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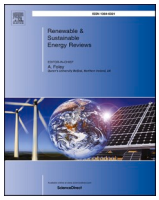

# Efficient sugar production from plant biomass: Current status, challenges, and future directions

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ABSTRACT

Lignocellulosic plant biomass is recognized as an alternative resource for sustainable production of liquid fuels, chemicals, and polymeric materials through the biorefining concept. The sugar platform is one of the promising approaches for commercial conversion of cellulose and/or hemicelluloses in plant biomass into the targeted products biologically and/or chemically through carbohydrate saccharification. However, efficient, and sustainable sugar production from lignocellulose remains a great challenge because of the natural recalcitrance of lignocellulose to deconstruction. This review aims to provide current understanding of the key barriers to lignocellulose saccharification, a critical assessment of the available technologies for sugar production from lignocellulose, including chemical and enzymatic approaches, and potential strategies to overcome the recalcitrance for commercial development. Special attention is paid to lignin-induced inhibition to enzymatic hydrolysis of lignocellulosic substrates and pretreatment/fractionation methods to overcome or decrease biomass recalcitrance. Perspectives and future research needs are also presented.

## 1. Introduction

Plant biomass (lignocellulose) has been well-recognized as a viable alternative feedstock for the production of commodity chemicals, liquid fuels, and synthetic materials through the biorefining concept. The gradual depletion of fossil resources and the increasing adverse environmental impacts, such as climate change contributed by greenhouse gas emission from the production and consumption of fossil-based fuels and products, increase the urgency for developing biorefineries using plant biomass [1]. As one of the most abundant renewable resources on Earth, approximately 200 billion dry metric tons of lignocellulosic biomass such as grasses, crops, and trees are produced annually via photosynthesis. Lignocellulose is not only renewable but also carbon neutral [2]. The utilization of lignocellulose does not emit net carbon dioxide into the atmosphere.

Chemically, lignocellulose is composed of polysaccharides (cellulose and hemicelluloses), polyphenols (lignin), and minor substances (e.g., extractives and inorganics). Depending on species, lignocellulosic biomass contains about 30–50% cellulose, 20–40% hemicelluloses, and 10–35% lignin [3]. Cellulose is a linear polymer of glucose linked by β-1, 4 glycosidic bond; hemicelluloses are branched copolymers of multiple sugars (i.e., xylose, mannose, arabinose, galactose, glucose, and uronic acid); and lignin is an aromatic polymer of three phenylpropane units (coniferyl, sinapyl, and *p*-coumaryl) linked by ether and carbon–carbon bonds.

There are primarily two platform technologies, thermochemical and biochemical or sugar (carbohydrate) platforms, to convert lignocellulose into chemicals, fuels, and materials through biorefineries [4]. In brief, using the thermochemical platform technologies, lignocellulose (both carbohydrate and lignin) is first broken down into small-molecule intermediates (e.g., syngas and biooil) via thermochemical processes (e.g., gasification and pyrolysis), and then the intermediates are upgraded into targeted products (e.g., chemicals and liquid fuels). Using biochemical or sugar platform technologies, however, the polysaccharides (cellulose and hemicelluloses) are hydrolyzed by enzymes or acids into monosaccharides (sugars), and then the sugars are converted into final products biologically or chemically. Lignin as a byproduct of the sugar platform is utilized separately. Therefore, efficient sugar production, i.e., with low cost, low energy input, and high yield, from lignocellulosic feedstocks is a crucial prerequisite to the

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success of the sugar platform technologies.

The sugar platform is attractive because a spectrum of products including chemicals, fuels, and polymeric materials can be successfully produced from sugars (both pentoses and hexoses). For example, furfural, hydroxymethylfurfural, levulinic acid, xylitol, sorbitol, mannitol, and amino acids are representative sugar-derived products, which are commodity chemicals and can also be used as precursors of downstream products [5,6]. Different forms of fuels, such as alcohols (e.g., ethanol and butanol), hydrocarbons, and hydrogen, can be produced from sugars by fermentation and reforming [4,7]. Biopolymers can also be synthesized from sugars biologically and/or chemically [8], such as polyhydroxyalkanoates (PHA) [9], polylactide (PLA) [10,11], polypropylene terephthalate (PPT) [12], and polyglutamic acids (PGA) [13]. Before petroleum-based nylon, nylon had been produced from furfural derived from xylose.

Sugars from sugar crops (e.g., sugarcane and sugar beet) and grain starch have been used for producing chemicals and fuels. For example, there is a long history of producing fuel ethanol from sugar cane sucrose in Brazil and corn starch in the United States. However, sucrose and starch are traditionally considered foods/feeds. Converting foods/feeds into fuels or chemicals is the center of the ethical debate of fuel vs food. Furthermore, producing sugar cane and corn needs more water, fertilizers, pesticides, and energy than producing lignocellulosic biomass. Therefore, future sugar production should use inedible lignocellulosic biomass, including naturally grown plants and dedicated energy crops for sustainable and economic biorefinery.

Producing sugars from lignocellulosic feedstocks (specifically, hydrolyzing cellulose in lignocellulose to glucose) is challenging with both technical and economic barriers.

There have been a number of good reviews on the subject. Some provided a good summary of the understanding of key barriers at the time [14–16]. Some addressed different pretreatment processes and their advantages and pitfalls [17–19]. Some focused on biorefinery process integration, energy efficiency and tech-economic analyses [3,20–22]. The present review has four unique perspectives: (1) Provides sufficient literature data and points out that cellulose accessibility is the key barrier to avoid confusion and misunderstanding in the existing literature. Specifically, it points out that removing hemicelluloses is much more effective and economically feasible than delignification for improving saccharification of plant biomass. (2) Provides a historical perspective, specifically, a brief overview of the origin of traditional (nonenzymatic) concentrated and dilute acid hydrolysis sugar production processes, followed by the introduction of recent studies on chemical saccharification of lignocelluloses. (3) Provides a brief history of the discovery of enzymatic saccharification of cellulose, followed by the introduction of nonproductive cellulase binding to lignin and provides a practical solution that was not discussed in previous reviews. (4) Focuses on recent studies on fractionation with consideration of producing reactive lignin for lignin valorization. This issue has not been emphasized in previous reviews, but it is critically important to biorefinery.

## 2. Anatomic and chemical structure of plant biomass

### 2.1. Anatomic structure and chemical composition of lignocellulosic biomass

Plant-biomass has a hierarchical structure in the radial direction. Using wood as an example, Moon et al. provided a pictorial description of the wood structure [23]. Wood matrix is constructed by vertically arranged cellular components (e.g., tracheids (fibers), vessels) with a small amount of horizontally oriented ray parenchyma. The cells are connected (bonded) at the middle lamella (ML), and the fiber cell wall consists of primary (P) and secondary (S1, S2, and S3) layers, as shown in Fig. 1A. Not only the thickness but also the chemical composition varies across the layers, as shown in Fig. 1B [24]. The combined ML and P are very thin but have a very high lignin concentration of approximately 70% with only a small amount of cellulose and hemicelluloses of approximately 15% each. The S2 layer has the greatest thickness and therefore counts for the largest portion of total cell wall mass (volume), also contains most cellulose, hemicelluloses, and lignin. However, lignin

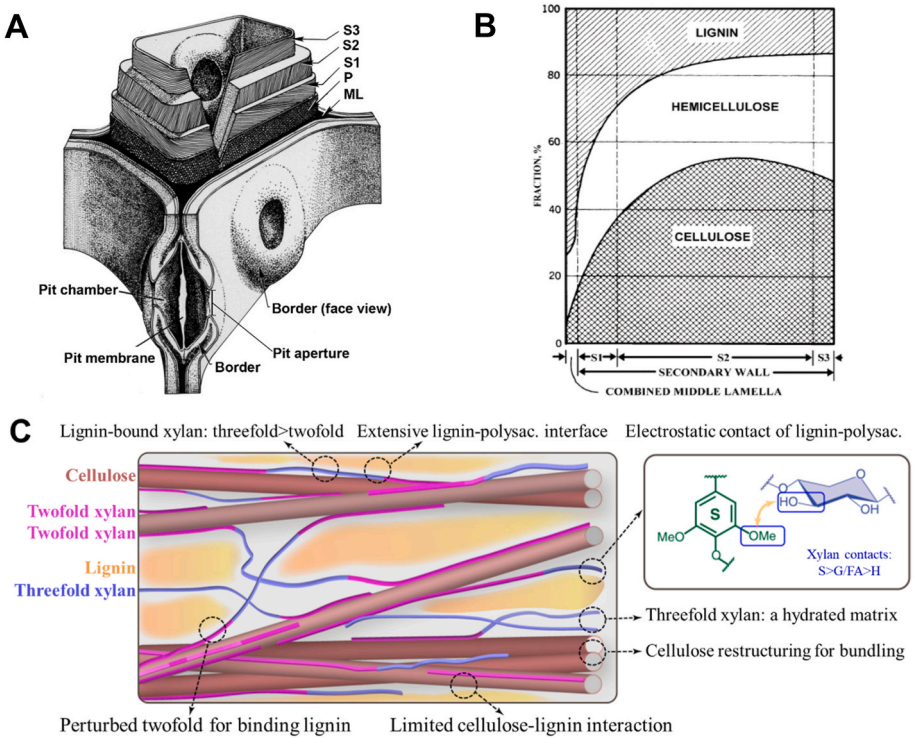



**Fig. 1.** Schematic diagram showing a wood cell structure (A) (illustrated by The USDA Forest Products Lab staff), component spatial distribution within a wood cell wall (B) ©McGraw-Hill [24], and lignin-polysaccharide packing in the secondary cell wall architecture (C) [25]. Permission to use all arts was granted.

has a lower concentration in S than in ML.

Typical chemical compositions of different biomass species are summarized in Table 1. In general, herbage has lower lignin but higher hemicelluloses and ash contents than wood. Softwoods have higher lignin contents than hardwoods have. Acetyl groups are linked to hemicelluloses, which can be easily released and play an important role in some pretreatments, as will be discussed later. The high ash content of herbage can present problems for pretreatment and chemical pulping, particularly using alkaline processes.

Cellulose coexists with hemicelluloses and lignin in plant cell walls as discussed above and shown in Fig. 1B. Within the matrix of a wood cell wall, it is generally understood that lignin is not chemically bonded to cellulose, rather to hemicelluloses to form the so-called lignin carbohydrate complexes (LCCs). A recent study further illustrated that lignin and xylan tend to form their separate phases in the secondary cell wall with limited interpenetration and chemical bonding or formation of LCCs, as shown in Fig. 1C [25]. Furthermore, xylan has two domains with the twofold xylan being coated on cellulose fibril surfaces and the threefold xylan crosslinking the cellulose fibrils [25,26]. The cellulose fibril matrices consist of elementary fibrils that were formed by 18–36 cellulose chains depending on the plant biomass [27–29]. A recent study indicated that cellulose in the secondary walls of cotton and poplar wood are highly bundled into large macrofibrils, which also limits cellulose accessibility to chemicals and enzymes [30].

Lignin plays critical biological and structural roles in plants. First, lignin protects the plant from biological and environmental degradations as it possesses anti-microbial, anti-UV, and antioxidant properties. Second, the hydrophobicity of lignin prevents water permeation through cell walls, which ensures the efficient transport of nutrients and photosynthesis products through the channel network consisting of lumens and pits. Third, as a natural binder, lignin binds fibers together to form a strong and tough wood matrix. All three roles that lignin plays contribute to the overall recalcitrance of lignocellulosic biomass to chemical and enzymatic cellulose hydrolysis.

From the plant cell wall structure (Fig. 1A), component distributions (Fig. 1B), and the functions of lignin, it is intuitive that efficient access and hydrolysis of cellulose by cellulases require the removal of lignin and/or hemicelluloses, at least partially. During biomass pretreatment, chemicals can enter an open lumen to access cell wall components from the S3 layer side to react with lignin and/or hemicelluloses. The chemicals can penetrate the cell wall to reach the middle lamella and be transferred into adjacent fiber lumens via the pits on the cell wall. Understanding of the anatomic structure of plant biomass, the location and distribution of cell wall components, and the chemical transfer into cell walls is important for developing effective pretreatment processes for sugar production from plant-biomass by enzymes or acids.

### 2.2. Chemistry of lignocellulose

The three major components in the plant cell wall are cellulose, hemicelluloses, and lignin. Their structure, building units, and interunit linkages are summarized in Table 2. Cellulose is a homogeneous polymer of glucose linked by β-1,4 glycosidic bond (Table 2). The number of anhydrous glucose units or the degree of polymerization (*DP*) is approximately 15,000 and 10,000 in cotton and wood cellulose, respectively [31]. Extensive inter and intramolecular hydrogen bonds are formed with and between cellulose chains via the hydroxyl groups at C2, C3, and C6 positions of the glucose units. Cellulose is an amphiphilic molecule. The three hydroxyls at the equatorial positions of each anhydrous glucose unit make cellulose hydrophilic in the equatorial direction, while the axial C–H bonds make cellulose hydrophobic in this direction [32,33]. Cellulose chains are stacked into a highly organized crystalline matrix via both the extensive hydrogen bonds and hydrophobic interaction [34,35]. Which contribute to the rigid crystalline structures of cellulose.

**Table 1**
Representative chemical compositions of lignocellulosic biomass [3,14,31].

| Biomass | Cellulose, % | Hemicelluloses, % | Lignin, % | Acetyl, % | Ash, % |
|---|---|---|---|---|---|
| Herbage | 30–45 | 20–40 | 10–20 | 3–6 | 5–18 |
| Hardwood | 35–50 | 20–30 | 18–25 | 2–5 | <1 |
| Softwood | 35–45 | 20–30 | 25–35 | 1–2 | <1 |

Hemicelluloses are a group of heterogeneous polysaccharides composed of multiple sugar units and acetyl groups (Table 2). The compositional units and structures of hemicelluloses vary greatly among plant species [36]. In angiosperms such as grasses and hardwood trees, *O*-acetyl-4-*O*-methylglucurono-D-xylan (sometimes called glucuronoxylan or simply xylan) is the principal hemicellulose, in which the β-1,4 linked D-xylopyranose units form the backbone with the branches of 4-*O*-methyl-D-glucopyranosyluronic acid α-1,2 linked to xylose units and acetyl group linked to xylose units at C2 or C3. Hardwood also contains a small amount (2–5%) of glucomannan, composed of D-glucopyranose and D-mannopyranose units at a ratio of 1:2 to 1:1 linked by β-1,4 glycosidic bond. Hemicelluloses in softwood (gymnosperms) are predominately galactoglucomannans that have the backbone of β-1,4 linked D-glucopyranose and D-mannopyranose units with the side chains of α-1,6 linked D-galactopyranose unit and acetyl group attached to the backbone at C2 or C3 of the D-glucopyranose and D-mannopyranose unit [31]. The ratio of galactose:glucose:mannose varies from 0.1:1:4 to 1:1:3. Softwoods also contain a small amount (5–10%) of arabinoglucuronoxylan, which has the backbone of the β-1,4 linked D-xylopyranose units with the branches of 4-*O*-methyl-D-glucopyranosyluronic acid and L-arobionfuranose linked to the backbone by α-1,2 and α-1,3 glycosidic bonds, respectively. The acetyl groups on hemicelluloses are easily cleaved under the hydrothermal condition with or without alkali or acid, which can be important to biomass processing. For example, in autohydrolysis such as hot water or steam explosion pretreatment of biomass, the cleavage of acetyl groups releases acetic acid to catalyze the hydrolysis of hemicelluloses. The side chains in hemicelluloses are generally substantial, which prevent hemicelluloses from the formation of crystalline structures via intermolecular hydrogen bonds as cellulose. Hemicelluloses function as a "glue" binding the microfibrils together via hydrogen bonding with cellulose.

