Simultaneous Pretreatment with Ultraviolet Light and Alkaline H$_2$O$_2$ to Promote Enzymatic Hydrolysis of Corn Stover

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Pretreatment processes are essential for the preparation of biofuels from lignocellulosic feedstocks. Based on alkaline H$_2$O$_2$ pretreatment, photocatalytic alkaline H$_2$O$_2$ pretreatment (U-AHP) was investigated to examine the effects of reaction time, alkali concentration, and H$_2$O$_2$ concentration on the enzymatic digestion of corn stover. The optimum process conditions were determined by orthogonal tests: 1% NaOH, 2% H$_2$O$_2$, and reaction time of 8 h. Under these conditions, the lignin removal efficiency of U-AHP was 90.2% and the saccharification yield was 94.7%. Furthermore, FT-IR, XRD, and SEM analyses showed that U-AHP pretreatment caused structural damage to the maize straw and increased the crystallinity of the cellulose, and it was speculated that the U-AHP pretreatment reaction was a complex mechanism, which might be a multiple synergistic reaction. This study shows that U-AHP pretreatment is a simple, green and effective method to promote lignin removal.

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Keywords: Corn Stover; Pretreatment; Enzymatic saccharification; Basic hydrogen peroxide; UV-catalyzed hydrogen peroxide

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INTRODUCTION

Biomass energy, the only renewable carbon source, is abundant. The net emission of carbon dioxide to the atmosphere in the process of biomass utilization is zero, such that biomass can be regarded as a source of green and clean energy. Biorefined chemicals can replace chemicals made from fossil energy (Chen et al. 2021). The lignocellulosic component of straw type contains a large amount of cellulose and hemicellulose, which is a carbohydrate-rich biomass resource (Gong et al. 2022). The sugars produced by the hydrolysis of lignocellulose can be used to manufacture biofuels and high-value-added chemicals. However, the efficiency of the biomass enzymatic digestion is low due to the network-protected structure composed of lignin and hemicellulose and the embedded crystalline structure of cellulose (Bhatia et al. 2018). It is, therefore, indispensable to pretreat lignocellulose.

Increasing the accessibility of cellulose and hemicellulose to exogenous enzymes is essential to improve lignocellulose utilization. Therefore, various pretreatment methods, including physical, chemical, and biological methods, as well as combinations of methods, have been explored (Niemi et al. 2017). Among the many pretreatment methods examined,
alkali is widely used for lignocellulose pretreatment due to its efficient lignin removal ability. The mechanism of action is to remove lignin and some hemicellulose from plant cell walls by saponification, which involves cleavage of ester and ether bonds in lignin and hemicellulose, thereby improving cellulose's accessibility to enzymes. Subsequently, the lignin fragments are solubilized and removed. In this process, high concentration alkali pretreatment generates substantial amounts of waste liquid that causes environmental hazards that cannot be ignored (Xu et al. 2020). Therefore, combining other methods with alkali pretreatment is a practical approach to reducing the alkali content in pretreatment.

