

Effects of ball milling on structural changes and hydrolysis of lignocellulosic biomass in liquid hot-water compressed carbon dioxide

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Abstract—Mechanical activation is an effective method for destroying the crystalline structure. Biomass, especially its hemicellulose, can be degraded in the green solvent of liquid hot-water compressed carbon dioxide. To improve the degradation of crystalline cellulose in liquid hot-water compressed carbon dioxide, pretreatment of camphorwood sawdust by mechanical activation with a stirring ball mill was studied. Ball milling parameters and their effects on structure were determined by SEM, XRD and FT-IR. The influence of milling parameters on cellulose conversion can be ranked as follows: ball milling speed > activation time > the mass ratio of ball to biomass. The optimum milling condition was obtained at ball milling speed of 450 rpm and mass ratio of 30 : 1 of ball to biomass for 2 h. In this condition, cellulose crystallinity of sawdust decreased from 60.93% to 21.40%. The cellulose conversion was 37.8%, which was nearly four times of raw material (10.2%). The glucose yield in the hydrolysate was 1.49 g·L⁻¹, which was nearly three times of that of raw material. It showed mechanical activation can destroy the crystalline structure of cellulose to promote degradation and the damage of lignocellulosic internal structure caused by ball milling is irreversible.

Keywords: Mechanical Activation, Biomass Pretreatment, Cellulose, Crystalline Structure, Carbon Dioxide Hydrolysis

INTRODUCTION

During this age of continued industrialization, the increasing shortage of traditional fossil fuels brings about a serious energy and ecological crisis. To decrease the reliance on fossil fuels, a world-wide effort has been directed to develop renewable and clean energy [1,2]. Currently, as one of the promising alternative energy sources, lignocellulosic biomass has attracted increasing attention. Utilization of biomass energy can mitigate the change of global climate and alleviate air pollution. Lignocellulosic biomass mainly consists of cellulose, hemicellulose and lignin. The three components associate with each other and form a complicated network structure through hydrogen bonds and covalent bonds. Cellulose is a linear homo-polymer of glucose linked by β -1, 4 glycosidic bonds. The highly-ordered and recalcitrant structure of lignocelluloses makes it difficult to dissolve in common solvents and thus restricts its efficient applications [3]. Acid hydrolysis is a popular process to degrade biomass. Commonly used acids include sulfuric acid or hydrochloric acid. A large amount of waste water and impurities will be produced in acid treatment process. And corrosion of equipment by acid is also undesirable in industry [4,5]. Enzymes can effectively catalyze the conversion of biomass without other chem-

icals and under mild processing conditions. However, the very low treatment rate and the long cycle processing and high cost lead to a long way to commercial application [6,7].

With an increasing requirement of environmental protection, supercritical carbon dioxide and water have attracted extensive attention as environmentally friendly solvents because of its non-toxic, non-corrosive, stable chemical properties and abundant source. Researches [8] have shown that carbon dioxide dissolved in hot water will increase the acidity of the solution and promote dissolution of biomass, especially hemicellulose. This process is more environmentally friendly because the process does not discharge harmful chemicals, compared with the hydrochloric acid and sulfuric acid catalytic hydrolysis, but has low cellulose conversion.

To improve the chemical reactivity of lignocelluloses, breaking the lignin seal and disrupting the crystalline structure are necessary to make the cellulose more accessible to degrading reagents. Many methods have been reported to pretreat lignocellulosic biomass, including physical methods, chemical methods, physicochemical methods, biological methods and their combinations [9-14]. Each method has its own advantages and disadvantages. Karimi et al. [15] reported 80.8% of xylose yield and 25.8% of glucose yield in enzymolysis reaction of straw pretreated with 0.5 wt% H₂SO₄ at 201 °C and 1.5 MPa for 10 min. R.C.N.R. Corrales et al. [16] treated sugarcane bagasse with CO₂ steam explosion for 15 min at 205 °C, and bagasse fiber crystallinity increased from 48.0% to 56.4% with 40.5% of hemicellulose degradation. Acid pretreatment and alka-

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line pretreatment are very effective; however, the high temperature and pressure are the main drawbacks. During the processes, hemicellulose degradation reduced the utilization of raw materials, and waste was produced causing environmental pollution. The main disadvantages of steam explosion are the incomplete degradation of lignin and the generation of some toxic compounds that could inhibit the subsequent hydrolysis.