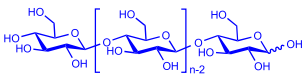
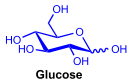
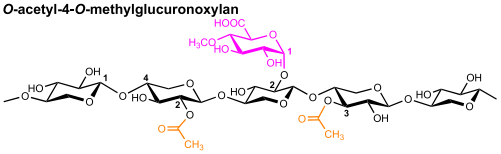
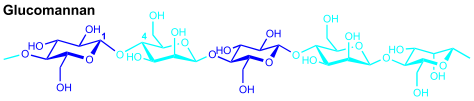
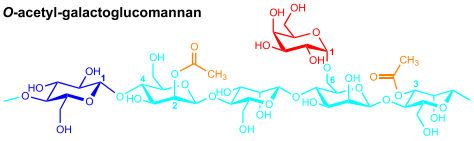
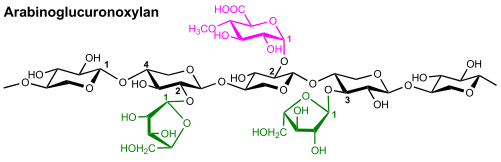
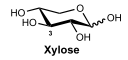
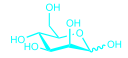
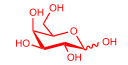
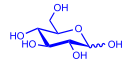
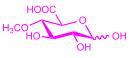
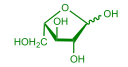
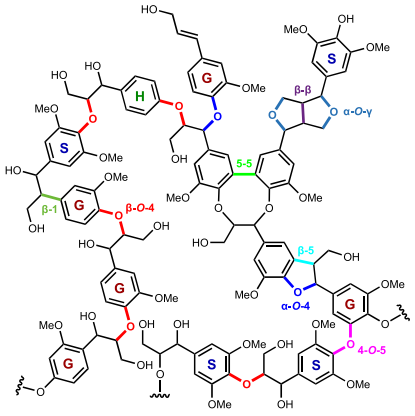
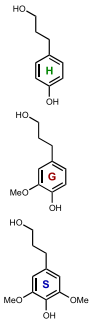
Lignin is biosynthesized from three primary precursors (*p*-coumaryl (**H**), coniferyl (**G**), and sinapyl (**S**) alcohols) by radical coupling. Since the radical coupling occurs among the monomers at multiple chiral sites, the resultant lignin has a very diverse and complex structure. The occurrence of the aromatic units in lignin varies greatly among different biomass species. Softwood lignin is comprised primarily of **G** units, while hardwood lignin contains both **G** and **S** units. The presence of **H** (4–15%) together with **G** and **S** units is a characteristic of grass lignin [37]. A small amount of other aromatic units, such as flavonoid and tricin, are also found in grass lignin [38]. The building units are primarily linked by C–*O*–C ether bonds and C–C bonds. The arylglycerol-β-aryl ether (β–*O*–4) linkage is the most abundant (50–80%) in native lignin. Other ether bonds in lignin include arylglycerol-α-aryl ether (α–*O*–4) linkage, diaryl ether (5–*O*–4) linkage, and resinol ether (α–*O*–γ) linkage. The primary C–C linkages in lignin are phenylcoumaran (β–5), biphenyl (5–5), 1,2-diarylpropane (β–1), and resinol (β–β) [39].

## 3. Chemical (acidic) sugar production

### 3.1. Reactions of cellulose, hemicelluloses, and lignin in acid-catalyzed processes

Acid-catalyzed hydrolysis is an important approach to produce sugars (monosaccharides and oligosaccharides) from polysaccharides (such as cellulose and hemicelluloses). When lignocellulosic biomass is treated under hydrothermal conditions in the presence of an acid, the glycosidic bonds linking the building sugar units in the polysaccharides

**Table 2**
Structure, building units, and interunit linkages of cellulose, hemicellulose, and lignin.

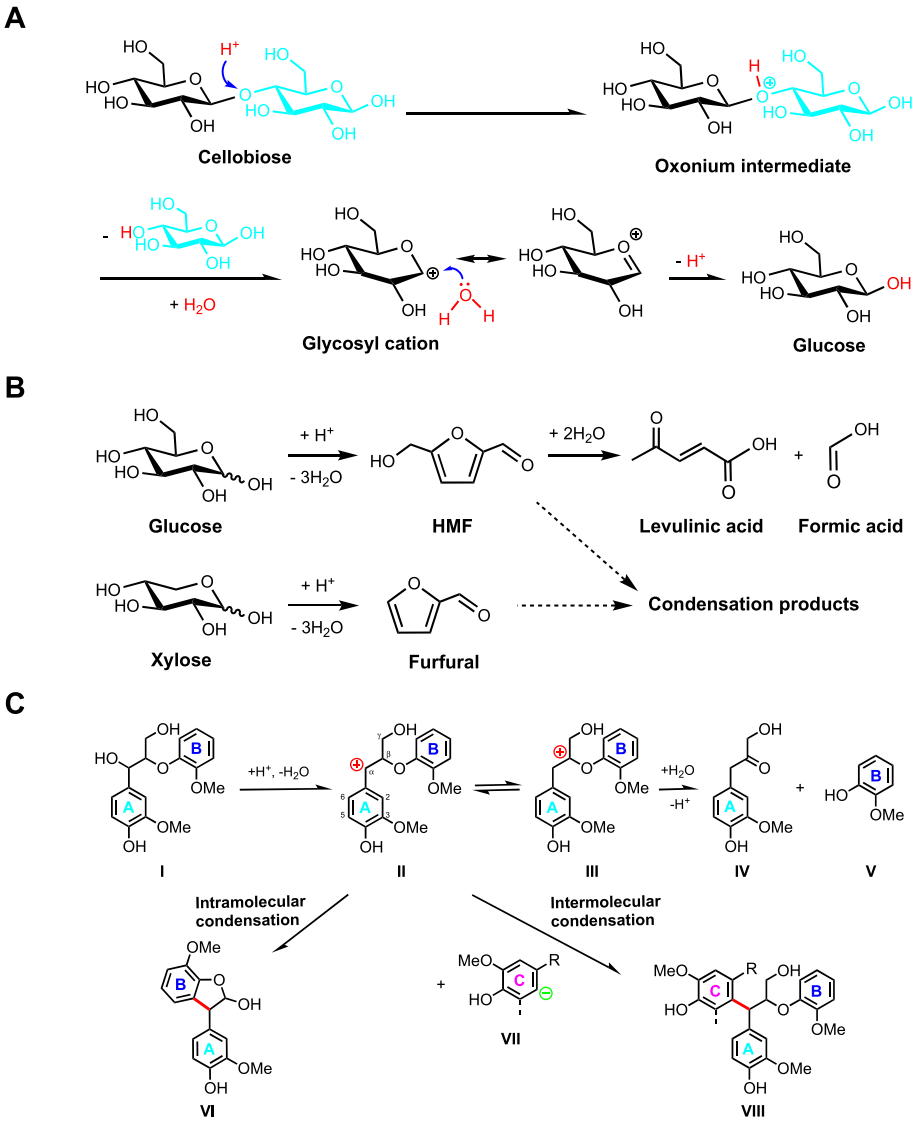
| Component | Structure | Building unit | Interunit linkage |
|---|---|---|---|
| Cellulose |  |  Glucose | β-(1, 4) glycosidic bond |
| Hemicelluloses[a] | *O*-acetyl-4-*O*-methylglucuronoxylan  Glucomannan  *O*-acetyl-galactoglucomannan  Arabinoglucuronoxylan  |  Xylose  Mannose  Galactose  Glucose  4-*O*-methylglucuronic acid  Arabinose  Acetyl | **Backbone:** β-(1, 4) glycosidic bond **Side chains:** α-(1, 2), α-(1, 3), α-(1, 6) glycosidic bonds **Acetyl:** Ester bond |
| Lignin[a] |  |  | **Ether bonds (C-O-C):** β-*O*-4, α-*O*-4, α-*O*-γ, 4-*O*-5 **Carbon-carbon bonds (C-C):** 5-5, β-1, β-5, β-β |

are cleaved to release sugars. The resultant monosaccharides can be further degraded (dehydrated) at high temperatures. Meanwhile, lignin experiences acid-induced depolymerization (cleavage of β–*O*–4 bond) and condensation (repolymerization).

Using cellobiose as an example, the acid-catalyzed cleavage of the glycosidic bond by hydrolysis is elucidated in Scheme 1A [40]. The protonation of glycosidic oxygen atom leads to an oxonium intermediate (conjugate acid). Then, the decomposition of the oxonium results in a glycosyl cation and a molecule of glucose. For most O-glycosidic bonds, the decomposition takes place only in one direction, i.e., splitting at the glycosidic center-oxygen bond rather than at the oxygen-aglycon bond. This is because the glycosyl cation from the former is more stable than the aglycon cation from the latter, as the cycle oxygen helps delocalize the charge of the glycosyl cation. The subsequent attack on the glycosyl cation by water leads to the formation of a new anomeric hydroxyl. Accordingly, complete hydrolysis of cellulose gives glucose as the only product. An excellent summary on the chemistry and mechanism of lignocellulosic biomass acid hydrolysis can be found in a recent review [41].

When hemicelluloses are exposed to acid under hydrothermal conditions, their acetyl groups are easily cleaved, releasing acetic acid. The branched sugar units (such as glucuronic acid, galactose, and arabinose) of hemicelluloses are usually cleaved before the backbone because the glycosidic bonds linking the branches to the backbones are easier to be cleaved than those in the backbones [31]. The hydrolysis of hemicelluloses produces a mixture of compositional sugars (i.e., xylose, mannose, galactose, glucose, and arabinose).

The sugars can undergo dehydration under acidic hydrothermal conditions, leading to decomposition products, as shown in Scheme 1B. For example, hexose dehydration gives hydroxymethylfurfural (HMF), and pentose dehydration forms furfural. HMF can be further decomposed by rehydration to produce levulinic acid and formic acid. These




**Scheme 1.** (A) Acid-catalyzed cleavage of glycosidic bond; (B) Acid-catalyzed sugar dehydration and degradation; and (C) Cleavage of β–*O*–4 linkage and condensation of lignin under acidic conditions.

sugar degradation products are important platform chemicals and have many applications in fine chemicals, pharmaceutics, solvents, fuels, and polymers. However, they are identified as inhibitors to many microorganisms for sugar fermentation, and the detoxification to remove the inhibitors can be expensive. In addition, HMF and furfural can undergo condensation at high acidity and temperature, leading to humin-like complexes.

Under acidic conditions, β–*O*–4 linkages in lignin can be cleaved, but this is usually not extensive enough to cause significant lignin depolymerization. Meanwhile, acid promotes lignin repolymerization (condensation). As a result, acidic processes usually generate highly condensed lignin. The cleavage of β–*O*–4 linkage is shown in Scheme 1C using a lignin model compound (I). The reaction is initiated by the protonation of benzyl alcohol followed by dehydration, leading to α–carbocation intermediate (II). The α–carbocation is transferred to β–carbocation (III), and the hydrolysis of the latter leads to the cleavage of β–*O*–4 linkage, generating Hibbert's ketone (IV) and guaiacol moiety (V) [42]. The reactive α–carbocation intermediate can also lead to condensation. As shown in Scheme 1C, electrophilic aromatic substitution at electron-rich C5 position by the α–carbocation intermediate leads to intramolecular condensation product (VI). Alternatively, the α–carbocation intermediate can attack the electron-rich carbon on the benzene ring, e.g., C6 of a lignin unit (VII), resulting in an intermolecular condensation product (VIII).

### 3.2. Concentrated acid hydrolysis

Concentrated acid hydrolysis of lignocellulose for sugar production started in the 1880s [43]. Significant development took place in the 1930s–1940s including the development of Hokkaido and Peoria processes. The Hokkaido process has three stages: a prehydrolysis stage using steam at 180–185 °C followed by impregnation using concentrated sulfuric acid at 80% at 10 °C or higher, then dilute the acid to hydrolyze the lignocellulosic solids at 100 °C [44,45]. The Peoria process was developed by the USDA Agricultural Research Service at the Peoria center [46]. The process involves at least two stages, i.e., a prehydrolysis stage to hydrolyze xylan to xylose using dilute sulfuric acid, for example at 1.9% and 121 °C for 50 min with a typical solid to acid solution ratio of 3:10, to achieve a very high xylose yield of over 90% theoretical, followed by a concentrated acid hydrolysis stage at low temperatures to achieve glucose yield of approximately 90% theoretical. Several variations of the Peoria process were evaluated by Purdue University [47], University of California at Berkeley, etc. Extensive studies were carried out by the Tennessee Valley Authority (TVA) from process scale-up, acid

recovery, to tech-economic analysis [48,49]. Acid recovery was achieved by recycling the dilute sulfuric acid but has difficulties in concentrating the dilute acid for reuse in the process.

Later, Clausen and Gaddy patented a single-stage concentrated acid hydrolysis process and achieved over 95% carbohydrate solubilization using an acid concentration of 70% at a temperature over 30 °C for 40 min [44]. A shorter reaction time should be used at higher temperatures, for example, 10 min at 60 °C, to avoid sugar degradation. To increase monomeric sugar yield, a dilution step was applied at 10–20 min to dilute the acid to approximately 30% to improve hydrolysis at 70–100 °C. Separation of the dissolved sugars in a sulfuric acid solution of high concentrations of approximately 30% has been difficult. Farone and Cuzens later patented a resin separation technology in the 1990s [50,51], the so-called Arkenol process, to achieve effective sugar and acid separation and recovery. Despite renewed interest in concentrated acid hydrolysis for sugar production, for example, BlueFire Renewables was established in 2010 to deploy the Arkenol technology with support from the U.S. Department of Energy loan guarantee from Export Import Bank of China [52] to build a plant in Fulton, Mississippi. No commercial success was achieved because of the difficulties in achieving economic acid recovery, excess equipment corrosion, and complicated process technology [53]. Nevertheless, even recently there are a few published research articles on using concentrated sulfuric acid hydrolysis partly because of very high sugar yield with minimal sugar degradation [54,55]. A recent study found that adding L-Cysteine at 10% into a concentrated sulfuric acid solution of 72 wt% significantly enhanced the dissolution of lignin and carbohydrates even at 60 °C [56]. These interesting results deserve exploration for biomass fractionation and sugar production.