Many physical (Muthuvelu et al. 2019) and chemical methods (Wu et al. 2021) have been investigated in combination with alkali pretreatment. Alkaline \( \text{H}_2\text{O}_2 \) pretreatment (AHP) is a promising method to make lignocellulose more amenable to enzymatic digestion (Dutra et al. 2018; Ho et al. 2019). \( \text{H}_2\text{O}_2 \) is used in large quantities for bleaching cotton fabrics and pulp, and its primary mechanism of action is the dissociation of \( \text{H}_2\text{O}_2 \) under alkaline conditions, generating highly reactive oxygen species (ROS), such as perhydroxylated anions (HO\(^{•-}\)) and free radicals (HO\(-\)). ROS depolymerizes lignin by attacking the side chain of lignin, and it reacts with ketene chromophores (such as quinone structure) in lignin to increase the dissolution of lignin and reduce the polymerization of lignin (Wang et al. 2019). Li et al. (2018b) pretreated corn stover at 30 °C, pH 11.5, and 5% (w/w) \( \text{H}_2\text{O}_2 \) concentration for 24 h, removing 91.5% of the initial lignin, destroying the dense structure of the straw, and doubling the exposed area of cellulose. Song et al. (2016) investigated the extraction of bamboo at 90 °C. They reported that a reaction time of 4 h and 1% NaOH AHP was influential in removing ash, organic solvent extracts, and acid-soluble lignin. Although AHP is effective in removing lignin, it requires high reaction temperatures, \( \text{H}_2\text{O}_2 \) concentrations, and significant reaction times. At room temperature, \( \text{H}_2\text{O}_2 \) has been reported to rapidly produce highly reactive oxygen species (ROS), such as hydroxyl radicals (HO\(-\)), under ultraviolet (UV) irradiation. Some researchers have found that the reactive substances produced in the UV/\( \text{H}_2\text{O}_2 \) system can degrade lignin derivatives in wastewater and even mineralize them to CO\(_2\) and H\(_2\)O (Ahmed et al. 2009; Wang et al. 2019). This technique has been applied by some researchers for the pretreatment of lignocellulose. Yang et al. (2018) used UV/\( \text{H}_2\text{O}_2 \) to pretreat lignocellulose (sisal residue) for the first time. The results showed that the lignin in the remaining sisal residue was effectively removed and the enzymatic hydrolysis efficiency was improved. However, the lignin removal efficiency could not be maintained at low pH through the pre-experiment of pretreatment of natural corn stalks, so it was considered to pretreat untreated natural corn stalks at low alkali concentration. An improvement in enzymatic efficiency made the UV/\( \text{H}_2\text{O}_2 \) method feasible for application in the lignocellulosic pretreatment process. Even though some studies have investigated the lignin removal efficiency and enzymatic performance of U-AHP on corn stover from a macroscopic perspective, the mechanism of pretreatment to improve enzymatic efficiency has rarely been investigated systematically.

In this study, the feasibility of U-AHP was evaluated to improve the enzymatic efficiency of corn stover. The effects of AHP and U-AHP on lignin removal and enzymatic digestion efficiency were examined. SEM, XRD, and FT-IR were used to characterize the different pretreatment samples to explain the principles of AHP and U-AHP reactions in terms of microstructure and physicochemical properties.
EXPERIMENTAL

Materials

The corn stalk used in the experiment came from Zhengzhou City, Henan Province, China. The collected corn stalks were washed, dried, crushed, passed through a 40-mesh sieve, dried, and stored. NREL method determined that the main components of corn stalk were 33.3% cellulose, 22.3% hemicellulose, and 19.0% lignin. The experimental reagent H$_2$O$_2$ (30%, w/w) was purchased from Yantai Shuangshuang Chemical Co., Ltd. (Shandong, China). The NaHSO$_3$, C$_6$H$_5$ONaOH, and NaKC$_6$H$_7$O$_6$ were from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). Concentrated H$_2$SO$_4$ (98%, W/w) was from Nanjing Chemical Reagent Co., Ltd. The C$_6$H$_8$O$_7$, Na$_3$C$_6$H$_5$O$_7$·2H$_2$O was from McLean (Shanghai, China), while the C$_7$H$_4$N$_2$O$_7$ (DNS) came from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). The NaHSO$_3$ and C$_6$H$_5$O were used without further purification. Cellic Ctec2 enzyme was obtained from Novozymes. The ultraviolet light source uses a 365 nm high-pressure mercury lamp with a power of 500 w (Beijing Tianmai Henghui Light Source Electric Co., Ltd.).

Corn Stover Pre-treatment

The corn stover feedstock was pretreated using the AHP and U-AHP methods, respectively. The AHP pretreatment was carried out in a 200 mL quartz reactor with 100 mL reaction solution and a solid-liquid ratio of 3% (w/w). Based on preliminary results, the specific parameters of AHP were designed for NaOH concentrations of 0, 0.2%, 0.4%, 0.6%, 0.8%, and 1% (w/w), H$_2$O$_2$ concentrations of 0, 0.5%, 1%, 1.5%, 2%, and 2.5% (w/w), and pretreatment times of 2, 4, 6, 8, and 10 h. For U-AHP pretreatment. The UV lamp was placed at a fixed position 10 cm from the quartz glass reactor for 2, 4, 6, 8, and 10 h of lateral irradiation. The rest of the conditions were the same as that for AHP. After pretreatment, the straw residue was separated from the reaction solution. The solid residue was rinsed with deionized water until the surface was neutral and then dried in an oven at 80 °C for 12 h for subsequent enzymatic reactions.