In recent years, mechanical activation technology was introduced to biomass treatment. Mechanical activation is usually achieved by high-energy ball milling. During a milling process, impact, collision, friction and shear forces are generated due to relative motion between milling media and samples. The action can remarkably change the crystalline structure and physicochemical properties of samples. It induces crystal defect and dislocation. At the same time, it reduces the particle size and increases the specific surface area and surface free energy, contributing to enhancing chemical reactivity [17]. In addition, a part of mechanical energy could be converted into internal energy of the milled solids and enhance the chemical reactivity of solids. The technology has been widely used in hydrometallurgy, mineral treatment, and synthesis of nanomaterials etc. Many studies have shown that mechanical activation does not require high temperature or pressure, and does not cause environmental pollution or loss of any of the main components of lignocellulosic biomass [18]. Therefore, mechanical activation is a simple, highly efficient and environmental friendly method for the pretreatment of lignocellulosic biomass. Ayla Sant'Ana et al. [19] reported that glucose yield increased from 95.4 mg/g (bagasse) to 338.6 mg/g (bagasse) in enzymolysis reaction with bagasse pretreated with planetary ball milling at 400 rpm for 60 min. Ago et al. [20] reported that mechanical activation treatment of native cellulose with 30-50 wt% water could cause a structural shift from cellulose I to cellulose II. Liao et al. [21] found that the cellulose crystalline structure of cassava residue was disrupted and the crystalline index was decreased from 44.2% to 19.5% with milling activation for 2 h. Hu et al. [22] found that *eucalyptus* pulp solubility in NaOH solution increased from 20% to 78% and the degree of polymerization reduced from 237 to 130 in milling activation for 90 min. However, most of the studies focused on the structural changes of high cellulose content biomass or pure cellulose in the milling process; only the effect of activation time was considered. Few works investigated the milling activation of complex natural lignocellulosic biomass and other milling factors such as ball milling speed, the mass ratio of the ball to the material, and the filling ratio. Based on the study of milling speed, the mass ratio of the ball to the material and the filling ratio, the optimal milling conditions and activation mechanism can be obtained to improve the energy efficiency.

This work focuses on improving the hydrothermal hydrolysis of cellulose catalyzed by compressed carbon dioxide after stirring ball milling. The effects of milling process parameters (ball milling speed, activation time and the mass ratio of the ball to biomass) on the morphology, distribution of particle size, specific surface areas and crystalline structure of camphorwood were studied. The significance order of three parameters was determined and process parameters were optimized to improve cellulose conversion in the subsequent hydrothermal hydrolysis catalyzed by compressed carbon dioxide. The degradation products were also analyzed to value the

Table 1. Contents of main composition in different biomass

Biomass	Cellulose (wt%)	Hemicellulose (wt%)	Lignin (wt%)
Camphorwood sawdust	36.3	30.1	23.7
Rape-straw	35.7	19.3	18.9
Microcrystalline cellulose	95.1	-	-

success of ball milling. Moreover, microcrystalline cellulose and rape-straw were treated under optimum milling conditions so as to compare the effect of mechanical activation on different lignocellulosic biomass.

EXPERIMENTAL

1. Materials

Camphorwood sawdust and rape-straw were collected from Chengdu region. The two types of sun-dried biomass samples were ground and screened to a particle size of 150-250 μm with moisture content of 7.5% and 10.1%, respectively. Microcrystalline cellulose was obtained from J&K Scientific LTD. The main chemical compositions of these biomass samples were determined according to the published procedure [8] as shown in Table 1.

2. Pretreatment by Mechanical Activation

Pretreatment of biomass by mechanical activation was conducted in a stirring ball mill. According to the experimental design, 30 g of raw samples was milled at different milling speed for different activation time in a 1,500 mL Teflon pot containing certain amount of zirconia balls (3.5 mm-diameter, specific gravity 6.1 kg/L and bulk density 3.6 kg/L) and no other additives were used during the milling. After activation, milling media and activated material were separated, and then the pot and balls were thoroughly cleaned and dried before the next milling experiment. Activated samples were sealed for characterization and hydrolysis. In addition, to compare with activated samples, a certain amount of raw samples without other additives were directly crushed for 30 min by a high-speed pulverizer (1,200 g capacity). The crushed samples were also sealed for storage.

3. Hydrothermal Hydrolysis of Lignocellulosic Biomass Catalyzed by Compressed Carbon Dioxide

Hydrothermal hydrolysis of lignocellulosic biomass catalyzed by compressed carbon dioxide was conducted in a 250 mL autoclave with an electromagnetic stirring device and an electric heating automatic temperature control device. In each experiment, 5 g of samples and 150 mL of deionized water were added. Then the system was heated to 180 $^{\circ}\text{C}$ with a heating rate of 5 $^{\circ}\text{C}/\text{min}$. After that, carbon dioxide was injected to the autoclave to 8 MPa. 60 min later, the autoclave was quenched to about 50 $^{\circ}\text{C}$. Finally, the reaction mixture was filtered to obtain the hydrolysate and the filter cake. The cellulose conversion was analyzed from the filter cake and the content of reducing sugar was obtained from the hydrolysate.

