Bioaugmentation of Corn Stalk Saccharification with *Aspergillus fumigatus* Under Low/High Solid Loading Culture

Zhiwei Song · Xuechen Wen · Tao Sheng · Caiyu Sun

Received: 24 November 2021 / Accepted: 21 January 2022 / Published online: 26 January 2022
© The Author(s), under exclusive license to Springer Science+Business Media, LLC, part of Springer Nature 2022

**Abstract**

In this study, pretreatment of corn stalks with the cellulosic fungus *Aspergillus fumigatus* CLL was performed to enhance its saccharification. The effect of feedstock loading (10 g/L and 20 g/L) on corn stalk pretreatment performance and the effects of *Trichoderma reesei* cellulase and commercial cellulase on the saccharification of pretreated corn stalks were investigated. The results indicated that with a solid loading of 10 g/L for 2 days at 30 °C, 25.2% lignin was degraded, whereas the degradation was only 2.8% under solid loading of 20 g/L. Pretreated corn stalks (solid loading 10 g/L for 2 days at 30 °C) saccharified by 300 U/L *T. reesei* cellulose exhibited 3.4 times higher sugar yields (67.9%) compared to the raw feedstocks, and the value was comparable with the saccharification performance of commercial cellulase (60.8%). These results demonstrated that *A. fumigatus* CLL not only decomposed the structure of corn stalks but also complemented the incomplete cellulase system of *T. reesei*. *A. fumigatus* CLL can be used to pretreat lignocellulosic materials to enhance the saccharification performance.

**Keywords** Biofuels · Cellulase system · Biopretreatment · Agricultural solid waste

**Introduction**

Lignocellulosic biomass is one of the most abundant resources on Earth [1], it represents a large reservoir of glucose and is an attractive renewable energy source. Saccharification of lignocellulosic biomass is one of the key steps in lignocellulosic biofuel production which affected by the synergistic effect of cellulose activity and the structure of lignocellulose feedstocks [2]. For saccharification, some efficient cellulases have been isolated and purified from *Trichoderma*, *Penicillium*, and *Aspergillus* spp. [3]. *Trichoderma reesei* cellulase is extensively used in commercial processes [4], but the β-glucosidases secreted by *T. reesei* are insufficient for effective cellulose conversion [5]. Some species of *Penicillium* have been reported to produce all enzymes in proper proportions, but their titers are very low [6]. The capacity of *Aspergillus fumigatus* to produce cellulase has not been compared with that of other well-known cellulytic fungi, such as *T. reesei*; however, *A. fumigatus* can grow on several substrates and produce hydrolytic enzymes of economic value [7]. More importantly, *A. fumigatus* was reported to have a good ability to synthesize cellulase β-glucosidase, which is a potential supplement required for the *T. reesei* cellulase system [8]. A prerequisite for the availability of holocellulose is the destruction of the rigid structure of lignin [9, 10]. At present, the main microorganism responsible for lignin degradation in nature is the white-rot fungus [11], but it takes a long time for white-rot fungi to degrade lignin. In recent years, *A. fumigatus* has been reported for its ability of lignin degradation [12]. Compared with white-rot fungi, *A. fumigatus* degrades lignin faster [13] and brings about substantial demethoxylation and dehydroxylation, making it a potential biopretreatment employee [14]. Hence, it is attractive to use of *A. fumigatus* to degrade lignin to destroy the structure of lignocellulose, while produce cellulase to couple with *T. reesei* cellulase enhanced the saccharification of lignocellulosic biomass. Nevertheless, to the best of our acknowledge, there are no reports on whether *A. fumigatus* pretreatment could supplement the incomplete cellulase system of *T. reesei*. Furthermore, solid loading has a significant influence on the pretreatment time cost. It is necessary to identify the effect of feedstock concentration on lignin degradation and structural changes to elucidate the mechanisms involved in the fungal pretreatment of corn stalks.
In this study, *A. fumigatus* CLL was utilized to degrade the lignin of corn stalks and to enhance the saccharification process. We then explored the feasibility of supplementing the *T. reesei* cellulase system with *A. fumigatus* CLL β-glucosidase. As solid loading greatly influences lignocellulose saccharification, *A. fumigatus* CLL was used to treat corn stalks under high solid loading (20 g/L) and low solid loading (10 g/L) conditions. Successively, the saccharification results obtained from *A. fumigatus* CLL cellulase and *T. reesei* cellulase were compared with that of commercial cellulase.

