

A Review of Lignocellulosic Biomass Pretreatment Technologies

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Abstract: Lignocellulose is the most abundant renewable resource on earth. However, owing to the tightly entangled structural characteristics, it is challenging to convert lignocellulose into bio-based products in the biorefinery process without pretreatment. Pretreatment can destroy the natural resistance structure of lignocellulosic biomass, which is conducive to its downstream enzymatic saccharification and fermentation process. Physical, chemical, and physicochemical pretreatments have been widely conducted for lignocellulosic biomass; several updated approaches and peculiar chemicals have also been proposed for these pretreatment methods in the recent years. Hence, this study comprehensively reviews the novel technologies and chemicals that were applied in the various pretreatments. In addition, the mechanisms, advantages, and disadvantages of the updated pretreatments are discussed to provide a reference for developing new pretreatment methods.

Keywords: lignocellulosic biomass; pretreatment; enzymatic hydrolysis; bioethanol

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1 Introduction

Long-term environmental effects, economic sustainability, and energy security concerns have recently motivated the study of biorefinery for biofuels using lignocellulose. Biofuels, such as biohydrogen, biomethane, bioethanol, biomethanol, and biobutanol, are renewable, sustainable, and economically viable energy sources^[1]. In China, bioethanol has been proposed to blend with gasoline to



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support vehicles ^[2]. Generally, bioethanol has a higher heat of vaporization and octane number than that of gasoline. Hence, it can be utilized as oxygenate for gasoline to elevate its oxygen content, allowing a better oxidation of hydrocarbons and reducing the amount of greenhouse gas emission ^[2-3].

In the traditional biorefining process, agricultural crops (grain, sugarcane, sugar beet, cassava, etc.) have been widely used in the production of "first-generation" bioethanol. However, whether to use crops as food resources or raw materials for biofuel remains conflicting ^[1], considering the shortage of food resources in developing countries. An estimated 135 million people in 55 countries globally have faced acute levels of hunger in 2019, according to the 2020 Global Report on Food Crises. Therefore, using food resources to produce biofuel is not a long-term feasible process. Owing to the high carbohydrate content (cellulose and hemicellulose), lignocellulosic biomass (agricultural and forestry residues) is an inexpensive and abundant feedstock for the production of "second-generation" bioethanol. There are several advantages of using lignocellulosic biomass as raw materials to produce bioethanol including abundant raw material sources, low cost, hygienic products, and the full utilization of natural resources to reduce unnecessary wastage. Therefore, the production of "second-generation" bioethanol from lignocellulosic biomass has become a popular research topic.

Compared to agricultural crops, lignocellulosic biomass cannot directly be used as raw material for bioethanol production owing to its resistive structure and the presence of a large amount of lignin. Hence, various pretreatment methods should be incorporated to breakdown the solid structure of lignocellulosic biomass to improve its accessibility for enzymatic hydrolysis ^[4]. The yield of fermentable monosaccharides that has been reported to result from the enzymatic hydrolysis of lignocellulosic materials without pretreatment was less than 10% ^[5], which is unfavorable for achieving the industrial production of bioethanol. Therefore, pretreatment is an essential step

for breaking down the resistant structure of lignocellulosic biomass to facilitate the subsequent enzymatic hydrolysis for sugar production.

Pretreatment refers to the use of physical, chemical, or biological methods to dissolve and separate one or more of the main components of lignocellulose, which can lead to the substances becoming easily hydrolyzed by enzymes. Generally, the pretreatment of lignocellulosic raw materials can achieve various effects ^[4], such as (1) breaking or removing the lignin by degrading its inherent linkage, (2) separation or removal of hemicellulose, (3) reducing the crystallinity of cellulose, (4) increasing the surface area and porosity of lignocellulose, which can increase the accessibility of enzyme to cellulose. A cost-effective pretreatment process must improve the sugar conversion in the subsequent step of enzymatic hydrolysis, reducing the degradation of the carbohydrates and the formation of inhibitors for hydrolysis and fermentation. Physical, chemical, and physicochemical pretreatments have been widely conducted for lignocellulosic biomass; several updated approaches and peculiar chemicals have also been recently proposed for these pretreatments.

Hence, the novel technologies and chemicals that are applied in the different pretreatments (physical method, chemical method, physicochemical method) are comprehensively reviewed in this paper. In addition, the mechanisms, advantages, and disadvantages of the latest pretreatments are discussed to offer an in-depth discussion and to provide a reference for exploring new pretreatment methods of lignocellulose.

2 The physical pretreatment

Physical pretreatment refers to directly reducing the particle size and crystallinity of lignocellulose through traditional mechanical grinding or irradiation ^[6]. The physical methods mainly include the traditional mechanical crushing method, using an ultrasonic and microwave assistance. The physical pretreatment for lignocellulosic materials has the advantages of increasing the specific surface area of raw materials,

destroying the crystallization zone, and reducing the crystallinity of cellulose, along with simple operation and lower environmental pollution. However, this process is not economically feasible for it usually requires a higher energy consumption.

