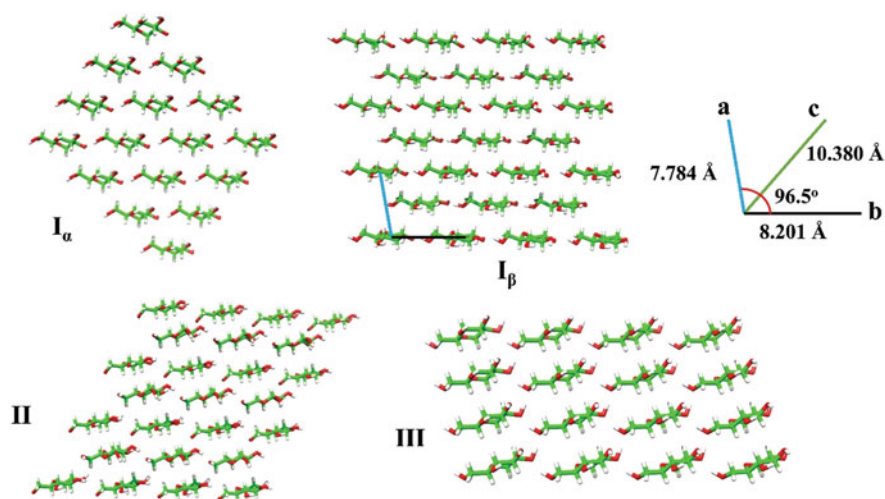


Fig. 2.4 Crystal structures of cellulose allomorphs. The end-on view of different cellulose crystals is shown. Carbon, oxygen, and hydrogen are shown in green, red, and white, respectively. The unit cell parameters for cellulose I_β are also presented

cellulose crystal give rise to four distinct crystalline allomorphs of cellulose. These are designated as cellulose I, II, III, and IV, respectively (Fig. 2.4). The difference is based on the crystal unit cell parameters such as unit cell length and unit cell angles, which defines the packing of cellulose chains in the crystal (Table 2.2).

Table 2.2 Different types of crystalline cellulose and their unit cell parameters

Cellulose crystal	Unit cell type	Unit cell parameters	References
I _α	Triclinic	a = 6.717, b = 5.962, c = 10.400, α = 118.08°, β = 114.80°, γ = 80.37°	Nishiyama et al. (2003)
I _β	Monoclinic	a = 7.784, b = 8.201, c = 10.380, γ = 96.5°	Nishiyama et al. (2002)
II	Monoclinic	a = 8.10, b = 9.04, c = 10.36, γ = 117.1°	Langan et al. (2001)
III	Monoclinic	a = 10.25, b = 7.78, c = 10.34, γ = 122.4°	Sarko et al. (1976)
IV	Orthogonal	a = 10.25, b = 7.78, c = 10.34	Gardiner and Sarko (1985)

Among all the cellulose allomorphs, cellulose I is predominantly found in nature. It is basically a mixture of two cellulose forms, i.e., I_α and I_β (Fig. 2.4). The I_β form of cellulose has a monoclinic unit cell and is mostly found in higher plants like wood, cotton, and animals, whereas the I_α form has a triclinic unit cell and is found in a wide range of microorganisms like bacteria (*Acetobacter xylinum*) and algae (*Glaucocystis nostochinearum*). The cellulose I_β form is gaining more interest, as this form is abundant in higher plants and serves as a potential feedstock for fermentable sugar generation. Cellulose II can be obtained from cellulose I by either alkali treatment or solubilization and regeneration (Mittal et al. 2011). The unit cell in cellulose II is also monoclinic as the I_β. However, the chains in cellulose I_β are parallel, whereas the chains in cellulose II are antiparallel. Cellulose I or II can be converted to cellulose III form by ammonia pretreatment which is more amorphous in nature. Allomorph III is subcategorized into cellulose III_I or III_{II} based on the source of the conversion process. Due to reduced crystallinity, both the II and III allomorphs have greater enzyme accessible surface area that enhances the saccharification process (Chundawat et al. 2011; Payne et al. 2015). Cellulose IV can be produced by high-temperature treatment of cellulose III. This form of cellulose is less characterized. However, reports suggest that it is structurally similar to cellulose I_β (Nishiyama 2009).

Some crucial properties of cellulose that influence its pretreatment and enzymatic scarification are described below:

- (a) *Crystallinity index (CrI)*: It provides a measure of the compactness and reactivity of the cellulosic material which can be quantified by an X-ray diffraction pattern. For example, the *CrI* for cellulose type I can be obtained using the following method of Jayme and Knolle (Lenz 1994):

$$CrI = 1 - \frac{h_{am}}{h_{cr}} = 1 - \frac{h_{am}}{(h_{tot} - h_{am})} \quad (2.1)$$

where h_{cr} is the height of the cellulose crystal in the 002 reflections with $2\theta = 22.5^\circ$, h_{am} is the height of the amorphous cellulose, and h_{tot} is defined as:

$$h_{\text{tot}} = h_{\text{cr}} + h_{\text{am}} \quad (2.2)$$

- (b) *Degree of Polymerization (DP)*: The degree of polymerization is another important parameter affecting enzymatic hydrolysis. It determines the availability of terminal and internal glycosidic bonds for both the exo- and endo-catalytic enzymes. Three specific DPs are generally considered such as the number average DP (DP_N), the weighted average DP (DP_W) and DP obtained from viscosity (DP_V). The governing formulae are given below:

