

Hydrothermal Pretreatment of Lignocellulosic Materials for Improving Bioethanol Production

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Abstract: With the continued depletion of non-renewable energy resources, it is essential to seek new methods of harnessing clean and renewable energy. In this regard, second-generation bioethanol derived from lignocellulosic biomass has attracted increasing attention in recent years. The choice of the pretreatment method of lignocellulose is critical to the subsequent bioconversion processes. Compared with other conventional chemical pretreatment methods, hydrothermal pretreatment is a simple, low-cost, and economically feasible process that requires water as the only reagent. This paper reviews the research efforts that have been made toward hydrothermal pretreatment of lignocellulosic biomass and focuses on the transformations involving cellulose, hemicellulose, and lignin during this process.

Keywords: hydrothermal pretreatment; bioethanol; biorefinery; lignocellulose



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1 Introduction

Fossil fuels are the primary sources of energy for transportation and manufacturing industries, and extensively utilized to generate electricity, which has brought convenience to modern life. Continued reliance on fossil fuels over the last century has led to an overconsumption of petroleum-derived products, which has adversely affected the environment^[1]. Therefore, it is crucial to develop renewable energy alternatives to petroleum as soon as possible. Bioethanol, a renewable and environment-friendly biofuel, has been widely used as an alternative to fossil fuels^[2]. It is derived from lignocellulose and presents several advantages such as cleaner burning compared to fossil fuels, low net greenhouse gas emissions, short cycle of growing plants, and providing employment in rural areas^[3]. In order to convert lignocellulose into bioethanol, cellulose and hemicellulose need to be converted or broken down into monosaccharides. However, native biomass is a complex structure



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of cellulose-hemicellulose-lignin, which is difficult to access by most enzymes and microorganisms only^[4]. The process of converting lignocellulose into bioethanol involves three steps, viz. pretreatment, enzymatic hydrolysis, and fermentation. As the initial process, pretreatment could significantly enhance the subsequent hydrolysis of cellulose by breaking plant cells in different degree through chemically altering and solubilizing lignin in a process involving the break of the lignin carbohydrate connections (LCC). Consequently, a proportion of hemicellulosic moieties are hydrolyzed, and crystallinity of cellulose is reduced^[5].

Hydrothermal pretreatment utilizes water as the only reaction solvent and the reaction temperatures lie in the range of 180~220°C^[6]. Thus, this process is both environment-friendly and technically attractive because it involves low chemical dosage and lower operational costs, in addition to improving the enzymatic hydrolysis of cellulose^[7]. Although the different parameters and mechanisms of hydrothermal pretreatment have been widely investigated, a detailed review of the structural variations of cellulose, hemicellulose, and lignin taking place during this process has not been reported. The objective of this review is to investigate the influence of pretreatment conditions (temperature and time) on the morphological changes of lignocellulosic fibers and the physicochemical variations of cellulose, hemicellulose and lignin taking place during the hydrothermal pretreatment process. In addition, the improvements in bioconversion efficiency have also been summarized in this paper.

2 Morphological variation

The cell wall of lignocellulose consists of a middle lamella (ML), primary wall (P) and secondary wall (S). The ML is comprised of lignin, hemicellulose, and a small amount of pectin substances while the P contains a high concentration of lignin and hemicellulose. Although the concentrations of lignin and hemicellulose in S are low, that of cellulose is the highest with a regular arrangement of microfibrils^[8]. This complex

structure makes lignocellulose recalcitrant toward deconstruction by enzymes or microbes. Biomass recalcitrance is one of the primary barriers to low-cost biological conversion of the lignocellulosic biomass to biofuels. Hydrothermal pretreatment reduces this recalcitrance by depolymerizing and solubilizing hemicellulose and lignin, which results in a breaking down of the rigidity of biomass by decreasing the physical barriers. In addition, the cell wall of different species and tissues undergoes highly complex and different changes after hydrothermal pretreatment, reflecting the variation in its chemistries^[9].

The biomass particles change differently before and after hydrothermal pretreatment at varying degrees of severity. Scanning electron micrograph (SEM) images given in Fig.1 and Fig.2 show these changes clearly. The samples treated under mild conditions (reflux and Soxhlet) exhibited similar color and morphologies to those of the parent biomass (Fig.1)^[10]. With the increasing severity of pretreatment conditions, the color of the samples became darker, and the microstructure of the biomass particles was partially destroyed. Under the severest condition (220°C, 15 min), droplets of lignin could be observed (arrows) on the surface of the biomass particles. These lignin droplets had dissolved and recondensed during the hydrothermal pretreatment.