### Methodology

#### Raw Materials and Inoculum

Corn stalks were obtained from the farm at Heilongjiang University, Harbin, Heilongjiang, China. The corn stalks were crushed, sieved through 60 sieves, then dried at 65 °C until weight kept constant for later use. The composition of lignocellulosic feedstocks was determined using the Van Soest method [15]. One-gram raw corn stalk contains 42.34 ± 3.41% cellulose, 32.77 ± 2.11% hemicellulose, 19.56 ± 5.41% lignin, and 5.33 ± 1.23% ash. *A. fumigatus* CLL was obtained from the Microbiology Laboratory of Heilongjiang University of Science and Technology and was maintained on potato dextrose agar (PDA) plates at 4 °C. The spores of *A. fumigatus* CLL that grew well on PDA were transferred to modified Martin medium and cultured for 2 days. Spore suspension (10^7 spores per mL) was added (2% v/w, corresponding to 2 × 10^5 spores/g feedstock) to high solid loading and low solid loading cultures.

*Trichoderma reesei* (DSM 768) was obtained from the Microbiology Laboratory of Heilongjiang University of Science and Technology, and *T. reesei* cellulase was produced and separated according to the method described by Zhao [16]. Briefly, *T. reesei* was cultured in the cellulase production medium ((NH₄)₂SO₄ 1.4 g/L; urea 0.3 g/L; KH₂PO₄ 2.0 g/L; MgSO₄·7H₂O 0.3 g/L; CaCl₂ 0.3 g/L; wheat bran, 20 g/L; soybean cake powder 5 g/L; Avicel, 8 g/L) in a shaking incubator at 29 °C and 120 rpm. Four days later, the culture medium was harvested at 8000 rpm for 10 min at 4 °C, and the supernatant was used as the source of cellulases. Commercial cellulase, composed of endoglucanase, exoglucanase, and β-D glucosidase, was purchased from Novozymes (1000 U/g).

#### Corn Stalks Degrade Under High Solid Loading Culture/Low Solid Loading Culture

Low solid loading culturing was carried out in 250-mL Erlenmeyer flasks containing 10-g corn stalks and 100-mL nutrient solution (peptone 5 g/L, yeast 2 g/L, MgSO₄·7H₂O 0.5 g/L, and KH₂PO₄ 1 g/L) at 30 °C for 20 days. High solid loading culturing was carried out in the 250-mL Erlenmeyer flasks containing 20-g corn stalks and 100-mL nutrient solution (peptone 5 g/L, yeast 2 g/L, MgSO₄·7H₂O 0.5 g/L, and KH₂PO₄ 1 g/L) at 30 °C for 60 days. Low solid loading culture/high solid loading culture samples were collected every 2/5 days to determine the composition of corn stalks, sugar yield, and cellulase and lignase activities. Corn stalks without fungal inoculation were used as the control.

#### Saccharification of Corn Stalks

We diluted the commercial cellulase and *T. reesei* cellulase to 100–350 U/L with citrate buffer (0.05 mmol/L, pH 4.5) for corn stalk saccharification. Saccharification of pretreated/untreated corn stalks was performed according to the method described by Zhao [16]. Briefly, commercial cellulase or *T. reesei* cellulase was mixed with different corn stalk samples (10 g/L) at 55 °C. The saccharification was carried out for 24 h, and the samples were collected every 3 h.

#### Hydrolysate Determination

The components of the hydrolysates were analyzed using high-performance liquid chromatography (HPLC) (1260 Infinity, Agilent Technologies, USA) equipped with a Bio-Rad Aminex HPX–87H column and a refractive index detector (RID). The operating temperatures of the column oven and detector were 70 °C and 55 °C, respectively. H₂SO₄ (2 mmol/L) was used as the mobile phase, with elution carried out at a 0.6 mL/min flow rate.

#### Scanning Electron Microscopy (SEM) Analysis

The surface structure of the corn stalks was determined using SEM (JEOL-JSM 6480 LVSEM at 20 kV). Samples were prepared by mounting them on specimen stubs using double-coated tape. Excess material was gently blown off and the sample was sputter coated with AuPd in the presence of argon gas using a Hummer I sputter coater.

#### FTIR Spectroscopic Analysis

FTIR spectra of the samples were obtained using a Bruker Tensor 27 FTIR spectrometer (Bruker Optics, Ettlingen, Germany), and a thin slice made of a mixture of 2 mg of the sample and 200 mg of KBr was used for scanning in the range of 400–4000/cm with a resolution of 1/cm [17]. Baseline and ATR corrections for penetration depth and frequency variations were applied using Spectrum One software supplied with the equipment.
Biomass Crystallinity (XRD Diffraction)

The crystalline indices of the samples were examined by XRD measurements performed on a Bruker D8 Advance diffractometer using Cu Kα radiation (λ = 0.1541 nm) at 30 kV and 30 mA. The sample of particle size less than 0.125 mm was scanned at the speed of 1°/min range from 2θ = 5–50° and with a step size of 0.05° at room temperature [17].