$$DP_N = \frac{M_n}{MW_{\text{glu}}} = \frac{\sum N_i M_i}{\sum N_i} / MW_{\text{glu}} \quad (2.3)$$

$$DP_W = \frac{M_w}{MW_{\text{glu}}} = \frac{\sum N_i M_i^2}{\sum N_i} / MW_{\text{glu}} \quad (2.4)$$

$$DP_V = \frac{M_v}{MW_{\text{glu}}} = \frac{\sum N_i \eta}{\sum N_i} / MW_{\text{glu}} \quad (2.5)$$

where M_i is the molar mass of a given fraction I with N_i number of moles with viscosity η and M_N , M_W , M_V , MW_{glu} being the average molecular weight, weight-average molecular weight, viscosity-average molecular weight, and molecular weight of anhydroglucose, respectively. Cellodextrins with DP ranging from 2 to 6 are generally water soluble (Klemm et al. 1998). Solubility decreases with increasing DP of the polysaccharide. For example, glucan chains with DP 7–13 are weakly soluble (Zhang and Lynd 2003). However, DP greater than 30 is insoluble in common solvents.

- (c) *Accessibility*: Cellulases need to bind effectively to the solid surface with proper orientation of the cellulose for the initiation of hydrolysis. The microcrystalline structure of cellulose along with particle size and shape determines the probability of interaction between the enzyme and the glycosidic bonds. Thus, cellulose accessibility is a key factor in the saccharification step.

2.2.2 Hemicellulose

It is a complex branched biopolymer of pentose (C5) and hexose (C6) sugars, mostly D-xylose, D-galactose, L-arabinose, D-mannose, and D-glucose, having a short length of around 50 to 200 units. However, the composition of hemicellulose varies from different sources due to its heterogeneous nature. Hemicellulose isolated from the cell wall of rice endosperm reveals that it is composed of mainly xylose, arabinose, and glucose forming the polysaccharide moieties xyloglucan, β -glucan, and arabinoxylan, respectively (Shibuva and Misaki 1978; Bhardwaj et al. 2020; Bhardwaj and Verma 2021). The β -glucan contains both the 1,3 and 1,4 glycosidic linkages. The xylose is attached to the C6 position of the 1,4 linked glucan backbone to form the xyloglucan. The arabinoxylan shows a complex branched structure

where the arabinose is connected to the 1,4 linked xylose chain. Acetate groups are attached to the hydroxyl functional groups of the sugar rings via an ester linkage. Hemicellulose mostly provides the bridge between core cellulosic parts with outer lignin. Researchers are focusing on hemicellulose for the production of value-added chemicals in biorefineries (Rambo et al. 2015).

2.2.3 Lignin

Lignin is a non-carbohydrate phenolic polymer, made up of a complex network of aromatic alcohols. The main components of this crosslinked heteropolymer are p-coumaryl, coniferyl, and sinapyl alcohols (Rubin 2008). The units are known as p-hydroxyphenyl, guaiacyl, and syringyl when present in a polymer chain. Lignin is bound to hemicellulose with covalent linkages to form an amorphous matrix that surrounds core cellulose microfibrils. Strong ether (C–O–C) and carbon–carbon (C–C) bonds in the lignin provide high mechanical strength to the plants. So far, lignin is unusable in the fermentation process for biofuel production and can be used as an alternative resource for value-added chemical production in the biorefinery (Radhakrishnan et al. 2021; Kumar et al. 2020).

2.3 Different Technologies for Lignocellulosic Biomass Pretreatment

For the production of lignocellulosic biofuels and other economically important chemicals, the first step is to overcome the heterogeneity of the raw biomass in a cost-effective manner. Several pretreatment techniques have been adopted to separate the cellulose from other biopolymeric mixtures followed by decrystallization of the cellulose microfibrils that will be subjected to the enzymatic hydrolysis in the subsequent steps. The primary goals of the pretreatment are the efficient separation of lignin, hemicellulose, and cellulose by disrupting the structural linkages and increasing the accessibility to the hydrolytic enzymes. Moreover, some other key targets in the pretreatment process are a reduction in the crystallinity index, the degree of polymerization of the cellulose, and particle size of biomass. Some of the key criteria for a “good” pretreatment method are (a) less degradation of the biomass, (b) lowering the inhibitory product formation, (c) minimizing energy input, (d) low-cost pretreatment agents and catalyst recycling, (e) overall cost-effectiveness. The pretreatment procedures can be broadly classified into three major categories: physical, chemical, and biological pretreatments. The chemical pretreatments are subcategorized into biochemical and thermochemical processes. The traditional physical and chemical pretreatment methods are discussed in the following sections.

2.3.1 Physical Pretreatment Process

The physical pretreatment processes are aimed to increase the overall surface area of the biomass and reduction in the particle size which in turn decreases the degree of polymerization of the material. The commonly available physical pretreatment methods are described below.