Fig.2 shows the SEM images of the different *Tamarix* samples. These images were obtained to further verify the material structural changes taking place as a result of the hydrothermal pretreatment. The regular and compact surface structure of the untreated material (Fig.2a) underwent gradual defibration with increasing hydrothermal temperature, and some structural breakdown and cracks could be clearly observed (Fig.2b, Fig.2c). Along with the temperature increased above 140°C, the surface became uneven and covered with small debris of perfectly granular and globular droplets. These droplets were identified as molten lignin, which was formed at the high temperature and condensed under compressed pressure (Fig.2d~Fig.2g)^[11]. Mechanistic investigations indicated that such thermochemical pretreatment exceeded the

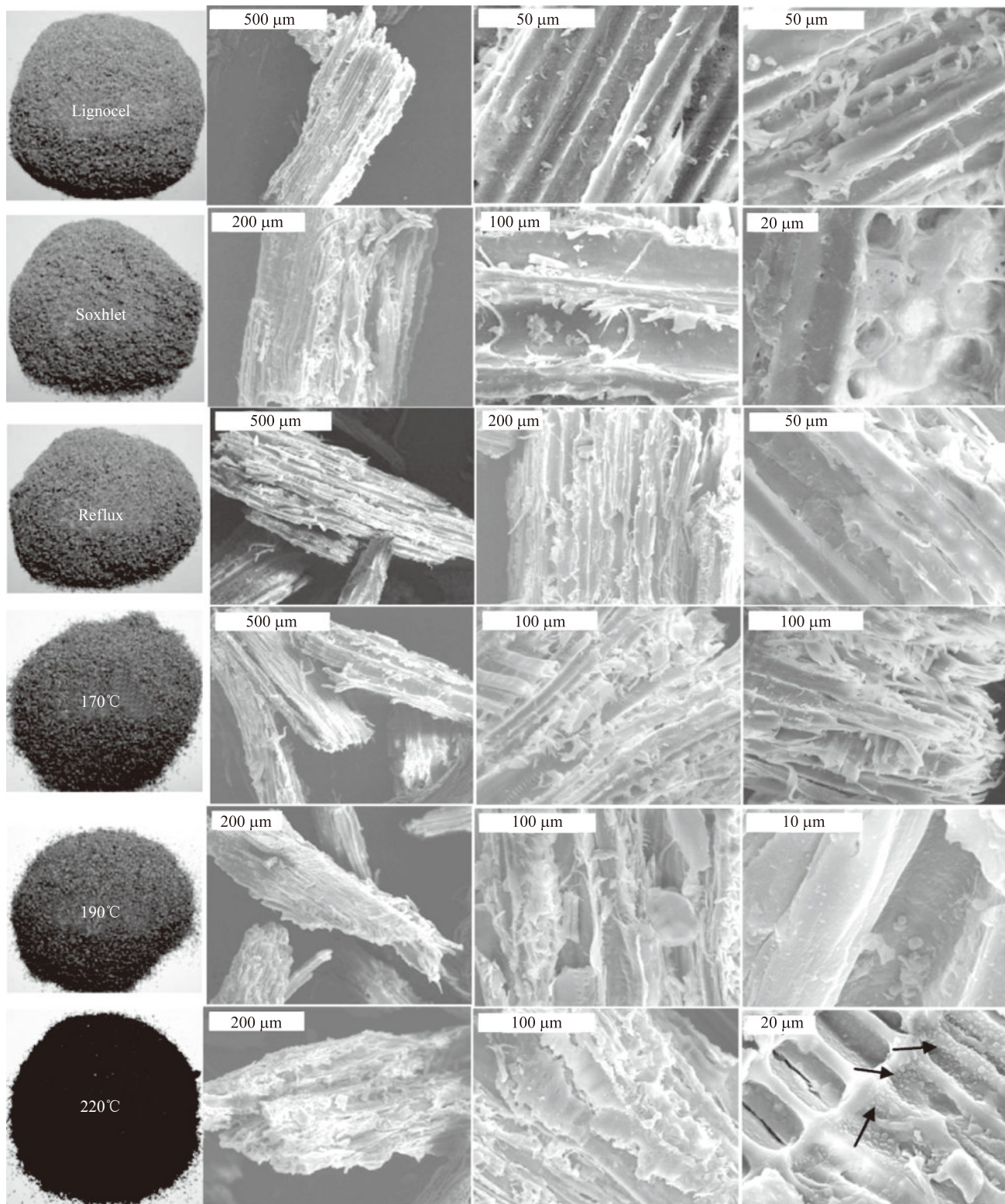


Fig.1 SEM images of the parent and representative hydrothermally treated biomass samples at increasing temperature and constant reaction time (15 min)

melting temperature of lignin, which resulted in the expansion and movement of lignin within the cell wall matrix. In an aqueous environment, the hydrophobic

lignin minimizes its contact surface area with water by coalescing and forming spheres. However, the natural pore structure of the cell wall limits such casual

aggregation of lignin. Therefore, small elliptic cylinders were formed instead of spheres under these conditions. If lignin exists in a larger void such as a delamination layer, cell corner, or pit, then the reshaping process of lignin formed flattened disks or spherical droplets^[12]. It was also observed that the molten lignin droplets left the cell wall matrix and moved into the hydrolysate

solution, redepositing on the surface of cellulose fibers during cooling.

