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Thread Rolling: An Efficient Mechanical Pretreatment for Corn Stover Saccharification

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Abstract: Sugar cane bagasse and corn stalks are rich in lignocellulose, which can be degraded into monosaccharides through enzymatic hydrolysis. Appropriate pretreatment methods can effectively improve the efficiency of lignocellulose enzymatic hydrolysis. To enhance the efficiency of enzymatic hydrolysis, thread rolling pretreatment as a physical pretreatment was applied in this study. The influence of raw material meshes size after pretreatment was also taken as the research target. Specific surface area analysis, Scanning electron microscope (SEM), X-rays diffraction (XRD), and Fourier transform infrared (FT-IR) were used for characterizations. The results showed that, the total monosaccharide recovery rates of the raw materials, 20–40 mesh, 40–60 mesh, and 60–80 mesh enzymolysis substrates were 17.6%, 34.58%, 37.94%, and 50.69%, respectively. The sample after pretreatment showed a better recovery of monosaccharide than that of the raw material. Moreover, the enzymolysis substrates with a larger mesh exhibited a higher recovery of monosaccharide than that of the enzymolysis substrates with smaller meshes. This indicated that thread rolling pretreatment can effectively improve the efficiency of enzymatic hydrolysis.

Keywords: pretreatment; enzymatic hydrolysis; corn stalks; bagasse; biomass

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1. Introduction

With the continuous depletion of petroleum resources, many energy problems have been brought about, and the search on new energy sources has increasingly become a concern of people [1]. Agricultural and forestry wastes (rice straw, bagasse, etc.) are rich in lignocellulose which makes them potential feedstocks for biobased fuels and chemical productions [2]. In addition, China, a country with large amount of agriculture productions, has extensive agricultural wastes. If there is no proper treatment, these agricultural wastes will bring crisis to the environment. Thus, the researches on the conversion of agricultural wastes to biofuels have both economic and environmental benefits.

Bioethanol is an important biobased energy, which can partly replace gasoline. Generally speaking, the conversion from lignocellulose to bioethanol mainly includes four key steps, which are pretreatment, enzymatic hydrolysis, fermentation, and distillation. Among them, the pretreatment of the feedstock cannot be overemphasized, because of the presence of noncarbohydrates and the compact structure of native biomass. Lignocellulose is mainly composed of cellulose, hemicellulose, and lignin, of which cellulose has the highest content. The intricate arrangements and connection structures of the three main components in the plant cell wall reduce the cellulose reactivity towards enzymatic hydrolysis. Thus, for efficient degradation of reducing sugars, it is imperative to choose a suitable pretreatment method [3].

Lignocellulose cannot be directly converted into fermentable monosaccharides, so it is necessary to carry out pretreatment to release monosaccharides before enzymatic hydrolysis and fermentation of ethanol [4]. Currently, various pretreatment methods have been developed to increase the reducing sugar recovery rate of lignocellulose. It is worth

noting that an effective pretreatment should be economical and effective for a variety of lignocellulosic biomass. Generally, pretreatment mainly includes four types, namely physical, chemical, biological, and combined pretreatment. The purpose of introducing chemical or biological actions in the pretreatment process is to change the chemical compositions of the feedstocks that can increase the accessibility of cellulose to cellulases [5]. However, this will cause some problems, such as serious chemical pollutions and high cost of the recovery of the chemical reagents [6]. To reduce environmental stress and the cost of chemical recovery, physic-chemical pretreatment such as acid immersion explosion, has attracted people attention. Explosion make up for the problems of chemical pretreatment to a certain extent, but there are still challenges to the issues of reaction temperature and pressure [7].