2.3.1.1 Milling

Milling is a mechanical process for lignocellulose pretreatment. The different types of milling used for the deconstruction of lignocellulosic biomass are ball milling, disk milling, two-roll milling, colloid milling, and hammer milling (Mood et al. 2013). The average particle size obtained from the milling process is around 10–30 mm. However, the high energy requirement of the process raises the production cost which is partially overcome by wet disk milling with comparatively lower energy consumption. In a comparative study on the effectiveness of wet disk and ball milling processes, it was found that the total glucose and xylose yield in the enzymatic hydrolysis step from the wet disk pretreated sugarcane bagasse were 49.3% and 36.7%, respectively, whereas the ball milling pretreated biomass gave rise to maximum yield of 78.7% and 72.1%, respectively (Hideno et al. 2009). Value-added chemicals such as methyl levulinate were made with a maximum yield of 64.92 mol%, using ball milling pretreatment by facilitating cellulose depolymerization (Chen et al. 2019).

2.3.1.2 Extrusion

Extrusion is another thermo-physical pretreatment method, where the biomass is passed through an extruder with moderate to high temperatures. The rapid mixing and excessive shearing in the chamber lead to the breakdown of the crystalline structure and produce short fibers which increase the enzymatic accessibility of the biomass in the hydrolysis step. For example, extrusion with temperature up to 80 °C, screw speed of 350 rpm, and moisture content of 40% provided a yield of 94.8% glucose conversion by the enzymatic hydrolysis from the pretreated soybean hulls (Yoo et al. 2011). Recently, extrusion has been combined with cellulases for obtaining sugar conversion yield of at least 94% at 50 °C (Gatt et al. 2019). This process doesn't produce any effluent which is an advantage in terms of disposal cost reduction. Continuous operating process without washing and the possibility of easy scale-up have made extrusion an industrially important pretreatment process.

2.3.1.3 Pyrolysis

Pyrolysis is the process of deconstruction of lignocellulosic biomass at high temperatures. This pretreatment approach has been adopted by many industries. When lignocellulosic materials are subjected to temperatures as high as 300–800 °C or more, the cellulose crystallinity is gradually decreased with the production of residual char and gaseous compounds (Fisher et al. 2002). For example, 50–55 (wt %) liquid fraction, 25–27 (wt%) char, and other gaseous compounds were produced from hardwood pyrolysis at a temperature of 200–275 °C for 30–45 min followed by 450 °C for 1 h (Ortega et al. 2011). However, the decomposition is significantly less at lower temperatures. It is reported that more than 80–85% cellulose conversion can be achieved by coupling mild acid hydrolysis with pyrolysis. Cellulose decomposition can also be performed by introducing sodium carbonate or zinc chloride as a catalyst in the process. Catalytic pyrolysis shows a significant rise in the organic liquid yield of 42 wt% in pretreated biomass than raw biomass at a process temperature of 600 °C (Persson and Yang 2019).

2.3.1.4 Microwave

The complex organization of cellulose and other components of the lignocellulosic biomass can also be subjected to microwave irradiation rather than conventional heating for the deconstruction process. Microwaves produce high-frequency electromagnetic field that generates heat when interacting with an object containing mobile charges. The difference with conventional heating is that heat transfer occurs through the surface of the objects, whereas microwave introduces high molecular collision in the cellulose because of dielectric polarization which leads to the breakdown of the cellulose ultra-structure, separation of lignin/hemicellulose and finally enhancing the accessibility of the material for the hydrolytic enzymes. For example, 30% and 12% lignin removal were obtained in corn straw and rice husk, respectively, with microwave pretreatment (Diaz et al. 2015). Currently, microwave-assisted pretreatment (with irradiation at 800 W and 152 °C for 45 s) was carried out using choline chloride and lactic acid deep eutectic solvent (ChCl: LA DES) where 85%–87% pure lignin was recovered while the cellulose existed in the solid fraction (Chen and Wan 2018). In another study, researchers have reported microwave pretreatment using choline chloride and oxalic acid dihydrate deep eutectic solvent (microwave irradiation at 800 W and 80 °C for 3 min) with a pure lignin recovery of 96% (Liu et al. 2017a).

2.3.1.5 Freeze Pretreatment

Freezing treatment is a newly introduced approach where the material is stored in the refrigerator for a specific time which significantly increases the cellulase

performance before the hydrolysis step. It is found that the enzymatic hydrolysis was enhanced from 48% to 84% on a freeze pretreated rice straw (Chang et al. 2011). Low environmental impact and absence of chemical hazards make the process promising.

The main advantage of the physical pretreatment method is that the particle size of the biomass is reduced which enables efficient heat and mass transfer during further processing. On the other hand, cellulose crystallinity and degree of polymerization are decreased, thus providing higher surface area and pore size during the hydrolysis step for improved sugar yield. However, the major limitations of these processes are the high energy requirement and equipment maintenance cost which make them economically inefficient.