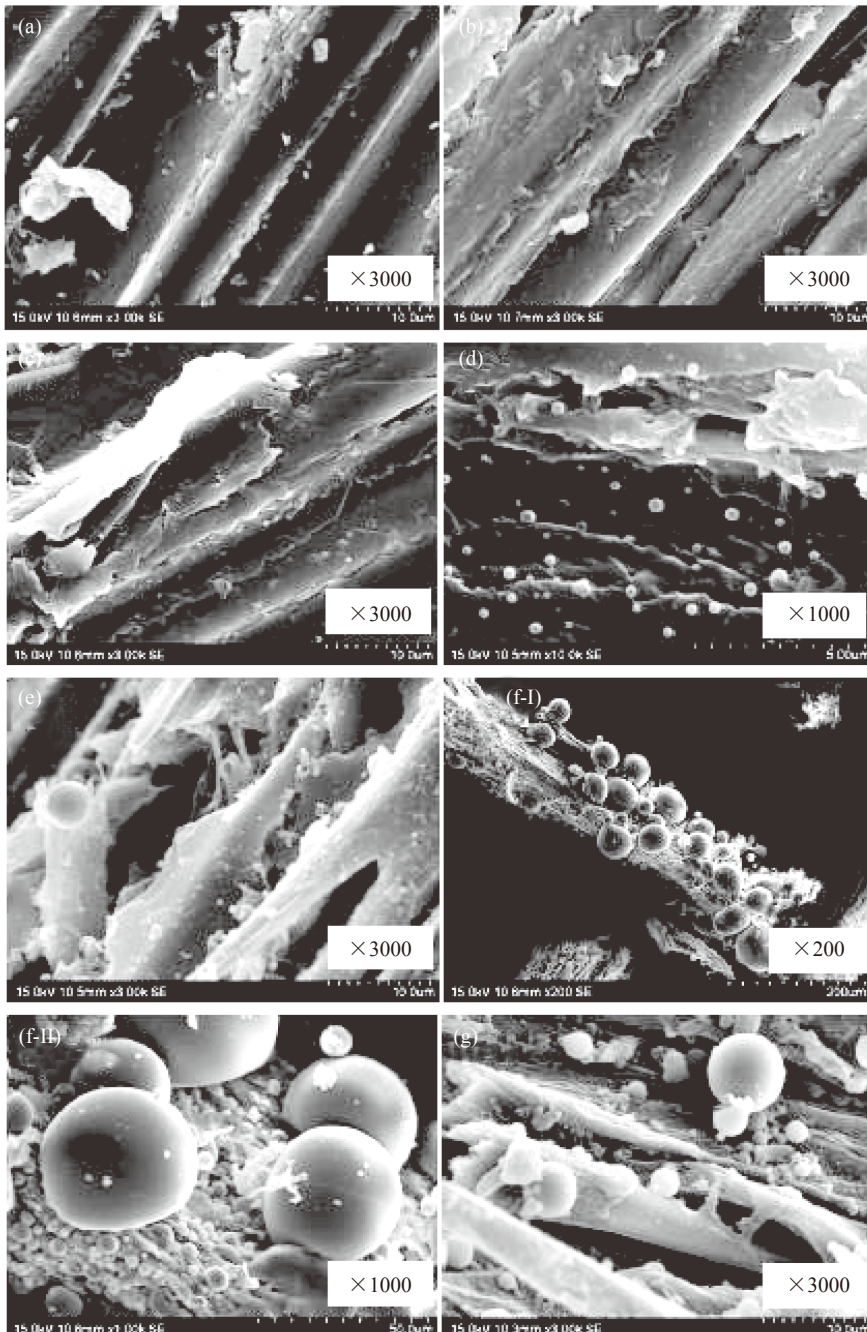
3 Physicochemical variations of three main components

3.1 Cellulose

The solid materials obtained after pretreatment contain

most of the cellulose and lignin, with varying amounts of hemicellulose (depending on the severity of the pretreatment conditions). As the main carbohydrate source, cellulose is used for hydrolysis and fermentation into ethanol. Second-generation bioethanol production methods include cellulose digestion after the pretreatment and fractionation processes. In order to obtain residues with high cellulose content after pretreatment, one of the major determinants of pretreatment efficiency is avoiding the solubilization of cellulose^[13]. However, the commonly employed pretreatment methods are limited by a low bioconversion utilization coefficient and high capital and operational costs, thus are unable to satisfy the criteria for commercial utilization of lignocellulose for bioethanol production.

Cellulose is a crystalline, linear polymer with a complex hydrogen-bonded network structure. Compared to its crystalline form, amorphous cellulose is significantly more reactive and the minimal temperature required to break the glycosidic bonds in its latter form is 150°C. Since amorphous cellulose is quite reactive, it produces more glucose monomers and oligomers at



(a) untreated raw material; pretreated *Tamarix* samples at different temperatures for 3 h:
(b) 100°C, (c) 120°C, (d) 140°C, (e) 160°C, (f-I) 180°C, (f-II) 180°C, (g) 200°C

Fig.2 SEM images of untreated and pretreated *Tamarix* samples

the same temperature compared to the crystalline form. Nevertheless, crystallinity has been suggested to be the most important factor in the following bioconversion process. Therefore, alterations in substrate characteristics after the mechanical pretreatment, such as the increase in available surface area or the reduction of particle size, may induce a decrease in crystallinity^[14].