The crystallinity index (CrI) was calculated according to the following formula.

\[
CrI(\%) = \frac{(I_{002} - I_{am})}{I_{002}} \times 100\%
\]

\[I_{002}\] is the intensity of the crystalline peak at 2θ = 18° and \[I_{am}\] is the intensity at 2θ = 22.5°.

Ligninase Activity Assay

Lignin peroxidase activity was measured by inspecting the oxidation of veratryl alcohol to veratraldehyde at 310 nm. One enzyme unit was defined as the amount of enzyme that oxidizes 1 μmol veratyl alcohol per min [18]. The activity of manganese peroxidase (MnP) was determined by measuring the amount of the Mn(III) malonate complex at 270 nm. One enzyme unit was equal to the amount of enzyme oxidizing one μmol Mn(II) to Mn(III) per min [19]. Laccase activity was measured by monitoring the oxidation of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) at 420 nm, and enzyme one unit was equal to the amount of enzyme oxidizing 1 μmol of ABTS per min [20].

Cellulase Activity Assay

According to previously reported protocols, the activities of endoglucanase, exoglucanase, and β-glucosidase were determined [21]. Briefly, endoglucanase activity was analyzed by incubating 0.2 mL of enzyme in CMC (1.8 mL), pre-dissolved in pH 7.0 buffer (50 mM potassium phosphate buffer). Pre-soaked Whatman No. 1 filter paper strip (1–3 cm) in 1.8 mL of the same buffer was used as a substrate for exoglucanase activity. The reducing sugars released due to the action of cellulase were analyzed using the 3,5-dinitrosalicylic acid (DNS) method [22]. One unit (U) of CMCase or FPase activity was the amount of enzyme that liberated 1 μmol of glucose per min under typical assay conditions. The activity of β-glucosidase was determined by incubating 0.2 mL of enzyme in 1.8 mL of 5 mM p-nitrophenyl-β-D-glucopyranoside (pNPG). One unit of β-glucosidase activity corresponds to the amount of enzyme that liberates 1 μmol of p-nitrophenol from pNPG per min under standard assay conditions [22].

Statistical Analysis

All samples were taken in triplicates. Mean values are presented with standard errors. Multiple comparison tests were performed using the Student t-test (significance level = 0.05). The experimental results were validated via statistical analysis using Spass V13.0.

Results and Discussion

Effect of Bioaugmentation on the Composition of Corn Stalks

Lignin is believed to be the main obstacle to lignocellulosic biomass saccharification [25]. The removal of lignin not only depolymerizes the hash structure of the corn stalks but also facilitates the contact of cellulase with holocellulose [25, 26]. In this study, as shown in Fig. 1a, during the first 30 days of high solid loading culture process, the degradation ratios of cellulose and hemicellulose were similar to that of lignin. After 30 days, the degradation ratio of lignin (36.32%) was higher than cellulose (28.39%) and hemicellulose (23.22%). Fifty days later, the lignin degradation ratio was 48.65%, whereas the degradation ratios of cellulose and hemicellulose were 40.11% and 31.41%, respectively. Compared to the high solid loading culture, the low solid loading culture’s corn stalk degradation ratio increased rapidly. As shown in Fig. 1b, the lignin degradation ratio increased rapidly in 2 days and gradually stabilized after 16 days; 25.2% lignin was degraded in 2 days, and the degradation ratios of cellulose and hemicellulose were 7.48% and 6.93%. After 10 days, the degradation ratio of lignin, cellulose, and hemicellulose were 40.61%, 18.01%, and 17.42% respectively. Twenty days later, the lignin degradation ratio reached 50.5%. Meanwhile, hemicellulose and cellulose degradation ratios reached 27.95% and 29%, respectively. While under high solid loading culture, the degradation ratios of lignin, cellulose, and hemicellulose were only 18.08%, 20.17%, and 21.41% in 20 days. The results indicate that

\[
\text{Saccharification ratio} = \frac{w_{\text{sugar released}} \times \text{sugar conversion factor}}{\text{biomass taken} \times \text{total cellulosics fraction}} \times 100\%
\]

In this formula, the sugar released is the sugar produced during saccharification (mg). The sugar conversion factor is the hydrolysis saccharification correction factor (glucose 0.9, xylose 0.88). Finally, the total cellulosic fraction is the fraction of cellulose and hemicellulose in corn stalks (mg) [23, 24].
A. fumigatus CLL can effectively degrade lignin under high/low loading culture conditions. Compared with high solid loading culture, less holocellulose is consumed during low solid loading culture, which is more conducive to subsequent saccharification and utilization of A. fumigatus CLL. In previous studies, some lignocellulosic fungi, especially white-rot fungi or brown-rot fungi, were used to pretreat cellulose feedstocks and some progress were obtained [27, 28]. In contrast, Aspergillus spp. have been reported to produce high levels of β-glucosidase [7]. More importantly, compared to white-rot fungi, lignin degradation products are fatty acids rather than aromatic monomers [15], indicating complete degradation of lignin [18].

