Overview of biomass pretreatment for cellulosic ethanol production

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Abstract: Bioconversion of lignocellulosic biomass to ethanol is significantly hindered by the structural and chemical complexity of biomass, which makes these materials a challenge to be used as feedstocks for cellulosic ethanol production. Cellulose and hemicellulose, when hydrolyzed into their component sugars, can be converted into ethanol through well established fermentation technologies. However, sugars necessary for fermentation are trapped inside the crosslinking structure of the lignocellulose. Hence, pretreatment of biomass is always necessary to remove and/or modify the surrounding matrix of lignin and hemicellulose prior to the enzymatic hydrolysis of the polysaccharides (cellulose and hemicellulose) in the biomass. Pretreatment refers to a process that converts lignocellulosic biomass from its native form, in which it is recalcitrant to cellulase enzyme systems, into a form for which cellulose hydrolysis is much more effective. In general, pretreatment methods can be classified into three categories, including physical, chemical, and biological pretreatment. The subject of this paper emphasizes the biomass pretreatment in preparation for enzymatic hydrolysis and microbial fermentation for cellulosic ethanol production. It primarily covers the impact of biomass structural and compositional features on the pretreatment, the characteristics of different pretreatment methods, the pretreatment study status, challenges, and future research targets.

Keywords: lignocellulosic biomass, pretreatment, cellulosic ethanol, enzymatic hydrolysis, fermentation, cellulose, hemicellulose, lignin

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1 Introduction

The transfer of crude oil-based refinery to biomass-based biorefinery has attracted strong scientific interest which focuses on the development of cellulosic ethanol as an alternative transportation fuel to petroleum fuels. The U.S. fuel ethanol industry represents an on-going success story for the production of renewable fuels. According to the BiofuelsDigest[1], U.S. ethanol production capacity reached 7.5 billion gallons by the end of 2007, a 40% increase over 2006, and national production capacity increased to 13.3 billion gallons at

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136 facilities in 2008 based on the completion of all existing projects. This industry forms an infrastructure from which future growth in cellulosic substrates utilization may occur. Demand for fuel ethanol is expected to increase. Currently practiced technologies in fuel ethanol industry are primarily based on the fermentation of sugars derived from starch and sugar crops and are quite mature with little possibility of process improvements. However, the conversion of starch and sugar crops to ethanol also has concerns since it draws its feedstock from a food stream.

Lignocellulosic biomass, including forestry residue, agricultural residue, yard waste, wood products, animal and human wastes, etc., is a renewable resource that stores energy from sunlight in its chemical bonds[2]. It has great potentials for the production of affordable fuel ethanol because it is less expensive than starch (e.g. corn) and sucrose (e.g. sugarcane) producing crops and available in large quantities. Lignocellulosic biomass typically contains 50%–80% (dry basis) carbohydrates that are polymers of 5C and 6C sugar units. Most carbohydrates can be processed either chemically or biologically to yield biofuels such as ethanol. The prerequisite in the utilization of lignocellulose for ethanol production is to efficiently yield a fermentable hydrolyzate rich in glucose from the cellulose content present in the feedstock. Employment of enzymes for the hydrolysis of lignocellulose is considered the prospectively most viable strategy to offer advantages over other chemical conversion routes of higher yields, minimal byproduct formation, low energy requirements, mild operating conditions, and environmentally friendly processing[3,4]. Although the enzymatic route has the highest cost at present, it has long-term potential for cost reductions compared to other more established routes such as concentrated acid and two-stage dilute acid hydrolysis[5]. The physicochemical and structural compositions of native lignocellulose are, however, recalcitrant to direct enzymatic hydrolysis of cellulose. Therefore, pretreatment step is invariably required to render the cellulose amenable to enzymatic attack prior to the enzymatic hydrolysis as represented in the schematic diagram of Figure 1[6].

The overall purpose of pretreatment is to break down the shield formed by lignin and hemicellulose, disrupt the crystalline structure and reduce the degree of polymerization of cellulose. Pretreatment has been viewed as one of the most expensive processing steps within the conversion of biomass to fermentable sugar[6]. With the advancement of pretreatment technologies, the pretreatment is also believed to have great potential for the improvement of efficiency and reduction of cost[7,8].