Enzymatic Reaction

The samples before and after pretreatment were digested with Cellic Ctec2 enzyme. The actual activity of the Cellic Ctec2 cellulase enzyme was based on the filter paper enzyme activity of 61 filter paper units (FPU/mL) at pH 4.8 and temperature 50 °C using Whatman No. 1 qualitative filter paper as the substrate (Eveleigh et al. 2009). 1 mL of Cellic Ctec2 enzyme reserve solution was diluted 1000 times to the sample of enzyme solution used for determination. Enzymatic digestion was performed by mixing 0.5 g of enzymatic sample with 25 mL of citric acid and sodium citrate buffer at pH 5. The substrate concentration was maintained at 2% (w/v) in the buffer. Next, cellulase was added at a load of 12.2 FPU per gram of dry biomass. The enzymatic digestion was performed at 50 ± 0.3 °C using a constant temperature water bath shaker at 150 rpm for 48 h. Microbial infection during enzymatic digestion was inhibited by supplementing the buffer with 1 g/L of tetracycline. At the end of the reaction, the samples were collected by centrifugation. The amount of reducing sugars in the enzymatic digestion products of the samples was determined using the 3,5-dinitrosalicylic acid (DNS) colorimetric method (Miller 1959). The absorbance of the samples at 540 nm was converted to glucose content using Eq. 1; the efficiency of the enzymatic digestion was calculated by Eq. 2.
The filtered sand core funnel was dried to a constant weight at 105 °C and weighed again (w1), calcined at 575 °C for 4 h, then cooled to room temperature and weighed again (w2). The lignin content was calculated from the absorbance using Eq. 5. Next, 5 mL of hydrolysis solution was added with 1.9 mL of 8% (w/w) sodium hydroxide and then diluted to 10 mL. The pH of the solution was made to be around 2 to 3. The samples were next filtered through a filter head and then analyzed for sugars by high-performance liquid chromatography (HPLC) using an Agilent-HPX-87H column (300 × 7.8 mm, Bio-rad, Richmond, CA, USA). Glucose and xylose were used as internal standards for cellulose and hemicellulose, and the calibration was carried out using Eqs. 4 and 5. The cellulose and hemicellulose contents were calculated using Eqs. 7 and 8. The hemicellulose and lignin removal efficiency were calculated using Eqs. 10 and 11, and the cellulose loss efficiency was calculated using Eq. 9, as follows:

\[ y_1 = 1205.5x_1 - 0.9551 (R^2 = 0.9999) \]  \tag{4}

\[ y_2 = 1154.2x_2 + 30.79 (R^2 = 0.9996) \]  \tag{5}

\[ L(\%) = \frac{w_1 - w_2}{300} \times 100 \]  \tag{6}

\[ C(\%) = \frac{2 \times \text{Glucose concentration(m/v)}(3+84) \times 0.9}{300(mg)} \times 100 \]  \tag{7}

\[ H(\%) = \frac{2 \times \text{Xylose concentration(m/v)}(3+84) \times 0.88}{300(mg)} \times 100 \]  \tag{8}

\[ \text{Cellulose loss efficiency(\%) } = \frac{C_R \times 300(mg) - C_P \times M_{mp}}{C_R \times 300mg} \times 100 \]  \tag{9}

\[ \text{Hemicellulose removal efficiency(\%) } = \frac{H_R \times 300(mg) - H_P \times M_p}{H_R \times 300(mg)} \times 100 \]  \tag{10}

\[ \text{Lignin removal efficiency(\%)} = \frac{L_P \times 300(mg) - L_R \times M_P}{L_P \times 300(mg)} \times 100 \]  \tag{11}

where \( y_1 \) is the mass of glucose (mg), and \( x_1 \) is the peak area of glucose determined in liquid phase.

In Eq. 4, \( y_2 \) is the mass of xylose (mg), and \( x_2 \) is the peak area of xylose determined in the liquid phase. \( R \) stands for untreated, \( P \) is after pretreatment, \( P_m \) is the remaining corn.
stover after the pretreatment, $C$ is cellulose content, $H$ is hemicellulose content, and $L$ is lignin content.