The effectiveness of cellulase is significantly reduced without pretreatment, closely relating to the intermolecular hydrogen bond in the crystalline region of cellulose. Although the lignocellulosic feedstock is more easily hydrolyzed after hydrothermal pretreatment, severe conditions such as high temperature and a longer duration time could induce the degradation of the carbohydrate. Hence, it is necessary to optimize the hydrothermal conditions according to the type, physical (particle size, moisture content, density, etc.) and chemical (contents of hemicellulose and lignin, degree of polymerization, crystallinity index, etc.) characteristics of the biomass^[15].

In the hydrothermal pretreatment process, the recovery of cellulose decreases while the amount of cellulose-derived sugars such as glucose, cellobiose, and cellulose oligomers in the liquid fraction increases with the severity of reaction conditions^[16]. According to the results from further analysis of the liquid product obtained at different temperatures, it could be confirmed that a few short glucose chains present in the amorphous section of cellulose are bonded to crystalline cellulose through hydrogen bonding, and may produce C4~C13 oligomers when the hydrothermal pretreatment was performed at 100°C. The minimum temperature at which the glucoside bonds of the short chains in the amorphous form underwent dissociation was determined to be ca. 150°C, while the hydrolysis of a part of the crystalline structure started at ca. 180°C. Because of the strong intra- and intermolecular hydrogen bonding, the efforts aimed at cleavage of the glucosidic bonds in the crystalline structure are limited during the hydrothermal pretreatment process. The extent of hydrogen bonds and chain lengths in the crystalline and amorphous

sections are different, which significantly affects the distribution of glucose oligomers in the hydrolysate. Although a larger amount of glucose and oligomers could be obtained from the hydrolysis of amorphous cellulose than crystalline cellulose under identical harsh conditions, the selectivity of glucose and oligomers in the primary liquid products did not show a monotonic trend with the degree of polymerization. After hydrothermal pretreatment at a high temperature, X-ray diffraction scans results found that crystalline cellulose changed to a smaller extent in comparison with the untreated eucalyptus. This observation suggested that high-temperature hydrothermal pretreatment had little influence on cellulose. Compared with the tightness and integrity structure of raw eucalyptus, hydrothermal pretreatment shortened the fiber length, increased the cellulose fragments, loosened the combinations between cellulose chains, and fractured the original structure to a large extent^[17]. Using wheat straw as raw materials, it was observed that the extent of hydrolysis of cellulose increased with increasing duration of pretreatment before 15 min. However, the hydrolysis of cellulose slowly decreased between 25 min and 60 min of pretreatment^[2].

3.2 Hemicellulose

Hemicellulose is a set of branched, amorphous heteropolymers that have a varying composition depending on the plant species, cell tissues, type of glycosidic bonds, composition of the side chains, and degree of polymerization. During the hydrothermal pretreatment, hemicellulosic carbohydrates are usually depolymerized to oligomers and monomers, and subsequently dissolved in water. Technically, the oligomers could be further hydrolyzed into C5 sugars by enzymes and fermented by a suitable microorganism to improve the ethanol yield^[18]. Degradation products have an inhibitory effect on enzyme hydrolysis and fermentation. Hence, the thermal degradation of monomers must be minimized during pretreatment^[19].

Some inhibitors influence the fermentation process and are mainly classified into three categories,