2.1 Mechanical crushing method

According to the different force modes, the basic force of material crushing includes grinding, shearing, extrusion, impact, and splitting. When mechanical crushing is used to treat lignocellulosic materials, large solid materials can be pulverized into an appropriate granularity to increase the specific surface area and reduce the crystallinity of cellulose, which can improve the efficiency of enzymatic hydrolysis. Jiang et al ^[7] reported that the enzymatic conversion of lignocellulose pretreated by the mechanical crushing method increased by 2–6 times compared to the unpretreated raw materials. Li et al ^[8] compared the efficiency of grinding and shearing pretreatments on rice straw for the fermentation of biogas. The results demonstrated that the grinding method was more effective than shearing method under the same particle size (25 mm), which increased the gas production by 12.5%. Wang et al ^[9] used a twin-screw extruder to prepare two types of pretreated corn stalks; one at 25% (*w/w*) of water content, named CS-DW, and the other at 23% (*w/w*) of water content, 2% (*w/w*) of glycerol, and 1 g/L NaOH, named CS-DW-GNa. The results demonstrated that the glucose hydrolysis yield can be increased from 25 g/L (CS-DW) to 34 g/L (CS-DW-GNa). Zheng et al ^[10] modified the screw extrusion method and found that lignin could be effectively removed by replacing the screw element in the extruder with the reverse element before processing corn stalks. Liu et al ^[11] compared the effects of three pretreatment methods (sulfonation, FPI grinding, and wet grinding) on the enzymatic hydrolysis efficiency of corn cob. They found that each method could significantly improve the enzymatic hydrolysis conversion of the pretreated material, among which wet grinding had an apparent advantage for enzymatic hydrolysis with a 96.7% yield. Overall, despite mechanical crushing

being the simplest and most direct pretreatment method for the biorefinery processes, several drawbacks remain. The mechanical crushing method requires a high energy consumption, which can increase the processing cost. In addition, cellulose in the lignocellulosic biomass is easily recrystallized and lignin/hemicellulose cannot be degraded after physical grinding, which remains an obstacle for enzymatic hydrolysis ^[12].

2.2 Ultrasonic pretreatment method

The ultrasonic pretreatment method is such that the surface material is impacted and sheared by the mechanical action and cavitation action produced by the medium (usually water) in an ultrasonic environment. Wang et al ^[13] adopted ultrasonic assisted pretreatment on eucalyptus samples and found that ultrasonic pretreatment changed the physical and chemical properties of eucalyptus samples, which can significantly improve its enzymatic hydrolysis. Zhang et al ^[14] pretreated sorghum stalks using the ultrasonic wave method and found that the enzymatic hydrolysis of the biomass can be improved from 10% to 33.7%. De Carvalho Silvello et al ^[15] reported that the ultrasonic pretreatment achieved a sugar yield of pretreated bagasse with 91.8% after enzymatic hydrolysis. Overall, the aforementioned studies demonstrated that ultrasonic pretreatment can enhance the catalytic activity of cellulase and promote the hydrolysis of lignocellulosic materials ^[16].

Various chemicals have been coupled with ultrasonic pretreatment to destroy the structure of lignocellulose by partially removing lignin and hemicellulose ^[17–20]. As reported by Mohapatra et al ^[21], it was found that the maximum delignification rates of hydrochloric acid pretreated Denanath and Hybrid Napier were 33.0% and 33.8%, respectively. While using the combination of hydrochloric acid and ultrasonic pretreatment for Denanath and Hybrid Napier, delignification rates of 80.4% and 82.1% can be achieved respectively. Patil et al ^[22] compared the effects of ultrasonic pretreatment and ultrasound-assisted alkali pretreatment on sawdust and found that the ultrasound-assisted alkali

pretreatment increased the monosaccharide yield by 1.25 times and shortened the treatment time by 4 h. SriBala et al ^[23] reported that the ultrasonic auxiliary alkali pretreatment with 10 wt% NaOH for 4 h changed the fiber morphology of the treated material, which improved in the digestion of glucose production by more than 2.5 times. When the lignocellulosic material was pretreated with chemicals by the ultrasonic, the ultrasonic caused an erosion on its surface, enabling the removal of lignin and hemicellulose by the chemicals, which significantly improves the enzymatic hydrolysis of biomass. However, the study regarding the ultrasonic pretreatment of lignocellulosic materials is only in the laboratory stage, and further research is needed to achieve industrial production.

2.3 Microwave pretreatment

A microwave is an electromagnetic wave with frequency of 300 MHz – 300 GHz. When applying microwaves to lignocellulosic biomass, the molecules in the substrate change their orientation repeatedly, which strengthens the collision between the molecules of polymers (cellulose, hemicelluloses, and lignin) to be degraded. Hence, the accessibility of enzymes to cellulose increases, and the efficiency of enzymatic hydrolysis can be improved. Conesa et al ^[24] evaluated the influence of microwaves with different powers (10.6, 8.5, 6.4, 4.3, and 2.1 W/g) on the sugar yield of pineapple residue and found that the sugar yield increased with the increase of microwave power. A higher microwave power and longer treatment resulted in the degradation of sugar and a low sugar conversion efficiency of enzymatic hydrolysis. Nuchdang et al ^[25] reported that a 30 min microwave treatment conducted at 120°C and a solid-liquid ratio of 5% (w/w) significantly increased the efficiency of enzymatic hydrolysis of *Brachiara mutica* from 316 to 750 mg/g. Ethaib et al ^[26] pretreated sago palm bark with a combination of the microwave and a dilute sulfuric acid pretreatment, which resulted in a lignin removal of 32% and an increase of the crystallinity from 29% to 47%. Both of these changes of sago palm bark can significantly improve its enzymatic digestibility. Tayeh

et al ^[27] reported that a sugar conversion of 90% was achieved from the enzymatic hydrolysis of olive solid waste pretreated by the microwave pretreatment with water and formic acid. Although microwave pretreatment methods have been widely investigated, they are only conducted in the laboratory owing to the high processing cost. Hence, it is difficult to apply microwave pretreatment to industrialized sugar production.