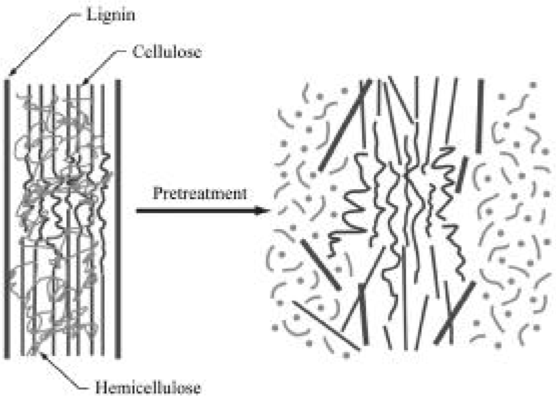


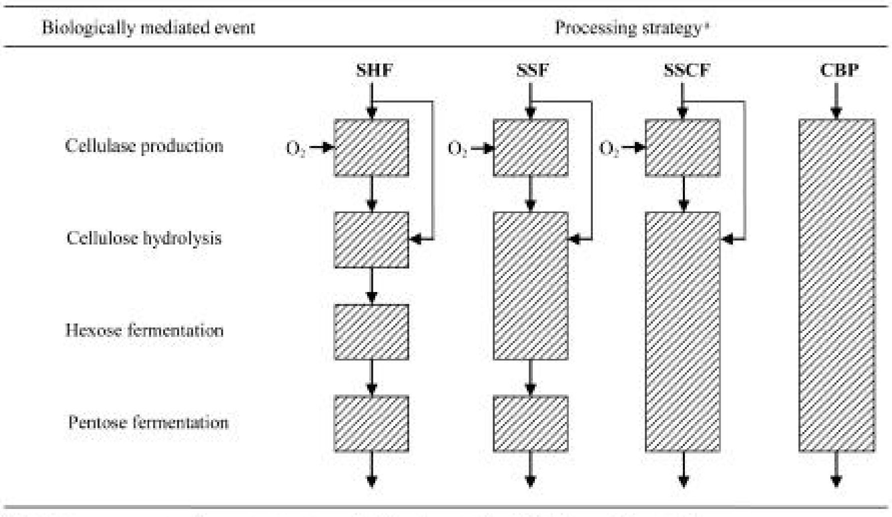
Figure 1 Schematic of pretreatment effect on lignocellulosic biomass[6]

Pretreatment techniques have been developed for various end uses of biomass feedstocks. The subject of this paper emphasizes the biomass pretreatment in preparation for enzymatic hydrolysis and microbial fermentation for cellulosic ethanol production. It primarily covers the impact of biomass structural and compositional features on the pretreatment, the action mode of different pretreatment methods, the pretreatment study status, challenges, and future research targets.

2 Conversion technology for cellulosic ethanol production

Production of ethanol from lignocellulosic biomass contains three major processes, including pretreatment, hydrolysis, and fermentation. Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic structural and chemical composition to facilitate rapid and efficient hydrolysis of carbohydrates to fermentable sugars[9]. Hydrolysis refers to the processes that convert the polysaccharides into monomeric sugars. The fermentable sugars obtained from hydrolysis process could be fermented into ethanol by ethanol producing microorganisms, which can be either naturally occurred or genetically modified. Cellulose in lignocellulosic biomass is usually organized into microfibrils, each measuring about 3 to 6 nm in diameter and containing up to 36 glucan chains having thousands of glucose residues. According to the degree of crystallinity, cellulose is classified into crystalline and paracrystalline (amorphous) cellulose. Cellulose can be hydrolytically broken down into glucose either enzymatically by cellulytic enzymes or chemically by sulfuric or other acids. Hemicellulose, a branched polymer composed of pentose (5-carbon) and hexose (6-carbon) sugars, can be hydrolyzed by hemicellulases or acids to release its component sugars, including xylose, arabinose, galactose, glucose and/or mannose. Hexoses such as glucose, galactose, and mannose are readily fermented to ethanol by many naturally occurring organisms, but the pentoses including xylose and arabinose are fermented to ethanol by few native strains, and usually at relatively low yields. Since xylose and arabinose generally comprises a significant fraction of lignocellulosic biomass, especially hardwoods, agricultural residues and grasses, it must be utilized to make economics of biomass ethanol processing feasible. The development of recombinant ethanogenic strains resulted in bacteria and yeasts capable of co-fermenting pentoses and hexoses into ethanol and other value-added products at high yields[10-14].

According to the consideration of cellulase production and the process configuration of hydrolysis and fermentation, four biologically mediated events, as shown in Figure 2, could occur in the course of the ethanol production from cellulosic biomass using enzymatic hydrolysis, including cellulase production, cellulose hydrolysis, hexose fermentation, and pentose fermentation[15]. Process configurations, including Separate Hydrolysis and Fermentation (SHF), Simultaneously Saccharification and Fermentation (SSF), Simultaneously Saccharification and Co-Fermentation (SSCF), and Consolidated Bioprocessing (CBP), proposed for the biological steps differ in the degree to which these events are integrated.



[a] Each box represents a bioreactor (not to scale). See the text for definitions of abbreviations.

Figure 2 Consolidation of biologically mediated events in cellulosic ethanol production[15]

After fermentation, ethanol can be recovered from the fermentation broth by distillation or distillation combined with adsorption or filtration, including drying using lime or a salt, addition of an entrainer, molecular sieves, membranes, and pressure reduction[16-19]. The distillation residual solid, including lignin, ash, enzyme, organism debris, residue cellulose and hemicellulose, and other components may be recovered as solid fuel or converted to various value-added co-products[20].