### 3.3. Dilute acid hydrolysis – Madison wood sugar process

Chemical sugar production from wood using dilute acid hydrolysis was developed as early as the 1930s, such as the well-known Scholler process [57]. This batch process requires a longer retention time of over 10 h and therefore results in inhibitory products to fermentation. The Madison wood sugar process was developed at the USDA Forest Products Laboratory (FPL), Madison, Wisconsin, to address the problems associated with the Scholler process by continuously flowing dilute sulfuric acid of approximately 0.5% at 150–185 °C to strip carbohydrates from wood in the form of sugars [58]. The process can produce a sugar yield of approximately 45–50% theoretical at a titer of approximately 5% using a total sulfuric acid charge on the wood of approximately 7% [58,59]. This was a pretty efficient process at the time and versatile to all wood species [59]. The process was further improved by TVA in large-scale trial runs with efficient percolation reactors [45,60]. A comparative economic analysis of the Madison wood sugar process and the Peoria process using concentrated sulfuric acid was also conducted by TVA [48]. However, economic commercial production was not achieved using dilute acid hydrolysis due to low sugar yield.

Studies continued using two-stage dilute acid hydrolysis at the USDA FPL until the 1970s. However, the work was more focused on reaction kinetics with limited performance improvement in terms of yield [61]. Although the FPL stopped working on acid hydrolysis for wood sugar production, the U.S. National Renewable Energy Laboratory (NREL) picked up where FPL left off with a focus on enzymatic hydrolysis after first-stage acid hydrolysis.

### 3.4. Direct saccharification of lignocellulose

Recently, Pan and Shuai patented a direct saccharification process of lignocellulosic biomass [62]. Different species of biomass (herbage, hardwood, and softwood) can be directly saccharified into sugars in a solvent of inorganic molten salt hydrate (IMSH), also called inorganic ionic liquid, without extensive size reduction, pretreatment, and enzymes. IMSH is a concentrated aqueous solution of specific inorganic salts (such as LiBr and $CaBr_2$) with a molar ratio of water to salt close to the coordination number of the salt cation, which is usually a low-viscosity solution at room or moderate temperature. Cellulose and lignocellulose can be hydrolyzed homogenously and therefore quickly in IMSHs because it can dissolve cellulose [63]. For example, poplar wood can be quickly and completely hydrolyzed in $LiBr{\cdot}3H_2O$ (61 wt% LiBr solution in water) under moderate conditions (e.g., 40 mM HCl as a catalyst at 110 °C) with sugar yield over 95% theoretical. Because IMSH enhances acidity compared with an aqueous acid solution at the same concentration, which allow reaction to be carried out at decreased acid loading at low temperatures to avoid undesired side reactions (such as sugar degradation to furans and lignin condensation). In addition, IMSH has a high boiling point and does not build much pressure at elevated temperatures, so no high-pressure reactor is needed for biomass processing. Moreover, IMSH usually has a low viscosity, which allows a high biomass loading up to 40–50% (w/v) to ensure a very high sugar titer in the hydrolysate without causing difficulties in mixing.

Pan's group demonstrated other advantages of the direct saccharification process using the IMSH. For example, lignin can be quantitatively recovered after cellulose and hemicelluloses are hydrolyzed [64]. Besides, the IMSH can cleave most lignin ether bonds [65] and produce an extensively depolymerized lignin (MW 2000–4000) with minimal condensation [66]. In addition, the lignin has a high purity (carbohydrate-free) and is soluble in many common solvents such as acetone, acetic acid, and dioxane. The favorable lignin structures and properties facilitate downstream valorization.

The biggest challenge of the IMSH-based direct saccharification is the separation of the salt from sugars. Recycling the salt is critical to the economic feasibility of the process, and residual salt in the sugar stream could inhibit the subsequent fermentation when salt concentration is high. Many technologies for the salt-sugar separation are available and have been evaluated, such as selective crystallization, precipitation, liquid-liquid extraction, ion exchange, and size-exclusion chromatography [62], but most of them are less efficient or too expensive for the separation of high-concentration salt-sugar mixture. For large-scale operations, simulated moving bed chromatography seems a promising and efficient technology for the sugar-salt separation [67,68]. In addition, because LiBr is not an inexpensive and largely abundant salt, alternative salts such as $CaBr_2$ should be explored for the scale-up of the IMSH-based saccharification.

Ionic liquid has also been used as a solvent for direct hydrolysis of lignocellulose. It was reported that a combination of peracetic acid (PAA) pretreatment with ionic liquid-HCl hydrolysis could efficiently saccharify lignocellulose [69]. Biomass was first pretreated with PAA at 80 °C for 3 h, dissolved in an ionic liquid (1-buthyl-3-methylimidazolium chloride or 1-buthyl-3-methylpyridium chloride) at 120 °C for 30 min, and then hydrolyzed by adding HCl, which led to a 70–80% cellulose conversion of bagasse. In another study [70], cellulose and corn stover were directly hydrolyzed in a chloride ionic liquid (1-ethyl-3--methylimidazolium chloride) containing acid catalyst (sulfuric or hydrochloric acid) with a glucose and xylose yield of 90% and 70–80%, respectively. The ionic liquid could be recovered by ion-exclusion chromatography, and the sugar was fermented to ethanol.

## 4. Enzymatic sugar production

Specific enzymes (cellulases) can hydrolyze cellulose to glucose. The chemistry of enzymatic hydrolysis of cellulose is the same as that of acidic hydrolysis of cellulose shown in Scheme 1A. The difference is that the carboxyl groups on the amino acids of cellulases provide the proton ($H^+$) required for cleaving the glycosidic bond of cellulose.

Enzymatic hydrolysis of cellulose was developed from a basic research program by the U.S. Army during World War II to understand the causes of degradation of military clothing in the South Pacific jungles [71,72]. *Trichoderma reesei* QM9414 was identified from this program and used to produce efficient cellulases. The development of

extracellular enzymes [73], genetic enhancement [74,75], identification of complex enzyme systems [76], and the discovery of thermophilic enzymes [77] have made commercial enzymatic sugar production from lignocelluloses promising [78].

Enzymatic sugar production from lignocellulosic substrates is a heterogeneous biochemical process with enzymes as catalysts for carbohydrate hydrolysis and is controlled by three limiting factors [79]: (1) The effective contact between the solid substrates and the catalysts in solution, i.e., the carbohydrates in the substrates and the enzymes in water—Therefore, the accessibility of carbohydrates, more specifically cellulose to cellulases, is critically important as cellulose is highly crystalline and inherently embedded in cell walls with surrounding hemicelluloses and lignin. (2) The activity of cellulases and the reactivity of the substrates, which refers to lignocellulosic substrate properties such as cellulose DP, crystallinity, and chemical composition (hemicelluloses and lignin) [15,79–81]—For a given cellulase, its performance is often reduced by the nonproductive binding to substrate lignin, depending on lignin physicochemical properties [82–84]. (3) The reaction conditions such as temperature and pH, which affect not only hydrolysis kinetics but also the rheological properties of reacting mixture and the activity of cellulases [82–84]—For example, the application of thermophilic enzymes can enhance the hydrolysis reaction rate and improve the rheological properties of the aqueous lignocellulosic systems. Based on these understandings, the key barriers to enzymatic sugar production and effective approaches to overcome these barriers will be discussed in this section.

### 4.1. Barriers to enzymatic sugar production

Nature produces plant biomass such as trees and agricultural residues as structural materials that have strong resistance to microbial deconstruction. At the macro level, the strong and tough biomass matrix prevents the enzymes from easy penetration and access to cellulose. At the micro-level, cellulose fibrils are coated and crosslinked by hemicelluloses and further protected by lignin in plant biomass, as shown in Fig. 1C. Lignin, a hydrophobic material, functions as a glue to prevent water/chemicals/fungi access to cellulose fibers. At the molecular level, cellulose chains are ordered and tightly hydrogen-bonded with limited access to water, chemicals, and enzymes [30]. All these factors contribute to barriers or recalcitrance of lignocellulosic substrates to enzymatic hydrolysis for sugar production.

***Cellulase activity and hydrolysis conditions:*** Cellulase activities are dictated by two factors: (1) The enzyme itself and the synergistic effect resulting from formulation using multiple complex enzymes—There are many excellent research reports and reviews on cellulase systems, for example, fungal cellulase [85], noncomplex cellulase systems [78,85], cellulosomes [86–88], thermophilic enzymes [89,90], and the recently developed Lytic Polysaccharide MonoOxygenases (LPMOs) [91–94]. Interested readers can refer to the literature on the subject. (2) The interactions between cellulase and substrates, for example, the nonproductive binding to substrate lignin [95]—There are also studies on understanding the factors affecting mass transfer between enzymes and substrates, such as the effects of cell wall nanoscale structure [96], enzyme molecule traffic jamming [97], and mechanism of cellulosomes [87] on enzymatic hydrolysis. This understanding can help to design better enzyme systems through improved formulation. Here, the discussion will be focused on nonproductive cellulase binding as it has been identified as a major cause of cellulase activity loss in processing lignocelluloses.

The enzymatic hydrolysis reaction conditions are optimized by the enzyme producers and are inherently dictated by enzymes themselves. For example, common fungal cellulase performs best at 50 °C while thermophilic enzymes are operated at 70 °C or higher. Typical fungal cellulase, such as *Trichoderma reesei* family, has the highest activity on **pure** cellulose substrates at approximately pH 4.8, i.e., near their isoelectric point (*pI*), as identified by commercial enzyme manufacturers. However, nonproductive cellulase binding can substantially affect cellulase activity for enzymatic cellulose saccharification of lignocellulosic substrates. pH mediation, i.e., using elevated pH > *pI* of enzymes in conducting enzymatic hydrolysis, can enhance electrostatic repulsion between cellulases and lignin as identified by Zhu's lab and has proven to be the most viable practical solution to substantially reduce nonproductive cellulase binding to substrate lignin to achieve great saccharification of cellulose [82,84,98], which will be discussed in detail later.

***Accessible surface area of substrate:*** The prerequisite of enzymatic hydrolysis is that cellulases must access and get attached to the cellulosic substrate first. It is apparent that the internal and external surface areas of the substrate are critical to cellulose accessibility to enzymes. This postulation is widely accepted [99–101], but for a long time, was only indirectly inferred from experimental data with multiple contributing factors to enzymatic hydrolysis [100–104].

Zhu's lab at the USDA Forest Products Laboratory demonstrated that the phenomenon of fiber hornification can be used to quantify the contribution of fiber external and pore surface area to cellulose accessibility while isolating contributions from other factors. They employed drying and wet pressing induced fiber hornification at varied degrees to isolate other factors which also affect enzymatic saccharification [105–107], such as chemical composition, degree of polymerization, and crystallinity.

Fiber hornification refers to irreversible loss of water-binding ability upon drying [108] as a result of the collapse of pores due to hydrogen bonding [109–111], which decreases internal surface areas and therefore limits cellulose accessibility to enzymes. Fiber hornification can be rigorously characterized by the percentage reduction in fiber saturation point (FSP) [112]. Due to time-consuming procedures involved in determining FSP such as using the solute exclusion technique, Zhu's lab defined the degree of hornification (DH) as the percentage reduction in water retention value (WRV) to measure the extent of hornification, as expressed by [106].

$$DH = \frac{WRV_{ND} - WRV}{WRV_{ND} - WRV_{CD}} \quad (1)$$

where WRV is a measure of water holding capacity in the pores excluding the water in the gross capillary such as lumens of a fiber sample [108]. It is determined by the difference between the wet weight and the oven-dry weight of a fiber sample divided by the oven-dry weight of the sample. The wet weight of the fiber sample is measured after the wet sample is centrifuged to exclude the free water, not in the pores such as film water on the fiber external surfaces [113,114]. Subscripts ND and CD denote never dried and completely dried, respectively.

The study by Zhu's lab showed (Fig. 2A) that substrate enzymatic digestibility (SED), defined as the percentage of substrate glucan enzymatically saccharified into glucose, of three lignocellulosic substrates, i.e., lodgepole pine (PLPS) and aspen (PASS) pretreated by SPORL process (Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose) [115], along with a bleached eucalyptus kraft pulp (BEP) sample, all decreased with the increase in the extent of drying [105]. Each set of samples was produced by air drying (AD), and/or heated drying at a low temperature of 105 °C (LHD) and a high temperature of 150 °C (HHD), for various times [105]. They also verified that DHs of a set of differently dried PLPS substrates correlated well to the cellulose accessibility to cellulase (CAC) measured by two different techniques (Fig. 2B) [106], i.e., solute exclusion [112,116] and protein absorption [117]. It is interesting to note that SED is only dependent on DH, not the drying method (Fig. 2A). Furthermore, for the three never-dried substrates, PLPS, PASS, and BEP evaluated, their SEDs were decreased from 54%, 85%, and 100% to 6%, 17%, and 26%, respectively, or by 89%, 80%, and 74%, respectively, when they are nearly completely dried with DH approaching 1. A similar phenomenon of decreasing SEDs due to

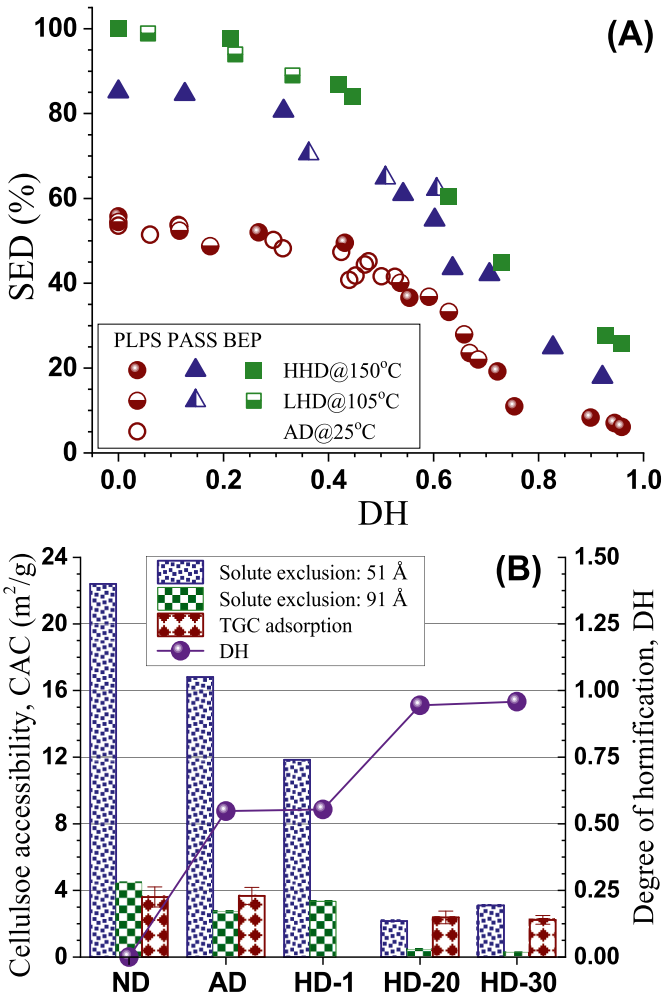



**Fig. 2.** Using drying induced fiber hornification to purposely collapse fiber pores through hydrogen bonding to validate that cellulose accessibility to cellulase (CAC) is the key barrier to enzymatic hydrolysis of plant-biomass cellulose. (A) Substrate enzymatic digestibilities (SEDs) of differently dried SPORL pretreated lodgepole pine (PLPS) and aspen (PASS), and bleached eucalyptus kraft pulp (BEP) fibers are all decreased with the increase in the degree of fiber hornification (DH), adapted from Ref. [105]; (B) DH is very well correlated with various measures of CAC by the solute exclusion of 90 Å and 51 Å, thioredoxin-green fluorescent protein-CBM3 (TGC) adsorption, of the never dried (ND), air-dried (AD) up to 24 h, heated dried at 150 °C for 1 min (HD-1); 20 min (HD-20), and 30 min (HD-30) PLPS [106]. © Wiley and Sons. All arts with permission to use.

wet-pressing-induced fiber hornification was also observed [107]. These experiments clearly indicate that cellulose accessibility dictated by pore surface area plays the most important role in enzymatic hydrolysis of cellulose. Other factors, including substrate chemical composition, DP, and wood species, play a secondary role as reflected from the observed relatively small differences in the SEDs of these three very different substrates after significant hornification (Fig. 2A).