4. Characterization

The morphology of biomass samples before and after activation was observed by scanning electron microscopy (JEOL, JSM-7500F, Japan). The distribution of particle size was then obtained

by a laser granularity analyzer (Rise-2002, China). The N_2 adsorption isotherms were measured at 77.35 K with an automated surface area analyzer (Quantachrome, USA). Before each measurement, the samples were degassed in vacuum at 120 °C for 3 h. Specific surface areas of samples were calculated by the Brunauer Emmett-Teller method. The chemical structure of biomass samples was characterized by Fourier transform infrared spectrometer (Thermo Electron, Nicolet-6700, USA). The changes in average hydrogen bond energy can be calculated by Eq. (1) [23]. The monosaccharide in the hydrolysate was determined by High performance liquid chromatography (Alltech, SSI-1500, USA) and the concentration of furfural and 5-hydroxymethylfurfural were determined according to the published material [24]. The reducing sugar in the hydrolysate was carried out by the DNS method [25]. The cellulose crystallinity was determined by a diffraction meter (HaoYuan, DX-2700, China). Crystalline index (CrI) of cellulose was calculated by referring to the diffraction intensities of the crystalline region and amorphous region according to the following Eq. (2) [26].

$$\Delta E = h c (v_1 - v_0) \quad (1)$$

where h is Planck's constant, c is the velocity of light, v_0 and v_1 are the wave numbers of the O-H vibration of unactivated and activated wood sawdust.

$$\text{CrI} = \frac{I_{200} - I_{am}}{I_{200}} \times 100\% \quad (2)$$

where I_{200} is the maximum intensity of the (200) lattice diffraction and I_{am} is the intensity of diffraction in the same unit at $2\theta = 18^\circ$.

RESULTS AND DISCUSSION

1. Orthogonal Experiments of Ball Milling Parameters

Many factors affected milling activation, mainly including mill-

Table 2. Analysis of single factor experiments

Experiment number	Milling speed (rpm)	Activation time (h)	The mass ratio of the ball to biomass	Cellulose conversion (%)
1	350	2.0	30 : 1	22.3
2	450	2.0	30 : 1	37.8
3	580	2.0	30 : 1	40.3
4	450	1.0	30 : 1	27.9
5	450	3.0	30 : 1	39.1
6	450	2.0	10 : 1	28.6
7	450	2.0	20 : 1	32.4

ing speed, activation time and the mass ratio of the ball to biomass. Table 2 and Table 3 present the single factor experiments and the orthogonal experiments containing three factors. Hemicellulose conversion was >97% even without milling [8]; therefore, cellulose conversion was as the evaluation index to discuss the effect of ball milling. In Table 2, it can be seen that cellulose conversion increased with an increasing intensity of milling activation. In Table 3, the results show a graded influence of various factors on wood cellulose conversion was milling speed>activation time>the mass ratio of the ball to biomass. Cellulose conversion of activated materials was about 38% in groups 5 and 9 experiments, which was nearly four times of those of non-activated materials (10.2%). Considering the energy consumption of milling and the cellulose conversion, the reasonable activation conditions were as follows: 450 rpm of milling speed, and 2 h of activation time and 30 : 1 of the mass ratio of the ball to biomass. The results showed that cellulose conversion of wood sawdust had a substantial increase after mechanical activation. It indicates that milling activation may change the structure and physicochemical properties of wood sawdust, resulting in

Table 3. Analysis of orthogonal experiments

Experiment number	Milling speed	Activation time	The mass ratio of the ball to biomass	Cellulose conversion (%)
1	1 (350 rpm)	1 (1.0 h)	1 (10 : 1)	13.6
2	350 rpm	2 (2.0 h)	2 (20 : 1)	21.8
3	350 rpm	3 (3.0 h)	3 (30 : 1)	24.0
4	2 (450 rpm)	1.0 h	20 : 1	26.6
5	450 rpm	2.0 h	30 : 1	37.8
6	450 rpm	3.0 h	10 : 1	27.3
7	3 (580 rpm)	1.0 h	30 : 1	25.8
8	580 rpm	2.0 h	10 : 1	30.5
9	580 rpm	3.0 h	20 : 1	38.6
k_{1j}	59.4	66.0	71.4	
k_{2j}	91.7	90.1	87.0	
k_{3j}	94.9	89.9	87.6	
K_{1j}	19.8	22.0	23.8	
K_{2j}	30.6	30.0	29.0	
K_{3j}	31.6	29.9	29.2	
R_j	11.8	8.0	5.4	

Note: k_{ij} (i represents the level number and $i=1, 2, 3$; j represents the column number and $j=1, 2, 3$) is the summation of test results with i level on j column. $K_j = k_{ij}/3$. R_j is the range of the levels on j column. $R_j = \text{Max}\{K_{1j}, K_{2j}, K_{3j}\} - \text{Min}\{K_{1j}, K_{2j}, K_{3j}\}$

Table 4. The main components of hydrolysate for raw material and activated material

Biomass	Reducing sugar (g·L ⁻¹)	Xylose (g·L ⁻¹)	Glucose (g·L ⁻¹)	Furfural (g·L ⁻¹)	5-HMF (g·L ⁻¹)
Raw material	4.43	3.65	0.51	1.20	0.26
Activated material ^a	6.02	4.16	1.49	1.72	1.05
Activated material ^b	5.83	3.82	1.74	1.90	0.97