Physical pretreatment refers to the environmentally friendly methods without using any chemical agents. It mainly acts on the surface of biomass through shear or compressive forces, thereby increasing the specific surface area of biomass and the contact area between biomass and enzymes [8]. The current physical pretreatment mainly includes mechanical pretreatment, microwave-assisted pretreatment, steam explosion, and hydrothermal pretreatment. Mechanical pretreatment is a fast, low-cost, and low-loss pretreatment method that the biomass is cut into small pieces by shear or compressive forces, thereby increasing the specific surface area of the substrate of enzymatic hydrolysis. However, the critical defects of mechanical pretreatment are its high energy consumption and low effects, especially for the wood biomass with dense and hard structures. Thus, we can emphasize the applications of mechanical pretreatment to the non-wood feedstocks with loose and soft structures. Thread rolling is a traditional process of tobacco sheet making. Its purpose is to crush the tobacco stems and provide uniformity and strength to the products. For non-wood fibers with loose structures, such as corn stalks, sugar cane bagasse, and wheat straw, thread rolling may be an ideal method of crushing. However, there are few studies on using thread rolling as the pretreatment of bioethanol production. At present, our enzymatic hydrolysis experiments on tobacco stems proved that it can effectively increase the enzymatic hydrolysis efficiency [9].

In this paper, two commonly used agricultural wastes, corn stalks and sugar cane bagasse, were processed by thread rolling. The processed samples were then separated into various meshes. The properties of the thread rolled feedstocks and the efficiency of the enzymatic hydrolysis were explored via sugar analysis, FT-IR, BET, SEM, and XRD technology. The aim of this work is to demonstrate the effects of thread rolling pretreatment on the subsequent enzymatic hydrolysis. This work will provide a reference for further exploration of physical pretreatment of biofuels production.

2. Materials and Methods

2.1. Materials

Corn stalks and sugar cane bagasse were obtained from Shenyang, China. Among them, bagasse comes from the residue left after the sugar extraction industry. Prior to the experiments, the corn stalks and sugar cane bagasse were decorticated, and grounded, respectively. The obtained particles were oven-dried at 55 °C to a constant weight. The composition analyses of the corn stalks and sugar cane bagasse (1:1, *w*/*w*) were performed according to the procedure established by National Renewable Energy Laboratory with glucose 39.12%, xylose 15.84%, arabinose 0.85% and lignin 25.10% [10]. H_2SO_4 (≥99%, AR) was purchased from Guanghua Sci-Tech Co., Ltd. (Guangzhou, China). Mixed-cellulase containing *β*-glucanase, cellulase, and xylanase was purchased from Imperial Jade Bio-technology Co., Ltd. (Ningxia, China). The glulose (≥99%, HPLC), xylose (≥99%, HPLC), and arabinose (≥99%, HPLC) used in HPLC were purchased from Sigma Chemical Company (St. Louis, MO, USA).

2.2. Thread Rolling Pretreatment

Firstly, the corn stalks and sugar cane bagasse (*w*/*w*, 1:1) were soaked in 40 °C water bath for 3~5 min to remove the dust. Then, the mixed feedstock was loaded into a rub

silk machine (the stock inlet consistency was 20~90%, the screw speed was 322 rpm, and the speed ratio was 2:7) and a certain amount of water was added to prevent clogging. The pretreated samples were air-dried and stored for subsequent processing. Then, the air-dried samples were separated by a grading sieve. The samples were divided into three levels, namely 20–40 mesh sample (40%), 40–60 mesh sample (26%), and 60–80 mesh sample (15%). Most of the remaining parts were larger than 20 mesh.

2.3. Enzymatic Hydrolysis

Enzymatic hydrolysis was carried out according to the method described in a literature [11]. In short, 1 g of the graded sample was washed three times with 10 mL of NaAc buffer to remove the residue on the surface. Then, 5 mL of mixed cellulase (45 FPU/g) solution and 0.5 mL of Tween-80 were added into the mixture. The total volume was adjusted to 20 mL with phosphate buffer. The substrates of the enzymatic hydrolysis were shaken in air atmosphere at 150 rpm at 50 °C for 48 h. After the reaction, the centrifuge tubes were soaked in a 90 °C hot water bath for 5 min to inactivate the enzyme protein. The hydrolytic residue was removed by centrifugation at 3000× *g* for 5 min. The centrifugal supernatants were collected for sugar analysis, and the residues were baked for later use. All experiments were carried out in biological triplicate. The formula for monosaccharide recovery is as follows:

$$\text{Monosaccharide recovery rate (\%)} = (\text{Monosaccharides (Enzymatic hydrolysis solution)}/(\text{Monosaccharides (Raw material)} \times 100$$