2.3.2 Physico-Chemical Pretreatment Process

2.3.2.1 Steam Explosion

Steam explosion is a thermo-mechano-chemical pretreatment method that involves the thermal steam-heating to degrade the lignocellulosic components, the mechanical shearing force due to sudden pressure drop, and chemical autohydrolysis for glycosidic bond cleavage. In this method, the biomass is subjected to saturated steam at a temperature of around 160 °C–260 °C at a high pressure of ~0.69 to 4.83 MPa for a few seconds to minutes followed by exposing it to the atmospheric pressure. This explosive decompression results in the disintegration of the lignocellulosic matrix. Hemicellulose hydrolysis along with lignin transformation at high-temperature treatment exposes the cellulose surface to the cellulolytic enzyme machinery in the subsequent steps. The efficiency of this process depends on the particle size, temperature, the residence time of the material, and total moisture content inside the chamber. The efficiency of the process can be greatly enhanced by the introduction of catalysts such as sulfuric acid (H₂SO₄), sulfur dioxide (SO₂), or carbon dioxide (CO₂) which accelerate hydrolysis and removal of hemicellulose. Steam explosion pretreatment at 177 °C for 5 min of banana lignocellulosic biomass gave rise to 91% glucose yield in enzymatic hydrolysis (Guerrero et al. 2017). Moreover, pretreatment including wheat straw, miscanthus, and poplar has shown the role of steam explosion in reducing hemicellulose content and breaking down the lignin cross-linkages for improved enzymatic hydrolysis (Auxenfans et al. 2017). The main advantage of this technology is that due to the partial lignin and hemicellulose solubilization, the sugar yield is improved during the enzymatic hydrolysis. On the other hand, no requirement of recycling and less environmental impact make this process industrially feasible. However, there is a possibility of forming fermentation inhibitors at high temperatures. In case of incomplete deconstruction of the biomass, soluble lignin may get precipitated and hinder effective hydrolysis. Considerable energy requirement due to the maintenance of high temperature and pressure increases the overall cost of the process.

2.3.2.2 Ammonia Fiber Explosion (AFEX)

It is a thermochemical pretreatment method that is based on the same principle as steam explosion. Here, the lignocellulosic biomass is treated with liquid ammonia at high pressure (1.72–2.06 MPa) and moderate temperature (60 °C–120 °C) for a specified time (~30 min). Then the pressure is swiftly dropped which results in the quick expansion of the compressed ammonia gas that breaks the matrix of the lignin–carbohydrate complex followed by degradation of the fibers. Ammonia pretreatment produces solid materials, whereas steam explosion gives rise to the slurry. It is notable that AFEX doesn't release any sugar due to less solubility of hemicellulose but it disintegrates the components to increase the surface area of the biopolymeric mixture for higher enzymatic accessibility. AFEX pretreatment is proven to be more effective for the pretreatment of low lignin-containing biomass such as alfalfa stems, wheat straw, rice straw, switchgrass, corn stover, etc. than hardwood and softwood materials with more lignin. AFEX process optimization can be done by varying the following parameters, i.e., the amount of ammonia to biomass loading ratio, water loading, temperature, the residence time of the biomass, and the moisture content. For example, ammonia to biomass loading ratio of 2:1 along with 120% moisture content and 5 min of residence time at 140 °C temperature were found to be optimal for bioethanol production from sorghum bagasse (Li et al. 2010). In a recent study, the efficacy of both the steam explosion and AFEX was explored for the production of animal feeds and biofuel feedstocks in a sugarcane-based biorefinery approach. It was shown that up to 3368 L and 4360 L of ethanol per hectare of sugarcane cultivated land could be generated from steam explosion and AFEX pretreated sugarcane residues, respectively (Mokomele et al. 2018). AFEX increases the effective surface for enzymatic hydrolysis and enhances the sugar yield. Besides, no inhibitory by-products are formed during the process, and no biomass washing or detoxification steps are required. However, this pretreatment method is not effective for high lignin-containing biomass and softwoods. The corrosive and hazardous properties of AFEX pretreatment require a controlled environment and costly equipment. The cost of ammonia is another concern that makes the process highly expensive at large scale (Agbor et al. 2011).

2.3.2.3 CO₂ Explosion

The CO₂ explosion is another explosion-based method similar to steam explosion and AFEX for the pretreatment of lignocellulosic biomass. In this process, supercritical CO₂ is used with a temperature and pressure greater than its critical temperature and pressure values (31 °C and 7.4 MPa, respectively). At this condition, liquid and gases can coexist, and CO₂ shows liquid-like density as well as gas-like transport properties with the ability to penetrate the lignocellulosic pores facilitating the deconstruction process. When it is used along with water, carbonic acid is formed which accelerates the polymer hydrolysis. After mild treatment, explosive release of

CO₂ due to the pressure drop destroys the architecture of hemicellulose and lignin and exposes enzyme accessible surface area for cellulose hydrolysis. It is shown that significantly higher amounts of reducing sugar (84.7%) are obtained from supercritical CO₂ (165 °C and 21.37 MPa for a residence time of 30 mins) pretreated aspen wood than the untreated biomass (14.5%) in the hydrolysis process (Kim and Hong 2001). In a recent study, supercritical CO₂ (50 °C–80 °C and 17.5–25.0 MPa pressure for 12 h–60 h) has been used for pretreating corn stover and corn cob, which resulted in around three to four-fold higher sugar yield than the untreated feedstocks during the enzymatic hydrolysis (Zhao et al. 2019). Low operating temperature with no inhibitory/toxic product formation and complete removal of CO₂ by depressurization have made this process promising for scale-up purpose. The CO₂ can be readily obtained as a by-product from many industrial processes. However, the low process efficiency for different lignocellulose resources and high-pressure equipment rises the capital cost significantly (Agbor et al. 2011).