3 Chemical pretreatment

A variety of chemical reagents (acid reagents, alkaline reagents, and strong oxidants) are used for chemical pretreatments to destroy the compact structure of lignocellulosic biomass for improving its enzymatic hydrolysis. The mechanism includes the chemical reagents dissolving and removing hemicellulose or lignin from lignocellulose, breaking the cell wall structure, decreasing the crystallinity of the substrate, and increasing the accessibility of enzymes to cellulose. Several commonly used chemicals have been utilized for chemical pretreatments, including acid, alkali, and oxidant ^[28].

3.1 Acid pretreatment

Acid pretreatment is the oldest and most thoroughly researched chemical pretreatment method. According to the type of acid, it can be divided into an inorganic acid (sulfuric acid, hydrochloric acid, nitric acid, and phosphoric acid) or organic acid (acetic acid, propionic acid, and oxalic acid). According to the concentration of the acid, the pretreatment can be concentrated acid pretreatment or dilute acid pretreatment. Among these, the dilute sulfuric acid pretreatment is the most widely used.

3.1.1 Concentrated sulfuric acid pretreatment

Concentrated sulfuric acid pretreatment usually refers to the pretreatment for lignocellulosic materials with a sulfuric acid concentration of more than 10% (w/V). It has been reported that the concentrated sulfuric acid pretreatment can be conducted at room temperature with a rapid reaction speed of cellulose, which can achieve an apparent effect for sugar production via

enzymatic hydrolysis. For example, Sasaki et al.^[29] impregnated cotton stalk with concentrated sulfuric acid for 30 min prior to microwave pretreatment and found that a glucose yield of 74.2% was achieved by the microwave pretreatment at 180°C for 3 min. Jang et al.^[30] reported that the pretreatment of lignocellulosic biomass with concentrated sulfuric acid can apparently destroy the structure of biomass to improve its enzymatic digestibility, which can reduce the production cost by approximately 55%. However, there are several disadvantages of concentrated acid pretreatment, such as high cost, difficulty of acid recovery, significant corrosion of equipment, generation of by-products that affect the enzymatic hydrolysis and fermentation, resulting in restrictive application of this method in practical production.

3.1.2 Dilute sulfuric acid pretreatment

Dilute sulfuric acid pretreatment usually refers to the pretreatment technology for lignocellulosic materials with a sulfuric acid concentration of 0.1%–1.5% (w/V) in a temperature range of 120–200°C. During dilute sulfuric acid pretreatment, the hemicellulose in the lignocellulosic material can be degraded to monosaccharides (mainly xylose), and the lignin can be partially degraded, which demonstrates the positive effect for enzymatic hydrolysis. Several studies have been conducted to evaluate the effect of dilute sulfuric acid pretreatment on enzymatic hydrolysis. For example, Bouza et al.^[31] pretreated fruit bunches with a thin dilute sulfuric acid and achieved a xylan recovery of 81.4% and a glucan conversion of 74.8% from the enzymatic hydrolysis. Karapatsia et al.^[32] reported that 75.04% of hemicellulose was removed from the biomass by the dilute sulfuric acid pretreatment, which resulted in an enzymatic hydrolysis efficiency of 69.69%. In addition, 78% of sugar conversion was achieved by pretreated corn stover, which was pretreated with a sulfuric acid loading of 0.8% for 10 min at 158°C^[33]. Lee et al.^[34] evaluated the effects of dilute acid pretreatment on the chemical composition and ethanol yield of rice straw. The obtained results demonstrated that dilute acid pretreatment significantly

improved the ethanol yield up to 0.20 g/g. Lee et al.^[35] pretreated *Acacia acuminata* with 0.05% dilute sulfuric acid at 200°C for 5 min and found that the maximum glucose yield of enzymatic hydrolysis was 94.9%. Kapoor et al.^[36] reported that the pretreatment of straw with dilute acid (0.35 wt%) at 160°C for 10 min could result in 83.3 g/L and 31.9 g/L of the final glucose and xylose concentrations after enzymatic hydrolysis. Chen et al.^[37] reported that 9.7% to 19.3% of lignin was removed by dilute acid pretreatment of corn stalk, which increased the yield of enzymatic hydrolysis glucose to 58.6%.

Dilute sulfuric acid pretreatment technology has been widely used in the biorefinery process of lignocellulosic materials; however, this pretreatment technology also has disadvantages^[38]. Generally, the low acid concentration used in the pretreatment process requires relatively harsh reaction conditions of pressure and temperature to break down the structure of lignocellulose. Additionally, during dilute acid pretreatment, the cellulose, hemicellulose, and lignin can be inevitably degraded into by-products, including organic acids, phenols, aldehydes, furans, and aromatic components. These degraded by-products may inhibit the subsequent enzymatic hydrolysis or microbial fermentation^[39–41]. Therefore, after the pretreatment operation, it is often necessary to set up a detoxification process to reduce or remove the influence of inhibitors in the hydrolysate for fermentation. Because enzymatic hydrolysis and fermentation processes have strict requirements for operating conditions, such as temperature and pH value, alkaline reagents may be added after pretreatment to neutralize the hydrolysate.