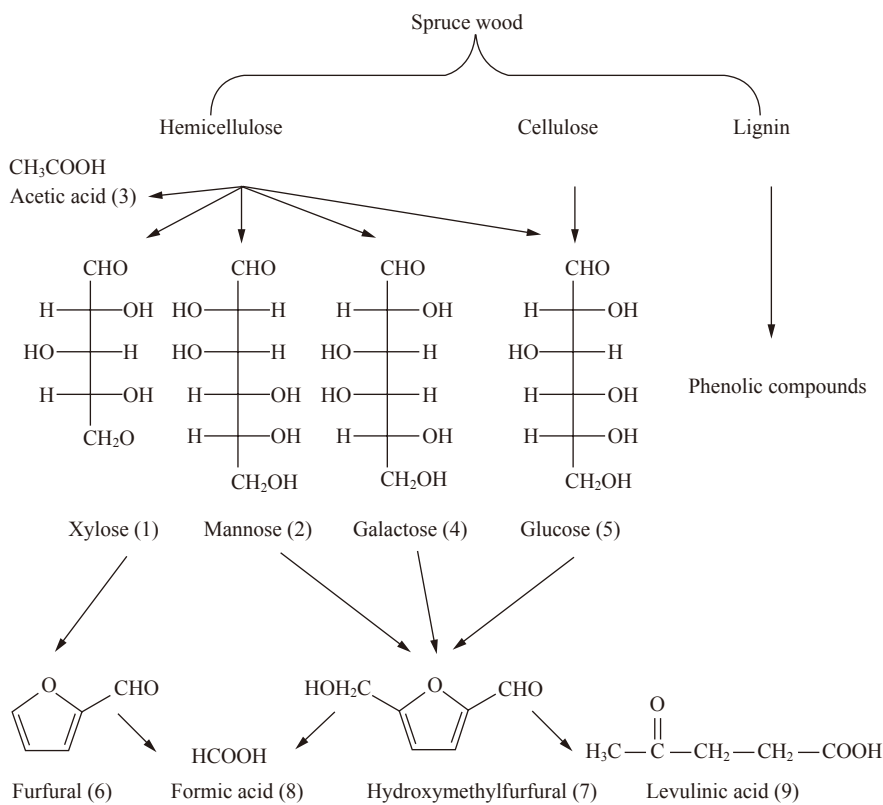


Fig.3 Reactions occurring during hydrolysis of lignocellulosic materials

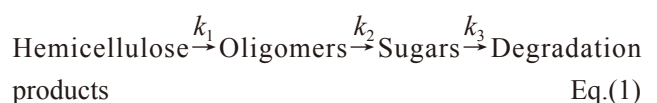
namely, carboxylic acids (e.g., formic acid, acetic acid, glucuronic acid, levulinic acid, ferulic acid, and coumaric acid), furan derivatives (e.g., furfural, hydroxymethylfurfural), and phenolic compounds (e.g., hydroquinone)^[20]. In the pretreatment process, autoionization of water generates hydronium ions, which contribute to the rupture of glycosidic bonds and the acetyl groups pull away from hemicellulose to form acetic acid in the aqueous solution. The furan derivatives and soluble phenolic compounds from lignin could be degraded further to form some polymeric material. Furfural and 5-hydroxymethylfurfural (HMF) are the direct degradation products of xylose and glucose. HMF could be further broken down to form levulinic and formic acids, while furfural degraded to formic acid^[21]. The main degradation pathways are illustrated in Fig.3^[22]. The furan derivatives and phenolic compounds will react further to form some polymeric material.

While the generated acetic acid promotes hydrolysis, the hydronium ions from acetic acid

dissociation have a stronger effect on hydrolysis than those produced from water. Weak acids make the intracellular environment acidic, which could explain why inhibitors restrain cell growth. The main influence of furan aldehydes on *Saccharomyce scerevisiae* is the inhabitation to the yeast growth. Phenolic compounds have strong inhibitory effect on the fermentation process, as low molecular weight phenolic compounds are strong toxins^[8]. The oligosaccharides generated in the hydrothermal pretreatment process could also inhibit the activity of cellulase; the degree of polymerization in the oligosaccharides is between 7 and 10, which was confirmed by the HPLC and MS^[17]. Most

of the hemicellulose content breaks down into oligosaccharides and dissolves in the pretreated liquid.

In order to design an appropriate pretreatment process, kinetic models could be used to identify and evaluate the different innovative configurations. As one of the components in lignocellulose, hemicellulose is mostly solubilized by hydrothermal pretreatments. In order to preserve sugars and avoid forming inhibitors of the enzymatic hydrolysis process, an understanding of the kinetics of hemicellulose hydrolysis is essential to propose conditions of solubilization^[23]. The pseudo-homogeneous kinetic models are usually used to express the mechanism of hydrolysis of polyxylose. However, the study on polyxylose dynamics is very difficult because the involved reaction steps are complex and there are many other influencing factors^[24]. Akinetic model that describes the hydrolysis of hemicellulose into xylooligomers is presented in Eq.(1)^[25].



3.3 Lignin

As an amorphous phenolic polymer, lignin plays the main role in strengthening the cell wall and protecting the plant from microbial damage. Lignin limits the rate of enzymatic hydrolysis in two possible ways; by acting as a physical barrier, it prevents access to the digestible parts of the substrate and appears to reduce cellulose hydrolysis by non-productive binding to cellulosic enzymes^[26]. While hydrothermal pretreatment removes only small amounts of lignin (the acid soluble fraction), it changes the lignin structure via its melting, coagulation, and subsequent repolymerization on the cellulose fibers. Therefore, it is impossible to extract lignin in its functional form from the hydrothermally pretreated solids^[27]. Depolymerization and repolymerization take place almost simultaneously. Spherical droplets formed on the surface of lignocellulosic materials are attributed to pseudo-lignin. Pseudo-lignin is considered to be formed as a result of repolymerization of the polysaccharide degradation products (such as furfural) or the polymerization of polysaccharides with lignin. It could be broadly defined as aromatic material which contains carboxyl, carbonyl, aliphatic, and aromatic structures. Pseudo-lignin yields a positive Klason lignin value and is not derived from native lignin. Furthermore, the change in its molecular weight is limited, $M_n \approx 1000$ g/mol and $M_w \approx 5000$ g/mol^[28].