3 Effects of biomass compositional and structural features on bioconversion of biomass

Structural and compositional features of lignocellulosic biomass form strong barriers to the biodegradation so that the pretreatment is a necessary step to alter the structure of biomass and increase the bio-digestibility of biomass. The enzymatic digestibility of native cellulose in biomass is usually less than 20% unless extremely excessive enzyme dose is used. The relationship between the structural and compositional features of biomass and the enzymatic hydrolysis rate has been the subject of extensive research and reviews, but is still unclear[15,21,22]. Such features, including cellulose crystallinity, specific surface area of cellulose, degree of polymerization, cellulose protection by lignin and hemicellulose, and degree of hemicellulose acetylation, are commonly considered rate-impacting factors[9,23-25]. However, opinions differ as to each factor's relative contribution to the native recalcitrance of biomass to enzymatic hydrolysis. The relationship among structural and compositional factors reflects the complexity of the lignocellulosic biomass matrix. The variability of these characteristics explains the change of enzymatic digestibility among different sources of biomass. It also indicates the fact that the enzymatic digestibility of biomass is substrate- and pretreatment method-specific, which means that one barrier is reduced and/or eliminated while another may become limiting. In principle, an effective pretreatment method or a combination of multiple pretreatments is expected to disrupt multiple barriers so that hydrolytic enzymes can access and hydrolyze cellulose more easily.

Since compositional and structural features are closely associated and the change in one feature may also lead to changes in the others, it is difficult even impossible to exclusively study the effect of some feature on the enzymatic digestibility of biomass. An ideal pretreatment technique should be able to maximize the recovery of available carbohydrates such as cellulose and hemicellulose while minimizing the degradation of sugars and the generation of possible inhibitors. The correlations between biomass features and enzymatic digestibility of cellulosic biomass are shown in Table 1. Again, there are contradictory conclusions on the effects of several features. The possible reasons would be: (1) these features are sensitive to pretreatment; (2) close associations among features make studies on the individual effect difficult even impossible; (3) the effects of different features are usually overlapped each other.

Table 1 Correlation between biomass features and enzymatic digestibility of biomass[26]

Biomass features	Relationship between features and digestibility
Cellulose crystallinity	Negative or no correlation
Degree of polymerization	Negative or no correlation
Specific surface area	Positive
Cellulose protection by lignin	Negative
Hemicellulose sheathing	Negative
Degree of hemicellulose acetylation	Negative or no correlation

4 Pretreatment technologies

Based on the application and type of pretreatment catalyst (liquid and steam water are not considered a catalyst in this paper), pretreatment techniques have generally been divided into three distinct categories, including physical, chemical, and biological pretreatment. Combination pretreatment by incorporating two or more pretreatment techniques from the same or different categories is also common[22, 27], but it is not grouped as an individual pretreatment category.

Various pretreatment technologies have been extensively studied to process different biomass for cellulosic ethanol production. However, none of those can be declared a "winner" because each pretreatment has its intrinsic advantages and disadvantages. An effective pretreatment is characterized by several criteria: avoiding size reduction, preserving hemicellulose fractions, limiting formation of inhibitors due to degradation products, minimizing energy input, and being cost-effective[28]. Except for these criteria, several other factors are also needed to be considered, including recovery of high value-added co-products (e.g., lignin and protein), pretreatment catalyst, catalyst recycling, and waste treatment. When comparing various pretreatment options, all the mentioned criteria should be comprehensively considered as a basis. In addition, pretreatment results must be weighed against their impact on the ease of operation and cost of the downstream processes and the trade-off between several costs, including operating costs, capital costs, and biomass costs[29,30].

4.1 Physical pretreatment

Physical pretreatment does not use chemical agents, and typically includes uncatalyzed steam explosion, liquid hot water pretreatment (LHW), mechanical comminution, and high energy radiation. The former two pretreatment methods are more common than the later.

4.1.1 Uncatalyzed steam-explosion

Uncatalyzed steam-explosion is also named autohydrolysis, in which only steam water is used. It is one of the most common pretreatment methods for lignocellulosic biomass. Extensive research work has been done on this method[22,27,31]. It is one of only a very limited number of cost-effective pretreatment technologies that have been advanced to pilot scale demonstration and commercialized application. Commercial steam-explosion equipment is available. In this method, biomass particles are rapidly heated by high-pressure saturated steam for a period time to promote the hemicellulose hydrolysis. This process is terminated by swift release of pressure, which renders the biomass undergo an explosive decompression. During the pretreatment, the hemicellulose is often hydrolyzed by organic acids such as acetic acids and other acids formed from acetyl or other functional groups, released from biomass. In addition, water, itself, also possesses certain acid properties at high temperature, which further catalyze hemicellulose hydrolysis[32]. Therefore, the degradations of sugars might happen during uncatalyzed steam-explosion due to acidic conditions[33,34].

The action mode of uncatalyzed steam-explosion is similar to that of acid-aid chemical pretreatment, except that, during steam-explosion, the biomass is heated rapidly by steam so that much less moisture exists in the reactor resulting in much more concentrated sugars in comparison. The key factors for uncatalyzed steam-explosion are treatment time, temperature, particle size and moisture content[35,36]. Uncatalyzed steam-explosion is typically conducted at a temperature of 160–270℃ for several seconds to a few minutes before pretreated contents are discharged into a vessel for cooling. Lower temperature and longer residence time are more favorable than higher temperature and shorter time[37], and the use of very small particles in steam explosion would not be favorable in optimizing the effectiveness of the process for improved economics[38].

The major physicochemical changes of lignocellulosic biomass during the uncatalyzed steam-explosion are attributed to the hemicellulose removal and lignin transformation. These changes improve the digestibility of biomass to enzymes. Enzymatic digestibility of pretreated poplar chips reached 90%, compared to only 15% hydrolysis of untreated chips[39]. In addition, the rapid thermal expansion opens up the biomass particle structure leading to the reduction of particle size and increased pore volume[40]; however, it was believed less important in enhancing the digestibility of steam exploded pretreated lignocellulosic biomass[41,42]. Although uncatalyzed steam-explosion can effectively improve the digestibility of the pretreated cellulose residue, it suffers from low hemicellulose sugar yield[37,43,44]. Therefore, it is still a problematic option for long-term ethanol production.