Physical size reduction can increase substrate external surface area and therefore improve substrate accessible area to cellulases [118]. Zhu's lab identified two classes of substrate physical size reduction [79], and found that complete physical deconstruction of cell walls (Class II) through extensive milling such as ball milling resulted in near-complete cellulose saccharification with an ultra-low cellulase loading of 5 and 15 FPU/g cellulose for bleached fibers [119] and pristine loblolly pine wood, respectively [80]. Mechanical energy consumption for complete physical deconstruction of cell walls of bleached fibers is approximately 1 MJ/kg, however, is prohibitively high of over 10 MJ/kg for pristine plant biomass based on laboratory studies [119]. Zhu's lab proposed to use the chemo-mechanical pulping approach to conduct post-chemical pretreatment of physical size reduction to reduce mechanical energy input [120]. They found that the effectiveness of size reduction on enzymatic saccharification is more effective on less or mild chemically pretreated wood. These understandings led to the development of the low-temperature mild deacetylation mechanical refining (DMR) process at the US DOE NREL to produce sugar streams not only inhibitor-free but also at very high yield with low mechanical energy input [121–123].

***Cellulose DP and crystallinity:*** In addition to the surface area and particle size, other substrate properties such as cellulose DP, crystallinity, and chemical composition inherently affect cellulose hydrolysis reaction [15,99]. Some researches indicated that cellulose DP has a negligible effect on enzymatic hydrolysis efficiencies [99,124,125], perhaps due to the relatively low DP of the substrates after pretreatment [126]. However, other studies indicated that a cellulose substrate with low DP hydrolyzes much faster [127,128], because the substrate has more ends (both reducing and nonreducing) for cellobiohydrolases to work on. Fig. 3A shows the effect of cellulose DP ($DP^{0.905} = 0.75[954 \cdot \log(viscosity)]$[129]) on the average rate of enzymatic hydrolysis (ARH) of organosolv ethanol pretreated poplar substrates during the first 12 h. It is apparent that ARH decreases linearly with cellulose

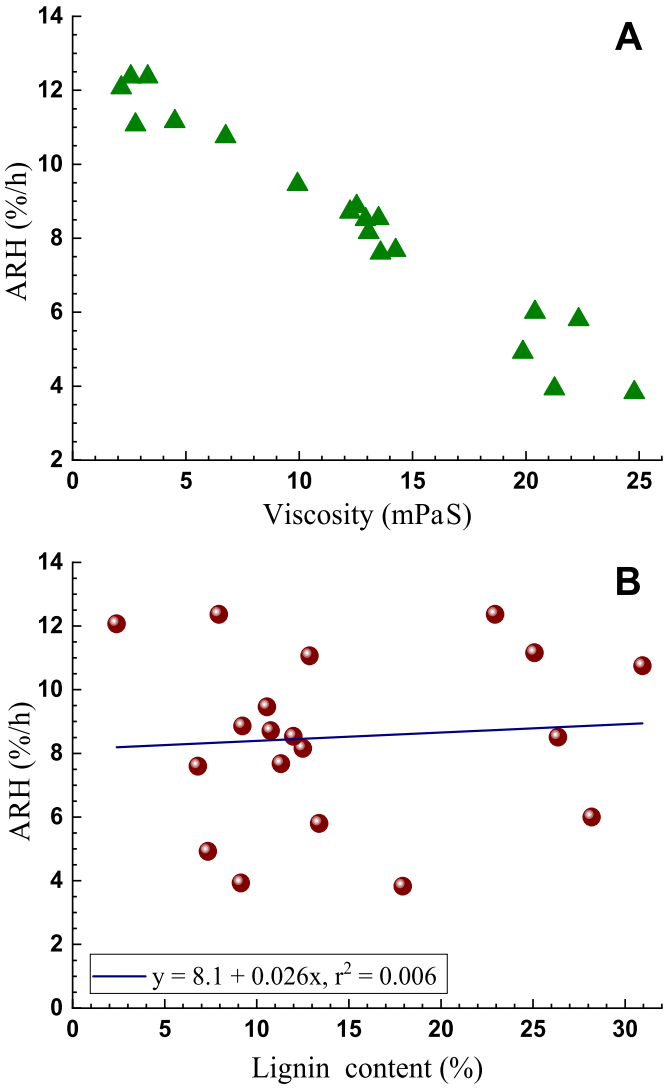



**Fig. 3.** Effect of cellulose DP (A) and lignin content (B) on enzymatic hydrolysis of organosolv ethanol pretreated poplar wood (redrawn based on the data in Refs. [130,131]).

DP. The substrates with lower cellulose DP also resulted in greater terminal hydrolysis efficiency [130,131].

It is known that disordered cellulose has a much faster hydrolysis rate than crystalline cellulose, which led to the suggestion that cellulose crystallinity negatively affects enzymatic hydrolysis [132–134]. Both negative [124] and no effect [127,135] of crystallinity on cellulose hydrolysis were reported [99]. Agarwal et al. demonstrated that decreasing substrate cellulose crystallinity through ball milling of loblolly pine wood had a substantial effect on improving substrate saccharification [80]. However, carefully examining the study indicates that the observed effect may well be due to the significant cell wall physical deconstruction by ball milling that made the wood highly accessible to enzymes, as discussed above, along with the reduction of cellulose crystallinity. Therefore, reported observation on crystallinity effect on substrate hydrolysis could well be contributed by other factors [99].

It should be emphasized that when dealing with a lignocellulosic substrate, not a pure cellulose one, the effects of some substrate properties, such as cellulose DP and crystallinity, on saccharification of the lignocellulosic substrate are less significant, compared with other substrate-related factors such as lignin and hemicelluloses [79]. Although different plant species have varied chemical composition and physical structure and thereby exhibit varied recalcitrance to enzymatic hydrolysis [136], often the chemical composition and therefore reactivity toward enzymatic hydrolysis were altered through chemical pretreatment/fractionation [79,81]. These will be addressed in detail in the following sections and the discussion on plant-biomass pretreatment or fractionation.

***Hemicellulose inhibition:*** Other components (hemicelluloses and lignin) in a lignocellulosic substrate dramatically inhibit the enzymatic hydrolysis of cellulose by affecting both cellulose accessibility and overall substrate reactivity. The effect of hemicelluloses is primarily from their physical blockage that limits cellulose accessibility to cellulases. Many studies have demonstrated that simply removing hemicelluloses from biomass can significantly enhance SED [15,79,137,138]. Removing hemicelluloses can facilitate enzyme access to cell wall cellulose from lumen or the side of S3 layer where hemicellulose concentration is the highest with low lignin concentration (Fig. 1B). Removing hemicelluloses is the working principle of many pretreatment technologies, such as hot water, steam, and dilute acid pretreatments [139,140]. When applying dilute acid to aspen (a hardwood), near-complete cellulose saccharification was achieved at a relatively low dosage of 10 FPU/g glucan, attributed to the removal of hemicelluloses [138]. These pretreatment methods are not designed to remove lignin as will be discussed below, and in fact, increased lignin content in substrates due to the enrichment after the removal of hemicelluloses.

***Lignin inhibition:*** Lignin is a primary inhibitor to enzymatic hydrolysis of lignocellulosic substrates. Although the inhibitory effects of lignin have been extensively studied, the inhibiting mechanisms are still not completely understood [16]. In general, lignin inhibits cellulases in three ways: (1) as a physical barrier to block or restrict cellulose accessibility to enzymes; (2) adsorbing enzymes nonproductively to compete with cellulose; and (3) deactivating enzymes. The inhibitory effect of lignin is dependent on the quantity, location, structure, and properties of the lignin in substrates.

It is easy to understand that more lignin in a substrate should give a greater inhibitory effect on decreasing cellulose accessibility and therefore SED. However, it should be noted that high lignin content does not always result in a low hydrolysis rate and yield, because the hydrolysability of a substrate is determined by many factors, as discussed above. For example, when poplar wood was pretreated by ethanol organosolv under various conditions, the average hydrolysis rates (AHRs) and SEDs of the resultant substrates were not correlated to substrate lignin content, as shown in Fig. 3B [130,131]. These substrates have a diverse chemical composition (hemicelluloses and lignin) and cellulose DP. As discussed earlier and in the pretreatment section below, hemicellulose removal played a critical role in dictating enzymatic saccharification [79,138]. For example, when aspen wood pretreated by dilute acid and sulfite with varied levels of delignification of 0–40%, lignin removal is not correlated to SED and lignin removal improving SED can only be seen when comparing substrates with the same level of hemicellulose removal [79], as will be further discussed in Section 4.2 and Fig. 5.

The location of lignin in a substrate is also important. A substrate has poor digestibility when cellulose is covered by lignin. For example, when wood chips were fiberized in the middle lamella by presteaming wood chips above the lignin glass transition temperature followed by disk milling, the resultant substrate was covered with lignin and therefore had a lower SED than the substrate with the same external surface area but fiberized in the secondary (S) layer (cellulose is exposed) through disk milling after presteaming below the lignin glass transition temperature at 134 °C [118]. In hydrothermal pretreatments (such as hot-water, steam explosion, and dilute acid methods), lignin migration out of cell walls was observed [141,142]. Lignin relocation is one of the contributing factors to the improved hydrolysis by these pretreatments.

Lignin inhibition is greatly dependent on its physicochemical properties. For example, lignin from different sources (e.g., from various biomass species, preparation methods and conditions) [143] and different lignin fractions from the same lignocellulosic substrate [144] showed varying inhibition to enzymatic hydrolysis. Selectively removing the highly inhibitory fraction without extensive delignification can dramatically improve SED [144]. Cellulose in a steam-exploded Douglas fir substrate with 43.2% lignin content had a SED of only 30.4% after 48 h hydrolysis with a cellulase loading of 20 FPU/g glucan. When the substrate was washed with 1% NaOH at room temperature, which reduced the lignin content to 35.5%, SED was increased to 54.2%. Further decreasing lignin content to 14.8% by harsh alkali-oxygen delignification improved saccharification to 71.2%. However, the hydrolysis improvement by unit lignin removal was decreased from 3.1 in cold alkaline extraction to 1.4 in alkali-oxygen delignification. It should be noted that alkali-oxygen delignification usually causes cellulose depolymerization, which should also contribute to the improved hydrolysis because decreasing cellulose DP favors enzymatic hydrolysis as discussed above. These results suggest that the lignin fraction extracted by 1% NaOH was more inhibitory than the fraction removed by oxygen-alkali.

Functional groups of lignin, e.g., phenolic hydroxyl, play a crucial role in inhibiting cellulases [143,145], and condensed phenolic moiety can be highly inhibitory to enzymatic hydrolysis of cellulose [146]. Blocking phenolic hydroxyl groups by etherification such as hydroxypropylation could reduce the inhibitory effect by 65–91% [143].

***Electrostatic repulsion for reducing lignin inhibition:*** Nonproductive adsorption (binding) of cellulases to lignin [95] through hydrophobic interaction [147,148] is a key process to lignin inhibiting enzymatic hydrolysis of cellulose, which is closely related to the overall hydrophobicity of lignin. It was reported that hydrophilic modification of lignin, e.g., by sulfonation and carboxylation, can reduce its hydrophobicity by 22–30% measured by contact angle and thereby remove the lignin inhibition by 76–96% [143]. This is one of the reasons why sulfite-based SPORL [115,149,150] and maleic acid hydrotropic fractionation (MAHF) [83,151] pretreatment of biomass result in outstanding enzymatic digestibility. The residual lignin in SPORL and MAHF substrates is partially sulfonated and carboxylated, respectively, and therefore is more hydrophilic and has less affinity to cellulases than substrates with more hydrophobic lignin.