The changes in the lignin structure contribute to the cleavage of the linkages between lignin and carbohydrates. This makes the cellulose fibrils vulnerable to the enzymatic action. In hydrothermal pretreatment, lignin impacted by hydrogen ion under acidic conditions transforms into benzyl alcohol structure intermediates. The ether bond is unstable and easily dissociated. Lignin fragments and carbocations of adjacent aromatic rings generate new C—C bonds. The syringil units in lignin have been found to be the most susceptible to degradation during hydrothermal pretreatment^[29]. The effects of lignin relocation or deposition are as important as its removal in improving cellulose digestibility. Irregular binding of enzymes

onto lignin is also influenced by the nature of the substrate. Lignin exerts a different inhibition influence on the different cellulases, but it has a lesser influence on xylanases and glucosidases^[19].

4 Bioconversion efficiency

As previously discussed, there occurs a simultaneous morphological variation of the cell wall with the physicochemical changes in cellulose, hemicellulose, and lignin during hydrothermal pretreatment. Under the combined action of the abovementioned factors, the bioconversion efficiency of lignocellulosic material is correspondingly improved. Table 1 summarizes the most recently published results on the bioconversion of lignocellulosic materials after hydrothermal pretreatment. The bioconversion efficiency of various materials was significantly improved after hydrothermal pretreatment. For example, a previous study reported that after 72 h of enzymatic hydrolysis, the digestibility was enhanced from 15.7% (untreated sugarcane bagasse) to 77.9% for the samples treated at 180°C for 20 min^[4]. Analogously, compared with the conversion of 48.9% of untreated prairie cord grass via hydrolysis, hydrothermal pretreatment at a high temperature

Table 1 Recent results on the bioconversion of biomass after hydrothermal pretreatment

Optimal condition		Raw materials	Bioconversion efficiency/%	Ref.
Temperature /°C	Time /min			
200	5	Corn stover	68.7	[2]
180	20	Sugarcane bagasse	77.9 (glucose)	[4]
80	5~10	Wheat straw	92.0	[19]
195	6~12			
180	20	Eucalyptus	80.5 (glucose)	[30]
260	60	Olive tree	76.0 (glucose)	[31]
210	10	Wheat straw	75.0 (glucose)	[32]
178	11.1	Oil palm fronds	92.8 (glucose)	[33]
190	17.2	Sugarcane bagasse	50.1	[34]
180	10	Wheat bran	97.5	[35]
200	10	Switchgrass	78.0	[36]
180	35	Miscanthus	77.9 (glucose)	[37]
188	40	Wheat straw	79.8	[38]
217/217.7	42/42.2	Rapeseed	69.0	[39]
170/200	0/40	Wheat straw	95.8	[40]

and short reaction time (210°C, 10 min) gave the highest conversion (94.5%)^[29]. Further enzymolysis of the optimized pretreated residue improved glucose conversion to 80.5%. This conversion percentage was an increase of 3.6 times compared with that of the raw residue^[30]. Thus, pretreatment does not result in a significant conversion of fibers into glucan, which might be explained by inefficient alterations in the structure of cellulose chains that limit the accessibility of cellulases.

Hemicellulose-derived sugar recovery in the prehydrolysate depends on the temperature and duration of the pretreatment process. It was found that the yield of the enzymatic hydrolysis step was enhanced as both pretreatment temperature and time increased. Pérez et al had found that the optimum conditions for maximum enzymatic hydrolysis yield were when the reaction was performed at 200°C for 40 min, and the xylan and acetyl group content remaining in the solid residue after pretreatment remarkably affected the degradability of substrate^[13]. Cellulose digestibility of the pretreated leaf portion of the switch grass gave a 32.5% greater glucose yield (77.4%) than that of the internode portion (44.9%)^[6]. Some results have suggested that the most efficient pretreatment conditions involve high temperatures (210°C) and relatively short reaction times (10 min), and the lignocellulose structure seems to be the most favored for enzymatic degradation. In this case, the pretreatment conversion rate reaches 97%^[5]. The optimum conditions for pretreatment as determined by Goh et al resulted in 92.8% glucose yield at 178°C for 11.1 min and a liquid-solid ratio of 9.6^[33]. It was found that the SSF (Simultaneous Saccharification and Fermentation) yield of the steam explosion pretreated materials showed a yield profile similar to that of enzymatic hydrolysis in relation to the studied pretreatment parameters, which increased with the increasing temperature. The maximum yield amounted to 60% of the theoretical value, corresponding to a final ethanol concentration of 17 g/L. The theoretical SSF yield is calculated by assuming that all of the potential glucose in the pretreated material is available for

fermentation at a fermentation yield of 0.51 g ethanol/g glucose^[24]. Bacterium is able to utilize all available sugars completely and produce ethanol. However, the bacterium first started to utilize glucose, followed by xylose and arabinose when a considerable amount of glucose (27%) had been depleted from the fermentation broth. In addition, the products of enzymatic hydrolysis after hydrothermal pretreatment are unnecessary proceeding detoxification.