**Crystallinity Determination (XRD)**

The corn stover samples were analyzed using a Bruker D8 Advance X-ray diffractometer, Germany, before and after pretreatment with a scanning voltage of 30 kV, a scanning current of 20 mA, and Cu as the test target. The scattering angle ($2\theta$) was varied from 5° to 50° at a scanning speed of $2^\circ$/min during data collection. The cellulose crystallinity (CrI) of corn stover was calculated using the following Eq. 12 (Segal et al. 1959),

$$\text{CrI} (%) = \frac{I_{002} - I_{\text{am}}}{I_{002}}$$

where $I_{002}$ is the lattice diffraction intensity observed at a $2\theta$ value of 24.3° corresponding to the plane (002), and $I_{\text{am}}$ is the lattice diffraction intensity observed at a $2\theta$ value of 18°.

**Fourier Transform Infrared (FT-IR)**

Functional group analysis of corn stover was performed using a VERTEX 70 Fourier transform infrared (FT-IR) spectrometer (Bruker, Karlsruhe, Germany) before and after pretreatment. A sample to KBr ratio of 1:150 was taken and mixed well with an agate lambda, pressed into a light-transmitting thin section, and then scanned in the range of 4000 to 400 cm$^{-1}$.

**Scanning Electron Microscope (SEM)**

The surface morphology of the corn stover was analyzed using a Czech Tescan MIRA LMS electron microscope (SEM) before and after pretreatment. The samples were fixed with conductive adhesive on the operating table, and the surface was sprayed with gold. Microscopic morphology scans were performed at 500x magnification.

**RESULTS AND DISCUSSION**

**Effect of H$_2$O$_2$ Concentration on Pretreatment of Corn Stover**

Choosing suitable H$_2$O$_2$ concentration is very important for the pretreatment of AHP and U-AHP. Under the same alkali concentration and reaction time, the mass loss of main fractions of corn stalk before and after pretreatment by AHP and U-AHP was calculated as shown in Fig. 1a. Lignin removal efficiency and enzymatic saccharification yield were used as the main indexes to evaluate the pretreatment effect. The lignin removal efficiency increased from 73.3% to 86.1% and from 88.5% to 93% for AHP and U-AHP, respectively, with increasing H$_2$O$_2$ concentration for the same reaction time and alkali concentration. The AP pretreatment was with a sodium hydroxide concentration of 1% (w/w). The lignin removal efficiency of alkali pretreatment (AP) alone was 62.0%. Thus, AHP showed better lignin removal than AP, with a minor difference in cellulose and hemicellulose loss. This suggested that the addition of hydrogen peroxide enhanced the efficiency of dilute alkali pretreatment and selectively removed lignin. This increase in efficiency may be because H$_2$O$_2$ generates free radicals (HOO•) in dilute alkali solutions, which assist the alkali pretreatment in breaking ether bonds between lignin, reducing lignin aggregation, and ultimately leading to lignin leaching (Li et al. 2018a; Yang et al. 2021).
Compared with the AHP method, U-AHP lignin removal was higher, possibly due to the generation of oxidative solid hydroxyl radicals (HO·) by hydrogen peroxide. These radicals can interact with the chromogenic groups in the lignin structure to produce hydrophilic carboxyl and hydroxyl groups, thereby promoting lignin solubilization (Yang et al. 2018).

Considering the economics of biorefining, seeking only high lignin removal can result in a significant loss of hemicellulose and cellulose, reducing the utilization of lignocellulose. The loss of hemicellulose and cellulose in AHP samples decreased gradually with increasing H₂O₂ concentration, but the hemicellulose loss in U-AHP samples continued to increase to 48.9%. An increase in H₂O₂ content during AHP reduced the alkali content in the reaction solution, resulting in a weaker stripping reaction of hemicellulose and cellulose by NaOH, which in turn, enhanced hemicellulose retention. However, during U-AHP, a large amount of lignin was removed due to UV irradiation, leading to the destruction of the lignin-carbohydrate complex (LCC) and promoting hemicellulose leaching (Oliveira et al. 2020).