3.1.3 Acetic acid pretreatment

Acetic acid is a mild and easily recovered organic acid, which is regarded as a novel acid in the pretreatment of lignocellulosic materials^[42]. Generally, the hemicellulose can be partially degraded into xylooligosaccharides (XOS), and an inherent structure of biomass can be changed after acetic acid pretreatment, which can improve the cellulose accessibility for enzymatic hydrolysis. For example,

Huang et al ^[43] used response surface analysis to study the factors influencing the preparation of XOS from poplar sawdust pretreated with acetic acid and found that 36.0% xylan was converted into XOS under the optimal treatment conditions, which was 6.5% acetic acid concentration at 170°C for 27 min. The enzymatic hydrolysis efficiency of the pretreated poplar sawdust was 51.0%, which was 80% higher than that of the hydrothermal pretreated substrate (23.8%). Lai et al ^[44] reported that acetic acid pretreatment significantly improved the accessibility of cellulose of poplar sawdust and was able to produce a high concentration of XOS. However, despite the destruction of the hemicellulose, the enzymatic hydrolysis efficiency remained low (28.3%). The accessibility and enzymatic hydrolysis efficiency of cellulose can be further improved by adding an alkali post-treatment. Wang et al ^[45] reported that the acetic acid pretreatment of poplar wood resulted in 55.8% of XOS yield; however, only up to 6.8% of lignin was removed. The high content of the residual lignin demonstrated the critical inhibition effect on the subsequent enzymatic hydrolysis.

As indicated in the aforementioned studies, despite achieving sufficient hemicellulose removal, the acetic acid pretreatment demonstrated a poor ability to remove the lignin. Therefore, significant research has been conducted to improve acetic acid pretreatment efficiency by adding various chemical reagents. Mota et al ^[46] reported that a hydrogen peroxide-acetic acid (HPAC) pretreatment demonstrated sufficient performances to remove lignin (45%–75%) for corn straw, bagasse, and eucalyptus bark, which increased the efficiency of enzymatic hydrolysis from 2.1 times to 20.8 times. Hao et al ^[47] reported that the HPAC pretreatment was more effective in removing lignin from lignocellulosic biomass than traditional concentrated sulfuric acid pretreatment. When HPAC with 50 mmol/L sulfuric acid as the catalyst was used to pretreat poplar wood, the enzymatic yields of glucose and xylose of the HPAC pretreated poplar wood reached 75.2% and 61.4% by xylanase and

cellulase, respectively. De Araújo Padilha et al ^[48] reported that a monosaccharide yield of 37 g/L of an enzymatic hydrolysis with an 80% yield was achieved from the hydrolysis of cashew residue using acetic acid (7.5% (w/V) solid content) pretreatment with a small amount of sulfuric acid as the catalyst.

The acetic acid pretreatment method is mild and efficient and has an exceptional performance on lignocellulosic raw materials. However, the acetic acid in the pretreatment waste liquid needs to be recovered, which increases the production cost. However, acetic acid recovery and sugar concentration can be separated by membrane separation (nanofiltration and reverse osmosis) and rotary evaporation technology.

3.1.4 Phosphoric acid pretreatment

To overcome the disadvantages of dilute sulfuric acid pretreatment, many researchers began to explore the use of phosphoric acid in the pretreatment of lignocellulosic materials. During phosphoric acid pretreatment, the hydroxyl group of cellulose reacts with phosphoric acid to form cellulose phosphate (Cellulose—O—H₂PO₃). The remaining hydroxyl and hydrogen ions in the cellulose chain form hydrogen bonds with water, which makes cellulose phosphate dissolve in water. The cellulose phosphate can be reduced to free phosphoric acid and amorphous cellulose in the phosphoric acid regeneration process, thus achieving an effective separation of the three components of lignocellulose ^[49]. Therefore, phosphoric acid is a solvent capable of dissolving crystalline cellulose to improve the enzymatic digestibility of lignocellulose ^[50–51]. Siripong et al ^[52] pretreated *Achyranthes aspera* and *Sida acuta* with concentrated phosphonic acid and found that the pretreatment removed most of the xylan and part of lignin, while most of the glucan remained. Nair et al ^[53] reported that the enzymatic hydrolysis efficiencies of 84%–90% can be achieved for wheat straw pretreated by phosphoric acid. Rojas-Chamorro et al ^[54] optimized the conditions for the phosphoric acid pretreatment of brewer grains and found that 63% of the sugar was dissolved during the pretreatment process at 155°C, which can achieve a

sugar production of 69% for ethanol fermentation. Bouza et al ^[31] pretreated the *Aspidium aspidiae* with 1.5% phosphoric acid with a solid-liquid ratio of 1:10 at 140°C for 60 min, which can achieve a final enzymatic hydrolysis efficiency of 68.61%.