^aActivated material : milling speed 450 rpm and the mass ratio of the ball to biomass 30 : 1 for 2 h

^bActivated material : milling speed 580 rpm and the mass ratio of the ball to biomass 30 : 1 for 2 h

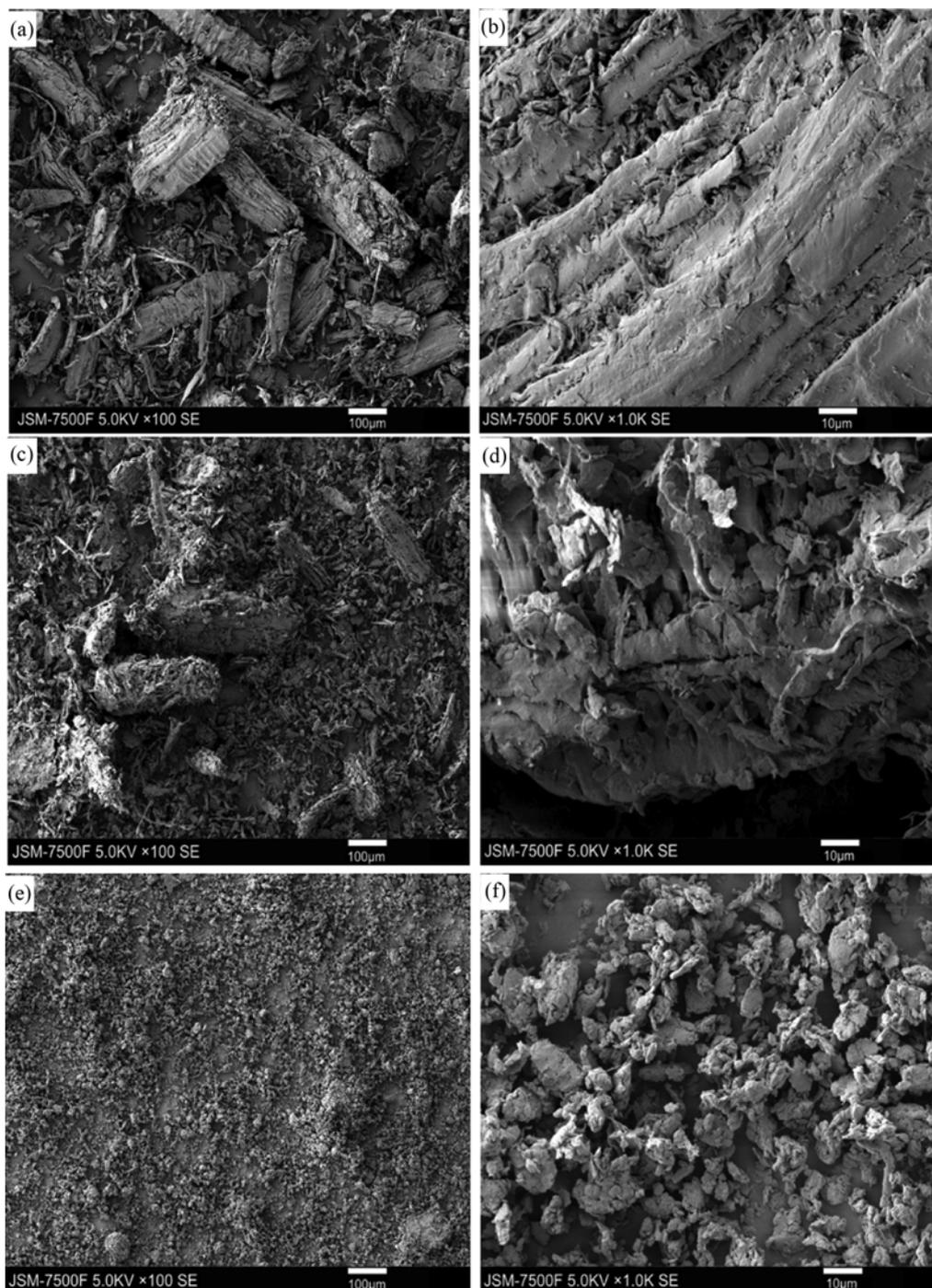


Fig. 1. SEM micrographs of activated camphorwood treated under different milling conditions with different magnifications. (a), (b) Untreated; (c), (d) at 450 rpm and the mass ratio of the ball to biomass 30 : 1 for 0.5 h; (e)-(f) at 450 rpm and the mass ratio of the ball to biomass 30 : 1 for 2 h.