As shown in Fig. 1b, combining AP and H₂O₂ oxidative pretreatment could improve the enzymatic efficiency with increased H₂O₂ concentration at the same reaction time and 1% NaOH concentration. At 2% H₂O₂ concentration, the highest enzymatic efficiency of AHP reached 68.6%. This value was 1.74 times that of AP, showing that the addition of H₂O₂ promoted the pretreatment effect of AP. The added H₂O₂ assisted AP in removing a large amount of lignin from corn stover during the AHP treatment, which increased the enzymatic accessibility of cellulose, improving the enzymatic efficiency, which is consistent with the results of the lignin removal efficiency analysis. At 2% H₂O₂ concentration, U-AHP showed 84.3% enzymatic efficiency, which was 113% higher than AP and 23.4% higher than AHP. This increase in efficiency can be attributed to the catalytic oxidation of lignin by H₂O₂ under UV light, which depolymerizes and dissolves lignin, destroys the corn straw structure, exposes cellulose and semi-fibers in it, increases surface area, and provides more binding sites for hydrolase. Due to the large amount of lignin removal, the ineffective binding between cellulase and lignin is reduced, which is more conducive to the enzymatic hydrolysis of corn stover (Song et al. 2016; Yang et al. 2021). However, adding excess H₂O₂ decreased the efficiency of enzymatic digestion. For example, the enzymatic efficiency of both AHP/U-AHP decreased slightly when the H₂O₂
concentration was 2.5%, probably because adding excess H$_2$O$_2$ resulted in more loss of hemicellulose and cellulose from the corn stover. Thus, AP can improve straw enzymatic efficiency with the help of UV/H$_2$O$_2$ photocatalysis at lower alkali concentrations compared to H$_2$O$_2$ oxidation assistance alone. Therefore, the results indicate that U-AHP is an effective pretreatment technology.

**Effect of Pretreatment Time on the Pretreatment of Corn Stover**

The effect of reaction time on the quality loss of corn stalk components pretreated by AHP and U-AHP was studied, as shown in Fig. 2a. The lignin removal efficiency of the AHP and U-AHP processes continued to increase with increasing pretreatment time, with maximum values of 81.6% and 90.1%, respectively. This led to a decrease in the relative lignin content and a relative increase in the cellulose and hemicellulose content of the samples. The data revealed that both AHP and U-AHP effectively removed lignin and hemicellulose from the straw structure and retained the cellulose component compared to untreated corn stover, consistent with previous reports (Yang et al. 2018). The pretreatment time for lignin removal was less for U-AHP than for AHP. The loss rate of cellulose and hemicellulose also required investigation, and excessive degradation of carbohydrates with increasing pretreatment time increased the cellulose and hemicellulose losses.

![Fig 2a](image1.png)

**Fig 2. a:** Effect of reaction time on the quality loss of corn stalk components after pretreatment with AHP and U-AHP; **b:** Effect of reaction time on sugar production from pretreated corn stalk by enzymatic hydrolysis

Figure 2b shows that the enzymatic glycation yield increased significantly with increasing pretreatment time. U-AHP reached a maximum enzymatic efficiency of 86.4% after 8 h of pretreatment, which was 35.5 times higher than untreated. Thereafter, the enzymatic efficiency decreased with time, mainly due to the excessive degradation of carbohydrates and correspondingly increased cellulose and hemicellulose losses. Meanwhile, the maximum enzymatic digestion percentage of AHP was 76.2%, which was 12.7 times higher than untreated. The enzymatic efficiency of the untreated was only 5.55%. The low enzymatic efficiency of untreated corn stover may be due to the winding surface of cellulose by lignin and hemicellulose, resulting in cellulase having poor access to cellulose. External cellulase can only hydrolyze a small portion of the sugar structure exposed on the corn stover's surface, not the cellulose's central crystalline region (Xue et al. 2021). With pretreatment, a substantial amount of lignin and some hemicellulose was removed, exposing the crystalline region of cellulose in the corn stover, considerably
increasing the accessibility of cellulase to the substrate, and leading to a significant improvement in the efficiency of enzymatic digestion.