In addition, modified phosphoric acid pretreatments, such as combined ethanol-phosphoric acid, concentrated phosphoric acid combined with hydrogen peroxide (PHP), and phosphoric acid-ultrasonic, have been conducted to maximally improve the enzymatic digestibility of biomass ^[55]. For example, Qiu et al ^[56] demonstrated that wheat straw pretreated by phosphoric acid combined with hydrogen peroxide improved the enzymatic hydrolysis efficiency when compared to neat phosphoric acid pretreatment. Wan et al ^[57] demonstrated that PHP at 50°C for 1 h can achieve 88%–96% of the glucose conversion for pretreated wheat straw after enzymatic hydrolysis. Compared to the other inorganic acids, phosphoric acid has the advantages of non-corrosive, safety, and low cost. The waste liquid obtained after pretreatment can also be used as raw material for making phosphate fertilizer, which will not cause environmental pollution.

3.2 Alkali pretreatment

Alkali pretreatment can remove lignin by saponifying the ester bonds between hemicellulose and lignin and weakening the hydrogen bonds between cellulose and hemicellulose. Chemical bonds between lignin carbohydrate complexes (LCC) in the feedstock can also be broken after the lignin is removed. Generally, the reaction conditions of alkali pretreatment are mild with low yields of inhibitors. During alkali pretreatment, most of the cellulose and hemicellulose are retained in the substrate, which is conducive to the subsequent bioconversion of carbohydrates ^[58].

NaOH and ammonia are commonly used in traditional alkali pretreatments. Lignocellulosic materials can swell during the NaOH pretreatment, which improves the accessibility of cellulase to cellulose. In addition, the decreased degree of polymerization and crystallinity of cellulose can improve its enzymatic digestibility. Ling et al ^[59]

demonstrated that the relative content of cellulose in *Phyllostachys pubescara* can increase after alkali pretreatment, which destroys the natural resistance structure of lignocellulosic materials for improving the enzymatic hydrolysis efficiency. Phitsuwan et al ^[60] found that NaOH pretreatment removed most of the lignin in elephant grass, which resulted in a 94% glucose yield. Ashoor et al ^[61] evaluated the effect of pretreatment with different concentrations of NaOH (0.5%, 1.0%, 1.5%, 2.0% (w/w)) on straw, and the results indicated that the pretreatment of straw with 1.5% NaOH had a higher cellulose conversion (91%). De Assis Castro et al ^[62] used 20–80 g/L NaOH to pretreat corn straw at 50–70°C and found that the enzymatic hydrolysis efficiency of cellulose was improved from 73% to 98%. NaOH pretreatment is a relatively mild pretreatment technology, which results in a reduced loss of sugar ^[63]. Owing to the relatively high cost and difficulty in disposing the produced wastewater, NaOH was not the first choice in the biorefinery industry ^[64].

Ammonia pretreatment is a method in which the raw material is immersed in a certain mass fraction of ammonia water solution to remove lignin and breakdown the anti-degradation properties of biomass ^[65]. Li et al ^[66] treated sugar beet pulp (SBP) with ammonia water and found that pores of different sizes formed on the surface of raw material, and the specific surface area increased after pretreatment. The maximum sugar yield of enzymatic hydrolysis was 2.42 times higher than that of the untreated material. Zhu et al ^[67] developed a step-by-step pretreatment method with ammonia and ethylenediamine for corn stalks and found that 95% of glucan and 85% of xylan in the corn stalks were retained after pretreatment. After pretreatment, the conversion efficiency of glucan and xylan could reach up to 94.18% and 72.81%, respectively. Zhao et al ^[68] optimized the conditions of liquid ammonia pretreatment for *Arundinopsis asparagus* and found that the change of enzymatic hydrolysis efficiency was dependent on the conditions of pretreatment. Sakuragi et al ^[69] pretreated six types of

hardwood materials with liquid ammonia and found that the enzymatic hydrolysis efficiency of the materials can effectively improve by using liquid ammonia pretreatment despite the content of lignin in hardwood being high. During ammonia pretreatment, organic components may be ammoniated to salt forms, which can provide nitrogen resources for the subsequent fermentation^[70-71].

Overall, chemical pretreatment is a common pretreatment method with a simple process and high efficiency. However, it has a significant impact on the subsequent enzymatic hydrolysis and fermentation process owing to the high concentration of the chemical reagents required. Therefore, it is necessary to adjust the pH value appropriately after pretreatment. In addition, the used acid and alkali reagents after pretreatment must be recycled; otherwise, the residue will cause pollution to the environment.

4 Physicochemical pretreatment

The physicochemical pretreatment is referred to a combination of a high temperature/pressure and chemical reaction on lignocellulose material. The commonly used physicochemical pretreatments include explosion (steam explosion and ammonia fiber explosion) and hydrothermal pretreatments.