2.3.2.4 Liquid Hot Water Pretreatment

Liquid hot water (LHW) pretreatment is an efficient pretreatment method for improving enzymatic hydrolysis in a chemical-free manner for biomass conversion. In this process, liquid water is used as a reaction medium at a high temperature (160 °C–220 °C), and pressure is controlled to keep the water in a liquid state. This method has been shown to be effective for the pretreatment of sugarcane bagasse, corncobs, corn stover, wheat straw, etc. For example, wheat pretreatment using LHW gave rise to glucose and xylose yields of 91% and 80%, respectively (Pérez et al. 2008). In another study, alkali-catalyzed (0.5% aqueous NaOH at 170 °C) LHW pretreated bamboo yielded 30.9 g/100 g reducing sugars during hydrolysis and 9.6 g ethanol/100 g bamboo after fermentation (Yang et al. 2019b). LHW pretreatment with corncobs for 10 min at 160 °C significantly enhanced solubilization of hemicellulose (pentose yield of 58.8%) and lignin removal (60%), resulting in 73.1% glucose recovery in the hydrolysis step (Imman et al. 2018). This is an environment-friendly approach with less or no inhibitory by-product formation due to low process temperature and results in significant sugar recovery from both hemicellulose and cellulose. But, there is high energy and water requirement, which limits the application of the process and it needs further optimization for commercial applications.

2.3.2.5 Irradiation

Irradiation is another approach for lignocellulosic biomass pretreatment. This is generally coupled with other conventional pretreatment processes. Application of electron beam or gamma (γ) rays along with mechanical crushing, thermal or chemical pretreatments has shown enhanced enzymatic hydrolysis in comparison with pretreatment in absence of such irradiation. Pretreatment with an electron beam

decreases the cellulose crystallinity and increases the enzymatic hydrolysis. In the case of ionizing irradiation, long- and short-lived radicals are formed that accelerate the deconstruction of biomass by chain scission and cross-linking (Khan et al. 2006). Hydrothermal pretreatment of sugarcane bagasse along with a 50 kGy electron beam has shown to increase the saccharification yield of glucose by 20% (Duarte et al. 2012). Application of electron beam (7.5 kGy) in mild alkali soaked *Artemisia ordosica* gave an increased yield of reducing sugars as 520.67 mg/g under optimal conditions (Xiang et al. 2017). In a recent study, significantly reduced particle size and low shear rate were achieved using γ -irradiation at 800 kGy along with thermal or dilute acid pretreatment where the sugar yield was found to be around 251 g/L (Liu et al. 2017b). Though electron beam or gamma (γ) rays enhance the enzymatic saccharification, the industrial application of irradiation is limited due to the high cost of installation and environmental concerns. However, these technologies can be used in combination with other methods for designing an optimized pretreatment process.

2.3.2.6 Ultrasonication

Ultrasonic radiation can also be used to deconstruct the complex architecture of lignocellulosic biomass. The basic mechanism of high-frequency ultrasonication in any liquid is the formation of bubbles followed by its sudden collapse at the solid surface, inducing a microjet streaming known as asymmetric cavitation. These cavities enhance the enzymatic accessibility at the substrate surface. At a fixed level of enzyme and substrate concentration, sonication at 40%, 60%, and 80% amplitudes (I_{diss} : 16.2, 32.2, and 43.4 W/cm², respectively) has shown increased enzymatic hydrolysis by 15%, 24%, and 54%, respectively, in comparison to the mechanical agitation alone (Szabo and Csiszar 2017). Ultrasonication can also be used along with other chemical pretreatment agents such as acid-alkali, ionic liquids, etc. For example, ultrasonication in combination with HCl pretreatment resulted in significantly higher delignification in deenanath grass (80.4%) and hybrid napier grass (82.1%) than that of acid pretreatment alone (Mohapatra et al. 2017). Another study shows that ultrasonication pretreatment along with NaOH in rice straw can degrade hemicellulose and lignin without much-affecting cellulose and generated 2.73 g/L of reducing sugars during hydrolysis (Wu et al. 2017). Ultrasonication is also a green approach and proved to be efficient at a laboratory scale. However, the main disadvantage is that it exhibits different efficacy toward a variety of lignocellulose biomass. Thus an optimized cost-effective technology development is still challenging. Moreover, it can be applied in combination with other methods for hybrid pretreatment of lignocellulose biomass.

2.3.3 *Chemical Pretreatment Process*

2.3.3.1 **Ozonolysis**

Ozone can be used as a potential oxidant to pretreat lignocellulosic biomass. It has the ability of delignification along with degradation of hemicellulose which leads to exposing the inner cellulosic core to the hydrolytic enzymes, accelerating the hydrolysis by 4–5 folds. Its powerful oxidant properties allow the breakdown and effective removal of lignin without producing any toxic by-products that may create inhibitory effects in downstream processes. As the process is operated in ambient conditions, no extra cost is required like other pretreatment methods which are carried out at high temperature and pressure. The enzymatic hydrolysis yield was increased from 29% to 88.6% after ozonolysis in wheat straw (García-Cubero et al. 2009). It was recently demonstrated that ozonolysis could effectively remove 20% lignin with 31% sugar yield through enzymatic hydrolysis from water-submerged municipal waste (Rosen et al. 2019). In another work, eucalyptus branches were subjected to ozonolysis with significant delignification (26.63%) and saccharification yield (68%) (Andersen et al. 2019). However, the high cost of ozone along with large requirements has limited its use on an industrial scale.