Effect of Alkali Concentration on Pretreatment of Corn Stover

The alkaline environment is a crucial factor affecting the lignin solubilization reaction of straw and the oxidation of lignin by H2O2 (Alvarez-Vasco and Zhang 2017; Cabrera et al. 2014). Under the condition of H2O2 concentration of 2% and illumination for 8 h, the alkali concentration was increased from 0 to 1%, and the mass loss of main fractions of corn stalk before and after pretreatment by AHP and U-AHP was calculated. As shown in Fig. 3.3a, the influence of alkali concentration on the mass loss of corn stalk fractions after pretreatment by AHP and U-AHP was studied. The concentration of HP pretreatment-H2O2 in the text is 2% (w/w). Lignin removal was not high with H2O2 pretreatment (HP) alone, suggesting that HP may remove only nonstructural extracts and small amounts of soluble sugars, consistent with previous reports (Cabrera et al. 2014; Yan et al. 2020). Under 1% NaOH, AHP and U-AHP showed the highest lignin removal with 80.8% and 87.2%, respectively, consistent with previous studies, indicating that AHP and U-AHP removed most of the lignin (Banerjee et al. 2012). At the same reaction time and H2O2 concentration, AHP and U-AHP pretreatments showed a significant trend of lignin reduction at alkali concentrations of 0 to 0.6%. The alkali concentration used in U-AHP pretreatment reaction was lower than that used in AHP pretreatment. Increasing the alkali concentrations further slowed down the rate of lignin removal.

The alkali concentration strongly affected the lignin leaching because ether bonds are easily broken in alkaline solutions. Therefore, with increasing alkali concentration, the breaking of the ether bonds between lignin molecules improves significantly, leading to lignin leaching from corn stover (Ma et al. 2020). Moreover, the ester bonds linking the lignin and hemicellulose molecules in the corn stover fraction are unstable in alkaline solutions. As the alkali concentration increases, the attached ester bonds break, promoting the leaching of lignin and hemicellulose from the corn stover (Ma et al. 2020). However, lignin removal reaches a saturation point when the lignin removal efficiency reaches a specific value (Huang et al. 2022).
The cellulose and hemicellulose loss gradually increases with increasing alkali concentration. Therefore, determining a suitable alkali concentration is necessary to improve the overall cellulose recovery. Further, determining the required alkali concentration can reduce the subsequent water demand and wastewater discharge, solving large-scale environmental problems due to wastewater (Dong et al. 2019a).

The enzymatic reaction of corn stover after pretreatment was carried out to understand the pretreatment effect further. Figure 3(b) shows the results of enzymatic digestion of pretreated corn stover by NaOH concentration. The enzymatic efficiency of H$_2$O$_2$ pretreatment (HP) alone (6.86%) was not significantly different from that without pretreatment (5.5%), which is consistent with the composition structure analyzed, and indicates that HP pretreatment has little effect on improving the accessibility of cellulase. On the contrary, the enzymatic efficiency of the purple H$_2$O$_2$ photocatalytic pretreatment (UV-HP) was 10.8%, which was slightly higher than HP. Further, Fig. 3(b) shows that the enzymatic efficiency of the samples increased with increasing alkali concentration under the same reaction conditions. The reason for this increased efficiency is that removing lignin in large quantities with increasing alkali concentration decreases the non-productive adsorption of lignin by cellulase (Dong et al. 2019b; Li et al. 2018a). On the other hand, cellulose undergoes a wetting and swelling reaction interacting with lignin removal under alkaline conditions, leading to the exposure of the crystalline zone of cellulose, making it easily accessible for cellulase. The increase in alkali concentration also intensifies the swelling of cellulose, improving the efficiency of enzymatic digestion (Li et al. 2018a,b; Shi et al. 2018; Yang et al. 2021). Maintaining a high alkaline loading promoted enzymatic digestion more efficiently than increasing the hydrogen peroxide loading. Adjusting the ratio of alkaline loading to hydrogen peroxide loading improved the substrate's enzymatic digestion efficiency.

**Orthogonal Test of U-AHP**

Based on the results of single-factor experiments, orthogonal tests were used to examine the factors affecting the enzymatic digestion of U-AHP pretreatment. The alkali concentration, hydrogen peroxide concentration, and pretreatment time, listed in Table 1, were selected as the primary factors of the orthogonal test. The results and data analysis of the orthogonal test are shown in Table 2. According to the data in Table 2, the factors influencing the enzymatic digestion of U-AHP pretreatment were in the following order: Alkali concentration > Pretreatment time > Hydrogen peroxide concentration. Further, the best pretreatment condition was found to be (A2B2C2), i.e., 1% NaOH, 2% H$_2$O$_2$, and an irradiation time of 8 h.