Sulfonation and carboxylation of residual lignin in substrates can also be utilized to enhance the electrostatic interaction (repulsion) between lignin and cellulase and therefore substantially reduce nonproductive cellulase binding to substrate lignin and thereby improve SED [82–84,98]. It is known that lignin contains a small amount of negatively charged groups such as hydroxyl and carboxyl groups. The charge of these groups in lignin increases with increasing pH. However, a

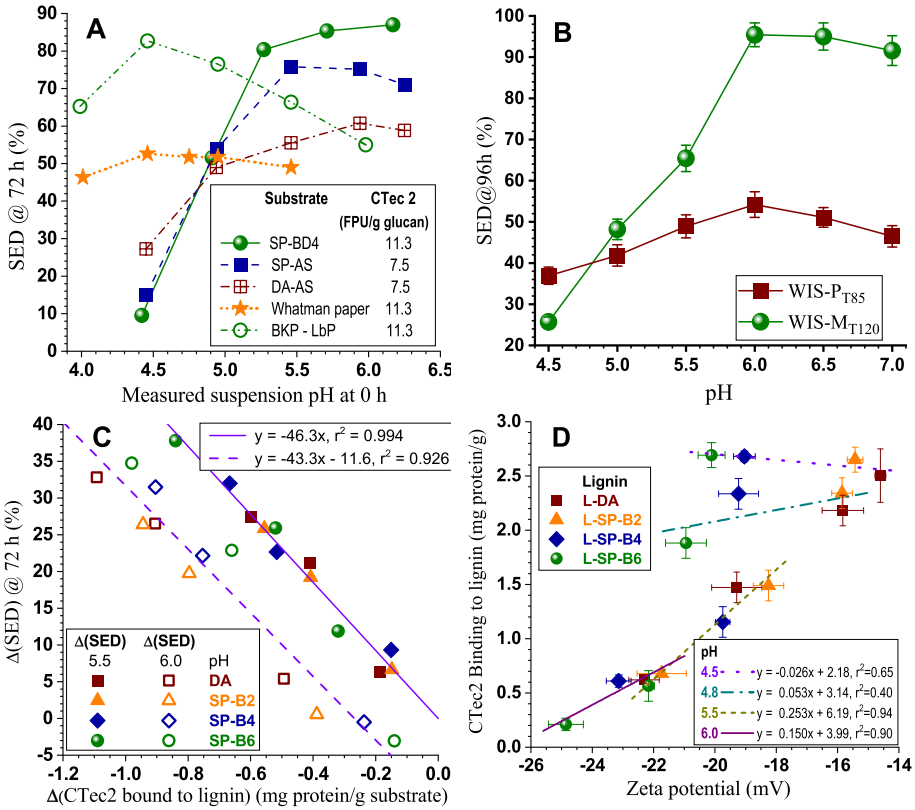



**Fig. 4.** (A–B) Effect of enzymatic hydrolysis pH on substrate enzymatic digestibility (SED): A: SP-BD4 and SP-AS are SPORL-treated lodgepole pine and aspen, respectively, DA-AS is dilute acid-treated aspen, BK-LbP is bleached kraft loblolly pine pulp; B: WIS-$P_{T85}$ and WIS-$M_{T120}$ are acid hydrotropic fractionated birch wood using *p*-TsOH and maleic acid, respectively. (C) Correlations between the increases in SED of four different lodgepole pine substrates with the decreases in the amount of nonproductive cellulase binding to the corresponding substrate lignin at two enzymatic hydrolysis pH levels; DA = dilute acid, SP2, SP4, SP6 = SPORL at sodium bisulfite loadings of 2, 4, 6% on wood. (D) Correlations of nonproductive cellulase bindings to four substrate lignin samples (same as in (C)) at different pH levels. (A, C, D) [84] and (B) [83] reproduced with permission from Wiley.

charged cellulase is required to initiate electrostatic interaction between cellulases and lignin. It is known that cellulases have an isoelectrostatic point (*pI*). A cellulase becomes positively charged when pH is lower than its *pI* and negatively charged when pH is higher than the *pI*. At pH = 4.8, Saddler's lab found that a positively charged mono-component cellulase with a greater *pI* > 4.8 was preferentially bound to lignin over a negatively charged cellulase with a lower *pI* < 4.8 [152]. Commercial cellulases are a cocktail of complex enzymes, however, they do have a bulk *pI* or a pH at which they are not charged. This *pI* (pH) is approximately equal to the common enzymatic hydrolysis pH of 4.8–5.0 suggested by enzyme manufacturers based on characterization studies using pure cellulose. Therefore, electrostatic interaction between lignin (or substrate) is essentially absent in most reported literature in which enzymatic hydrolysis studies were conducted at pH 4.8–5.0, suggested by enzyme manufactures for **pure**cellulose materials such as Whatman paper and bleached kraft pulps as shown in Fig. 4A. With this understanding, Zhu's lab purposely used elevated pH of 5.5–6.5 from commonly practiced pH of 4.8–5.0 and demonstrated that enzymatic digestibility of sulfonated (SPORL) and carboxylated (MAHF) lignocellulosic substrates can be increased by approximately 100%, as shown in Fig. 4A and B [82–84], respectively.

Most lignocellulosic substrates reported in the literature are not sulfonated nor carboxylated. As a result, the enhancement in SED by the repulsive electrostatic interaction between lignin and cellulase was not observed, especially when enzymatic hydrolysis was widely practiced at a pH of 4.8–5.0 near the bulk cellulase *pI* (with minimal cellulase charge). These conventional substrates have lower surface charges derived from the native negatively charged groups than the sulfonated and/or carboxylated substrates used by Zhu's groups [82–84]. For example, the zeta potential of lignin from a diluted acid lodgepole pine wood was −16 mV and −22 mV at pH 4.8 and 6.0, respectively, in comparison with −21 mV and −25 mV at the corresponding pH for sulfonated lignin from the same lodgepole pine but pretreated by SPORL [84]. Furthermore, the ratio of the amount of cellulase binding to lignin at pH 5.5 to that at pH 4.8 was decreased with increasing lignin sulfonation [84], i.e., increasing lignin sulfonation decreased nonproductive cellulase binding to lignin at the same pH. The gains in SEDs from elevating hydrolysis pH of four lodgepole pine substrates pretreated by dilute acid (no sulfonation) and SPORL at different sulfite loadings (with different extent of sulfonation) are linearly correlated to the decrease in the amount of cellulase nonproductively bound to these substrates, as shown in Fig. 4C. It is interesting to note that at pH 4.5 and 5.0, or approximately equal to the cellulase *pI* at which cellulases are not much charged, the amounts of nonproductive cellulase bound to the lignin in these four substrates are not (much) affected by the extent of lignin sulfonation or surface charge, as shown in Fig. 4D. Furthermore, the amount of cellulase bound to lignin was slightly lower at pH 5.0 than pH 4.5, indicating the minor positive effect of negative cellulase charge at pH 5.0. This cellulase charge effect is further observed when pH was increased to 5.5 and 6.0 at which the amount of cellulase bound to lignin were decreased significantly with the increase in the extent of lignin sulfonation (or lignin charge). Fig. 4C and D further illustrate that enzymatic hydrolysis of lignocellulosic substrates should be conducted at elevated pH to facilitate electrostatic repulsion interaction between cellulase and substrate lignin. As shown in Fig. 4A and B, even for conventional substrates such as dilute acid pretreated substrates without lignin sulfonation and carboxylation, SED can be improved by approximately 20% simply by raising enzymatic hydrolysis pH to 5.5–6.5 from the commonly practiced 4.8–5.0.

***Other strategies for reducing lignin inhibition:*** There are many strategies to overcome the lignin inhibition of cellulases. It is apparent that the most effective way is to remove lignin from the substrate. However, no delignification method that can extensively remove lignin from lignocellulosic biomass is simple and inexpensive. Besides, extensive delignification usually reduces overall sugar yield due to sugar degradation under severe conditions required for delignification. In addition, the sugar degradation products are mostly fermentation inhibitors. Chemical modification to block the inhibitory groups or reduce

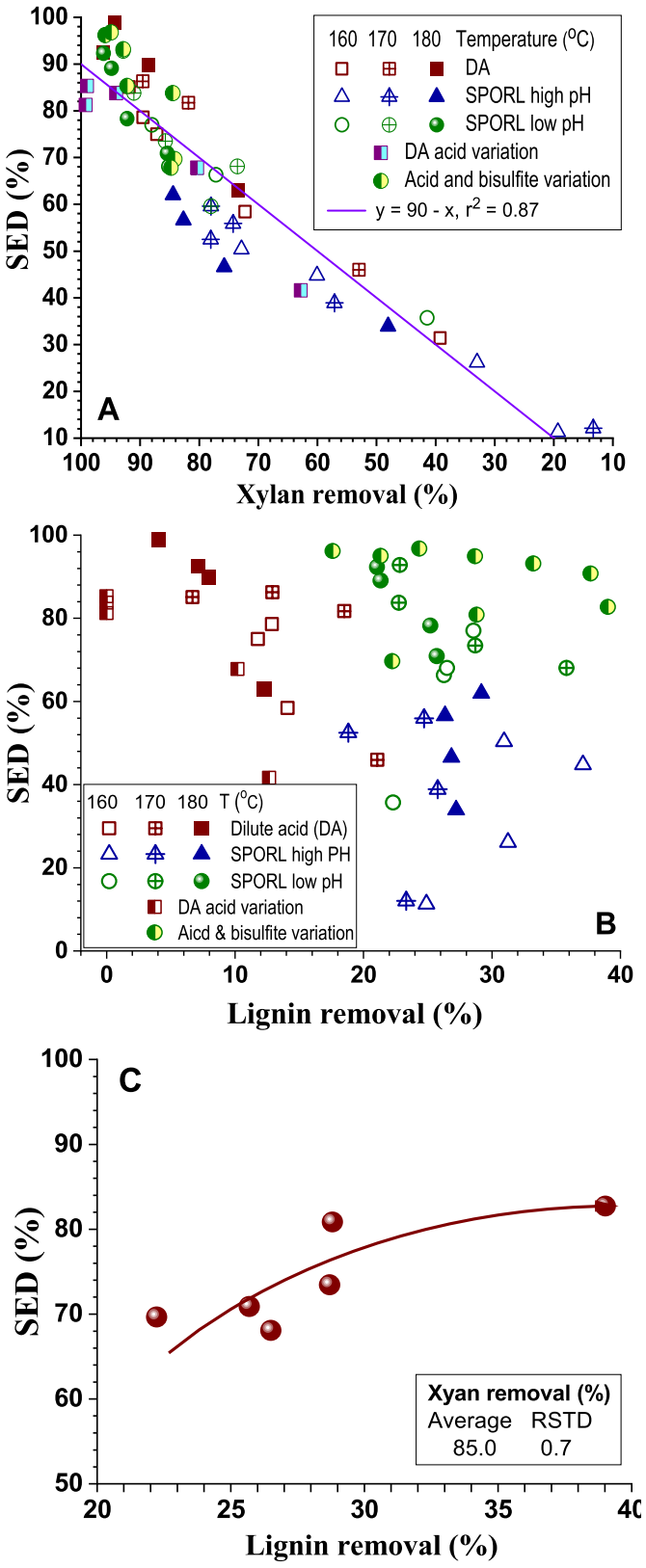



**Fig. 5.** Effects of xylan and lignin removal on substrate enzymatic digestibility (SED) of dilute sulfuric acid and acidic sulfite (SPORL) pretreated aspen of 51 samples. A: Effect of xylan removal; B: Effect of delignification; C: Effect of delignification under constant xylan dissolution of approximately 85% (6 samples) with relative standard deviation (RSTD) of 0.7%. All data from Zhu et al. [138] and plots adapted from Leu and Zhu [79].

hydrophobicity of lignin is another effective strategy to reduce or remove the lignin inhibition after the pretreatment, but it is expensive and economically less attractive/practical. Selecting pretreatment chemicals that can reduce lignin hydrophobicity, such as sulfite in SPORL process [115,149,153–155] that gets lignin partially sulfonated or maleic acid in hydrotropic fractionation that carboxylates lignin [83,156–158], is an economically feasible technology. Adding metal salt to form a complex with lignin was also found effective to reduce lignin effect on enzymatic hydrolysis of pure cellulose and sulfonated lignocellulosic substrates [159,160]. Blocking lignin using exogenous proteins [144,161] or surfactants [162–164] to prevent lignin from adsorbing cellulases have been widely studied. Many studies demonstrated the positive results of treating the substrates before loading cellulases with proteins [144,161] and surfactants [162–164]. However, using protein is certainly an expensive option and using surfactant may cause downstream processing problems due to foaming.

Zhu's group found that lignosulfonate (LS) can be a less expensive and native surfactant to block substrate lignin to reduce nonproductive cellulase binding and improve lignocellulosic enzymatic saccharification [98]. Later they found that the lower molecular weight fraction of a commercial LS, often with a greater degree of sulfonation, is more effective [165]. Furthermore, they found that the effectiveness of LS in enhancing SED also depends on substrate lignin, i.e., the same LS can enhance the enzymatic hydrolysis of a lodgepole pine substrate prepared with an alkaline method much more significantly than a SPORL pretreated substrate from the same wood. The enhancement for a dilute acid-treated aspen wood was much less significant despite high lignin content. Other lignin samples were found to have a similar effect as LS [166,167]. Especially, carboxylated lignin can substantially improve enzymatic hydrolysis of a carboxylated substrate [83]. It is interesting to note that LS can also enhance enzymatic hydrolysis of pure cellulose substrate, similar to the effect of surfactant. The optimal pH for enhancement, however, varies with the LS applied [168].

It is apparent that using elevated pH of 5.5–6.5 is the most convenient and practical solution to reduce nonproductive cellulase binding to lignin to improve enzymatic sugar production from plant biomass. Even with conventional pretreatments such as dilute acid (Fig. 4A), SED can be improved by approximately 20%. The improvement of SED can be as high as 100% for sulfonated and carboxylated substrates (Fig. 4A and B).

### 4.2. Pretreatment/fractionation

Pretreatment or fractionation of lignocellulose is essential to remove the barriers (recalcitrance) posed by the tight structure of lignocellulose and cell wall components (lignin, hemicelluloses, and cellulose) for effective enzymatic production of cellulosic sugars. In most cases, pretreatment is a step that is unaffordable to skip, though it is an energy- and cost-intensive operation. Otherwise, enzymatic sugar yield would be too low to be feasible for a biorefinery. Pretreatment not only dictates the enzymatic digestibility of a lignocellulosic substrate but also impacts the downstream sugar fermentation due to potential inhibitor formation in the pretreatment, co-product potential from lignin and hemicelluloses, wastewater treatment, and the economics of the entire biorefinery.

Reducing or removing biomass recalcitrance via pretreatment can be achieved by destroying the cell wall structure of feedstock biomass, increasing surface area by size reduction, removing hemicelluloses and/or lignin, and disrupting the crystalline structure of cellulose, and/or pre-hydrolyzing cellulose (shortening cellulose chains). Accordingly, physical, chemical, physio (thermo)chemical, and biological methods have been developed for biomass pretreatment.

Physical pretreatment often refers to physical size reduction through mechanical means to increase substrate surface area. As briefly discussed earlier, there are two classes of size reduction [79]: to fiberize the feedstock to fiber or fiber bundle level through milling and refining similar to mechanical wood pulping [118], and to deconstruct the cell

wall through milling of fibers or using more intensive milling such as ball milling [80], which can be energy-intensive depending on the level of cell wall deconstruction but extremely effective in improving cellulose enzymatic saccharification. For woody biomass, size reduction to wood chip level with minimal energy input is always needed. Using post-chemical/hydro-thermal pretreatment size reduction is effective in improving enzymatic saccharification with moderate energy input [120,123]. Combining physical and chemical treatment such as in steam explosion with/without additional catalysts or chemicals is also very effective to achieve needed size reduction and removal of hemicelluloses and/or lignin inhibition with relatively low energy input especially for woody biomass [169–171].

Chemical pretreatment can be implemented using acidic or basic reagents in aqueous or organic media, as discussed below. Dilute acid process is the most extensively investigated pretreatment method [138,172–174], which was developed from dilute acid sugar production processes such as the Madison wood sugar process [58] by substitution acid hydrolysis with enzymatic hydrolysis in the later 1970s to early 1980s [175,176]. Several mineral acids and carboxylic acids were used, but sulfuric acid remains the most used acid for its low cost. Typical reaction conditions vary with acid concentration from sub to a few percent, temperature from 110 to 190 °C for 10–120 min. Dilute acid hydrolysis relies on removing hemicelluloses to improve cellulose enzymatic saccharification. Digestibility of dilute acid pretreated substrates was found to have an excellent correlation with xylan removal, independent of individual pretreatment conditions in an earlier study [177]. This was later verified even with partial delignification using sulfite [79,138], suggesting hemicellulose removal is indeed critical to improving enzymatic digestibility. As shown in Fig. 5A, SED of dilute sulfuric acid and sulfite (SPORL) [115] pretreated aspen wood was linearly correlated with the amount of xylan dissolution, independent of pretreatment condition, chemistry, and the extent of delignification (0–40% for the 51 substrates presented). Furthermore, SED is not correlated to delignification (Fig. 5B). Only when comparing the substrates with the same level of xylan dissolution, the moderate positive effect of delignification on SED was seen (Fig. 5C). This indicates that delignification is not the critical factor to improve enzymatic saccharification at least for aspen, and potentially for most hardwood species and herbaceous plant biomass, contrary to the common belief that lignin is the main barrier for biomass bioconversion.