Lignin degradation is closely related to that of ligninases [29]. Therefore, the trend of change in ligninase activity in high/low-solid-loading cultures should be clarified. The major enzymes associated with lignin-degrading fungi are lignin peroxidase (EC 1.11.1.14), manganese peroxidase (EC 1.11.1.13), and laccase (EC 1.10.3.2) [30], as shown in Fig. 1c and Fig. 1d. During the low solid loading culture process, the laccase (Lac) activity increased in the first 10 days, reached maximum (15.6 U/mL) after 10 days, and then decreased to 1.2 U/mL at 20 days. The trend of lignin peroxidase (Lip) activity was similar to that of Lac activity; the maximum Lip activity of 14.6 U/mL was obtained after 10 days. Twenty days later, the activity of Lip was just 1.1 U/mL. Unlike Lac and Lip, manganese peroxidase (Mnp) activity reached peak after 8 days and the maximum activity was 16.3 U/mL. Twelve days later, the Mnp activity was only 10% of the peak.

In contrast to the low solid loading culture, the lignase activity of the high solid loading culture peaked at 25 days and decreased quickly. Although the peak lignase activity of the high solid loading culture was similar to that of the low solid loading culture \( (p > 0.05) \), the peak time for lignase was doubled, which indicates that the low solid loading culture is more conducive for ligninase activity of A. fumigatus CLL. The better lignin degradation performance obtained in low solid loading might be attributed to the increased contact of ligninase with corn stalks, and the presence of proteins has been reported to induce high ligninase production [31]. In the low solid loading culture, peptones are dissolved in the liquid medium, which increase the contact of the protein with A. fumigatus CLL and enhance its ligninase activity. Furthermore, a certain level of readily available carbon sources is necessary to induce and maintain ligninase activity [32]. It can be inferred that low solid loading culture provided soluble oligosaccharides for A. fumigatus CLL and the available carbohydrates enhanced the synthesis of ligninase.

**Effect of Bioaugmentation on the Structural Features of Corn Stalks**

The degradation of lignin not only released holocellulose from the lignin package but also loosened the structure of lignocellulose raw materials for subsequent use [33]. As shown in Fig. 2a, the raw corn stalks showed a dense layer of lignin structure, and 1 day later, some breakage was observed on the corn stalks surface (Fig. 2b), suggesting that the corn stalks started to degrade. Two days later, the
destruction of the corn stalk surface enhanced. Meanwhile, the cellulose and hemicellulose were exposed to lignin, and the structure was relatively complete (Fig. 2c). Ten days later, as shown in Fig. 2d, the surface structure of the corn stalks was completely destroyed.

The FTIR results are presented in Fig. 3a. The functional groups of corn stalks showed obvious changes during
treatment with A. fumigatus CLL. Compared to the untreated raw materials, the 1512/cm band showed evident absorption in the first 2 days, and corresponded to the aromatic skeleton of lignin vibration C=C [34]. In addition, the characteristic peaks near the 1266/cm waveband appear to be significantly weakened, which corresponded to the C-O bond [35]. Obvious absorption appeared at 2919–2922/cm and the wide absorption band between 3300 and 3500/cm corresponds to the –OH tensile vibration signal, which is significantly increased after CLL pretreatment, indicating that cellulose and hemicellulose are exposed [36]. As shown in Fig. 3b, the XRD results indicated that the crystalline index of raw corn stalks was 0.43. When pretreated with A. fumigatus CLL for 1 day, the crystalline index slightly declined to 0.36, which coincides with the chemical composition changes of corn stalks. Two days later, the crystallinity index of rice straw dramatically declined to 0.35; 14.2% crystallinity reduction of rice straw was achieved. Upon further increasing the operational time to 10 days, the crystalline index of pretreated rice straw decreased stepwise to 0.33, which indicated that pretreatment with A. fumigatus CLL not only decomposed the structure of corn stalks but also decreased their crystallinity.