5 Conclusions

Hydrothermal pretreatment, an effective and environmentally friendly method, has been extensively employed as no chemicals are required in this simple process. This method removes a majority of hemicellulose and a small part of lignin, thus enabling the fractionation of biomass, and also causes a morphological variation of the cell wall. The effectiveness of hydrothermal pretreatment for lignocellulosic materials prior to their hydrolysis and after ethanol fermentation was compared for both treated and untreated materials. However, it is necessary to conduct further research in order to improve the effectiveness of the hydrothermal pretreatment process, since the bioconversion efficiency is still not satisfactorily high. Recently, hydrothermal pretreatment has been combined with other pretreatment procedures (acidic, alkaline, organosolv, etc.) as pioneering new technologies.

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Reference

- [1] Shen F, Liu R. Research on solid-state ethanol fermentation using dry sweet sorghum stalk particles with active dry yeast[J]. *Energy & Fuels*, 2008, 23 (1): 519-525.
- [2] Saha B C, Yoshida T, Cotta M A, et al. Hydrothermal pretreatment and enzymatic saccharification of corn stover for efficient ethanol production[J]. *Industrial Crops and*

- Products, 2013, 44: 367-372.
- [3] Hahn-Hägerdal B, Galbe M, Gorwa-Grauslund M F, et al. Bio-ethanol—the fuel of tomorrow from the residues of today[J]. Trends in Biotechnology, 2006, 24(12): 549-556.
 - [4] Zhuang X, Yu Q, Yuan Z, et al. Effect of hydrothermal pretreatment of sugarcane bagasse on enzymatic digestibility[J]. Journal of Chemical Technology and Biotechnology, 2015, 90 (8): 1515-1520.
 - [5] Cybulska I, Lei H, Julson J. Hydrothermal pretreatment and enzymatic hydrolysis of prairie cord grass[J]. Energy & Fuels, 2009, 24(1): 718-727.
 - [6] Hu Z, Ragauskas A J. Hydrothermal pretreatment of switchgrass[J]. Industrial & Engineering Chemistry Research, 2011, 50(8): 4225-4230.
 - [7] Rocha G J M, Silva V F N, Martín C, et al. Effect of xylan and lignin removal by hydrothermal pretreatment on enzymatic conversion of sugarcane bagasse cellulose for second generation ethanol production[J]. Sugar Tech, 2013, 15(4): 390-398.
 - [8] Xiao L P. Mechanism research on hydrothermal utilization process of lignocellulosic biomass[D]. Beijing: Beijing Forestry University, 2014.
 - [9] Zhang H, Thygesen L G, Mortensen K, et al. Structure and enzymatic accessibility of leaf and stem from wheat straw before and after hydrothermal pretreatment[J]. Biotechnology for Biofuels, 2014, 7(1): 74.
 - [10] Nitsos C K, Matis K A, Triantafyllidis K S. Optimization of hydrothermal pretreatment of lignocellulosic biomass in the bioethanol production process[J]. Chem Sus Chem, 2013, 6(1): 110-122.
 - [11] Xiao L P, Sun Z J, Shi Z J, et al. Impact of hot compressed water pretreatment on the structural changes of woody biomass for bioethanol production[J]. Bioresources, 2011, 6 (2): 1576-1598.
 - [12] Donohoe B S, Decker S R, Tucker M P, et al. Visualizing lignin coalescence and migration through maize cell walls following thermochemical pretreatment[J]. Biotechnology and Bioengineering, 2008, 101(5): 913-925.
 - [13] Pérez J A, Ballesteros I, Ballesteros M, et al. Optimizing liquid hot water pretreatment conditions to enhance sugar recovery from wheat straw for fuel-ethanol production[J]. Fuel, 2008, 87(17): 3640-3647.
 - [14] Alvira P, Tomás-Pejó E, Ballesteros M, et al. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review[J]. Bioresource Technology, 2010, 101(13): 4851-4861.
 - [15] Petchpradab P, Yoshida T, Charinpanitkul T, et al. Hydrothermal pretreatment of rubber wood for the saccharification process[J]. Industrial & Engineering Chemistry Research, 2009, 48(9): 4587-4591.
 - [16] Petersen M Ø, Larsen J, Thomsen M H. Optimization of hydrothermal pretreatment of wheat straw for production of bioethanol at low water consumption without addition of chemicals[J]. Biomass & Bioenergy, 2009, 33(5): 834-840.
 - [17] Kont R, Kurašin M, Teugjas H, et al. Strong cellulase inhibitors from the hydrothermal pretreatment of wheat straw[J]. Biotechnology for Biofuels, 2013, 6(1): 135.
 - [18] Min D, Xu R, Hou Z, et al. Minimizing inhibitors during pretreatment while maximizing sugar production in enzymatic hydrolysis through a two-stage hydrothermal pretreatment[J]. Cellulose, 2015, 22(2): 1253-1261.
 - [19] Ruiz H A, Ruzene D S, Silva D P, et al. Evaluation of a hydrothermal process for pretreatment of wheat straw—effect of particle size and process conditions[J]. Journal of Chemical Technology & Biotechnology, 2011, 86(1): 88-94.
 - [20] Alvira P, Tomás-Pejó E, Ballesteros M, et al. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review[J]. Bioresource Technology, 2010, 101(13): 4851-4861.
 - [21] Yu Q, Zhuang X, Lv S, et al. Liquid hot water pretreatment of sugarcane bagasse and its comparison with chemical pretreatment methods for the sugar recovery and structural changes[J]. Bioresource Technology, 2013, 129: 592-598.
 - [22] Palmqvist E, Hahn-Hägerdal B. Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition[J]. Bioresource Technology, 2000, 74(1): 25-33.
 - [23] Santucci B S, Maziero P, Rabelo S C, et al. Autohydrolysis of hemicelluloses from sugarcane bagasse during hydrothermal pretreatment: a kinetic assessment[J]. Bioenergy Research, 2015, 8(4): 1778-1787.
 - [24] Ma J. The mechanism of topochemical dissolving of hemicellulose during hydrothermal pretreatment of poplar[M]. Beijing: Beijing Forestry University, 2015.
 - [25] Pronyk C, Mazza G. Kinetic modeling of hemicellulose hydrolysis from triticale straw in a pressurized low polarity water flow-through reactor[J]. Industrial & Engineering Chemistry Research, 2010, 49(14): 6367-6375.
 - [26] Trajano H L, Engle N L, Foston M, et al. The fate of lignin during hydrothermal pretreatment[J]. Biotechnology for Biofuels, 2013, 6(1): 110.
 - [27] Aita G M, Kim M. Pretreatment Technologies for the Conversion of Lignocellulosic Materials to Bioethanol[J]. Acs Symposium, 2010, 1058: 117-145.
 - [28] Hu F, Jung S, Ragauskas A. Pseudo-lignin formation and its impact on enzymatic hydrolysis[J]. Bioresource Technology, 2012, 117(4): 7-12.
 - [29] Cybulska I, Brudecki G, Lei H. Green Biomass Pretreatment for Biofuels Production[M]. Springer