2.3.3.2 **Acid Hydrolysis**

Acid pretreatment of lignocellulosic biomass has been extensively used in industries. Strong acids like sulfuric, nitric, and hydrochloric acid are employed to remove lignin and hydrolyze the hemicellulose and cellulose for providing greater accessibility to the enzymes. Acid pretreatment can be performed in either concentrated acid at low temperature or a diluted acid at high temperature. The main advantages of this technique are the solubilization of hemicellulose and the precipitation of lignin at the same time. For example, acid pretreatment of coastal bermudagrass with 1.2% H_2SO_4 at 140 °C for 30 min retention time gave rise to a total sugar yield of 94% in the hydrolysis step (Redding et al. 2011). However, during the process, the degradation of monosaccharides leads to the formation of many unwanted inhibitory by-products such as 5-hydroxymethylfurfural (HMF) and 2-furfuraldehyde (Saha 2004). Moreover, high-temperature treatments sometimes produce furfural which gets degraded into formic or levulinic acids. These inhibitory compounds create a severe impact affecting the efficiency of the hydrolytic enzymes. Toxicity, acid recovery, and corrosiveness of the equipment are some of the considerable drawbacks of this approach. Current industrial processes are focused on reducing the inhibitory by-products by using diluted acid treatments.

2.3.3.3 Alkali Hydrolysis

Alkaline pretreatment is one of the common industrial methods for delignification. Alkaline agents like sodium hydroxide (NaOH), ammonium hydroxide (NH₄OH), calcium hydroxide (Ca(OH)₂), potassium hydroxide (KOH), etc. are used for pretreatment of lignocellulosic residues. Alkaline pretreatment induces solvation and saponification. These cause the deformation of complex networks of lignin, decrystallization of cellulose, cellulose swelling, and fractionation of lignin by breaking the ester linkages between lignin and other hemicelluloses in the complex network, thus promoting the solubilization of these components and enhancing the activity of the catalytic enzymes. For example, NaOH pretreatment (4–40 g/100 g dry straw) was found to decrease xylan content up to 46.37% and resulted in 64.55% glucose yield (Wan et al. 2011). Moreover, sodium carbonate (Na₂CO₃) pretreatment was found to yield significant total sugar (71.7%), glucan (76.1%), and xylan (73.2%) from rice straw (Yang et al. 2012). Alkaline pretreatment is very much effective for lignin removal and cellulose decrystallization and thereby increasing the enzyme accessible surface area. The energy requirement is also low due to the low process temperature. However, long residence time makes the neutralization step very difficult before the downstream and increases the overall cost of the process.

2.3.3.4 Organosolv Pretreatment

In this approach, lignocellulosic biomass is heated in the presence of organic liquids and a water mixture (ethanol-water, ethylene glycol, benzene-water, etc.). A significant amount of lignin and some amount of hemicellulose get dissolved in the process while the solid cellulose part is left in the medium. A wide range of organic compounds such as ethanol, methanol, glycerol, tetrahydrofurfuryl alcohol, dimethyl sulfoxide, ethers, ketone, etc. has been used to study the solvent extraction process (Thring et al. 1990). Pretreatment of wheat straw using aqueous glycerol has shown cellulose recovery of 95% along with 70% lignin (Sun and Chen 2007). For a purpose of utilizing whole lignocellulose biomass in biorefinery, ethanol organosolv pretreatment (50% (v/v) ethanol and 1% (w/v) sulfuric acid catalyst) was carried out with simultaneous generation of organosolv lignin (12 g), furfural (7.9 g), glucose (37.1 g), and hemicellulose-derived sugars (11.4 g) (Choi et al. 2019). In a recent study, hybrid Pennisetum (HP) biomass was pretreated with different organosolv (tetrahydrofurfuryl alcohol (THFA), γ -valerolactone (GVL), ethanol, and acetone) under 100 °C for 2 h which resulted in significant-high glucan yield (80.8%), and THFA was reported to be most effective among the organosolv for improved hydrolysis and lignin recovery (Tan et al. 2019). In another work, the use of ethanol-water resulted in 86% glucan and 62% lignin removal in *Eucalyptus nitens* bark (Romaní et al. 2019). This method is effective for the pretreatment of softwood materials with high lignin content and also for efficient fractionation of pure

cellulose, hemicellulose, and lignin. Organic solvents with a low boiling point can be easily recovered and recycled. However, solvents with high boiling points need high-pressure conditions, and the huge cost of the solvents makes the process expensive. The solvents must be well separated before the downstream process or else the growth of the fermentative microbes can be inhibited.

2.3.3.5 Oxidative Lime Pretreatment (OLP)

Lime is an effective oxidative agent for lignocellulose pretreatment for reducing the lignin content and enhancing better cellulose recovery. The efficiency of the delignification of thermal lime pretreatment can be greatly enhanced by introducing oxygen as an oxidant in the process. OLP effectively removes lignin and hemicellulose acetyl content from the biomass which makes the substrates more accessible to hydrolysis. Recent studies have shown that 68% and 98% of total xylan and glucan have been recovered, respectively, when switchgrass is pretreated using OLP at 110 °C and 6.89 bar for 240 min in combination with milling (Falls and Holtzapple 2011). In another study, lime pretreatment of corn stover resulted in 87.5% lignin removal along with a yield of 93.2% glucose and 79.5% xylose during the hydrolysis (Kim and Holtzapple 2005). One major disadvantage of this process is that a huge amount of water is required for washing out the calcium salts. Moreover, the cost of downstream processing also makes it expensive.