4.1 Steam explosion pretreatment

For steam explosion pretreatment, saturated water steam at 160–260°C is used to heat the biomass at 0.69–4.83 MPa for approximately 10–600 s. Generally, steam explosion pretreatment can be divided into the two following stages: the steam phase cooking process and blasting process^[72]. During the steam phase cooking process, a high-pressure steam is applied into the lignocellulose, resulting in hemicellulose degradation, lignin softening, partial degradation, and cellulose crystallinity reduction. During the blasting process, the high-pressure water vapor is instantly released, which results in a rapid drop of pressure around the materials. The high-pressure condensed water in the cell boils rapidly to form a flash, which generates a high shear force to break the intra or

intermolecular hydrogen bonds between the cellulose that makes up the cell wall. After destroying the cell wall of biomass, the major component in biomass can be fractionated, which is beneficial for improving its enzymatic digestibility. Liu et al^[73] pretreated corn stalks with a steam explosion at 160°C for 48 s; the recovery of glucan and xylan was 93.4% and 71.6%, respectively. After enzymatic hydrolysis, the yield of glucose, xylose, and the total sugar reached up to 77.3%, 62.8%, and 72.3%, respectively, which was significantly higher than that of the untreated materials. Jiang et al^[74] studied the influence of steam explosion pretreatment on the enzymatic hydrolysis of wheat straw and found that the structure of wheat straw was destroyed by a low-pressure steam explosion, which can effectively increase the efficiency of enzymatic hydrolysis from 20% to 63%. Zhu et al^[75] compared changes in the enzymatic hydrolysis yield of the steam explosion pretreated corn stalks before and after water washing and found that the enzymatic hydrolysis efficiency of steam explosion pretreated corn stalks after water washing was as high as 89.2%, while for the unwashed corn stalks it was only 74.7%. Pielhop et al^[76] found that strengthening the steam explosion pretreatment intensity is beneficial for softening the lignin so as to breakdown the raw material and improve the digestibility of cellulose.

Recently, steam explosion pretreatment by adding chemical reagents has been proposed to improve the steam explosion pretreatment for the enzymatic hydrolysis of lignocellulosic materials. Chu et al^[77] attempted to impregnate poplar with O₂ and Na₂CO₃ under relatively mild conditions (110 and 135°C) prior to steam explosion pretreatment; the results demonstrated that the removal of lignin was 58% and the recovery of carbohydrate was over 80% in this pretreatment process. Silva et al^[78] treated bagasse with organic acid (citric acid) and NaOH catalyzed steam explosion and found that the hemicellulose of bagasse pretreated by organic acids was effectively degraded, leading to 86.5% of hemicellulose removal in the substrate. Steam explosion has the advantages of

saving energy, protecting the environment, has a high enzymatic hydrolysis efficiency, and can be widely applied. It is widely used in the pretreatment of various lignocellulosic materials. However, steam explosion pretreatment requires a high mechanical precision, equipment strength, and a high investment cost ^[79].

4.2 Ammonia fiber explosion pretreatment

The ammonia fiber explosion method overcomes the disadvantages of the high operating temperature of traditional steam explosion methods; however, it prolongs the reaction time. The typical process of ammonia fiber explosion pretreatment (AFEX) is performed at 90–100°C for 20–30 min, using approximately 1–2 kg/1 kg solid of ammonia dosage ^[80]. After AFEX, part of hemicellulose and lignin are degraded, resulting in a decreased crystallinity of cellulose and enhanced accessibility of cellulose for cellulase. Abdul et al ^[81] pretreated an oil palm empty fruit bunch (OPEFB) with AFEX, and the results demonstrated that the structure of OPEFB changed significantly compared to the untreated raw materials; the glucose yield of OPEFB increased from 0.15 g/g to 0.53 g/g. Zhang et al ^[82] compared the pretreatment of the mechanical grinding method and the AFEX method on switchgrass; the results demonstrated that there was no significant difference in the number of products by the fermentation. However, more glucan can be produced with the AFEX method; the fermentation of xylose was restricted under this condition.

Compared to the steam explosion pretreatment, AFEX has the advantages of (1) not producing inhibitors during pretreatment; (2) the residual ammonium salt in the pretreatment solution can be used as a nitrogen source for microorganisms; (3) the process conditions are easy to adjust and control. However, the AFEX approach is limited owing to the high cost of ammonia gas and the potential emission of volatile gas.

4.3 Hydrothermal pretreatment

Hydrothermal pretreatment, also known as liquid hot

water pretreatment or autohydrolysis, is a novel method to pretreat biomass by maintaining water in the liquid state under high temperatures (120–260°C) ^[83]. During hydrothermal pretreatment, the hemicellulose was nearly completely degraded, which can break down the natural resistance structure of lignocellulose to improve its enzymatic hydrolysis. For example, De Barros et al ^[84] conducted hydrothermal pretreatment on spelt straw and found that the enzymatic conversion rate of raw cellulose was 52.16% under the optimal pretreatment conditions of 180°C for 10 min. Nitsos et al ^[85] found that the removal of hemicellulose of 70%–85% can be achieved for three biomass feedstocks (poplar twigs, grapevine, and pine sawdust) after pretreating them with the hydrothermal method. Compared to the untreated biomass, the enzymatic hydrolysis yields of poplar twigs and grapevine were significantly improved from 10% to 49% and 77%, respectively, while the enzymatic hydrolysis efficiency of the pretreated pine sawdust was only 16%. Wu et al ^[86] pretreated wheat straw using the hydrothermal method combined with alkali post-extraction and found that the efficiency of enzymatic hydrolysis increased to 83.7%, which was significantly higher than that of the hydrothermal pretreatment alone. In addition, Wu et al ^[87] attempted to add a small amount of AlCl₃ and FeCl₃ in the hydrothermal pretreatment of wheat straw and found that with the increased concentrations of AlCl₃ and FeCl₃ from 0 to 20 mmol/L, the enzymatic hydrolysis efficiencies of pretreated wheat straw increased from 49.7% to 62.1% (AlCl₃) and 66.6% (FeCl₃), respectively.