Hydrothermal methods that include hot water, steaming, and pure steam explosion (without an acid as a catalyst) are also classified as acidic pretreatments, because the acetyl groups on hemicelluloses are released as acetic acid under the hydrothermal conditions, catalyzing the hydrolysis and dissolution of hemicelluloses. These pretreatments are efficient for herbaceous biomass (e.g., wheat straw), but are usually not effective to woody biomass, in particular, softwoods, because of the low acetyl content and high lignin content of woody biomass. For example, it was demonstrated that hot water/steaming is an efficient pretreatment/fractionation for co-production of bioethanol (from cellulose), animal feed (molasses from hemicelluloses), and solid fuel (from residual lignin) from wheat straw [178] after the pretreatment at 180 °C for 5–15 min. About 143 kg (48 gallons) of bioethanol, 353 kg of lignin-rich solid biofuel, and 420 kg of feed (65% dry matter (DM) molasses) could be produced from 1 metric ton of wheat straw (86% dry matter).

Alkaline pretreatment relies primarily on delignification, swelling of biomass fibers, and disruption of lignin structure and linkages [53]. A variety of chemicals have been used [179] such as sodium hydroxide [121,180–182], carbonate [183], lime, and ammonia [184,185]. In general, alkali pretreatment is less effective in removing plant biomass recalcitrance unless extensive delignification is implemented but has the advantage of eliminating fermentation inhibitors such as furans formed by acid-catalyzed dehydration of solubilized sugars, especially in dilute acid pretreatment. Another example, using NaOH to extract acetyl groups effectively eliminated the formation of acetic acid [186], a key fermentation inhibitor to most microbes. However, NaOH recovery requires expensive capital investment and remains a key barrier to NaOH pretreatment.

Sulfite-based pretreatment is one of the most effective chemical pretreatment methods, including steam explosion with $SO_2$ [169–171,188–190], organosolv pretreatment using $SO_2$ [191], along with SPORL that was developed by modifying acidic sulfite pulping using a higher temperature [115,192,193], or extended reaction of 4–6 h but at low chemical dosages (Fig. 6A) [187], or higher chemical loadings similar to those used in sulfite pulping but with a shorter reaction time (Fig. 6B) [194]. These sulfite pretreatments can effectively remove the conversion barriers of the most recalcitrant feedstock, softwoods. Lignin sulfonation is an important factor that contributed to the effectiveness of these processes, but these processes have significantly different sulfonation degrees. The organosolv pretreatment has the highest $SO_2$ loading of typically 12% in the pretreating chemical solution (or 48% on wood, assuming liquor to wood ratio of 4:1 L/kg) [195], but only produced a moderate lignin sulfonation. $SO_2$ loading in steam explosion is the lowest with a typical value of 2.5% on wood, which resulted in a low degree of sulfonation [169,189]. $SO_2$ loading in SPORL is 2–4% for hardwoods and 6–10% for softwoods [115,187,196–198], and can be as high as 30% on wood (similar to sulfite pulping) for pretreatment conducted at low temperatures of 120 °C to reduce furan formation [194,199]. A pH profiling scheme in pretreatment was also developed to reduce furan formation using SPORL [153]. The high degree of lignin sulfonation from SPORL resulted in the most effective pretreatment among the three sulfite processes. $SO_2$ organosolv relies on the substantial dissolution of lignin and hemicelluloses to achieve great enzymatic digestibility of the resultant substrates as will be discussed further later. The comparative study indicated that SPORL is much more effective than $SO_2$ catalyzed steam explosion when applied to most recalcitrant feedstock such as softwoods, mainly attributed to dissolution of lignin as well as a higher degree of lignin sulfonation [193].

Solvent (organic solvents and ionic liquids) based pretreatments are also the most efficient ones in terms of overcoming biomass recalcitrance [200–202], such as co-solvent systems using tetrahydrofuran (THF + $H_2SO_4$) [203], γ-valerolactone (GVL + $H_2SO_4$) [204,205], ionic liquids (ILs) [206–208], deep eutectic solvents (DESs) [209–212], acid hydrotropes [151,156,158,213], and traditional organosolv processes using ethanol [214,215] or formic acid [216,217]. All these solvent systems primarily rely on the extensive dissolution of both hemicelluloses and lignin to improve cellulose accessibility and thereby enzymatic saccharification, though decrystallization and depolymerization of cellulose in some of the processes also contribute to the improved enzymatic digestibility of cellulose. IL and DES systems are two families of solvents [201,218–221] for processing plant-biomass, and both processes have been extensively studied as reported in the

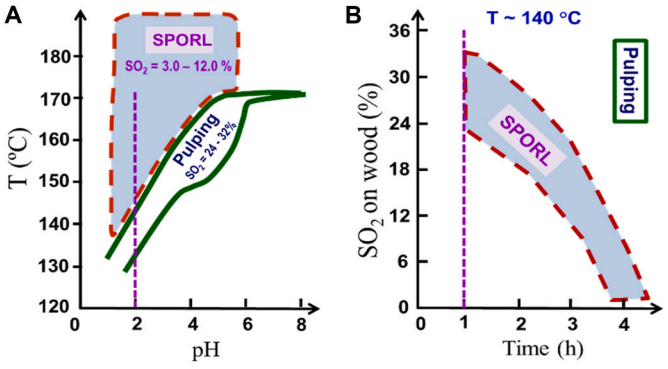



**Fig. 6.** Operating conditions for SPORL in comparison with those for sulfite pulping. SO2 loadings are percentage of oven dry wood. A: *T-pH* diagram under low sulfite loadings (adapted from Ref. [187]); B: $SO_2$-*t* diagram including high sulfite loadings similar to those in sulfite pulping.

literature. Readers are referred to the cited references to gain in-depth information about these two processes in terms of typical operating conditions and performance. The most challenging issue of all solvent-based pretreatments is the cost and recovery of the solvents and therefore most of the processes have low technology readiness level (TRL).

In comparison, acid hydrotropic fractionation (AHF) is a very new process recently developed by the Zhu's group at the USDA Forest Products Laboratory [151,213] with limited work reported and low TRL. However, it has shown some promising results. This is especially true for maleic acid (MA) hydrotropic fractionation (MAHF) because MA is a U. S. Food and Drug Administration (FDA) approved indirect food additive (21CFR175-177). The key interesting features include rapid fractionation of only 20–30 min at atmospheric pressure and 120 °C or lower with high selectivity in retaining cellulose while dissolving hemicelluloses and lignin. Separation of dissolved lignin can be achieved simply by diluting the resultant spent liquor using water below the minimal acid hydrotropic concentration (MHC). When using MA, both the cellulose and lignin are carboxylated, advantageous for further processing and utilization such as mechanical fibrillation for producing cellulose nanomaterials and enzymatic cellulose saccharification [83,151,158], because carboxylation can enhance the lignin lubrication effect in fibrillation and reduce nonproductive cellulase binding to the substrate at elevated pH in enzymatic saccharification, as discussed earlier [83,151,158]. More importantly, AHF especially MAHF results in lignin with a low degree of condensation to facilitate lignin valorization. Readers are referred to a recent minireview on AHF for details [222]. It should be pointed out that AHF is very different from traditional sulfonic aromatic salt-based hydrotropic fractionation that has proven not effective for its long reaction time at high temperatures [223,224].

Energy efficiency is an important factor in selecting pretreatment processes. A complete energy efficiency analysis requires consideration of all process details, including chemical recovery, which depends on the process design details. However, conceptual analyses can be carried out to understand key factors contributing to process energy efficiency. Without considering heat recovery from pretreatment spent liquor, Zhu and Zhuang [3] found that thermal energy usage for chemical pretreatment mainly depends on two factors: (1) the pretreatment temperature and (2) biomass solids loading. The effects of these two factors on energy consumption for heating biomass slurry for pretreatment are shown in Fig. 7. The effects of temperature and solids loading are most significant at solids loading below 10%. Pretreatment temperature is

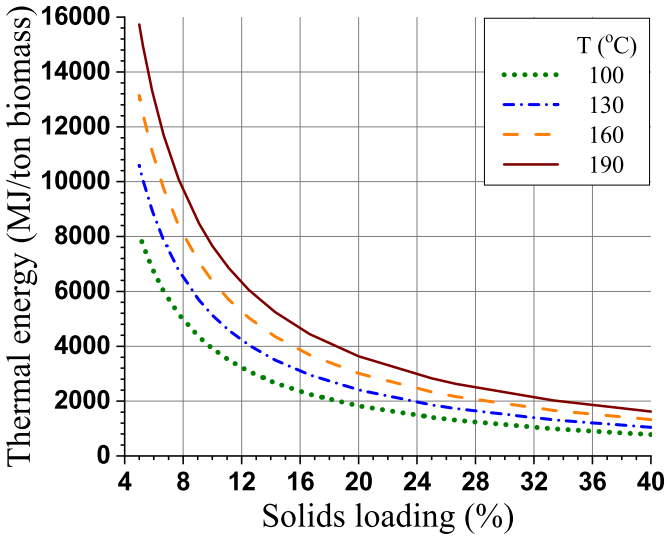



**Fig. 7.** Effects of pretreatment temperature and plant biomass solids loading on thermal energy requirements for chemical pretreatment based on thermodynamic calculations.

inherently pretreatment process dependent. In this sense, low temperature processes such as AHF or MAHF [151,213] are advantageous compared with steam explosion [178,225,226], which is often carried out around 190 °C. Solids loading is primarily dependent on biomass type. In general, woody biomass can be treated at high solids loadings of approximately 25% or greater by most treatment methods such as alkaline wood pulping [180] and sulfite [194,198] processes, which are highly effective on wood chips, whereas bulky herbaceous and agricultural biomass require treatment at low solids loadings of approximately 10%. Some solvent processes may also require low solids loadings for pretreatment to be effective.

Some level of plant biomass physical size reduction/physical pretreatment is always required, which can be energy intensive for woody biomass for their large size and physical integrity [118]. Processes such as alkaline wood pulping [180] and sulfite [194,198] that are effective on wood chips need less energy than some solvent processes that require significant size reduction. Post chemical treatment size reduction is very effective to reduce energy requirement for wood size reduction without affecting enzymatic hydrolysis efficiency [120].

### 4.3. Comparisons of different pretreatment/fractionation processes

To better understand the differences among the common pretreatment processes discussed in Section 4.2, Table 3 summarizes the mechanism, pros and cons, and TRLs of these processes. To provide reasonable comparisons among different pretreatment/fractionation processes in removing lignocellulose recalcitrance for enzymatic sugar production, Table 4 lists processing conditions and the resultant SEDs of two major categories of plant biomass: nonwoody herbage and woody biomass. Table 4 is certainly not meant to be exhaustive. We selected recent literature with the most comprehensive data, especially pretreatment conditions, xylan (hemicelluloses) and lignin removal, and enzyme dosages. The literature-reported enzyme dosages were in different units and were converted to the same unit (FPU/g substrate glucan) whenever possible with available data. Considering the enzyme dosages applied, the data in Table 4A and B clearly indicate the following:

(1) Alkaline pretreatment, including both NaOH [227] and Ammonia [228], is not effective partly due to low hemicellulose removal without significant delignification, as discussed earlier.
(2) THF [229], GVL [230], and traditional organosolv processes [231,232] are very effective due to their ability for simultaneous dissolution of significant amounts of hemicelluloses and lignin; however cellulose formylation by concentrated formic acid pretreatment [216] inhibited substrate enzymatic hydrolysis [217]. GVL system has excellent fractionation performance for materials production [204], but may be too expensive compared with other solvents for commercial biomass fractionation at the scale of thousands of tons per day.
(3) SPORL process [115,194,197] is most robust for treating woody biomass, especially those with high lignin content, such as forest residues from softwood species [187,198], due to both lignin sulfonation and significant hemicellulose dissolution. Solvent-based systems such as $H_2SO_4$ or $SO_2$ + ethanol organosolv [215,233], GVL [230], and THF [229] need much higher chemical loadings to achieve substantial lignin dissolution than SPORL in addition to their nearly complete dissolution of hemicelluloses to reach a similar level of saccharification.
(4) IL-based systems [208,234,235] performed well, but not as well as SPORL or organosolv systems under similar cellulase loadings. However, the Ionosolv process using low-cost hydrogen sulfate IL systems [236,237] developed at the Imperial College has advantages in terms of environmental impact. It should be noted that the reported substrate enzymatic digestibility of the Ionsolv process was obtained using air-dried samples [234,235,237],

**Table 3**
Mechanism, pros and cons, and technology readiness levels of common pretreatment processes.

| Process | Mechanism | Pros | Cons | TRL |
|---|---|---|---|---|
| Physical size reduction | Physical deconstruction of cell wall | No chemicals involved<br>No need of wastewater treatment<br>No chemical changes to lignin | Energy intensive<br>Low saccharification efficiency unless broke call walls (Class II [79]) | 5 |
| Hydrothermal:<br>Hot water/ steam<br>Steam explosion | Hemicellulose removal | No chemicals needed | Low saccharification efficiency unless under extremely high temperatures.<br>Energy intensive for steam explosion | 6–7 |
| Dilute acid | Hemicellulose removal | Low acid loading but effective in removal hemicelluloses | Producing inhibitors for fermentation.<br>Corrosion of equipment<br>Condensed lignin | 6 |
| Alkaline | Delignification and some hemicellulose removal | Compatible with pulp mills (scalable) Good chemical recovery technology when using NaOH<br>No fermentation inhitors | Capital intensive for NaOH recovery<br>Low saccharification efficiency using weak alkalinity chemicals such as carbonate. | 6 |
| Sulfite:<br>SPORL<br>$SO_2$ steam<br>$SO_2$ ethanol | Hemicellulose removal<br>Delignification<br>Lignin sulfonation | High saccharification efficiency and effective on most difficult feedstock<br>Compatible with sulfite mills (SPORL)<br>Lignin coproduct in existing market (SPORL only) | $SO_2$ air emission<br>Difficulties in chemical recovery unless using Mg as base | 7 |
| Solvent-based:<br>Organic solvents<br>IL, DES, GVL | Delignification and/or hemicellulose removal | High saccharification efficiency<br>Relatively low toxicity chemicals<br>High-quality lignin from GVL and ethanol organosolv | High solvent usage<br>Long reaction time using DES<br>Solvent recovery challenges | 7 (Organic solvent)<br>4 (IL,DES, GVL) |
| Acid hydrotrope:<br>Maleic acid | Hemicellulose removal<br>Delignification<br>Lignin carboxylation | High saccharification efficiency<br>Maleic acid FDA approved indirect food additive<br>Ease in chemical recovery<br>Reactive and light color lignin<br>Xylan directly dehydrated into furan | High chemical usage | 4 |

which reduced digestibility due to drying-induced fiber hornification, as well illustrated by Zhu's group [105,106] and discussed earlier. Although the imidazolium cation-based IL systems performed well with extended reaction time [207,238], these systems are much more expensive than hydrogen sulfate IL systems [236,237] for commercial applications.