2.4. Physicochemical Characterization of the Corn Stalks and Sugar Cane Bagasse

Sugar analysis was determined by a high-performance liquid chromatography coupled with a refractive index detector (RID) and a Bio-rad Aminex HPX-87H (300 × 7.8 mm) column. 5 mM of H_2SO_4 was employed as the eluent with a flow rate of 0.5 mL/min at 60 °C. Calibration curves of xylose, glucose, and arabinose were established for the quantitative calculation.

Chemical bonds in the molecules of the corn stalks and sugar cane bagasse were measured with an FT-IR spectrophotometer (Tensor 27, Bruker Optics, Germany) using a KBr disc containing finely ground samples (1%). They were recorded in the absorption mode in the range of 4500–500 cm^{-1}.

XRD patterns of the sample were detected in the 2θ range of 5° to 40° by X'pert Powder X-ray diffractometer (Karlsruhe, Germany) with a Cu K_α radiation. The crystallinity index (CrI) of the cellulose-rich residues was calculated using the formula:

$$\text{CrI} = (I_{020} - I_{am})/I_{020} \times 100$$

where I_{020} is the intensity of the crystalline portion of cellulose at about 2θ = 22.5° and I_{am} represents the amorphous portion at about 2θ = 18.0°.

The surface morphology of the sample was analyzed with a scanning electron microscopy (SEM; S-4300, Hitachi, Tokyo, Japan). Firstly, complete fibers from the enzymatic hydrolysis residues were selected and stacked on the conductive glue. Then the conductive glue was fixed on an iron observation table. All specimens were coated with Au before observation.

The specific surface area of the sample were calculated by the Brunauer-Emmett-Teller (BET) method on ASAP 2020 M instrument (American).

3. Results

3.1. Mechanism of the Thread Rolling Pretreatment

Standard mechanical treatment mainly includes three kinds of forces, including shearing, crushing, and cutting [12]. However, different from beating and boll milling, the mechanical action of thread rolling is mainly shear, but less crushing and cutting. Figure 1

showed a mechanism of the thread rolling machine. The thread rolling room consists of two rollers with gears (Figure 1C), one is driven by an electric motor, and the other is driven with connection to produce the shearing action. After the starting material enters into the feeding mouth of the thread roller room, the fibers are broken by the shear stress. Unlike ball milling, the rolling action is rare during the thread rolling, because there is a gap of 1 mm between the two gear rollers. Thus, the crystallization of the fibers will not be affected. On the other hand, due to the large size of the gear on the thread rollers, the cutting effect is less than that of disc grinding [13]. Cracking, separation, and brooming of the fibers mainly occur under shearing, which can bring a fluffy structure to the treated sample. The substrate with a fluffy structure and many pores is beneficial to its wetting and the diffusion of cellulase, during the enzymatic hydrolysis. Thus, it can be seen that thread rolling is a promising mechanical pretreatment that suitable for non-wood feedstocks cracking.

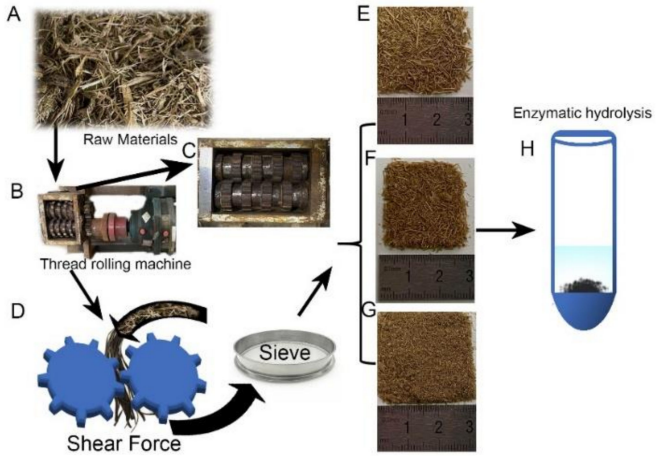

Figure 1. Mechanism of the thread rolling pretreatment ((**A**) Corn stalks and sugar cane bagasse raw material; (**B**,**C**) Thread rolling mechine; (**D**) Schematic diagram of the mechanism of thread rolling; (**E**) 20–40 mesh sample; (**F**) 40–60 mesh sample; (**G**) 60–80 mesh sample; (**H**) Enzymatic hydrolysis reactor).