**Effect of Bioaugmentation on the Saccharification of Corn Stalks**

To evaluate effect of cellulase on the saccharification of pretreated/unpretreated corn stalks, commercial cellulase and T. reesei cellulase were used to hydrolyze corn stalks that were pretreated for one day under low solid loading culture. One-gram pretreated corn stalk contains 42.90 ± 6.41% cellulose, 33.52 ± 5.22% hemicellulose, 17.98 ± 3.78% lignin, and 5.60 ± 2.11% ash. As shown in Fig. 4a, the saccharification ratio increased as the commercial cellulase concentration increased from 100 to 300 U/L; however, when the commercial cellulase concentration increased to 350 U/L, saccharification ratio barely increased. The maximum saccharification ratio (42.8%) was obtained with 300 U/L commercial cellulase. The results of sample hydrolysis using T. reesei cellulase are shown in Fig. 4b. The peak saccharification ratio also obtained with T. reesei cellulase concentration of 300 U/L (45.6%). It is worth noting that the peak saccharification ratios with both T. reesei cellulase and commercial cellulase were obtained after 24 h and then maintained stability. It has been reported that cellulase is more susceptible to end-product inhibition caused by glucose. Once glucose accumulates in the medium in a higher amount, its high concentration can either block the active site for the substrate [37]. Feedback inhibition inhibits cellulase hydrolysis during lignocellulosic biomass saccharification. Previous studies have found that the low β-glucosidase activity of T. reesei reduced the efficiency of lignocellulosic hydrolysis [38]. The catalytic efficiency of T. reesei cellulase was lower than that of commercial cellulase composed of multiple fungal cellulase cocktails [39, 40]. It is worth noting that the saccharification performance of T. reesei cellulase was comparable with commercial cellulase in this study when A. fumigatus CLL β-glucosidase was supplied to the T. reesei cellulase system. Additionally, in previous studies, some species of A. fumigatus have been reported to produce β-glucosidase with high activity [41]. Therefore, it is can be inferred that the β-glucosidase produced by A. fumigatus CLL supplements the cellulase system of T. reesei and enhanced the saccharification performance of T. reesei cellulase.

The cellulase activities of the following: untreated samples saccharified by 300 U/L T. reesei cellulase (group I); pretreated sample saccharified by 300 U/L commercial cellulase (group II); pretreated sample without external cellulase (group III); pretreated sample saccharified by 300 U/L T. reesei cellulase (group IV) were investigated at under culture conditions of 10 g/L feedstock, 55 °C, 130 rpm, for 24 h. The activities of endo-glucanohydrolase (shorted for EG), exo-glucanohydrolase (shorted for CBH), and β-glucosidase (shorted for BG) were determined every 6 h. As shown in Table 1, EG, CBH, and BG activities were observed in the untreated sample saccharified by T. reesei cellulase, with the peak activities of EG (0.229 ± 0.016 U/mL) and CBH (0.216 ± 0.009 U/mL) being much higher than that of BG (0.087 ± 0.007 U/mL) (p < 0.05). It is commonly believed that T. reesei has a poor ability to produce β-glucosidase, and the saccharification of lignocellulose
feedstocks was accomplished by the synergy of endo-glucanohydrolase, exo-glucanohydrolase, and β-glucosidase. The lack of β-glucosidase reduced the hydrolysis efficiency of lignocellulose, indicating that external β-glucosidase supplementation is indispensable for the saccharification by T. reesei cellulase [42]. Compared to the untreated sample saccharified by T. reesei cellulase, the pretreated sample saccharified by T. reesei cellulase demonstrated a higher β-glucosidase activity (0.318 ± 0.009 U/mL). This suggests that the addition of strain A. fumigatus CLL not only enhanced the β-glucosidase activity, but also completed the cellulase system of T. reesei cellulase. Meanwhile, the cellulase activity of the four treatments cellulase activity exhibited a pronounced drop after 18 h. Eighteen hours later, for the untreated sample saccharified by T. reesei cellulase, the activities of endo-glucanohydrolase, exo-glucanohydrolase, and β-glucosidase were approximately 64.2%, 51.9%, and 49.4% of the peak, respectively. For the pretreated sample saccharified by T. reesei cellulase, the activities of endo-glucanohydrolase, exo-glucanohydrolase, and β-glucosidase were approximately 61.9%, 68.7%, and 63.2% of the peak, respectively. Similar results were obtained for the sample saccharified by commercial cellulase, which is consistent with the results of saccharification ratio.

The pretreatment of lignocellulose accompanied by the loss of holocellulose [42], since appropriate pretreatment time is a key factor in improving the saccharification ratio of lignocellulosic feedstocks [43]. In this study, corn stalks (10 g/L) degraded by strain A. fumigatus CLL under high/low solid loading culture conditions were saccharified by 300 U/L T. reesei cellulase/commercial cellulase to investigate the effect of pretreatment duration on the saccharification performance of feedstocks. As shown in Fig. 5a, the saccharification ratio of the untreated sample was just 20.18%. With the extension of the pretreatment duration, the saccharification ratio gradually increased and peaked after 2 days. The maximum saccharification ratio was 68.4%, which was 3.38-fold that of the untreated sample (p < 0.05). After extending the pretreatment time to 10 days and 16 days, the saccharification ratios were 41.3% and 32.4%, respectively. Different from low solid loading culture, the maximum saccharification ratio (60.9%) under high solid loading culture was obtained at 10th day (Fig. 5b). Furthermore, after extending the pretreatment duration to 30 days and 50 days, the corn