2.3.3.6 Ionic Liquid Treatment

The chemical pretreatments discussed above mainly consist of derivatizing solvents which cause chemical modification to the cellulose, reducing cellulose yield in the pure form. In recent years, Ionic Liquids (ILs) are considered a non-derivatizing solvent and have shown huge potential for the pretreatment of lignocellulosic biomass. ILs are eco-friendly and often referred to as “Green Solvents” (Wasserscheid and Keim 2000). The specialty of IL is that cellulose and lignin can be solubilized simultaneously. Ionic liquids are organic salts generally composed of a relatively large cation and a smaller anion, with a melting point of less than 100 °C (Rogers and Seddon 2003). IL has been proved to be a potential pretreatment agent due to its high thermal stability, low vapor pressure, and non-derivatizing solvent properties. Several ILs such as 1-ethyl-3-methylimidazolium-acetate ([EMIM][Ac]), 1-ethyl-3-methylimidazolium diethyl phosphate ([EMIM][DEP]), 1-allyl-3-methylimidazolium-chloride ([AMIM][Cl]), and 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]) are some of the effective ILs to dissolve lignocellulosic biomass (Fig. 2.5) (Zhao et al. 2008). However, [EMIM][Ac] shows one of the best promising cellulose recovery among all the ionic liquids (Sun et al. 2009).

Pretreatment of wheat straw at 120 °C for 6 h using [EMIM][Ac] resulted in near 100% (w/w) sugar recovery (da Costa Lopes et al. 2013). [EMIM][Ac] along with buffer solution is used to successfully remove ~50% lignin from the wood chip

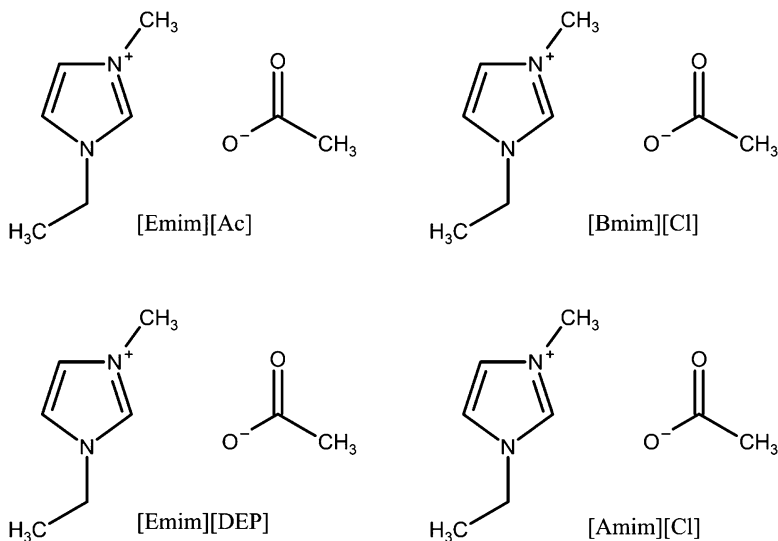


Fig. 2.5 Chemical structures of some imidazolium based ionic liquids are shown. 1-ethyl-3-methylimidazolium-acetate ([EMIM][Ac]), 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]), 1-ethyl-3-methylimidazolium diethyl phosphate ([EMIM][DEP]), and 1-allyl-3-methylimidazolium-chloride ([AMIM][Cl])

biomass (Moniruzzaman and Ono 2012). To study the enzymatic saccharification, IL pretreated eucalyptus was subjected to enzymatic hydrolysis, and a nine-fold rise in cellulose conversion was observed (Uju et al. 2012). A high glucose yield of around 95.2% was found when the sugarcane bagasse was pretreated with IL and regenerated in water followed by enzymatic hydrolysis. Studies have been performed to optimize the process parameters of IL pretreatment which reported that dissolution can be enhanced by 50% using 67% aqueous-[EMIM][Ac] solvent mixture at 150 °C for 90 min, generating ~81% fermentable sugar (Fu and Mazza 2011). Several computational, as well as experimental studies, have been performed to understand the underlying mechanism of the process. The key factors governing the interaction between IL and lignocellulosic biomass are the cationic and anionic properties which vary with different ILs, the temperature, and the time of the pretreatment process. ¹³C high-resolution NMR spectroscopy of cellulose oligomers in [BMIM][Cl] has revealed that the cellulose chains adopt specific conformational changes in IL environment (Moulthrop et al. 2005). Another study using ¹³C and ^{35/37}Cl NMR relaxation in [BMIM][Cl] revealed that the hydroxyl protons of the cellulose chains interact with the Cl⁻ ions during the dissolution (Remsing et al. 2006). High temperature (~450 K similar to the industrial pretreatment temperature) vacuum molecular dynamics simulation study of crystalline I_β cellulose has shown a temperature-dependent behavior of cellulose microcrystal where significant deviation in the crystal unit cell parameters has been found due to the change in the rotamers of the hydroxymethyl group conformation (Bergensträhle et al. 2007). This

