Bioaugmentation of Corn Stalks Saccharification with *Aspergillus Fumigatus* Under Low/High Solid Loading Culture

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Abstract

Decomposed the dense structure of lignocellulosic feedstocks and hydrolysis lignocellulose into monosaccharide were essential prerequisite for bio-energy production at this level. In this study, a cellulosic fungi *Aspergillus fumigatus* CLL was conducted to pretreated the corn stalks under high/low solid loading culture to enhanced the cellulase saccharification performance. The results indicated that *A. fumigatus* CLL decomposed the corn stalks effectively under high/low solid loading culture, what's more, *A. fumigatus* CLL completed the *T. reesei* cellulase system and promoted the corn stalks saccharification performance. 25.2% lignin was degraded after *A. fumigatus* CLL treated just for two day under low solid loading culture with holocellulose loss less than 10%. Meanwhile, the β-glucosidase of *A. fumigatus* CLL complemented the incomplete cellulase system of T. reesei, the maximum saccharification ratio of sample saccharified by *T. reesei* cellulase combined *A. fumigatus* CLL was comparable with the sample saccharified by commercial cellulase. Compared with raw corn stalks, the saccharification ratio of pretreated sample increased 3.1-3.4 fold. These results demonstrated that *A. fumigatus* CLL can be used for pretreatment of lignocellulosic materials to enhanced the saccharification performance.

Introduction

Lignocellulosic biomass is one of the most abundant resources on the Earth [1] which represents a large reservoir of glucose and an attractive renewable energy source which can be a potential feedstock for the biofuels and other high-value chemical products[2]. The utilization of lignocellulosic biomass to produce biofuels will bring significant opportunities for carbon-neutral economy [3] The hydrolysis of lignocellulosic feedstocks into monosaccharides is one of the key steps in the lignocellulosic biofuels production. Lignocellulosic biomass saccharification affected by the structure of lignocellulose feedstocks and the activity of cellulase[4], cellulose and hemicellulose entangled together with lignin formed a rigid matrix in plant biomass which is recalcitrant to cellulase attacks[5], in addition, the holocellulose hydrolysis performed with the synergism of multiple cellulase which increased the saccharification cost and reduced process operability of lignocellulosic biofuels[6].

It has been generally agreed that effectiveness of pre-treatment and saccharification determines the yield of biofuels in the fermentation step. In previous studies, number of excellent physical/chemistry pretreatment methods such as acid or alkali treatment, hot water soak, steam explosion and ammonia fiber expansion have been developed[7]. However, high energy consumption, high equipment requirements, heavy pollution and the production of downstream fermentation inhibitors during physical and chemical pretreatment should not be ignored[8]. On the other hand, the saccharification of lingnocellulose still remains as one of the critical bottle-necks. The enzymatic hydrolysis of cellulosic feedstocks achieved by cellulase cocktail which composed of endoglucanases, exoglucanases and β-glucosidases. Although many fungi have been reported for high-yield cellulase production, *Trichoderma reesei* cellulase is most extensively used in commercial processes, and frequently used in biomass saccharification[8, 9]. The amount of β-glucosidases secreted by *T. reesei* was insufficient for effective cellulose conversion and can be relieved by adding external β-glucosidases[10], but the addition of β-glucosidase increases the cost of saccharification. As a ubiquitous filamentous fungal, *Aspergillus fumigatus* was reported to have a good ability to synthesize cellulase β-glucosidase[11, 12], the high β-glucosidase activity of served it being a potential good supplement for *T. reesei* cellulase in lignocellulose saccharification. Meanwhile, the genus *Aspergillus* sp. has been reported to have the highest degradative capacity for aromatics [13]which suggest with ability of lignin degradation and decomposed the structure of lignocellulose feedstocks which facilitated the lignocellulose saccharification[14].

In this study, *A, fumigatus* CLL was promoted to decompose the structure of corn stalks to enhance the saccharification performance of corn stalks, what's more, explored the feasibility of supplement β-glucosidase of to *T. reesei* cellulase system. The solid loading has a great influence on the lignocellulose saccharification, *Aspergillus fumigatus* CLL was conducted to treat corn stalks under high solid loading (20 g/L) culture and low solid loading (10 g/L) culture. In addition, the saccharification results obtained from *A, fumigatus* CLL cellulase and *T. reesei* cellulase were compared with the sample saccharification by commercial cellulase saccharification in this study.