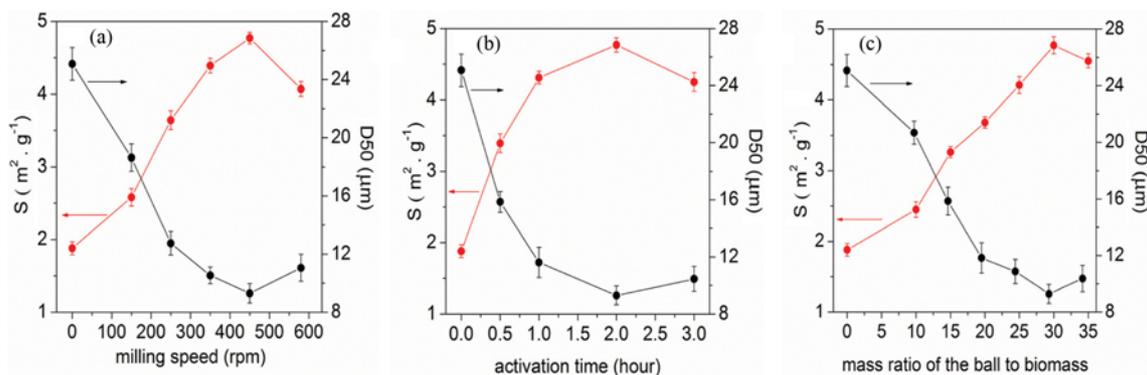


Fig. 2. Specific surface area and particle size distribution of camphorwood sawdust at different milling conditions. (a) The mass ratio of the ball to biomass 30 : 1 and activated for 2 h; (b) milling speed 450 rpm and the mass ratio of the ball to biomass 30 : 1; (c) milling speed 450 rpm and activated for 2 h.

enhanced chemical reactivity of lignocellulosic biomass and making it more accessible to reagents to improve cellulose conversion.

2. The Main Components of Hydrolysate

The experimental results show that the cellulose conversion increased after activation. To further confirm the effect of milling activation, the main components of hydrolysate were measured. The results are shown in Table 4. After activation, the reducing sugar yield was $6.02 \text{ g}\cdot\text{L}^{-1}$. It increased by 36% compared to that of raw material. The glucose yield (mainly from cellulose) was $1.49 \text{ g}\cdot\text{L}^{-1}$, which was about three times of that of the raw material under milling speed 450 rpm and the mass ratio of the ball to biomass 30 : 1 for 2 h. However, the increase of xylose (mainly from hemicellulose) content was not obvious because the hemicellulose conversion yield was very high (>97%) even without activation. In addition, furfural (mainly from xylose) yield and 5-HMF (mainly from glucose) yield increased with the increase of intensity of ball milling. Therefore, it means the high conversion of cellulose after activation and indicates that milling activation may change the structure and physicochemical properties of the cellulose to promote the hydrolysis.

3. Effects of Mechanical Activation on Surface Morphology and Particle Size

3-1. SEM Analysis

The surface morphologies of milled wood sawdust under different activation conditions can be observed from SEM photographs. Fig. 1 shows that the surface morphology of wood sawdust was significantly changed during milling activation. From Fig. 1(a) and Fig. 1(b), one can observe that unactivated wood sawdust consisted of different particles with compact structure and rough surface. With an increasing intensity of mechanical activation, the compact fiber bundles of wood sawdust are split and fractured, contributing to the generation of small irregular particles (Fig. 1(c) and Fig. 1(d)). In Fig. 1(e) and Fig. 1(f), the fibrous structure of wood sawdust is completely destroyed, and the particle size of wood sawdust decreases sharply and becomes more uniform. These results indicate the decrease in particle and ordered structure, split and destruction of compact fiber bundles were induced by milling activation.