**Table 1. Orthogonal Test Factors and Levels**

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Table 2. Experimental Design and Results

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Effect of Pretreatment on the Structure of Corn Stover

The absorbance peak with a wave number of 3400 cm⁻¹ was attributed to O-H group stretching vibration (containing hydrogen bonds). Relative to the untreated sample peak. The peak appeared narrow and shifted to the right due to the breaking of chemical bonds with hemicellulose and lignin in the cellulose framework because of pretreatment. With the degradation of a large and partial quantity of lignin hemicellulose degradation, the relative content of cellulose increases, consistent with previous reports (Wang et al. 2021). The peak at 1632 cm⁻¹ was ascribed to the bending absorbance of water in crystalline cellulose (Zhao et al. 2013). Further, the peaks at 1733 cm⁻¹ and 1249 cm⁻¹ corresponded to the C=O and C-O stretch, respectively. The disappearance of these two absorbance peaks after pretreatment indicated that the ester bonds in hemicellulose and lignin were effectively cleaved by AHP and UV- AHP pretreatment. The absorbance peaks around 1500, 1430, and 1600 cm⁻¹ were due to the vibrations of the aromatic ring skeleton of lignin (Sun et al. 2002). Thus, the disappearance or the intensity of the absorbance peak decreased after pretreatment indicates that a large amount of lignin (possessing a benzene ring structure) was removed after pretreatment (Rehman et al. 2014).

The characteristic XRD peaks of cellulose I were located at 2θ = 14.8°, 15.2°, 20.7°, 21.9°, and 34.7°. The characteristic XRD peaks of cellulose II were located at 20.6° and 22.3° (Liu et al. 2019). The diffraction peaks of HP samples, AP, were similar to those of untreated samples, indicating that the cellulose in the corn stover crystal structure was unchanged. In contrast, the diffraction peaks of the AHP/U-AHP samples were significantly different from the untreated samples. The crystal type of cellulose was partially converted from cellulose I to cellulose II during pretreatment. Both were coexisting (Wang et al. 2018). In addition, the shape of the diffraction peaks at 16.1° and 20.9° changed from broad to narrow and then gradually sharpened, and the CrI of the pretreated samples increased significantly with the enhancement of pretreatment (Bernardinelli et al. 2015).
Fig. 4. Effect of pretreatment on the microstructure of corn stover; a: FTIR spectra before and after pretreatment; b: Crystallinity of cellulose analysis of corn stalk before and after pretreatment; c: SEM of surface structure of corn stalk before and after pretreatment. In the figure, U is untreated corn stalk; W is washed corn straw; HP was pretreatment with H$_2$O$_2$ oxidation; AP is alkali pretreatment; AHP is alkaline hydrogen peroxide pretreatment; U-AHP is photocatalytic alkaline H$_2$O$_2$ pretreatment. U-AHP is photocatalytic alkaline H$_2$O$_2$ pretreatment.

The morphology of the pretreated corn stover changed compared to the untreated. Before pretreatment, tiny amounts of dust impurities were attached to the surface of the samples, and the cellulose skeleton was dense and neatly arranged in three dimensions. Further, cracks and tiny pores appeared on the surface of the HP samples. However, this phenomenon only existed on the contact surface. The overall three-dimensional structure remained unchanged after pretreatment, primarily due to the removal of a small amount of lignin and the precipitation of extracts (waxes, oligosaccharides, and others). The surface of the AP samples was rough and folded, forming a furrow-like structure and exposing the cellulose crystal structure. The pore structure and specific surface area increased, facilitating the combination of cellulase with cellulose and significantly improving the enzymatic efficiency. Notably, the AHP and U-AHP samples had collapsed steric structures due to removing a large amount of lignin and part of hemicellulose. Some fibers
also exhibited severe fractures, increasing the surface area of the exposed cellulose, which further increased the accessibility of cellulase to cellulose. This chain of events led to a significant improvement in enzymatic digestion efficiency. Further, the SEM data revealed that AHP pretreatment damaged corn stover more than HP and AP pretreatment alone. A synergism existed between H$_2$O$_2$ and NaOH. U-AHP enhanced the synergistic effect of H$_2$O$_2$ and NaOH through the unique properties of H$_2$O$_2$ under UV.