3.2. The Changes of the Properties of the Thread Rolled Fibers

The morphology of the fiber is crucial for the followed enzymatic hydrolysis, because it involves the specific surface area of the substrate. We first test the surface area of the raw materials and the thread rolled samples as the contrastive substrates for enzymatic hydrolysis by BET (Table 1). The corresponding morphologies were then observed by SEM. Obviously, the shearing of the thread rolling pretreatment cloud made the fibers separate and brooming, which induced a significant improvement of both surface area and pore size. After screening, the fibers were crushed into various dimensions. However, both the specific surface area and the pore size were consistent with the mesh number. The particles of the 60–80 mesh sample, which having smaller size were not tight-packed, and a fluffy structure of the sample was maintained. As discussed above, this result was attributed to the special mechanical treatment of the thread rolling.

Table 1. The data of the BET analysis.

Samples	Samples	Specific Surface Area (m^2/g)	Pore Volume (cm^3/g)	Pore Size (nm)
entry 1	Raw Material	3.16	0.002	2.88
entry 2	20–40 mesh	5.26	0.003	3.31
entry 3	40–60 mesh	7.86	0.006	4.62
entry 4	60–80 mesh	10.58	0.010	5.32

The microstructure of the raw material (mixed corn stalks and sugar cane bagasse) and thread rolled samples (20–40 mesh, 40–60 mesh, 60–80 mesh) were shown in Figure 2.

It can be seen from Figure 2a that the cell wall of the fibers of the raw material was intact. In this situation, the carbohydrates in the microfibrils could not be effectively contacted with the cellulase, which led to a poor enzymolysis effect. However, after thread rolling, the cell wall of the fiber was broken to a certain extent. As shown by the red arrows in Figure 2b–d, many defects of cell wall could be clearly observed on the surface of the thread rolled fibers. The existence of these cell wall defects was conducive to the penetration and adsorption of cellulase protein, thereby promoting the efficiency of enzymatic hydrolysis.