3-2. Distribution of Particle Size and Specific Surface Area

Mechanical activation can decrease particle size and increase the

specific surface area. Fig. 2 presents the particle size distribution and specific surface area of wood sawdust under different activation conditions. D50 is the median diameter that the percentage of the cumulative particle size distribution of the sample reaches 50%. With the increasing intensity of milling, coarse particles became finer and the fraction of small particles increased, then D50 showed a gradual declining trend. However, the particle size did not reduce anymore but increased to some extent beyond certain condition. Under 450 rpm of milling speed, 30 : 1 of the mass ratio of the ball to biomass and 2 h of activation time, the D50 decreased from $25.07 \mu\text{m}$ to $9.29 \mu\text{m}$ and the specific surface area increased from $1.79 \text{ m}^2\cdot\text{g}^{-1}$ to $4.88 \text{ m}^2\cdot\text{g}^{-1}$. It increased the accessibility of biomass to reaction reagent to reach 37.8% of cellulose conversion. The cellulose conversion of non-activated material was only 10.2%. In Fig. 2(a), it can be seen the D50 decreased from $18.63 \mu\text{m}$ to $10.53 \mu\text{m}$ and the specific surface area increased from $2.58 \text{ m}^2\cdot\text{g}^{-1}$ to $4.39 \text{ m}^2\cdot\text{g}^{-1}$ with the increase of milling speed from 150 rpm to 350 rpm. The increasing impingement and shear forces improve the milling efficiency. But when the speed increased from 450 rpm to 580 rpm, the D50 increased from $9.29 \mu\text{m}$ to $11.05 \mu\text{m}$. The reason was probably that some powder and balls close to the cylinder wall moved along with a circular motion at excessive speed which decreased the mechanical forces between balls and biomass. In Fig. 2(b), one can observe that the effect of activation time on the D50 and the specific surface area is similar to that of the milling speed. The D50 decreased from $15.85 \mu\text{m}$ to $9.29 \mu\text{m}$ and the specific surface area increased from $3.39 \text{ m}^2\cdot\text{g}^{-1}$ to $4.88 \text{ m}^2\cdot\text{g}^{-1}$ with the increase of activation time from 0.5 h to 2.0 h. After 2 h, grain refinement reached maximum level and the materials were dispersed relatively uniform. With a further increase in activation time, plastic deformation based on van der Waals forces caused the powder to coalesce and aggregate, leading to the particle size being increased [27]. This result is consistent with Yang's study [28] that aggregation occurred when *morus alba* wood was milled without no other additives for more than 4 h. In Fig. 2(c), one can observe that when the mass ratio of the ball to biomass was less than 15 : 1, the decrease of particle size was not noticeable. It is possible that the collision frequency and the impact energy were weak as fewer balls were used with smaller milling area. When the mass ratio of the ball to biomass increased to 20 : 1-30 : 1, the contact area and

collision frequency increased with high milling efficiency. Thus, the particle size decreased significantly. Yet, there is an optimum for the mass ratio of the ball to biomass at a fixed speed. When the mass ratio of the ball to biomass was high enough, the particle size increased gradually. Probably the filling ratio of the reactor was too high and the movement space of balls decreased, which decreased the collision frequency. Therefore, the milling efficiency was reduced.

4. Hydrolysis of Activated and Untreated Wood Sawdust

For a general liquid-solid reaction, the smaller particle size induces less mass transfer resistance to increase reaction. From above results, we found that the particle size of biomass became smaller and the hydrolysis action increased after ball milling. To further investigate the effect of milling on the hydrolysis, wood sawdust (150-250 μm , CrI=60.93%) with and without ball milling having the same size was compared in the hydrothermal hydrolysis catalyzed by compressed carbon dioxide.

As shown in Table 5, the cellulose conversion of materials obtained by direct crushing was significantly lower than that obtained by milling with same particle size. Comparing the two methods, both had the same particle size of materials after pretreatment, but the cellulose crystallinity was remarkably lower after stirring ball milling. It shows that milling activation could destroy the internal structure of lignocellulosic biomass. That's why activated biomass has higher hydrolysis conversion.

5. XRD Analysis

The above results show that the decrease of particle size was not

Table 5. Hydrolysis of activated and unactivated wood sawdust with similar particle size

Pretreatment method	Particle size (μm)	Cellulose crystallinity (%)	Cellulose conversion in hydrolysis ^a (%)
Direct crushing	48-56	54.13	12.9
	56-62	56.34	13.2
Ball milling	48-56	21.72	41.5
	56-62	23.18	38.7

^aReaction condition: 180 °C and 8.0 MPa CO₂ for 1 h

the main factor affecting the hydrolysis of cellulose and the decrease of cellulose crystallinity may be the key factor. Fig. 3 presents the XRD patterns of wood sawdust after treatment under different activation conditions. All the diffraction patterns contain the (101), (10 $\bar{1}$) and (200) planes and no new diffraction peaks appeared after milling activation. The location of diffraction peaks belongs to cellulose I according to 2θ [19]. The result is consistent with the study of Huang et al. [29] who showed that the crystal type of cellulose could not be changed by dry milling. The cellulose crystallinity of wood sawdust decreased from 60.93% to 21.40% after mechanical activation under the optimum condition, indicating that the crystalline structure of lignocellulosic was significantly damaged by mechanical forces. In Fig. 3(a), the broadening of diffraction peaks and a clear decrease in peak intensity of (200) plane and cellulose crystallinity with the increasing milling speed can be seen. The (101) and (10 $\bar{1}$) planes disappeared gradually when milling speed increased from 250 rpm to 450 rpm. Further increase in milling speed to 580 rpm, the shape of diffraction peak and the crystallinity were essentially unchanged. This is because the velocity of balls and collision frequency are proportional to milling speed [30], and the internal energy of milled biomass obtained is proportional to mechanical energy $E=0.5 m v^2$ provided by milling process, and v is calculated by $v=2 \pi n r$ (where m is the mass of balls, n is the milling speed and r is the motion radius). The mechanical energy is only 0.77 J when the milling is carried out at 250 rpm, resulting in the low milling efficiency due to the weaker impingement and shear forces. When the speed is increased to 450 rpm, the mechanical energy is 2.51 J and is beneficial to break the lignin wrap and disrupt the crystalline structure. Some glycoside bonds among cellulose can be broken and the amorphous region is increased. In Fig. 3(b), it can be seen that the effect of activation time on the changes of diffraction peaks is similar to the milling speed. At a fixed speed, the formula $E=P t$ (where P is output power of ball mill) shows that if output power is the same, energy obtained from balls is proportional to the activation time. As a result, wood sawdust will also obtain high energy in milling. With an increase in activation time, the crystallinity of lignocellulose was decreased. But when activation time was increased from 2 h to 3 h, the crystallinity only was decreased 1.55%. The reason is prob-