Hydrothermal pretreatment is a green pretreatment method that uses water as the medium and does not require the use of other chemical reagents ^[88]. This method has been widely used owing to its advantages, such as having a strong adaptability, short reaction cycle, less by-products, and significantly improved enzymatic hydrolysis efficiency. However, the pretreatment of lignocellulosic raw materials using the hydrothermal method requires a large amount of water. In addition, it has been reported that the high

temperature of hydrothermal treatment can also lead to the formation of pseudo lignin, which works against the improvement of enzymatic hydrolysis.

Although various pretreatment methods have been conducted for lignocellulosic biomass to improve its enzymatic digestibility, they have advantages and disadvantages, which are summarized in Table 1.

5 Novel pretreatment

Although the traditional pretreatment methods can effectively destroy the natural resistance structure of lignocellulosic materials and improve the efficiency of enzymatic hydrolysis, they have certain disadvantages, such as a complex operation, high requirements for equipment, large energy consumption, high cost, a long cycle, and pollution to the environment. Therefore, researchers have developed new pretreatment methods to overcome the shortcomings, which have the characteristics of relatively mild treatment conditions, low equipment requirements, low energy consumption, and green environmental protection. Common novel pretreatment methods include ionic liquid pretreatment, supercritical carbon dioxide pretreatment, and deep eutectic solvent pretreatment.

5.1 Ionic liquid pretreatment

Ionic liquid (IL) is a liquid salt composed of organic

cations and organic or inorganic anions at or near room temperature (<100°C). Ionic liquid has been proved to be an effective solvent for biomass pretreatment, which can effectively destroy the three-dimensional network structure between cellulose, hemicellulose, and lignin, thus improving the efficiency of subsequent enzymatic hydrolysis. Financie et al ^[89] found that ionic liquid pretreatment can significantly remove the lignin in oil palm leaf, leaving only 8.5% of lignin in the pretreated raw material. After pretreatment, the enzymatic hydrolysis efficiency of the oil palm leaf was significantly improved. Hashmi et al ^[90] compared the ionic liquid ([C4mim] [OAc]) pretreatment with the hydrothermal pretreatment of bagasse. The results demonstrated that the increase of hydrothermal pretreatment intensity is beneficial to the enzymatic hydrolysis of cellulose but limited its crystallinity. [C4mim][OAc] pretreatment significantly reduced the lignin content of bagasse; the digestibility of glucan and xylan reached 97.4% and 98.6%, respectively, which was significantly higher than that of the hydrothermal pretreatment (62.1% and 57.5%). Xu et al ^[91] developed a simple and economical pretreatment method by combining an aqueous solution of choline ornithine ([Cho] [Orn]) and a metal salt (FeCl₂). The results demonstrated that the removal of

Table 1 A summary of the advantages and disadvantages of different pretreatment methods for lignocellulosic biomass

Pretreatment	Advantages	Disadvantages
Mechanical	<ol style="list-style-type: none"> 1. Reducing cellulose crystallinity 2. Changing the fiber morphology of the treated material 	<ol style="list-style-type: none"> 1. High power and energy consumption 2. High processing cost 3. Low enzymatic hydrolysis efficiency
Alkaline	<ol style="list-style-type: none"> 1. Removing hemicelluloses and lignin 2. Increasing accessible surface area 3. High glucose and xylose yield after enzymatic hydrolysis 4. Low formation of inhibitors for fermentation 	<ol style="list-style-type: none"> 1. Long residence time required 2. Irrecoverable salts formed and incorporated into biomass 3. Water pollution
Acid	<ol style="list-style-type: none"> 1. High glucose yield after enzymatic hydrolysis 2. Obtaining the xylose without enzymes 3. Reduced hemicellulose content in substrate 	<ol style="list-style-type: none"> 1. Solvents need to be drained and recycled 2. Reactor corrosion 3. Formation of inhibitors 4. Generation of degradation for fermentation products
Steam explosion and hydrothermal	<ol style="list-style-type: none"> 1. Causing lignin transformation and hemicellulose solubilization 2. Higher yield of glucose and hemicellulose in the two-step method 3. High enzymatic hydrolysis efficiency and wide application range 	<ol style="list-style-type: none"> 1. Generation of toxic compounds 2. Partial hemicellulose degradation 3. Low concentration of monosaccharide in pretreatment liquor
AFEX	<ol style="list-style-type: none"> 1. Increasing accessible surface area 2. Low formation of inhibitors 	<ol style="list-style-type: none"> 1. Not efficient for raw materials with high lignin content 2. High cost of large amount of ammonia

lignin and hemicellulose after pretreatment can increase by 28% and 53%, respectively. Chang et al ^[92] explored the effects of different types of surfactants (non-ionic, anionic, cationic, and bio-based surfactants) on the pretreatment of ionic liquids. The results demonstrated that pretreatment with a bio-based surfactant and ionic liquid can effectively remove the lignin of 26.1% in the straw feedstock, and the cellulose conversion rate reached 86.5%. However, the improvement effect of cationic surfactants is not significant.

Regarding the disadvantages of ionic liquids, the major issue is how to recover the ionic liquids owing to its high cost and potential toxicity. Researchers have currently used the electrochemical method, membrane filtration, organic solvent extraction, vacuum distillation, and other technologies to achieve the recovery of ionic liquids.