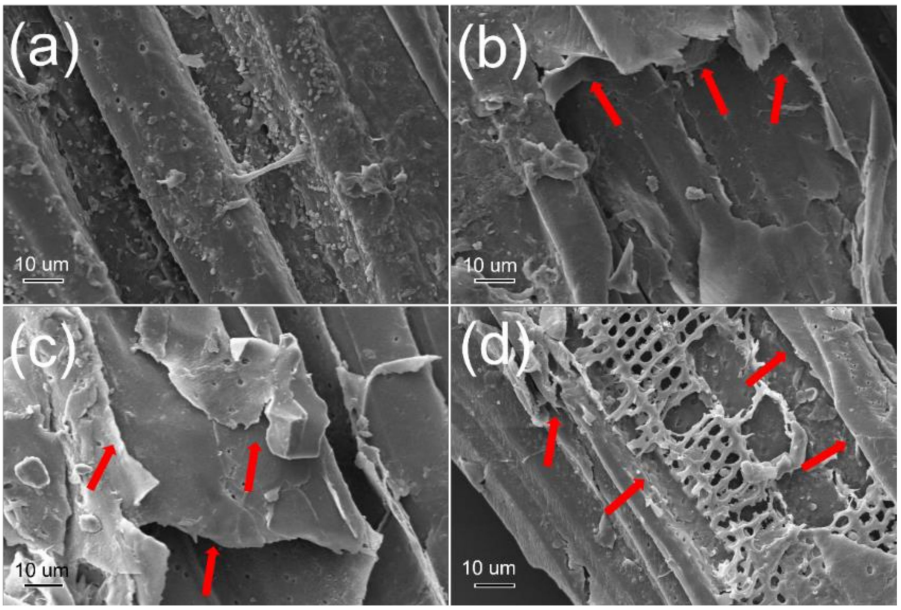

Figure 2. SEM images of the raw material and thread rolling treated fibers ((**a**) Raw materials; (**b**) 20–40 mesh enzymatic residue; (**c**) 40–60 mesh enzymatic residue; (**d**) 60–80 mesh enzymatic residue).

Figure 3 shows the XRD patterns of the thread rolled samples with various meshes. It can be seen that no significant difference can be found in all the samples. All of them exhibited the well-known characteristic diffraction peaks of cellulose I at 2θ around 14.5°, 16.5°, and 22.5°, which were attributed to the planes of (1 1 0), (1 1 0) and (2 0 0), respectively [14]. At the same time, the crystallinity of the 20–40 mesh sample, 40–60 mesh sample, and 60–80 mesh sample residue had little difference, which were 45.81%, 46.72%, and 47.53%, respectively. It indicated that there was no relationship between the mesh number and the crystallinity. Thus, it can be inferred that the crystallinity of cellulose was not changed significantly by thread rolling. This result was consistent with the above discussion on the mechanism of the thread rolling pretreatment.

3.3. Enhanced Biomass Saccharification under Thread Rolling Pretreatment

Enzymatic hydrolysis efficiency was mainly expressed by calculating the yields of the three main monosaccharides (glucose, xylose, and arabinose) released from the cellulase hydrolysis of grass biomass [15]. The recovery rates of enzymatic hydrolysis of the monosaccharides were shown in Figure 4. Compared with the raw materials, the mixed corn stalks and sugar cane bagasse sample pretreated by thread rolling followed by sieving showed a higher enzymolysis efficiency. Among them, the 60–80 mesh sample had the highest recovery rate of enzymatic hydrolysis of reducing sugar. Compared with the untreated raw material, the corresponding recovery rate of glucose, xylose, and arabinose of 60–80 mesh sample were increased by 2.78%, 1.98%, and 2.28%, respectively. It was because the 60–80 mesh sample had a higher specific surface area (10.58 m^2/g) as discussed above (Table 1). On the other hand, due to the less rolling and cutting during the thread rolling, the smaller particles of the 60–80 mesh sample did not accumulate compactly, which was conducive to the improvement of enzymatic hydrolysis.

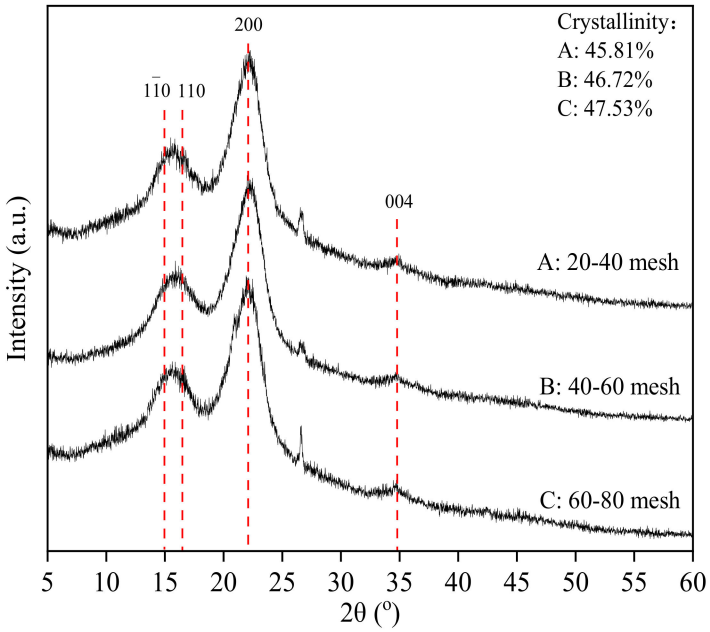

Figure 3. XRD patterns of the thread rolled samples with various meshes.