(5) In general, DES systems [211,239,240] require a very long pretreatment/fractionation time of 4 h or longer to be effective, which can be a serious issue from a process intensification point of view. For example, DES TEBAC/LA, with over 70% dissolution of both xylan and lignin [239], a very high enzyme loading of 67 FPU/g glucan is needed to achieve SED of 89% after wheat straw being treated for 10 h at 100 °C. When using DES ChCl:glycerol: $FeCl_3$ to treat hybrid pennisetum at 120 °C for 4 h, which dissolved over 90% xylan and 74% lignin, a SED of 92% was achieved only after using a higher enzyme dosage of 18 FPU/g glucan [211]. Similarly, a ChCl/LA system after fractionating eucalyptus wood for 6 h at 110 °C achieved hemicellulose and lignin dissolution of 89% and 82%, respectively, and resulted in a SED of 94% only at a cellulase loading of 22 FPU/g glucan [240]. All these studies using different DES systems performed poorly, compared with the MAHF treatment of wheat straw for 60 min at 120 °C with lower xylan dissolution of 76% and delignification of 57% [157], which achieved a similar SED of 89% at a much lower enzyme dosage of only 10 FPU/g glucan. While DES systems have many applications [201], their merit for fractionating lignocelluloses may be overstated based on the performance discussed above and the amount of solvent needed.

(6) AHF pretreatments [213,222] including MAHF [151] are effective in improving enzymatic saccharification of herbaceous biomass and hardwoods due to dissolution of both hemicelluloses (xylan) and lignin. The AHF using aromatic sulfonate acids performed at the same level of DES systems, but with a much shorter reaction time. MAHF with lignin carboxylation reduced nonproductive binding of cellulases to lignin, which substantially reduces cellulase loading to 10 FPU/g substrate glucan or lower while achieving 90% or higher cellulose saccharification [83]. For example, birchwood fractionated at 120 °C for 30 min resulted in approximately 65% of the dissolution of xylan and lignin and achieved a SED of 90% at a cellulase loading of only 7.5 FPU/g glucan. Furthermore, the dissolved lignin can be used as a biosurfactant to substantially reduce nonproductive cellulase binding [83].

Table 3 also lists the TRL of above pretreatment processes. In general, old processes such as dilute acid reached much higher TRLs simply because of enough research and development were invested in these old processes. SPORL is probably the only process that is new but reached TRL of 7 partly because it was developed from a sulfite wood pulping with excellent scalability. The world first commercial flight using certified woody biojet fuel was demonstrated using cellulosic sugars from softwood forest residue processed by SPORL [241,242].

## 5. Lignin from pretreatment/fractionation for valorization

Sugar-platform-based biorefineries focus on the utilization of carbohydrates (cellulose and hemicelluloses), and little or insufficient attention has been paid to lignin valorization in the past. However, it has been well-recognized that using lignin to create new revenue is critical to the sustainable and economic operation of biorefineries [250]. Lignin has been mostly proposed as a solid fuel for producing heat and power such as in pulp mill operations. Burning lignin is not only a low-value proposition but also can lead to SOx, NOx, and POx emissions [251–253] because the lignin from the enzymatic sugar production is usually contaminated with residual enzymes and yeasts. There is a great deal of literature on lignin valorization [250,254,255] and extensive studies on the utilization of technical lignin such as kraft lignin and lignosulfonate from the pulp and paper industry [256,257]. Readers are encouraged to refer to the related literature for more information. Here we will briefly discuss the properties of lignin from different pretreatment/fractionation processes to facilitate downstream coproducts development and help to select a particular pretreatment/fractionation for biorefinery deployment.

It is difficult to define what is good or ideal lignin, dependent on applications, but in general, lignin with high purity (low contamination), limited condensation (i.e., low degree of crosslinking via carbon–carbon bonds), high reactivity (e.g., more functional groups), narrow molecular weight distribution, and good solubility in common

**Table 4**
Comparisons of the performance among different pretreatment/fractionation processes in terms of dissolutions of hemicelluloses and lignin as well as substrate enzymatic digestibility (SEDs), along, with dissolved lignin molecular weight and β–O–4 ether aryl linkage content. **A**: Herbaceous biomass; **B**: Woody biomass.

| A: Herbaceous biomass | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Process | Biomass | Fractionation condition | Xylan; lignin removal (%) | Enzyme loading [a] | SED (%) | Mw (Da); PDI of DL | β–O–4 of DL (%) | Ref. |
| Dilute acid | Wheat straw (20–40 mesh) | T = 120 °C; t = 60 min | | CTec 2 = | | | | [243] |
| | | $H_2SO_4$ = 1% | 71; 5 | 20 | 44 | | | |
| | | $H_2SO_4$ = 3% | 86; 2 | 18 | 46 | | | |
| Dilute acid + bleaching | | $H_2SO_4$ = 1% + $NaClO_2$ @T = 75 °C; t = 180 min | 77; 94 | 14 | 90 | | | |
| Hydrothermal Steam | Wheat straw | T = 190 °C; t = 10 min | | CTec 2 (+LPMO) = | | | | [226] |
| | | Solids = 20% | 86; ~0 | 10 | 77 | | | |
| IBUS | Wheat straw (5–10 cm) | T = 180–200 °C; t = 5–15 min | 67; 13 | Celluclast 1.5 L + Novozymes 188 (5:1, v/v) = 4.1 FPU/g solids | EtOH yield = 67% | | | [178] |
| Alkaline (NaOH) | Wheat straw (40 mesh) | T = 121 °C; t = 60 min | | Celluclast 1.5 L = | | | | [227] |
| | | NaOH = 2% | 31; 84 | 35 FPU/g solids + β-G = 61.5 | 75 | | | |
| | | NaOH = 4% | 42; 89 | CBU/g solids | 87 | | | |
| Alkaline (Ammonia) | Rice Straw | T = 69 °C; t = 10 h $NH_3$ = 21 wt% | 32; 66 | Celluclast 1.5 L = 15 + β-G = 30 CBU | 74 | | | [228] |
| Organosolv | Wheat Straw (1–8 mm) | Formic acid = 72 wt %; t = 15 min | | | | | 44[b] | [216] |
| | | T = 120 °C (flow-through) | 65; 57 | 10 FPU/g solids (=16) | 58 | | 38 | |
| | | | | 20 FPU/g solids (=33) | 71 | | | |
| | | T = 130 °C (flow-through) | 75; 79 | 20 FPU/g solids (=33) | 75 | | 37 | |
| THF | Corn stover | T = 150 °C; t = 25 min | | Accelerase 1500 = 17 mg protein/g glucan | | | | [229] |
| | | THF = 50 v/v %; $H_2SO_4$ = 0.5% | ~75; ~95 | | 97 | | | |
| IL | Wheat straw (0.5 mm) | [emin][$HSO_4$] = 41.3 wt% | | CTec 2 ≈ | | | | [208] |
| | | T = 131 °C; t = 88 min | 82; 6 | 20 | 76 | | | |
| | Miscanthus | T = 120 °C; t = 8 h | 96; 80 | CTec 2 = | 80 | 4000; 4.9 | 13 | [237] |
| | | [TEA][$HSO_4$] = 80 wt % | 91; 70 | 0.2 mL/g solids | 70 | | | |
| | | | 93; 56 | | 56 | | | |
| DES | Wheat straw (60 mesh) | TEBAC/LA (1:9) | | Celluclast 1.5 L = 35 FPU/g solids (= 67) | | | | [239] |
| | | T = 100 °C for 10 h | ~73; ~73 | + β-G = 158 CBU/g solids | 89 | | | |
| | Sugar cane bagasse | TEBAC/LA (1:9); t = 4 h | | Celluclast 1.5 L = | | | | [209] |
| | | T = 100 °C | 62; 67 | 35 FPU/g solids (= 62) | 75 | | | |
| | | T = 120 °C | 74; 89 | 35 FPU/g solids (= 51) | 88 | | | |
| | | | | 15 FPU/g solids (= 22) | 72 | | | |
| | | T = 140 °C | 89; 94 | 35 FPU/g solids (= 50) + β-G = 82 CBU/g solids | 85 | | | |
| | Hybrid pennisetum | T = 120C; t = 6 h ChCl:glycerol:$FeCl_3$ (mole) = | | CTec 2 = 15 FPU/g solids or | | | | [211] |
| | | 62:124:0 | 15; 56 | 28.9 | 42 | | | |
| | | 62:124:1 | 94; 79 | 18.5 | 100 | | | |
| | | t = 4 h; 62:124:1 | 92; 74 | 18.2 | 92 | | | |
| | | t = 2 h; 62:124:1 | 90; 67 | 19.1 | 71 | | | |
| | | t = 1 h; 62:124:1 | 65; 44 | 24.0 | 40 | | | |
| MAHF | Wheat straw hammer milled (8 mm long) | Maleic acid = 40 wt% | | CTec 3 = | | 13,200; 3[b] | 51[b] | [157] |
| | | T = 80 °C; t = 120 min | 66; 38 | 10 | 42 | 8100; 2.2 | 50 | |
| | | Maleic acid = 60 wt% | | | | | | |
| | | T = 100 °C; t = 30 min | 67; 54 | 10 | 78 | 7010; 1.9 | 38 | |
| | | T = 120 °C; t = 60 min | 76; 57 | 10 | 89 | 5730; 1.9 | 25 | |
| AHF (*p*-TsOH) MAHF (MA) | Switch grass (10 mm) | *p*-TsOH = 40 wt% | | CTec 3 = | | 13,090; 2.7[b] | 47[b] | [158] |
| | | T = 80 °C; t = 60 min MA = 50 wt% | 57; 42 | 10 | 51 | 6070; 2.2 | 33 | |
| | | T = 90 °C; t = 90 min *p*-TsOH = 60 wt% | 67; 42 | 10 | 57 | 7010; 1.9 | 38 | |
| | | T = 80 °C; t = 90 min MA = 60 wt% | 76; 58 | 10 | 54 | 2300; 1.6 | 21 | |

(*continued on next page*)

**Table 4** (*continued*)

| A: Herbaceous biomass | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Process | Biomass | Fractionation condition | Xylan; lignin removal (%) | Enzyme loading [a] | SED (%) | Mw (Da); PDI of DL | β–O–4 of DL (%) | Ref. |
| | | T = 120 °C; t = 30 min | 79; 58 | 10 | 79 | 3070; 1.6 | 29 | |
| **B: Woody biomass** | | | | | | | | |
| Dilute acid SPORL | Poplar chips | T = 175 °C; t = 24 min | | CTec 3 = | | | | |
| | | $H_2SO_4$ = 0.2%; | 83; 3 | 10 | 69 | | | [197] |
| | | + $NaHSO_3$ = 4% (on wood) | 87; 28 | 10 | 97 | | | [197] |
| SPORL | Douglas fir residue chips | T = 165 °C; t = 75 min | 79; 35 | CTec 2 = | | | | [198] |
| | | $H_2SO_4$ = 2.2%; $NaHSO_3$ = 12% (both on wood) | | 15 | 91 | | | |
| | | T = 145 °C; t = 240 min; | 92; 45 | CTec 3 = | | | | [187] |
| | | $SO_2$: free = 2.3% or total = 6.6%; Ca $(HSO_3)_2$ = 6.5% (on wood) | | 15 | 97 | | | |
| Organosolv (Acetone) | Birch particles (2 mm) | Acetone = 50 wt%+ $H_2SO_4$ = 3.9 g/L | | Accelerase TRIO = 10 FPU/ | | | | [231] |
| | | T = 140 °C; t = 120 min | 92; 86 | g solids (= 14) | ~90 | | | |
| Organolsolv (Ethanol) | Birch residue | $SO_2$ + ethanol + $H_2O$ (12 + 44 + 44) wt %; T = 150 °C; t = 30 min | 80; 93 | CTec2 = 8 | ~95 | | | [233] |
| Organosolv (GVL) | Hardwood (3–10 mm) | GVL = 80% + $H_2SO_4$ = 75 mM | | | | | | [230] |
| | | T = 120 °C; t = 60 min | 79; 77 | | | | | |
| | | Without alkali incubation | | 15 | 55 | | | |
| | | With alkali incubation | | 15 | 99 | | | |
| Organosolv (Ethanol) | Poplar (<1/4″) | 50% (v/v) ethanol with 1.25 wt% $H_2SO_4$ | 90; 85 | Celluclast 1.5 L = 20 + | ~97 | | | [130] |
| | | T = 180 °C; t = 60 min | | Novozymes 188 = 40 IU/g cellulose | | | | |
| | Lodgepole pine chips | 65% (v/v) ethanol with 1.1 wt% $H_2SO_4$ | 95; 83 | Celluclast 1.5 L = 20 + | ~97 | | | [244, 245] |
| | | T = 170 °C; t = 60 min | | Novozymes 188 = 40 IU/g cellulose | | | | |
| Alkaline oxidation Steam explosion | Birch chips | $Na_2CO_3$ = 26.5 g/L | | Celluclast 1.5 L = 10 FPU/g | | | | [246] |
| | | T = 120 °C; t = 1200 min | ~100; NA | solids + Novozymes 188 | ~95 | | | [246] |
| | | Steam + $H_2SO_4$ = 5 g/L | | | | | | |
| | | T = 200 °C; t = 5 min | ~100; NA | | ~95 | | | |
| $SO_2$ steam explosion | Douglas fir Wood chips | 4.5 wt% $SO_2$; T = 195 °C; t = 4.5 min | | Celluclast 1.5 L = 40 + Novozymes 188 = 40 or 80 IU/g cellulose | ~33 or ~57 | | | [225] |
| | Spruce | 5 wt% $SO_2$; T = 205 °C; t = 6–7 min | | CTec 2 = 10 FPU/g solids) + β-G | EtOH yield = 72% | | | [247] |
| THF | Poplar wood chips | THF = 50 wt%; t = 15 min | | | | | | [248] |
| | | $H_2SO_4$ = 0.25%, T = 150 °C | ; 65 | | | 9400; 5.4 | 41 | |
| | | $H_2SO_4$ = 0.5%, T = 150 °C | ; 69 | | | 8750; 5.6 | 35 | |
| | | = 160 °C | ; 76 | | | 3900; 3.0 | 16 | |
| | | = 180 °C | ; 94 | | | 3300; 2.8 | 1 | |
| IL | Eucalyptus *red grandis* | $[N_{2220}][HSO_4]$ = 80 wt% | | Novozymes NS-22201 = 0.5 | | | | [235] |
| | | T = 120 °C; 60 min | 36; 48 | mL/g solids | 10 | 5259; 3.1 | 30 | |
| | | T = 120 °C; 120 min | 79; 59 | | 16 | 4344; 3.1 | 20 | |
| | | T = 120 °C; 240 min | 90; 74 | | 63 | 4181; 2.7 | 14 | |
| | | T = 150 °C; 60 min | 100; 92 | | 100 | 3029; 2.7 | 5 | |
| | Pine | [HBim][$HSO_4$] = 80 wt% | | Novozymes NS-22201 = 0.5 | | | | [234] |
| | | T = 120 °C, t = 60 min | 64; 40 | mL/g solids | 8 | | 22 | |
| | | T = 150 °C, t = 30 min | 68; 49 | | 18 | | | |
| | | T = 150 °C, t = 60 min | 85; 79 | | 67 | | 10 | |
| | | T = 170 °C, t = 30 min | 94; 81 | | 76 | 4000, 2.5 | 10 | |
| | | T = 170 °C, t = 60 min | 99; 77 | | 83 | | 5 | |
| | Pine | [DMBA][$HSO_4$] = 80 wt% | | CTec 2 = | | | | [237] |
| | | T = 170 °C, t = 80 min | 98; 70 | 0.5 mL/g solids | 76 | 4500; 2.2 | 4 | |
| DES | Eucalyptus saw dust | ChCl/LA (1:10) | | CTec 2 = | | | | [240] |
| | | T = 90 °C; t = 6 h | 64; 39 | 15 FPU/g solids (= 36) | 57 | 1790; 1.4 | 27 | |
| | | T = 100 °C; t = 6 h | 69; 45 | 15 FPU/g solids (= 35) | 74 | 1825; 1.4 | 23 | |
| | | T = 110 °C; t = 6 h | 89; 82 | 15 FPU/g solids (= 22) | 94 | 1990; 1.3 | 12 | |
| AHF (*p*-TsOH) | Poplar fibers | *p*-TsOH = 50 wt% | | | | | | [249] |
| | | T = 90 °C; t = 112 min | 80; 84 | CTec 3 = 20 | 93 ± 2 | | | |
| MAHF (MA) | Birch fibers | MA = 50 wt% | | | | 14,830; 4.6[b] | 65[b] | [151] |
| | | T = 100 °C; t = 30 min | 69; 48 | CTec 3 = 10 | 76 ± 4 | 2380; 1.8 | 54 | |
| | | T = 100 °C; t = 60 min | 70; 49 | CTec 3 = 10 | 89 ± 3 | 1640; 1.5 | 27 | |
| AHF (*p*-TsOH) | | MA = 60 wt%; t = 30 min | | CTec3 = | | 14,830; 4.6[b] | 65[b] | [83] |
| | | T = 110 °C | 58; 48 | 10 | 85 | 1600; 1.5 | 41 | |
| | | T = 120 °C | 67; 63 | 5 | 71 | 1520; 1.5 | 27 | |
| | | | | 7.5 | 90 | | | |
| | | | | 10 | 98 | | | |
| | | *p*-TsOH = 60 wt%; t = 20 min T = 85 °C | 61; 63 | 10 | 54 | 3290; 2.4 | 4 | [83] |