| Table 1 The cellulase activity during the corn stalk saccharification |
|--------------------------|-----|-----|-----|-----|-----|
| Time (h) | 0   | 6   | 12  | 18  | 24  |
| T. reesei cellulasea  
(group I) | EG  | 0.229±0.016 | 0.208±0.010 | 0.174±0.009 | 0.147±0.009 | 0.081±0.007 |
| | CBH | 0.216±0.009 | 0.197±0.008 | 0.156±0.013 | 0.112±0.008 | 0.065±0.008 |
| | BG  | 0.087±0.007 | 0.078±0.007 | 0.079±0.005 | 0.043±0.004 | 0.013±0.003 |
| Commercial cellulasea  
(group II) | EG  | 0.242±0.014 | 0.216±0.009 | 0.187±0.011 | 0.155±0.008 | 0.077±0.007 |
| | CBH | 0.203±0.011 | 0.174±0.010 | 0.135±0.009 | 0.102±0.008 | 0.067±0.007 |
| | BG  | 0.287±0.009 | 0.225±0.012 | 0.187±0.010 | 0.158±0.008 | 0.104±0.006 |
| A. fumigatus CLL cellulasea  
(group III) | EG  | 0.198±0.011 | 0.188±0.010 | 0.174±0.010 | 0.153±0.016 | 0.134±0.013 |
| | CBH | 0.122±0.013 | 0.107±0.010 | 0.095±0.009 | 0.076±0.008 | 0.058±0.008 |
| | BG  | 0.279±0.019 | 0.257±0.023 | 0.241±0.011 | 0.226±0.014 | 0.196±0.017 |
| T. reesei cellulase and A. fumigatus CLL cellulasea  
(group IV) | EG  | 0.265±0.010 | 0.246±0.010 | 0.207±0.009 | 0.182±0.009 | 0.128±0.007 |
| | CBH | 0.233±0.009 | 0.218±0.011 | 0.176±0.009 | 0.157±0.008 | 0.101±0.006 |
| | BG  | 0.318±0.009 | 0.287±0.010 | 0.233±0.010 | 0.201±0.011 | 0.143±0.005 |

*aThe mean value of cellulase activity (U/mL)*
The biopretreatment with *A. fumigatus* CLL in this study is destroying the corn stalk structure, and enhanced the sugar yield after hydrolysis by *T. reesei* cellulase in theory. Our results indicated that the β-glucosidase of *A. fumigatus* CLL completed the cellulase system of *T. reesei*. Reduced the solid loading of *A. fumigatus* CLL improved lignin degradation rate while avoid the loss of holocellulose. Analysis of FTIR and XRD revealed the mechanisms how the biological pretreatments improved the sugar yield from corn stalk hydrolysis by cellulase. This study provided a promising method for lignocellulose biopretreatment and enzymatic hydrolysis.

**Conclusions**

The biopretreatment with *A. fumigatus* CLL in this study is destroying the corn stalk structure, and enhanced the sugar yield after hydrolysis by *T. reesei* cellulase in theory. Our results indicated that the β-glucosidase of *A. fumigatus* CLL completed the cellulase system of *T. reesei*. Reduced the solid loading of *A. fumigatus* CLL improved lignin degradation rate while avoid the loss of holocellulose. Analysis of FTIR and XRD revealed the mechanisms how the biological pretreatments improved the sugar yield from corn stalk hydrolysis by cellulase. This study provided a promising method for lignocellulose biopretreatment and enzymatic hydrolysis.

**Author Contribution** All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Zhiwei Song. The experiments of biopretreatment and enzyme were carried out by Xuechen Wen. The experiments of SEM and FTIR were carried out by Tao Sheng. The experiments of XRD were carried out by Caiyu Sun. The first draft of the manuscript was written by Zhiwei Song and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Funding** This work was financially supported by the National Natural Science Foundation of China (Nos. 51678222 and 51908200).

**Data Availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Ethics Approval and Consent to Participate** Not applicable.