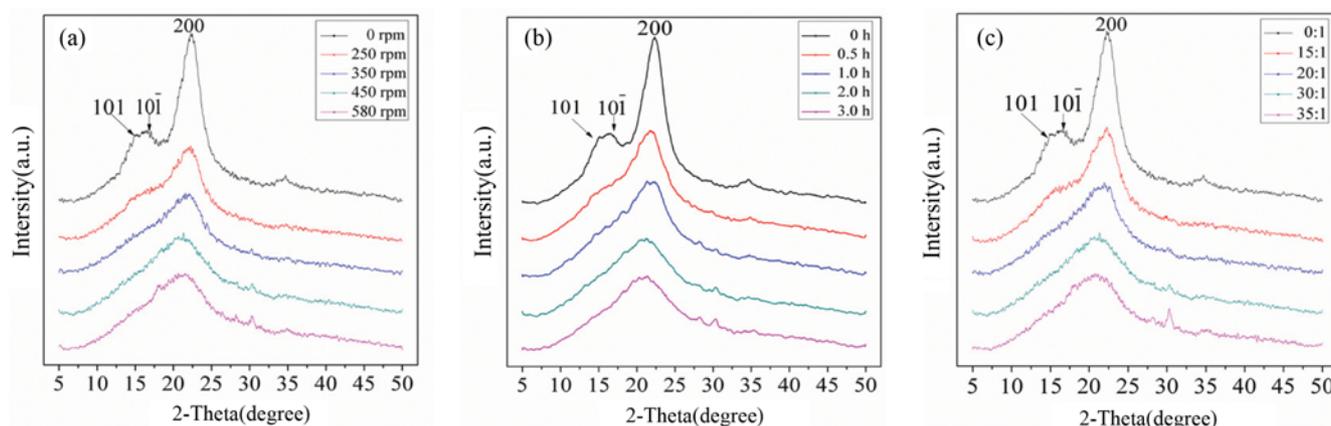


Fig. 3. XRD patterns of the (a) mass ratio of the ball to biomass 30 : 1 and activated for 2 h; (b) milling speed 450 rpm and the mass ratio of the ball to biomass 30 : 1; (c) milling speed 450 rpm and activated for 2 h.

ably due to the aggregation of particles. In Fig. 3(c), with the increase of the mass ratio of the ball to biomass, the lignocellulosic becomes more amorphous. The collision frequency increases between balls and biomass contributing to an increase in the internal energy of the biomass. However, when the mass ratio of the ball to biomass was increased to 35 : 1, the final crystallinity of biomass was 22.57%, higher than that of 30 : 1. It shows that there is an optimum mass ratio of the ball to biomass in milling. When the mass ratio of the ball to biomass is too high, the filling ratio is too high and there is not enough space for balls to move, resulting in the mechanical forces being decreased and the amorphous trend is weakened. On the contrary, when the ratio is too small, the collision frequency is reduced and good milling results cannot be achieved.

6. FTIR Analysis

To obtain more information about the changes of lignocellulosic molecular groups during milling activation, FTIR analysis was used. Fig. 4 presents the FTIR spectra of camphor wood sawdust before and after milling pretreatment. The band at 896 cm^{-1} was assigned to the β -glycosidic linkages between glucose units in cellulose [31]. The absorption at $1,056\text{ cm}^{-1}$ is due to C-O-C asymmetric valence vibration from cellulose. The absorption at $1,603\text{ cm}^{-1}$ is attributed to aromatic skeletal vibrations of lignin. The absorp-

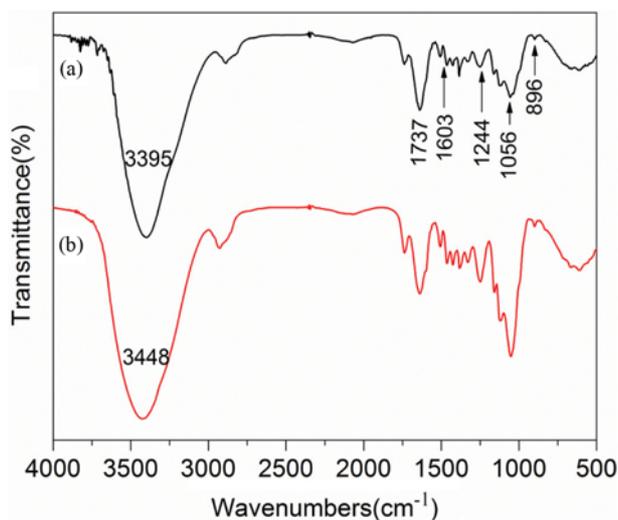


Fig. 4. FTIR spectroscopy of camphor wood sawdust activated under different milling conditions (a) untreated; (b) at milling speed 450 rpm and the mass ratio of the ball to biomass 30 : 1 for 2 h.