[a] Unit in FPU/(g substrate glucan) unless indicated.
[b] Data for MWL of the feedstock as reference.

solvents (including water) is always desirable. Lignin from sugar production can be classified into two categories, the dissolved (isolated) lignin (DL) from biomass during pretreatment/fractionation and the residual lignin (RL) in the solid residues after saccharification (both acidic and enzymatic hydrolysis). The following general descriptions can be applied to DL and RL.

(1) DL has better purity (many are carbohydrates free) than RL. The latter is typically contaminated with unhydrolyzed cellulose, enzymes, and yeasts. As a result, RL is usually not considered as a good lignin for co-product development due to its impurity.
(2) In general, DL has a lower molecular weight (and likely narrow distribution) due to the extensive depolymerization during the pretreatment than the corresponding RL. The RL usually has a higher molecular weight and higher density (condensed) of carbon–carbon linkages inherited from native lignin and/or newly formed during the pretreatment.
(3) Acidic processes (both pretreatment and hydrolysis, in particular, high-temperature ones) lead to more lignin condensation, as described in Scheme 1C, than basic processes.
(4) Most organosolv processes produce a high-quality DL, e.g., high purity, low and narrow molecular weight, good thermal properties, and excellent solubility in organic solvents [214].
(5) Sulfite-based SPORL process not only produces less inhibitory substrate for enzymatic hydrolysis, as discussed above, but also has the potential to produce lignosulfonate [258,259], the most successful commercial lignin product so far.
(6) Maleic acid hydrotropic fractionation also produced readily enzymatically digestible substrates, it also produced carboxylated lignin (DL and RL) with light color lignin and low extent of condensation and low MW with narrow distribution [83,157,158]. This lignin can be attractive for a variety of applications.
(7) Some ionic liquid-based processes that can dissolve and depolymerize lignin produce high-quality lignin like organosolv lignin, while others may cause significant lignin condensation [260].
(8) The direct saccharification process using molten salt hydrate, as described above, can produce unique lignin with high purity, low and narrow molecular weight, limited condensation, and high reactivity (more phenolic hydroxyl from extensive depolymerization and demethylation) [66].

Several recent studies on biomass pretreatment/fractionation evaluated lignin properties as listed in Table 4 in response to the interest in lignin valorization [83,158,235,237,240,248,261]. The characterization of DL includes lignin molecular weight and distributions using gel permeation chromatography (GPC) and chemical structures using 2D $^{1}H–^{13}C$ nuclear magnetic resonance (NMR) spectroscopy. Lignin glass transition temperature was also analyzed in some studies using differential scanning calorimetry (DSC) [261]. 2D $^{1}H–^{13}C$ NMR analysis can semi-quantitatively determine the lignin units and their linkages. Unfortunately, quantification of the extent of lignin condensation (new C–C bond formation) has not been implemented and is not available in literature, despite the fact that it can be done theoretically.

Some studies showed that the fractionated DL with high amount of β–O–4 ether aryl linkages is favorable for producing lignin aromatics through depolymerization [262,263]. A process that can isolate lignin with high residual β–O–4 linkage is usually operated under mild conditions that are not severe enough to extensively cleave β–O–4 linkages and very likely do not cause significant lignin condensation either, both of which are favorable to down-stream lignin valorization. Therefore, high residual β–O–4 linkages in lignin can be an indication of good reactivity for depolymerization under mild conditions [263–265]. For example, native lignin contains an abundant of β–O–4 linkages (up to 70 per 100 aromatic units) and is easy to cleave. Traditional fractionation processes such as wood pulping often result in condensed lignin (increased density of C–C linkages) [266] and β–O–4 linkages in DL are always decreases as pulping proceeds [267–269], which limited the potential of technical lignin for efficient valorization. In general, increasing pretreatment/fractionation severity decreases β–O–4 linkages of DL, and lignin condensation occurs as fractionation severity increases, similar to that observed in wood pulping [267–269].

It is certainly desirable if a pretreatment/fractionation process can isolate a reactive DL (e.g., more β–O–4 and less C–C linkages) while generate a readily digestible substrate. However, most processes cannot achieve both. Hydrogen sulfate IL system can achieve over 90% glucan conversion but result in a significant decrease (cleavage) in β–O–4 linkages of DL. For example, when pine was pretreated using [HBim][$HSO_4$] of 80 wt% at 170 °C for 30 min, near complete conversion of cellulose (based on using undried sample, the data in Table 4 were from air-dried sample) was obtained [234], but the resultant DL had a β–O–4 linkage content of only 10% (Table 4). Similarly, the DL from THF fractionation had low β–O–4 linkages of 15% [248] at conditions for over 90% delignification to achieve excellent saccharification [229]. DES systems with long reaction of several hours also substantially decreased β–O–4 linkages and probably well condensed lignin. For example, the ChCl/LA DES system achieved SED of 94% at cellulase loading of 22 FPU/g glucan but resulted in low β–O–4 linkages of 15% after eucalyptus was pretreated at 110 °C for 6 h [240].

In comparison, MAHF was able to preserve more β–O–4 linkages or reactivity. For example, birch wood fractionated at 110 °C for 30 min resulted in a dissolved lignin with β–O–4 linkages of 41%, comparing with 65% β–O–4 in birch MWL, and a SED of 85% at a lower cellulase loading of 10 FPU/g glucan [83]. As another example, MAHF of wheat straw at 100 °C for 30 min resulted in a dissolved lignin with β–O–4 linkages of 38% (51% in wheat straw MWL) and SED of 78% at cellulase loading of 10 FPU/g glucan [157].

The extent of lignin condensation can be indirectly seen from the molecular weight distributions of DL. The stronger high molecular weight peak observed in THF lignin [248] is indicative of a higher degree of lignin repolymerization (condensation), compared to a weaker shoulder observed in MAHF lignin [83]. Because quantitative information on lignin condensation are not available in literature, β–O–4 linkages can be used as an indirect indication of lignin reactivity [263–265]. The amount of β–O–4 linkages in DLs from different fractionation processes with respect to their corresponding substate digestibility was compared. The data for MAHF samples fall to the most top left corner as shown in Fig. 8, i.e., higher β–O–4 linkage content and greater SED than those from other processes. It should be noted that a lower cellulase dosage was used in the MAHF study than other solvent systems. The results clearly indicate the advantages of MAHF over other solvent systems.

## 6. Perspectives and future research needs

Economic sugar production from lignocellulosic plant biomass remains a challenge to sustainable biorefineries. Pretreatment/fractionation is effective to remove the strong recalcitrance of lignocellulose to deconstruction for hydrolyzing carbohydrates into sugars. However, pretreatment/fractionation is expensive. An ideal pretreatment/fractionation should have the following desired features: (1) producing a readily hydrolysable cellulosic substrate and a high recovery yield of all carbohydrates (cellulose and hemicelluloses) with no or minimal formation of fermentation inhibitors, (2) capable of separating or fractionating hemicelluloses and “good” lignin from cellulose for value-added co-products development, (3) using low-cost, recyclable, and environmentally benign chemicals with low energy input and costs for

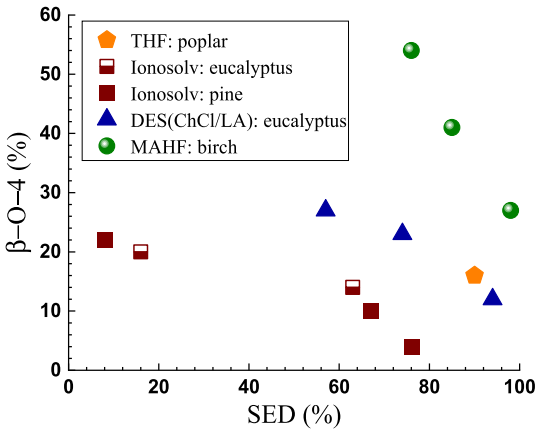



**Fig. 8.** Comparisons of pretreatment/fractionation selectivity in terms of achieving high substrate enzymatic digestibility (SED) while preserving dissolved lignin ether aryl β–O–4 linkages. Except for MAHF, β–O–4 of MWL were not available. SED of THF was unavailable and estimated at 90% from Nguyen [229]. Ionosolv SED data were obtained using air dried samples from Refs. [234,235,237] and estimated to shift right by 15–30%. DES data from Ref. [240]. MAHF data used a much lower cellulase dosage of 10 FPU/g glucan and β–O–4 of MWL = 65% from Ref. [83].

capital and operation, and (4) of course easily scalable up to thousands of tons biomass/day. Unfortunately, none of the existing pretreatment technologies meets all these criteria. Probably, there will never be a such ideal pretreatment. However, these criteria should be used to guide selecting, evaluating, and developing pretreatment/fractionation technologies. For example, while most solvent-based fractionation processes can produce good sugar yield and favorable lignin properties, some solvent systems such as IL and DES have the versatility of using a variety of chemicals with low toxicity and potential recoverability. However, the great amounts of solvent (especially nonaqueous solvents, such as DES) used in some processes are still a significant challenge for commercial implementation, as reflected in the low TRLs, because a highly efficient chemical (solvent) recovery (99% or greater) is difficult but required for producing commodity sugars using these solvent systems. At the scale of over 1000 tons biomass per day, even a minor inefficiency in chemical recovery would result in a great amount of chemicals released to the environment, which can be a great concern, especially when the full toxicity and total environmental risk of these chemicals have not been fully evaluated [270–273]. This key challenge has been underestimated by the research community in choosing solvent systems, especially nonaqueous solvent systems.

Enzyme cost remains significant. Developing efficient cellulases can reduce the extent of pretreatment/fractionation and thereby reduce the cost for pretreatment/fractionation and provide greater flexibility for selecting environmentally friendly and recyclable chemicals. With advances in microbiology, developing new enzymes can be one of the most fruitful strategy to achieve sustainable biorefinery [274]. The discovery of oxidative cleavage of glycosidic bonds by Lytic Polysaccharide MonoOxygenases (LPMOs) in 2010 [91] is a great example that shows novel enzymes can improve lignocellulose saccharification [275–277].

With advances in enzymology, novel enzymes can facilitate the implementation of the novel concept of integrating the production of sugars with cellulose nanomaterials (CNMs) outlined by Zhu's group [278], in which the crystalline cellulose that is more difficult to be hydrolyzed is used for producing CNMs, rather than glucose, so that the extent of pretreatment and hydrolysis can be decreased to reduce processing and enzyme costs. Furthermore, CNMs are high value and can be used for a variety of applications [23,279,280]. A recent study indicate that multifunctional enzymes are better suited for integrating CNM with sugar/biofuel production [281].

For successful commercial lignin valorization, one key aspect is to identify the key chemical and physical properties required for a specific lignin co-product. Despite difficulties, several pretreatment and fractionation technologies can tune the resultant lignin properties. Unfortunately, existing literature contains very limited information on desired lignin properties for a lignin co-product, which impedes the development of value-added lignin products. There has been much research focus on lignin depolymerization for producing lignin aromatics for biochemicals [263,265,282]. However, attention should also be paid to using lignin as a polymer for biomaterials production, which can substantially decrease the extent of further processing for commercialization. Recently, lignin nanoparticles (LNPs) attracted great interest in the research community [283–286].

Finally, it should be emphasized that despite most fruitful research being focused in the downstream such as developing co-products and novel robust enzymes, pretreatment remains the bottleneck for biorefinery. The research community and funding agencies should not overlook this upstream process, because it affects everything in downstream and dictates the successful commercialization of biorefinery. Small improvements in pretreatment/fractionation, such as those made by the recent maleic acid hydrotropic fractionation process [151,156] over traditional solvent processes in terms of easing chemical recovery and decreasing environmental impact by using an indirect food additive at a lower loading, can yield substantial benefits to the overall process runnability, environmental sustainability, and economics of the entire biorefinery.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Zhu and Pan are the co-inventors of the SPORL process. Zhu is also a co-inventor of the acid including maleic acid hydrotropic fractionation process. Pan is a co-inventor of the inorganic molten salt hydrate sugar process.

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