4.1.2 Liquid hot water (LHW) pretreatment

In liquid hot water pretreatment, pressure is utilized to maintain water in the liquid state at elevated temperatures[45-48]. Biomass undergoes high temperature cooking in water with high pressure. LHW pretreatment has been reported to have the potential to enhance cellulose digestibility, sugar extraction, and pentose recovery, with the advantage of producing prehydrolyzates containing little or no inhibitor of sugar fermentation[49]. The sugar-enriched prehydrolyzates can be directly fermented to ethanol. It has been shown to remove up to 80% of the hemicellulose and to enhance the enzymatic digestibility of pretreated biomass materials such as corn fiber [50] and sugarcane bagasse[51]. Perez et al.[52] used LHW to pretreat wheat straw and obtained maximum hemicellulose-derived sugar recovery of 53% and enzymatic hydrolysis yield of 96%. Perez et al.[53] continued to optimize process variables (temperature and residence time) in LHW pretreatment of wheat straw and achieved 80% and 91% xylose recovery and enzymatic hydrolysis, respectively. LHW reduces the need for neutralization of liquid streams and conditioning chemicals since acid is not added[6,47,52]. Additionally, biomass size reduction is not needed because the particles are broken apart during pretreatment; therefore, LHW appears attractive for large scales[32].

During LHW pretreatment, the cleavage of *O*-acetyl and uronic acid substitutions from hemicellulose produces acetic acid and other organic acids which help catalyze the hydrolysis of polysaccharide such as hemicellulose into soluble oligosaccharides first, and then monomeric sugars. Under acidic condition, these monomeric sugars are subsequently partially degraded to aldehydes such as furfural and 5-HMF which are inhibitors to fermenting microorganisms. In additional, hot water has an unusually high dielectric constant and enables to dissolve almost all hemicellulose and a certain amount of lignin, depending on the temperature. Therefore, hot water, itself, plays a role like an acid to hydrolyze hemicellulose to release sugars and acids[54].

4.1.3 Mechanical comminution

Biomass materials can be comminuted by various chipping, grinding and milling. The milling can be further detailed into hammer- and ball-milling (wet, dry, vibratory rod/ball milling)[55,56], compression milling[57,58], ball milling/beating[59], agitation bead milling [60], pan milling[61], and other types of milling (fluid energy milling, colloid milling, two roll milling)[62,63]. In addition, attrition [55] and disk refining[59] were also used for pretreatment. Biomass was also pretreated by simultaneous ball milling/attrition and enzymatic hydrolysis[64,65]. Vibratory ball milling was found to be more effective than ordinary ball milling on the improvement of biomass digestibility when used to pretreat spruce and aspen chips[66]. Among all the mechanical comminution techniques, the compression milling is the only process that has been tested in production-scale[27].

Mechanical comminution primarily disrupts cellulose crystallinity, decreases the degree of polymerization, and increases the specific surface area of cellulosic biomass by breaking down the biomass into smaller particles, rendering the substrate more amenable to subsequent enzymatic hydrolysis. However, the mechanical comminution is time-consuming, energy-intensive and expensive; furthermore, mechanical comminution is much less effective than chemical pretreatments since it does not result in lignin removal, which has been proved to significantly restrict accessibility of cellulose and inhibit cellulases. Therefore, it is seldom used at present as a pretreatment method exclusively.

4.1.4 High energy radiation

Digestibility of cellulosic biomass has been enhanced by the use of high energy radiation methods, including γ-ray[67,68], ultrasound[69-71], electron beam[72,73], pulsed electrical field[74], UV[75], and microwave heating[76-79]. The action mode behind the high energy radiation could be one or more changes of features of cellulosic biomass, including increase of specific surface area, decrease of the degrees of polymerization and crystallinity of cellulose, hydrolysis of hemicellulose, and partial depolymerization of lignin. However, these high energy radiation methods are usually slow, energy-intensive, and prohibitively expensive[80,81]. They also appear to be strongly substrate-specific[75]. As a result, high energy radiation techniques lack commercial appeal based on current estimation of overall cost.

4.2 Chemical pretreatment

Chemical pretreatments were originally developed and have been extensively used in the paper industry for delignification of cellulosic materials to produce high quality paper products. The possibility of developing effective and inexpensive pretreatment techniques by modifying the pulping processes has been considered[62]. Chemical pretreatments that have been studied to date have had the primary goal of improving the biodegradability of cellulose by removing lignin and/or hemicellulose, and to a lesser degree decreasing the degree of polymerization (DP) and crystallinity of the cellulose component. Chemical pretreatment is the most studied pretreatment technique among pretreatment categories. This paper mainly reviews seven common chemical pretreatment techniques, including catalyzed steam-explosion, acid, alkaline, ammonia fiber/freeze explosion, organosolv, pH-controlled liquid hot water, and ionic liquids pretreatments.