change affects the stable hydrogen bonding network in the cellulose which facilitates the formation of hydrogen bonding between the anionic species of the IL and the hydroxyl group of cellulose chains. At the same time, the hydrophobic interaction occurs between the imidazolium ring of the cation and cellulose. These interactions break the stable inter- and intra-hydrogen bonds present within the lignocellulosic biomass, leading to the dissolution of the complex (Wahlström and Suurnäkki 2015). IL pretreatment increases the porosity of the biomass which makes it highly susceptible to enzymatic hydrolysis. Despite having so many attractive features of ILs, the main challenges of IL pretreatment are its high cost, recyclability, and recovery of ILs. To optimize the process, researchers are trying to use different binary mixtures along with IL during the pretreatment. In a recent study, it has been reported that the molecular behavior of [Emim][Ac]/water mixtures and its interaction with cellulose microcrystal reveals that 50–80% [EMIM][Ac] can be effective for both cellulose and lignin dissolution process (Manna et al. 2021; Manna and Ghosh 2019; Shi et al. 2014). Researchers have also used several organic solvents (N, N-dimethylacetamide, N,N-dimethylformamide, dimethyl sulfoxide, and pyridine) as diluents to improve the viscosity of IL for process optimization (Yang et al. 2019a). Progress has been made for a simultaneous pretreatment and hydrolysis process for cost-effective IL pretreatment technology (Husson et al. 2018).

2.4 Current Challenges in the Pretreatment Methods

At a process level, pretreatments are a multi-scale and non-homogeneous system where LCB interacts with the other components of physical and chemical agents. The complex heterogeneous nature of the LCB affects the overall reaction process leading to changes in yield in different pretreatment processes. This complexity has limited many of the traditional physical and chemical pretreatment processes in an experimental stage. Some of the key problems in pretreatment processes are given below:

1. Each pretreatment method has its own limitations. Some limitations of the traditional pretreatment methods are summarized in Table 2.3. However, a proper evaluation method needs to be developed in terms of economic feasibility and productivity to access the pretreatment processes.
2. The understanding of a physical or chemical pretreatment technology on the basis of the reaction mechanism is limited. More research needs to be done on the fundamentals to reveal the influence of particular pretreatment on the structure and digestion of cellulose and hemicellulose. The knowledge can be utilized to develop a suitable reaction model or optimization of the existing technologies.
3. Most of the pretreatment technologies primarily consider the cellulose hydrolysis, rate of hydrolysis, overall glucose yield, and lignin removal for the effectiveness of the process. However, it doesn't provide insight into the physical chemistry perspective in the reaction process. Thus, further, development is necessary.

Table 2.3 Overview of the challenges in some common physical and chemical pretreatment methods

Method	Challenges
Mechanical pretreatments	<ul style="list-style-type: none"> • Very expensive due to high energy input and operating costs • Actual performance varies with the type of LCB • Economically infeasible compared to other pretreatment methods
Wet oxidation	<ul style="list-style-type: none"> • Overall hydrolysis yield is lower than yields obtained in the steam pretreatment
Liquid hot water	<ul style="list-style-type: none"> • Water consumption and energy inputs are very high • Selection of the appropriate temperature is important
Ozonolysis	<ul style="list-style-type: none"> • Ozone generation and supply are crucial • High-operation cost
Steam explosion	<ul style="list-style-type: none"> • Incomplete deconstruction of LCB leads to precipitation of lignin components making it less digestible by enzymes. Also, some amount of xylan degraded and lost • Generation of inhibitors at higher temperatures • Loss of soluble sugars due to extensive washing
Super critical CO ₂	<ul style="list-style-type: none"> • Less effectiveness of pretreatment • Capital cost is very high due to operational conditions with high pressures and energy input for carbon dioxide liquefaction
Acid pretreatment	<ul style="list-style-type: none"> • The amount of water required for washing is very high • Formation of enzyme inhibitors • Corrosiveness of the acid affects the reactor lifespan
IL	<ul style="list-style-type: none"> • High cost of IL • Recycling of the IL needs to be optimized • Inhibitory effects against hydrolytic enzymes
AFEX	<ul style="list-style-type: none"> • High cost of ammonia • High equipment maintenance cost • The corrosive properties affect the reactor
Organosolv	<ul style="list-style-type: none"> • Requirement of high process pressure • Cost of organic solvents and their recycling are high • Controlled environment is required due to explosion hazards and environmental concerns

4. Even if some methods are effective to the laboratory scale, the scale-up becomes more challenging due to the high cost, the requirement of optimal process conditions, and involved environmental concerns.

2.5 Conclusion

Pretreatment is the key step in the deconstruction of LCB for producing any biofuel or value-added chemicals. The effects of the traditional pretreatment methods on the different components of LCB have been discussed in detail. The crystallinity of the cellulose and removal of lignin are crucial for the effective enzymatic hydrolysis and overall sugar yield. Though few of the processes can help significant yield, the scale-

up for industrial application is still a challenge to overcome. The challenge can be addressed in two ways. Either the existing techniques should be improved, or else research should be done on developing new eco-friendly, cost-effective, efficient pretreatment methods. Besides, an investigation needs to be done on LCB components to design efficient separation methods. Moreover, when a single pretreatment technique is used, it raises various technological drawbacks, environmental concerns, high-operation costs, and compromised productivity. Thus, the initiative should be taken to combine multiple pretreatment methods including physical and chemical processes. For example, mechanical crushing–electronic radiation–alkali treatment, mechanical crushing–microwave–chemical processing, microwave-IL treatment, mechanical crushing–chemical treatment–steam explosion, and others. By means of a combined pretreatment method, the deconstruction of lignocellulosic biomass can be significantly improved in terms of accessible surface area to the enzyme, efficient hydrolysis, and production of biofuel and value-added chemicals in a cost-effective manner.

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