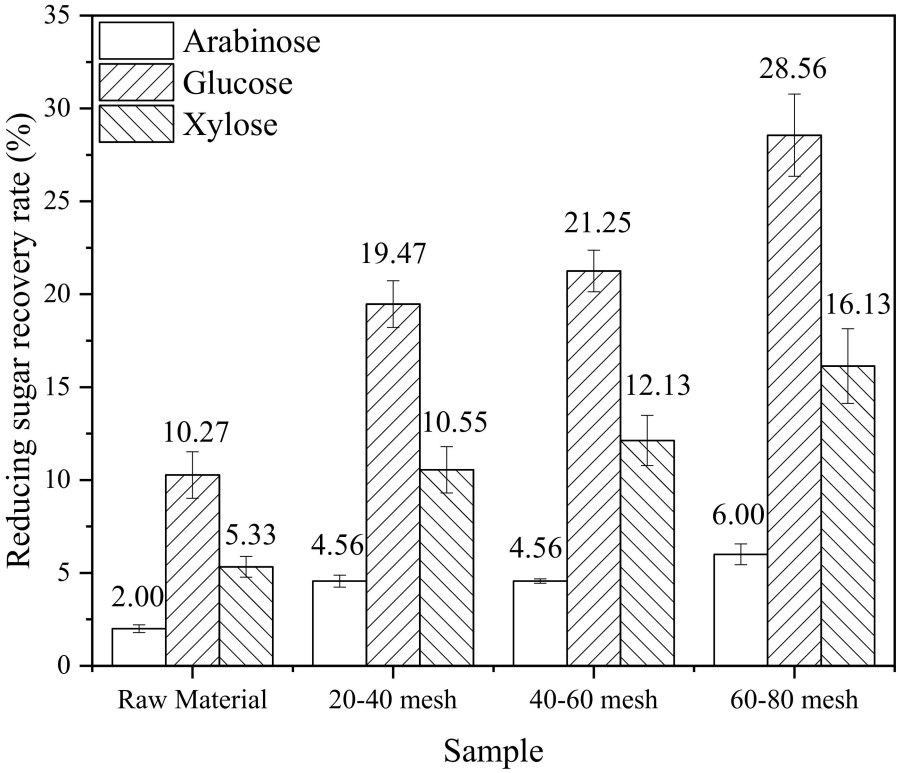

Figure 4. Enzymatic hydrolysis recovery rates of monosaccharides after thread rolling and sieving.

The concentration of glucose in the substrate plays a key role in the fermentation of bioethanol. It also can be seen from the results that the recovery rate of glucose was the highest, followed by xylose, and that of arabinose was the lowest. The effect of thread rolling on the glucose recovery rate was greater than that of the mesh number. Between the raw material and the 20–40 mesh sample, the difference of the glucose recovery was 9.2%. However, it was only 1.78% between the 20–40 mesh sample and the 60–80 mesh sample. The recovery rate of xylose and arabinose exhibited the same trends. This indicated that the pretreatment of thread rolling could effectively increase the recovery rates of

enzymatically hydrolyzed reducing sugars. At the same time, the lower the particles after sieving, the higher the recovery rates. Compared with the untreated sample, the monosaccharide (glucose, xylose and arabinose) recovery rates of the sample after the thread rolling pretreatment were increased by about 2–3 times, which was consistent with the results of other mechanical splintered pretreatments [16]. The standard deviation of three repetitions was shown in Figure 4. The error mainly comes from operation and detection during the experiment. The higher the content, the greater the absolute value of the error, which was mainly caused by the baseline fluctuation of the HPLC during the detection process. Overall, the errors were within the allowable range of errors.

In order to study the structural changes of the mixed corn stalks and sugar cane bagasse samples during the thread rolling and enzymatic hydrolysis, XRD and FT-IR analyses of the enzymolysis residues were performed and the results are shown in Figure 5. It can be seen from XRD patterns (Figure 5a) that, both allomorph and crystallinity unchanged during the enzymolysis. It might be because the enzymatic hydrolysis occurred in both crystalline regions and amorphous regions. In addition, it has been proved that the cellulose crystallinity was not the decisive reason for the difference in enzymatic hydrolysis results.