4.2.1 Catalyzed steam-explosion

Catalyzed steam-explosion is very similar to uncatalyzed steam-explosion on their action modes, except that some acidic chemicals (gases and liquids), primarily including SO_2, H_2SO_4, CO_2, oxalic acid, etc. are used as catalysts to impregnate the biomass prior to steam-explosion. It is recognized as one of the most cost-effective pretreatment processes. SO_2- and H_2SO_4-impregated steam-explosions have been tested in pilot-scale[82,83]. Compared to uncatalyzed steam-explosion, catalyzed steam-explosion has more complete hemicellulose removal leading to more increased enzymatic digestibility of biomass with less generation of inhibitory compounds[84]. A number of studies on the catalyzed steam-explosion pretreatment have been reported[85-87]. SO_2 appears more appealing than H_2SO_4 in steam-explosion since the former requires milder and much less expensive reactor material, generates less gypsum, yields more xylose, and produces more digestible substrate with high fermentability. Although several researchers found CO_2-explosion (with or without steam) is effective[88-90], Dale and Moreira[91] observed that it is less effective than ammonia fiber/freeze explosion (AFEX). Hohlberg et al.[92] and Mamers and Menz[93] also concluded that CO_2 is less effective than SO_2 in steam-explosion. SO_2-impregantaed steam-explosion is the only known pretreatment technique that can effectively make softwoods more digestible[94], even though it was found less effective for softwood than hardwood and herbaceous biomass[95]. However, SO_2 is highly toxic and may present negative safety, heath and environmental impacts. At the same time, the catalyzed steam-explosion also generates some inhibitors derived from the degradation of carbohydrates. Certain detoxification strategies might be needed if inhibitors are detrimental to downstream processes. Additional limitations of catalyzed steam-explosion include destruction of a portion xylan faction and incomplete disruption of the lignin-carbohydrate matrix [96].

4.2.2 Acid pretreatment

Acid pretreatment method was derived from the concentrated acid hydrolysis such as concentrated H_2SO_4 and HCl hydrolysis, which had been a major technology for hydrolyzing lignocellulosic biomass for fermentable sugar production[97,98]. The concentrated acid hydrolysis had been temporarily commercialized in the World War II. Concentrated acid has been initially applied to remove hemicellulose either in combination with hydrolysis of cellulose to glucose or prior to dilute acid hydrolysis of cellulose. Even though it is powerful and effective for cellulose hydrolysis, concentrated acid is toxic, corrosive and hazardous and requires reactors that need expensive construction material resistant to corrosion. Additionally, the concentrated acid must be recovered and recycled after hydrolysis to render the process economically feasible[99]. Therefore, it has phased out gradually.

Alternatively, dilute acid pretreatment has received numerous research interests. It has been successfully developed for pretreatment of lignocellulosic biomass. Several different acids, including dilute sulfuric acid[35,100-104], dilute nitric acid[105,106], dilute hydrochloric acid[107,108], dilute phosphoric acid[109-111], and peracetic acid[9,112-114], have been reported in the literature. The action mode of dilute acid is to solubilize hemicellulose and remain lignin and cellulose intact so that the enzymatic digestibility of cellulose is enhanced. The oligomeric hemicellulosic saccharide could be completely hydrolyzed into monosaccharides by adjusting pretreatment conditions, but also the sugar degradation products will be generated during oligomer hydrolysis. The major advantage of dilute acid pretreatment over steam-explosion is significantly higher xylose yield. Using batch dilute sulfuric acid pretreatment process, xylose yield was showed to approach 80%–90% of theoretical value[115-117].

Dilute acid pretreatment has been applied to a wide range of feedstocks, including softwood, hardwood, herbaceous crops, agricultural residues, wastepaper, and municipal solid waste. It performed well on most biomass materials. Of all acid-based pretreatment methods, sulfuric acid has been most extensively studied since it is inexpensive and effective. Dilute sulfuric acid has some important disadvantages: (1) corrosion that mandates expensive materials of construction; (2) acidic prehydrolyzates must be neutralized before the sugars proceed to fermentation; (3) gypsum has problematic reverse solubility characteristics when neutralized with inexpensive calcium hydroxide; (4) formation of degradation products and release of natural biomass fermentation inhibitors are other characteristics of acid pretreatment; (5) disposal of neutralization salts is needed; (6) biomass particle size reduction is necessary. Plus, the current sulfuric acid price has increased quickly so that the economically feasibility of dilute acid pretreatment might need to be reconsidered.

4.2.3 Alkaline pretreatment

Alkaline pretreatment is one of major chemical pretreatment technologies receiving numerous studies. It employs various bases, including sodium hydroxide[118-121], calcium hydroxide (lime)[23,122,123], potassium hydroxide[9], aqueous ammonia[124,125], ammonia hydroxide[126], and sodium hydroxide in combination with hydrogen peroxide or others[127-129]. Alkaline pretreatment is basically a delignification process, in which a significant amount of hemicellulose is solubilized as well. The action mechanism is believed to be saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components, for example, lignin and other hemicellulose. Alkaline pretreatment also removes acetyl and various uronic acid substitutions on hemicellulose that reduce the accessibility of hemicellulose and cellulose to enzymes[9]. Alkaline pretreatment of lignocellulosic materials causes swelling, leading to decreased DP and crystallinity, increased internal surface area, disruption of the lignin structure, and separation of structural linkages between lignin and carbohydrates[130].