tion at $1,737\text{ cm}^{-1}$ is due to carbonyl groups of uronic acid from hemicellulose [32]. Compared with the IR spectrum of original wood sawdust, no new absorption peaks appear in the IR spectra of activated samples, indicating that no new functional groups were generated during milling. However, the hydroxyl group O-H was shifted to a higher wave number from $3,395\text{ cm}^{-1}$ of the raw material to $3,448\text{ cm}^{-1}$ of the activated material and stretching vibration intensity increased. This may be due to the breakage of intramolecular and intermolecular hydrogen bonds in lignocelluloses by impingement and shear during milling, resulting in an increase of free hydroxyl groups and then a part of free hydroxyl groups reformed intermolecular hydrogen bonds [33]. The results showed that the hydrogen bond energy of the activated wood sawdust under the optimum condition increased $1.05 \times 10^{-21}\text{ J}$, compared with unactivated wood sawdust. Therefore, it demonstrates that some mechanical energy was converted into internal energy of biomass under mechanical activation and weakened the structural stability of lignocelluloses, promoting the subsequent hydrolysis reactions.

7. Effect of Ball Milling on the Hydrolysis of Different Biomass

The structures and components of different raw materials are different. The effect of the mechanical activation may be diverse. Rape-straw, camphorwood and microcrystalline cellulose may represent three different types of biomass: grasses, woody plant and regenerated cellulose. Rape-straw and microcrystalline cellulose were activated under optimum milling conditions and hydrolyzed in the same conditions as camphorwood sawdust. The crystallinity and cellulose conversion of biomass are shown in Table 6.

Although different biomass has different composition and cellulose crystallinity (Table 1 and Table 6), after mechanical activation, the crystallinity was significantly decreased and cellulose conversion was increased by 3–4 times compared with those of raw materials. Although the content of cellulose in rape-straw and camphorwood was almost the same, because of the lower cellulose crystallinity in rape-straw, the cellulose conversion of rape-straw in hydrolysis was higher than that in camphor wood after activation. In addition, cellulose conversions of camphorwood (10.2%) and microcrystalline cellulose (9.4%) were similar without pretreatment, but after activation, the cellulose conversion of camphor wood (37.8%) was significantly higher than that of microcrystalline cellulose (29.6%). This may be due to the lower degree of polymerization and crystallinity of grasses, compared with woody plant and regenerated cellulose [34]. The changes in the internal structure and properties of herbaceous cellulose are more remarkable than woody plant and regenerated cellulose under the same activation conditions, resulting in a higher cellulose conversion.

Table 6. Cellulose conversion of different biomass before and after activation

Biomass	Cellulose crystallinity before activation (%)	Cellulose crystallinity after activation ^a (%)	Cellulose conversion in hydrolysis ^b before activation (%)	Cellulose conversion in hydrolysis after activation (%)
Rape-straw	57.26	18.51	13.6	45.2
Camphorwood sawdust	60.93	21.40	10.2	37.8
Microcrystalline cellulose	90.72	25.23	9.4	29.6

^aActivation condition : milling speed of 450 rpm, mass ratio of 30 : 1 of ball to biomass for 2 h

^bReaction condition: 180 °C and 8.0 MPa CO₂ for 1 h

CONCLUSIONS

Mechanical activation pretreatment can promote hydrolysis of lignocellulosic biomass in liquid hot-water compressed carbon dioxide. In this process, not only is the particle size reduced, but also partial mechanical energy is converted into the internal energy of biomass, which decreases the cellulose crystallinity. The change of internal structure of biomass caused by mechanical activation is irreversible.

The influence of ball milling parameters on cellulose hydrolysis was ranked as follows: ball milling speed > activation time > the mass ratio of ball to biomass. The optimum milling condition was obtained at 450 rpm of ball milling speed and 30 : 1 of the mass ratio of ball to biomass for 2 h. In this condition, cellulose crystallinity of camphor wood sawdust decreased from 60.93% to 21.40% and the specific surface area increased from $1.79 \text{ m}^2 \cdot \text{g}^{-1}$ to $4.88 \text{ m}^2 \cdot \text{g}^{-1}$. The cellulose conversion was 37.8% in liquid hot-water catalyzed by compressed carbon dioxide, which was nearly four times of that of the raw material. The glucose yield in the hydrolysate was $1.49 \text{ g} \cdot \text{L}^{-1}$, which was nearly three times of that of the raw material.

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