**Mechanism Analysis**

The synergistic effect in the alkaline H$_2$O$_2$ pretreatment was primarily due to the interaction between the alkaline pretreatment and the oxidative pretreatment with H$_2$O$_2$. The active components of the reaction consist of OH$^-$ and highly reactive oxygen species (HOO$^-$, O$_2^-$, HO$^-$). The pathways for the generation of the reactive oxygen species are shown in (R1), (R2), and (R3). The pathways of action of AHP and U-AHP are summarized in Fig. 5 and Table 3.

\[
\begin{align*}
H_2O_2 & \xrightarrow{\text{uv}} 2HO. \quad \text{(R1)} \\
H_2O_2 + OH^- & \rightarrow HOO^- + H_2O \quad \text{(R2)} \\
H_2O_2 + HOO^- & \rightarrow HO. +O_2^- +H_2O \quad \text{(R3)}
\end{align*}
\]

(1) In the alkali H$_2$O$_2$ pretreatment, the nucleophilic reaction of OH$^-$ with lignin moieties (Table 3) broke up the lignin molecules. Simultaneously, the introduced hydrophilic groups made lignin more liquidophilic. Cellulose and hemicellulose underwent a wetting and swelling reaction in the polar NaOH solutions (Yang et al. 2021). These changes promote penetration of the reaction solution into the crystalline region of the cellulose, enlarge the diameter of the microfibers, and reduce the surface porosity, reducing the crystallinity of cellulose. The swelling of cellulose disrupted the straw structure, facilitating removal of lignin and hemicellulose. Thus, there is a synergistic mechanism of lignin solubilization and microfibril morphological changes during alkali pretreatment (Ji et al. 2014).

**Table 3. Important Responses of AHP and U-AHP Pretreatment of Corn Stover**

<table>
<thead>
<tr>
<th>Components</th>
<th>Active Fraction</th>
<th>Reaction Structures Involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin</td>
<td>ROS</td>
<td>Any structure</td>
</tr>
<tr>
<td></td>
<td>OH$^-$</td>
<td>Phenolic α/β aryl ether structure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-phenolic β-o-4 structure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methyl-aryl ether structure</td>
</tr>
<tr>
<td></td>
<td>HOO$^-$</td>
<td>Lignin color-emitting groups</td>
</tr>
<tr>
<td>Cellulose</td>
<td>OH$^-$</td>
<td>Cellulose swelling</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>ROS</td>
<td>Lignin-Carbohydrate Complex(LCC)contain ester bonds, ether bonds</td>
</tr>
</tbody>
</table>

Notes: ROS is reactive oxygen species; AHP is alkaline H$_2$O$_2$ pretreatment; U-AHP is photocatalytic alkaline H$_2$O$_2$ pretreatment.

(2) Lignin degradation was facilitated by adding H$_2$O$_2$ during AHP. H$_2$O$_2$ generated perhydroxyl anions (HOO$^-$) that selectively attacked the colored groups in corn stover, including (quinone structures), side chain conjugated double bonds, and benzene rings, while reacting with specific groups produced during lignin depolymerization to dissolve lignin. NaOH and H$_2$O$_2$ synergize lignin removal and enzymatic digestion by adding H$_2$O$_2$.

to the alkali pretreatment (Yan et al. 2020).

(3) U-AHP assisted reaction under ultraviolet (UV) irradiation generated hydroxyl radicals (HO\(^-\)) and superoxide anion radicals (O\(_2^-\)) than AHP and other highly reactive oxidants (Wang et al. 2019). Non-selective, advanced oxidation processes (AOPs) remove phenolics from lignin and colored groups from wastewater (Ahmed et al. 2009; Carvalho et al. 2020).

CONCLUSIONS

1. This study compared the pretreatment ability of alkaline H\(_2\)O\(_2\) pretreatment (AHP) and ultraviolet light photocatalytic AHP (U-AHP) for corn stover. U-AHP was more effective than AHP in removing lignin, resulting in effective enzymatic digestion of the pretreated corn stover.

2. The highest enzymatic efficiency of 88.3% was obtained under the following conditions: 1% NaOH, 2% H\(_2\)O\(_2\), and 8 h irradiation time.

3. Based on the micro-structure characteristics of straw, the collaborative mechanism between AHP and U-AHP preprocessing is speculated. The mechanism of improving the efficiency of enzymolysis was discussed, including the crystallization change of cellulose, the removal of lignin and the destruction of surface structure, which provided a theoretical basis for the subsequent pretreatment of lignocellulose.

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