The effectiveness of alkaline pretreatment varies, depending on the substrate and treatment conditions. In general, alkaline pretreatment is more effective on hardwood, herbaceous crops, and agricultural residues with low lignin content than on softwood with high lignin content[131]. Millet et al.[66] observed that the digestibility of NaOH-treated hardwood increased from 14% to 55% with the decrease of lignin content from 24%–55% to 20%. However, slight effect of dilute NaOH pretreatment was found for softwoods with lignin content greater than 26%. Kim and Holtzapple[23] used lime to pretreat corn stover and obtained maximum lignin removal of 87.5% at 55℃ for four weeks with aeration. Using lime pretreatment at ambient conditions for up to 192 h, Playne[132] enhanced the enzyme digestibility of the sugarcane bagasse from 20% to 72%. He also concluded that lime would be the choice chemical based on the cost of chemicals. Using alkali chemicals (NaOH, $Ca(OH)_2$ and KOH) to pretreat rice straw in 24 h at 25℃, the authors found that NaOH (6% chemical loading, g/g dry rice straw) was the best alkali chemical to achieve 85% increase of glucose yield by enzymatic hydrolysis. Aqueous ammonia is also a common alkali chemical for alkaline pretreatment. The delignification efficiencies of corn cob and switchgrass were improved by 80% and 85%, respectively by ammonia recycled percolation (ARP) pretreatment[133]. ARP was somewhat less efficient in pretreatment of softwood-based pulp mill sludge[134]. A recent study achieved the enzymatic digestibility of the ARP treated corn stover of 90% with an enzyme loading of 10 FPU/g-glucan[135].

In comparison with other pretreatment technologies, alkali pretreatment usually uses lower temperatures and pressures, even ambient conditions. Pretreatment time, however, is recorded in terms of hours or days which are much longer than other pretreatment processes. A significant disadvantage of alkaline pretreatment is the conversion of alkali into irrecoverable salts and/or the incorporation of salts into the biomass during the pretreatment reactions so that the treatment of a large amount of salts becomes a challenging issue for alkaline pretreatment.

4.2.4 Ammonia fiber/freeze explosion (AFEX)

AFEX is conceptually similar to the steam explosion. In AFEX, the biomass is exposed to hot liquid ammonia under high pressure for a period time, and then the pressure is suddenly released. This swift reduction of pressure opens up the structure of lignocellulosic biomass leading to increased digestibility of biomass. AFEX pretreatment simultaneously delignify and solubilize some hemicellulose while decrystallizing cellulose, but does not significantly remove hemicellulose as acid and acid-catalyzed steam-explosion pretreatments[6,136,137]. Thus, both micro- and macro-accessibilities of cellulose to the cellulase are affected.

Typically, operation conditions of AFEX include aqueous ammonia dosage, (1–2 kg ammonia/kg dry biomass), temperature (＜90℃), pH values (＜12.0), and pretreatment time (＜30 min). The composition of the AFEX pretreated materials was essentially the same as the original[138]. AFEX has been applied to various lignocellulosic materials, including rice straw, municipal solid waste, newspaper, sugar beet pulp, sugar cane bagasse, corn stover, switchgrass, miscanthus, aspen chips, etc. Herbaceous crops and agricultural residues are well suited for AFEX. However, the AFEX works only moderately and is not attractive for the biomass with high lignin content such as hardwood, softwood and newspaper[22]. The yields of enzymatic hydrolysis of AFEX-pretreated newspaper (18%–30% lignin) and aspen chips (25% lignin) were reported as only 40% and below 50%, respectively[22]. The important advantages of AFEX include: (1) producing negligible inhibitors for the downstream biological processes, so water wash is not necessary[136]; and (2) requiring no particle size reduction[139]. However, ammonia must be recycled after the AFEX pretreatment based on the considerations of both the ammonia cost and environmental protection. Therefore, both ammonia cost and the cost of recovery processes drive up the cost of the AFEX pretreatment[140].

4.2.5 Organosolv

The organosolv process is a delignification process, with varying simultaneous hemicellulose solubilization. In this process, an organic or aqueous organic solvent mixture with or without an acid or alkali catalysts is used to break the internal lignin and hemicellulose bonds[138,141-143]. Usually, the presence of catalyst can increase the solubilization of hemicellulose and the digestibility of substrate is also further enhanced[144]. The organic solvents used in the organosolv process include methanol, ethanol, acetone, ethylene glycol, triethylene glycol, tetrahydrofurfuryl alcohol, glycerol, aqueous phenol, aqueous n-butanol[143,145]. For economic reasons, the use of low-molecular-weight alcohols such as ethanol and methanol has been favored[146]. The usual operation temperature of organosolv is in the range of 150–200℃. Aziz and Sarkanen[147] found that the addition of catalyst was unnecessary for satisfactory delignification when temperature is higher than 185℃. Organic solvents are costly and their use requires high-pressure equipment due to their high volatility. The used solvents should be recovered and recycled to reduce the operation costs. Removal of solvents from the pretreated biomass is necessary because the residual solvents may be inhibitors to enzymatic hydrolysis and fermentation. The main advantage of organosolv over other chemical pretreatments is that relatively pure, low molecular-weight lignin is recovered as a by-product[148]. The organosolv process is still too complex and expensive to attract industrial interests.