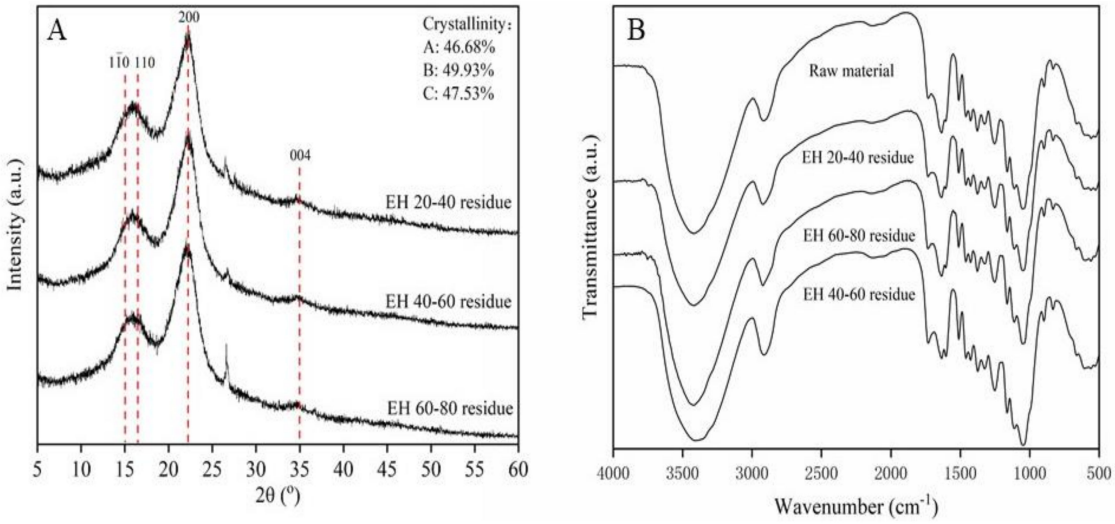

Figure 5. (**A**) XRD patterns and (**B**) FT-IR spectra of mixed corn stalks and sugar cane bagasse samples treated by thread rolling and enzymatic hydrolysis residues (EH means enzymatic hydrolysis).

The peak assignments of the FT-IR were conducted according to the previous studies [14,17–19]. It can be seen that the sample pretreated by thread rolling did not show an obvious difference, indicating that the pretreatment did not change the chemical properties of the samples, which was consistent with other physical pretreatments. The peaks at 898 cm^{-1}, 1737 cm^{-1}, 1500 cm^{-1} represented the β-glycosidic linkages between the glucose units in cellulose, the stretching of C=O in hemicelluloses, and the aromatic skeletal vibrations respectively. Other characteristic peaks such as 1159 cm^{-1} and 1460 cm^{-1} were caused by β-glycosidic linkages C–O–C stretching vibration and C–H deformation associated with aromatic ring vibration and aromatic C–H in-plane deformation.

4. Conclusions

Thread rolling is a pretreatment of biomass saccharification using non-wood fibers as the raw materials, such as corn stalks, sugar cane bagasse, and wheat straw. As a simple mechanical pretreatment, thread rolling has the advantages of clean, rapid and high crushing efficiency. The crystallinity of the raw material did not change after thread rolling, but the cell wall was torn. The torn fibers could be accumulated into a relatively fluffy structure, which was conducive to the wetting and contact with the cellulase of the enzymolysis substrate. The thread rolling pretreatment could effectively improve the monosaccharide recovery of the subsequent enzymatic hydrolysis. After screening, the

enzymatic hydrolysis effects of the 20–40 mesh sample, 40–60 mesh sample, and 60–80 mesh sample were improved by 16.98%, 20.34%, and 33.09%, respectively. In a word, thread rolling is a potential economic mechanical pretreatment of biomass saccharification.

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