- Netherlands, 2013: 87-106.
- [30] Xu S H, Wu S B, Wei W Q. The changes of three-components in eucalyptus during processes of pre-processing in high temperature liquid water and enzymolysis[J]. Chemistry and Industry of Forest Products, 2013, 33(1): 21-26.
 - [31] Cara C, Romero I, Oliva J M, et al. Liquid hot water pretreatment of olive tree pruning residues[J]. Applied Biochemistry and Biotechnology, 2007, 137-140(1): 379-394.
 - [32] Ballesteros I, Negro M J, Oliva J M, et al. Ethanol production from steam-explosion pretreated wheat straw[J]. Applied Biochemistry & Biotechnology, 2006, 130(1): 496-508.
 - [33] Goh C S, Lee K T, Bhatia S. Hot compressed water pretreatment of oil palm fronds to enhance glucose recovery for production of second generation bio-ethanol [J]. Bioresource Technology, 2010, 101(19): 7362-7367.
 - [34] Da Cruz S H, Dien B S, Nichols N N, et al. Hydrothermal pretreatment of sugarcane bagasse using response surface methodology improves digestibility and ethanol production by SSF[J]. Journal of Industrial Microbiology & Biotechnology, 2012, 39(3): 439-447.
 - [35] Merali Z, Collins S R A, Elliston A, et al. Characterization of cell wall components of wheat bran following hydrothermal pretreatment and fractionation[J]. Biotechnology for Biofuels, 2015, 131(1): 226-234.
 - [36] Suryawati L, Wilkins M R, Bellmer D D, et al. Simultaneous saccharification and fermentation of Kanlow switchgrass pretreated by hydrothermolysis using *Kluyveromyces marxianus* IMB4[J]. Biotechnology and Bioengineering, 2008, 101(5): 894-902.
 - [37] Zhang T, Wyman C E, Jakob K, et al. Rapid selection and identification of Miscanthus genotypes with enhanced glucan and xylan yields from hydrothermal pretreatment followed by enzymatic hydrolysis[J]. Biotechnology for Biofuels, 2012, 5 (1): 56.
 - [38] Pérez J A, Ballesteros I, Ballesteros M, et al. Optimizing liquid hot water pretreatment conditions to enhance sugar recovery from wheat straw for fuel-ethanol production[J]. Fuel, 2008, 87(17): 3640-3647.
 - [39] López-Linares J C, Romero I, Cara C, et al. Experimental study on ethanol production from hydrothermal pretreated rapeseed straw by simultaneous saccharification and fermentation[J]. Journal of Chemical Technology and Biotechnology, 2014, 89(1): 104-110.
 - [40] Pérez J A, González A, Oliva J M, et al. Effect of process variables on liquid hot water pretreatment of wheat straw for bioconversion to fuel-ethanol in a batch reactor[J]. Journal of Chemical Technology and Biotechnology, 2007, 82(10): 929-938. PBM