5.2 Supercritical CO₂ pretreatment

Pure substances form a uniform fluid at the gas-liquid interface at a certain temperature and pressure, which is called supercritical fluid (SCF). The SCF has both liquid and gas properties, such as a low viscosity, high diffusion coefficient, strong solubility, and significant fluidity. Supercritical CO₂ (scCO₂) refers to the CO₂ fluid formed from the above critical temperature of 31.7°C and pressure of 7.38 MPa; scCO₂ is the most commonly used fluid owing to its ease of availability, non-toxicity, non-combustibility, and being environmental-friendly. Recently, scCO₂ has demonstrated an extensive application prospect in biomass pretreatment ^[93]. For scCO₂ pretreatment, it is simple to assimilate into the porous structure of biomass, and the rapid relief of structure damage owing to CO₂ has excellent permeability characteristics. The structure changes of the biomass after scCO₂ pretreatment can be favorable in improving its enzymatic hydrolysis.

Putrino et al ^[94] pretreated green coconut fiber with scCO₂ and found that scCO₂ can effectively reduce the content of phenolic substances from the raw material and break the hydrogen bond between cellulose and

hemicellulose. However, this method cannot enhance the enzymatic hydrolysis efficiency of cellulose; the maximum sugar yield of green coconut fiber treated with scCO₂ for 5 h was only 54%. De Carvalho Silvello et al ^[95] studied the effect of scCO₂ pretreatment on enzymatic hydrolysis of bagasse; the results indicated that the total conversion of cellulose could be increased by 14.3%. Zhang et al ^[14] compared the effects of scCO₂ pretreatment and ultrasonic pretreatment and found optimal scCO₂ pretreatment conditions of sorghum stalk were 60°C and 20 MPa for 36 h, which increased the enzymatic digestibility of sorghum stalk by 43.8%. Zhao et al ^[96] pretreated various crop residues (including corn stalks, corn cobs, and sorghum stalks) with scCO₂ and found that the enzymatic hydrolysis efficiency of pretreated raw materials can increase by 3–4 times that of raw materials. In addition, it was found that water plays an important role in the process of scCO₂ pretreatment (wetting, softening, and swelling). CO₂, as a greenhouse gas, is significant for maintaining the stability of the carbon cycle in the biosphere when it is used as a solvent for chemical reactions during the pretreatment process.

5.3 Deep eutectic solvent pretreatment

Researchers have recently discovered that urea (hydrogen bond donor) and choline chloride (ChCl, hydrogen bond acceptor) can be composed as types of solvents with melting points less than room temperature, and these have been named as deep eutectic solvents (DES) ^[97]. This solvent is non-toxic, biodegradable, and the atomic utilization rate of the synthesis process reaches 100%. DES is considered to be a new type of green ionic liquid owing to its physical and chemical properties being significantly similar to other ionic liquids. There have been several reports regarding the positive effect of DES as an additive in the pretreatment of lignocellulose. According to Nor et al ^[98], the crystallinity of cellulose can decrease after lignocellulose is pretreated with ChCl/urea at 110°C. This phenomenon occurs because the cellulose can be destroyed at high temperatures. Vigier et al ^[99] found that a solvent with sufficient

energy is necessary to disrupt the strong hydrogen bond network of cellulose and the bonds between the carbohydrate and lignin to improve its enzymatic digestibility by enzymes. Lin et al.^[100] studied the effects of different molar ratios of ChCl/lactic acid solvents on the enzymatic hydrolysis efficiency of bamboo; the results demonstrated that with an increasing molar ratio of ChCl/lactic acid, the efficiency of enzymatic hydrolysis increased by 42.7%. Shen et al.^[101] pretreated *Eucalyptus urophylla* with ChCl/lactic acid and found that DES can significantly remove lignin and hemicellulose; the glucose yield of cellulose reached up to 94.3% under the optimal pretreatment conditions (DES solvent molar ratio 10 : 1 at 110°C for 6 h), which was 9.8 times higher than that of the untreated materials. In addition, the results of the experiments on the recoverability of the DES solvent demonstrated that the recovery of the DES solvent was greater than 90%, which is promising for further reuse.

DES are significantly common compounds with abundant sources and low prices. Therefore, DES is expected to achieve large-scale industrial production. However, not all DES can effectively dissolve lignin. It is necessary to detect the dissolution and depolymerization of DES for lignin in biomass prior to pretreatment.

6 Conclusions

Although the existing pretreatment technology has made significant progress and has a relatively ideal pretreatment effect, industrialization of these methods is limited owing to high energy consumption, complex processing, and low target product yield. Considering the concept of green chemical industry and cleaner production, it is imperative to develop efficient and environmentally friendly pretreatment methods, which is critical for the industrialization of bioethanol production from lignocellulosic biomass. In addition, the high cost of enzyme production and the requirement of higher enzyme dosage for the hydrolysis of biomass are considered to be major hurdles for the economic viability of sugar platforms.

To promote the industrialization of bio-refinery, major studies should be conducted, including (1) developing the pretreatment to simultaneously separate the cellulose, hemicellulose, and lignin with a high purity for further application, (2) optimizing the enzyme producing strains for advanced cellulose production to improve the enzymatic hydrolysis of pretreated biomass, and (3) preparing various enzyme cocktails for a specific biomass to improve its enzymatic hydrolysis.

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