**Consent for Publication** Not applicable.

**Competing Interests** The authors declare no competing interests.

**References**

1. Bara NR, Shah A (2016) Techno-economic analysis of cellulose dissolving ionic liquid pretreatment of lignocellulosic biomass for fermentable sugars production. Biofuel Bioprod Bioref 10(1):70–88. https://doi.org/10.1002/bbb.1622
2. Capolupo L, Faraco V (2016) Green methods of lignocellulose pretreatment for biorefinery development. Appl Microbiol Biotechnol 100(22):9451–9467. https://doi.org/10.1007/s00253-016-7884-y
3. De França PD, Pereira N Jr, De Castro AM (2018) A comparative review of recent advances in cellulases production by *Aspergillus, Penicillium* and *Trichoderma* strains and their use for lignocellulose deconstruction. Curr Opin Green Sust 14:60–66. https://doi.org/10.1016/j.coegsc.2018.06.003
4. Rai R, Kaur B, Singh S (2016) Evaluation of secretome of highly efficient lignocellulolytic *Penicillium* sp. Dal 5 isolated from rhizosphere of conifers. Bioresource Technol 216:951–967. https://doi.org/10.1016/j.biortech.2016.06.040
5. Juhász T, Egyházi A, Réczey K (2005) β-Glucosidase production in the rhizosphere of conifers. Bioresource Technol 216:958–967. https://doi.org/10.1016/j.biortech.2016.06.040
6. Adsu M, Ghule J, Shaikh H, Singh R, Bastawde K, Gokhale D, Varma A (2005) Enzymatic hydrolysis of delignified bagasse...
22. Akhtar N, Goyal D, Goyal A (2013) Characterization of microwave-alkali-acid pre-treated rice straw for optimization of ethanol production via simultaneous saccharification and fermentation (SSF). Energ Convers Manage 141:133–144. https://doi.org/10.1016/j.enconman.2016.06.081
23. Jiang LQ, Wu YX, Wang XB, Zheng AQ, Zhao ZL, Li HB, Feng XJ (2019) Crude glycerol pretreatment for selective saccharification of lignocellulose via fast pyrolysis and enzyme hydrolysis. Energ Convers Manage 199:111894. https://doi.org/10.1016/j.enconman.2019.111894
24. Zabed HM, Akter S, Yun J, Zhang G, Awad FN, Qi X, Sahu J (2019) Recent advances in biological pretreatment of microalgae and lignocellulosic biomass for biofuel production. Renew Sust Energ Rev 105:105–128. https://doi.org/10.1016/j.rser.2019.01.048
25. He YC, Xia DQ, Ma CL (2013) Enzymatic saccharification of sugarcane bagasse by N-methylmorpholine-N-oxide-tolerant cellulase from a newly isolated Galactomyces sp. CCZU11-1. Bioresearch Technol 135:18–22. https://doi.org/10.1016/j.biortech.2012.10.156
26. He YC, Liu F, Gong L, Di JH, Ding Y, Ma CL, Zhang DP, Tao ZC, Wang C, Yang B (2016) Enzymatic in situ saccharification of chestnut shell with high ionic liquid-tolerant cellulases from Galactomyces sp. CCZU11-1 in a biocompatible ionic liquid-cellulose media. Bioresearch Technol 201:133–139. https://doi.org/10.1016/j.biortech.2015.11.034
27. Zhang J, Ren X, Chen W, Bao J (2012) Biological pretreatment of corn stover by solid state fermentation of Phanerochaete chrysosporium. Front Chem Sci Eng 6(2):146–151. https://doi.org/10.1007/s11705-012-1220-6
28. Gupta R, Mehta G, Khasa YP, Kuhad RC (2011) Fungal delignification of lignocellulosic biomass improves the saccharification of cellulose. Biodegradation 22(4):797–804. https://doi.org/10.1007/s10532-010-9404-6
29. Zhang S, Xiao J, Wang G, Chen G (2020) Enzymatic hydrolysis of lignin by ligninolytic enzymes and analysis of the hydrolyzed lignin products. Bioresearch Technol 304:122975. https://doi.org/10.1016/j.biortech.2020.122975
30. Martinho V, Dos Santos Lima LM, Barros CA, Ferrari VB, Passarini MRZ, Santos LA, De Souza Sebastianes FL, Lacava PT, De Vasconcellos SP (2019) Enzymatic potential and biosurfactant production by endophytic fungi from mangrove forest in Southeastern Brazil. AMB Express 9(1):1–8. https://doi.org/10.1186/s13568-019-0850-1
31. De Souza Silva CMM, De Melo IS, De Oliveira PR (2005) Ligninolytic enzyme production by Ganoderma spp. Enzyme Microbial Technol 37(3):324–329. https://doi.org/10.1016/j.enzmictec.2004.12.007
32. Khelifi E, Ayed L, Bouallagui H, Touhami Y, Hamdi M (2009) Effect of nitrogen and carbon sources on Indigo and Congo red decolourization by Aspergillus aliuscens strain 121C. J Hazard Mater 163(2–3):1056–1062. https://doi.org/10.1016/j.jhazmat.2008.07.000
33. Rouches E, Herpoël-Gimbert I, Steyer JP, Carrere H (2016) Improvement of anaerobic degradation by white-rot fungi pretreatment of lignocellulosic biomass: a review. Renew Energy 95:179–198. https://doi.org/10.1016/j.renene.2015.12.017
34. Sheng T, Zhou L, Gao LF, Liu WZ, Cui MH, Guo ZC, Ma XD, Ho SH, Wang AJ (2016) Lignocellulosic saccharification by a newly isolated bacterium, Ruminoclostridium thermocellum M3 and cellular cellulase activities for high ratio of glucose to cellobiose. Biotechnol Biofuels 9(1):1–11. https://doi.org/10.1186/s13068-015-0585-z
35. Choi J, Yang C, Fujitsuka M, Tojo S, Ihee H, Majima T (2015) Proton transfer of guanine radical cations studied by time-resolved
resonance Raman spectroscopy combined with pulse radiolysis. J Phys Chem Lett 6(24):5045–5050. https://doi.org/10.1021/acs.jpclett.5b02313