4.2.6 pH-controlled liquid hot water

As mentioned in the LHW pretreatment under the category of “Physical Pretreatment”, during LHW pretreatment, the pH value of contents in the reactor can usually drop to below 4 leading to the inhibitor formation due to the carbohydrate degradation. In order to control the pH value of the liquid hot water between 5 and 7, some bases such as KOH are usually added into LHW pretreatment process with its role to maintain the pH value not as a catalyst in alkaline pretreatment. This method is termed pH-controlled hot water pretreatment[149]. In this pretreatment method, the base differs in function from chemicals added as catalysts in chemical pretreatment methods with its role to maintain the pH value constant above 5 and below 7 in order to minimize hydrolysis to monosaccharides[150]. Mosier et al.[151] pretreated corn fiber using pH-controlled liquid hot water at 160℃ and a pH value above 4.0 and found that 50% of the fiber was dissolved in 20 min. The pretreatment also enabled the subsequent complete enzymatic hydrolysis of the remaining polysaccharides to monosaccharides. The carbohydrates dissolved by the pretreatment were 80% soluble oligosaccharides and 20% monosaccharides with ＜1% of the carbohydrates lost to degradation products. Mosier et al.[152] continued to optimize the pH-controlled liquid hot water for the pretreatment of corn stover and they got the optimal pretreatment conditions to be 190℃ for 15 min. At the optimal conditions, 90% of the cellulose was hydrolyzed to glucose by 15FPU cellulase/g-glucan. Both the xylose and the glucose in this undiluted hydrolyzate were shown to be fermented by recombinant yeast 424A (LNH-ST) to ethanol at 88% of theoretical yield. It seems that this technology is more promising than the LHW pretreatment, but more research is needed to obtain its performance data and economic feasibility analysis.

4.2.7 Ionic liquids (ILs) pretreatment

ILs technique has recently received extensive research attention on the cellulose dissolution[153-158]. ILs shows promise as efficient and “green” novel cellulose solvents. They can dissolve large amounts of cellulose at considerable mild conditions and feasibility of recovering nearly 100% of the used ILs to their initial purity makes them attractive[159]. This technology was used to direct dissolution of cellulose in the commercially Lyocell process as a modern industrial fiber-making[160].

As cellulose solvents, ILs possesses several advantages over regular volatile organic solvents of biodegradability, low toxicity, broad selection of anion and cation combinations, low hydrophobicity, low viscosity, enhanced electrochemical stability, thermal stability, high reaction rates, low volatility with potentially minimal environmental impact, and non-flammable property. The dissolution mechanism of cellulose in ILs involves the oxygen and hydrogen atoms of cellulose hydroxyl groups in the formation of electron donor-electron acceptor (EDA) complexes which interact with the ILs[161]. Upon interaction of the cellulose-OH and ILs, the hydrogen bonds are broken, resulting in opening of the hydrogen bonds between molecular chains of the cellulose[161]. The interaction finally results in the dissolution of cellulose. Solubilized cellulose can be recovered by rapid precipitation with some anti-solvents such as water, ethanol, methanol, or acetone. The recovery cellulose was found to have the same DP and polydispersity as the initial cellulose, but significantly different macro- and micro-structure, especially the decreased degree of crystallinity[162].

The previously used ILs include N-methylmorpholine-N-oxide monohydrate (NMMO)[163], 1-n-butyl-3-methylimidazolium chloride (BMIMCl)[164-167], 1-allyl-3-methylimidazolium chloride (AMIMCl)[165,168,169], 3-methyl-N-bytylpyridinium chloride (MBPCl) and benzyldimethyl (tetradecyl) ammonium chloride (BDTACl)[159]. Using 1-butyl-3-methylimidazolium chloride (BMIMCl) for pretreatment, Dadi et al.[164] found that the initial enzymatic hydrolysis rate and yield of pretreated Avicel-PH-101 were increased by 50- and 2-fold in comparison with untreated Avicel. Kuo and Lee[163] also observed that the 1, 3-N-methyl-morpholine-N-oxide (NMMO) pretreated sugarcane bagasse has 2-fold higher enzymatic hydrolysis yield as compared to untreated bagasse. Using BMIMCl to treat raw and steam-exploded wheat straw, Liu and Chen[166] obtained significant improvement of enzymatic hydrolysis yield. They found BMIMCl modified the structure of wheat straw by decreasing the DP and crystallinity and partially solubilizing cellulose and hemicellulose.

Application of ionic liquids has opened new ways for the efficient utilization of lignocellulosic materials in such areas as biomass pretreatment and fractionation. However, there are still many challenges in putting these potential applications into practical use, for example, the high cost of ILs, regeneration requirement, lack of toxicological data and knowledge about basic physico-chemical characteristics, action mode on hemicellulose and/or lignin contents of lignocellulosic materials and inhibitor generation issues. Further research and financial support are required to address such challenges.

4.3 Biological pretreatment

Biological pretreatment employs wood degrading microorganisms, including white-, brown-, soft-rot fungi, and bacteria to modify the chemical composition and/or structure of the lignocellulosic biomass so that the modified biomass is more amenable to enzyme digestion[170-172]. Fungi have distinct degradation characteristics on lignocellulosic biomass. In general, brown and soft rots mainly attack cellulose while imparting minor modifications to lignin, and white-rot fungi are more actively degrade the lignin component[173]. Present research is aimed towards finding those organisms which can degrade lignin more effectively and more specifically. White-rot fungi were considered the most promising basidiomycetes for bio-pretreatment of biomass and were the most studied biomass degrading microorganisms[174].

Hwang et al.[175] studied biological pretreatment of wood chips using four different white-rot fungi for 30 days and found that the glucose yield of pretreated wood by *Trametes versicolor* MrP 1 reached 45% by enzymatic hydrolysis while 35% solid was converted to glucose during fungi incubation. A Japanese red pine *Pinus densiflora* (softwood) was pretreated biologically by white-rot fungi of *Ceriporia lacerata*, *Stereum hirsutum*, and *Polyporus brumalis* and it was found that *S. hirsutum* is the most effective to degrade lignin and improve the enzymatic digestibility of wood[171]. Keller et al.[176] reported that their preliminary tests showed a 3- to 5-fold improvement in enzymatic digestibility of corn stover after pretreatment with *Cyathus stercoreus*; and a 10- to 100-fold reduction in shear force needed to obtain the same shear rate of 3.2 to 7.0 rev/s, respectively, after pretreatment with *Phanerochaete chrysosporium*. Zhang et al.[177,178] screened 35 isolates of white-rot fungi for the biological pretreatment of bamboo for enzymatic saccharification. They observed that *Echinodontium taxodii* 2538, *Trametes versicolor G*20 and *Coriolus versicolor B*1 were the most promising white-rot fungi for highly selective lignin degradation and significant improvement of enzymatic saccharification. Degradation of lignin by white-rot fungi is a co-oxidative process, thus accompanying carbon source is necessary, usually from cellulose and hemicellulose. In order to reduce and eliminate the sugar loss during bio-pretreatment, the fungal strains with preference of lignin degradation such as *P. ostreatus*, *C. subvermispora*, *Cyathus stercoreus*, and genetically modified *P. chrysosporium* were developed to produce less cellulase activity[179,180].

The biological pretreatment appears to be a promising technique and has very evident advantages, including no chemical requirement, low energy input, mild environmental conditions, and environmentally friendly working manner[138]. However, its disadvantages are as apparent as its advantages since biological pretreatment is very slow and requires careful control of growth conditions and large amount of space to perform treatment[31]. In addition, most lignolytic microorganisms solubilize/consume not only lignin but also hemicellulose and cellulose[171,172,181,182]. Therefore, the biological pretreatment faces technoeconomic challenges and is less attractive commercially.

5 Current status and challenges

Biomass pretreatment, for the purpose of improving the bio-degradability of biomass materials, has been studied for years. Although extensive research has been done to develop numerous effective pretreatment techniques on various lignocellulosic biomass feedstocks, none has been commercialized for cellulosic ethanol production due economic feasibility. Only a few of the reported pretreatment methods such as dilute acid and steam-explosion have been tested on a demonstration scale. One of the most famous demonstration scale cellulose ethanol facilities is held by Iogen Corporation in Canada. Iogen Corporation is using modified steam-explosion pretreatment to improve the enzymatic digestibility of straw to produce cellulosic ethanol with yield of 340 litres per tonne of fibre[183]. The U.S. Department of Energy has also been supporting several biofuel programs through bioenergy laboratories or centers such as National Renewable Energy Laboratory (NREL), Great Lakes Bioenergy Research Center (GLBRC), and Oak Ridge National Laboratory (ORNL) to develop various pretreatment methods, collect process performance date, and evaluate their economic feasibility. Currently, the research of pretreatment is facing several technoeconomic challenges, including environmental impact, delignification, co-fermentation of hexose and pentose into ethanol, hydrolyzate detoxification, hemicellulose and cellulose separation, pretreatment product digestibility, energy demand, and processing costs. Future research should address these challenges to promote the commercialization of cellulosic ethanol.

6 Future work

Extensive research has been done on the development of advanced pretreatment technologies to prepare more digestible biomass to ease bioconversion of biomass into cellulosic ethanol. An ideal cost-effective pretreatment method might have several characteristics[27,184,185]: (1) maximum fermentable carbohydrate recovery; (2) minimum inhibitors produced from carbohydrate degradation during pretreatment; (3) low environmental impact; (4) low demand of post-pretreatment processes such as washing, neutralization, and detoxification; (5) minimum water and chemical use; (6) low capital cost for reactor; (7) moderately low energy input; (8) relatively high treatment rate; and (9) production of high value-added by-products. Therefore, the future research on pretreatment would be focused on the following areas. First, reduction of water and chemical use; Second, recovery of carbohydrates and value-added by-products to improve the economic feasibility; Third, development of clean delignification yielding benefits of co-fermentation of hexose and pentose sugars with improved economics of pretreatment; Fourth, fundamental understanding of pretreatment mechanisms and the relationship between the biomass structure features and enzymatic hydrolysis; Fifth, reduction of the generation of inhibitors such as furfural, 5-HMF and acetic acid which could significantly inhibit enzymatic hydrolysis and fermentation of biomass[27, 184].

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