36. Su Y, Yu X, Sun Y, Wang G, Chen H, Chen G (2018) Evaluation of screened lignin-degrading fungi for the biological pretreatment of corn stover. Sci Rep-UK 8(1):1–11. https://doi.org/10.1038/s41598-018-23626-6

37. Krogh KB, Harris PV, Olsen CL, Johansen KS, Hojer-Pedersen J, Borjesson J, Olsson L (2010) Characterization and kinetic analysis of a thermostable GH3 β-glucosidase from Penicillium brasiliannum. Appl Microbiol Biotechnol 86(1):143–154. https://doi.org/10.1007/s00253-009-2181-7

38. Lynd LR, Weimer PJ, Van Zyl WH, Pretorius IS (2002) Microbial cellulose utilization: fundamentals and biotechnology. Microbiol Mol Biol Rev 66(3):506–577. https://doi.org/10.1128/MMBR.66.3.506-577.2002

39. Wen Z, Liao W, Chen S (2005) Production of cellulase/β-glucosidase by the mixed fungi culture Trichoderma reesei and Aspergillus phoenicis on dairy manure. Process Biochem 40(9):3087–3094. https://doi.org/10.1016/j.procbio.2005.03.044

40. Druzhinina IS, Kubicek CP (2017) Genetic engineering of Trichoderma reesei cellulases and their production. Microb Biotechnol 10(6):1485–1499. https://doi.org/10.1111/1751-7915.12726

41. Liu D, Zhang R, Yang X, Zhang Z, Song S, Miao Y, Shen Q (2012) Characterization of a thermostable β-glucosidase from Aspergillus fumigatus Z5, and its functional expression in Pichia pastoris X33. Microb Cell Fact 11(1):1–15. https://doi.org/10.1186/1475-2859-11-25

42. Zhou S, Raouche S, Grisel S, Navarro D, Sigoiollet JC, Herpoël-Gimbert I (2015) Solid-state fermentation in multi-well plates to assess pretreatment efficiency of rot fungi on lignocellulose biomass. Microb Biotechnol 8(6):940–949. https://doi.org/10.1111/1751-7915.12307

43. Shuang Qi T, Ren Yong Z, Zhi Cheng C (2018) Review of the pretreatment and bioconversion of lignocellulosic biomass from wheat straw materials. Renew Sust Energ Rev 91:483–489. https://doi.org/10.1016/j.rser.2018.03.113

44. Wang P, Chang J, Yin Q, Wang E, Zhu Q, Song A, Lu F (2015) Effects of thermo-chemical pretreatment plus microbial fermentation and enzymatic hydrolysis on saccharification and lignocellulose degradation of corn straw. Bioresource Technol 194:165–171. https://doi.org/10.1016/j.biortech.2015.07.012

45. Wei W, Jin Y, Wu S, Yuan Z (2019) Improving corn stover enzymatic saccharification via ferric chloride catalyzed dimethyl sulfoxide pretreatment and various additives. Ind Crop Prod 140:111663. https://doi.org/10.1016/j.indcrop.2019.111663

46. Shi F, Xiang H, Li Y (2015) Combined pretreatment using ozonolysis and ball milling to improve enzymatic saccharification of corn straw. Bioresource Technol 179:444–451. https://doi.org/10.1016/j.biortech.2014.12.063

47. He YC, Ding Y, Xue YF, Yang B, Liu F, Wang C, Zhu ZZ, Qing Q, Wu H, Zhu C (2015) Enhancement of enzymatic saccharification of corn stover with sequential Fenton pretreatment and dilute NaOH extraction. Bioresource Technol 193:324–330. https://doi.org/10.1016/j.biortech.2015.06.088

48. Wang FQ, Xie H, Chen W, Wang ET, Du FG, Song AD (2013) Biological pretreatment of corn stover with ligninolytic enzyme for high efficient enzymatic hydrolysis. Bioresource Technol 144:572–578. https://doi.org/10.1016/j.biortech.2013.07.012

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.