Method And Materials

Raw materials and inoculum

Corn stalks obtained from the farm of Heilongjiang University, Harbin, Heilongjiang, China. The corn stalks were crushed and sieved through 60 sieves, then dried at 65 °C until weight kept constant for later use. The composition of lignocellulosic feedstocks was determined by ANKOM automatic fiber analyzer (https://www.ankom.com/product-catalog/ankom-200-fiber-analyzer) and shown in Table 1. Lignocellulosic fungi *A. fumigatus* CLL was obtained in the Microbiology Laboratory of Heilongjiang University of Science and Technology. *A.fumigatus* CLL 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 the modified Martin medium and cultured for 2 days. Spore suspension (10^7 spores per mL) was added (2% v w^{-1}, corresponding to 2×10^5 spores g^{-1} feedstock) for high solid loading culture and low solid loading culture.

Table 1
The composition of raw corn stalks

	Cellulose[a]	Hemicellulose[a]	Lignin[a]	Ash and silicate[a]
corn stalks	42.34±3.41%	32.77±2.11%	19.56±5.41%	5.33±1.23%
[a]The mean value of lignocellulose composition				

Trichoderma reesei(DSM 768)was obtained in the Microbiology Laboratory of Heilongjiang University of Science and Technology, and the cellulase of *T. reesei* cellulase was produced and separated according the method described by Zhao[15]. Briefly, *T. reesei* was cultured in the cellulase production medium ($(NH_4)_2SO_4$ 1.4 g/L; urea 0.3 g/L; KH_2PO_4, 2.0 g/L; $MgSO_4•7H_2O$, 0.3 g/L; $CaCl_2$, 0.3 g/L; wheat bran, 20 g/L; soybean cake powder 5 g/L; Avicel, 8 g/L) at 29 ℃ with a speed of 120 rpm. 4 days later, harvested the culture medium by at 8000 rpm for 10 min at 4 ℃, the supernatant was used as the source of cellulases[15]. The commercial cellulase was purchased from Novozymes (1000 U/g) which composed of endoglucanase, exoglucanase, and β-D glucosidase

Corn stalks degrade by under high solid loading culture/low solid loading culture

Low solid loading culture was carried out in the 250 mL Erlenmeyer flasks contained 10 g corn stalks and 100 mL nutrient solution (Peptone 5 g/L; Yeast 2 g/L; $MgSO_4•7H_2O$ 0.5 g/L and KH_2PO_4 1 g/L) at 30 ℃ for 20 days. High solid loading culture was carried out in the 250 mL Erlenmeyer flasks contained 20 g corn stalks and 100 mL nutrient solution (Peptone 5 g/L; Yeast 2 g/L; $MgSO_4•7H_2O$ 0.5 g/L and KH_2PO_4 1 g/L) at 30 ℃ for 60 days. Low solid loading culture/high solid loading culture samples were taken every two/five days to determine the composition of corn stalks, sugar yield, cellulase and lignase activities. Corn stalks without fungus inoculation were conducted as control.

The saccharification of corn stalks

Diluted the commercial cellulase and *T. reesei* cellulase to 0.6-6.0 g/L with citrate buffer (0.05mmol/L, pH4.5) for future corn stalks saccharification. The saccharification of pretreated/untreated corn stalks was performed according the method described by Sheng[16] . Briefly,. mixing the commercial cellulase/ *T. reesei* cellulase with different corn stalks samples (10/1, v/w) at 55°C, the saccharification was carried out for 24 hours and the samples were collected every 3 hours.

Analysis methods

Sugar was performed by HPLC described by Sheng[16]. The structure of corn stalks was determined by JSM6480 scanning electron microscope. The changes in functional groups of corn stalks was determined by Perkin Elmer Spectrum 100 FT-IR Spectrometer. The cellulase activities determined according to the method described by Ghose[17]. The lignase activities were determined according to the method described by Morgan[18]. All tests were replicated three times, and the mean was described with standard deviation.

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

In this formula, W_{sugar} released is sugar production during saccharification (mg), sugar conversion factor is hydrolysis saccharification correction factor (Glucose 0.9, Xylose 0.88) ,total cellulosic fraction is the fraction of cellulose and hemicellulose in corn stalks (mg) [19, 20]. All tests were replicated three times, and the mean was described with standard deviation.

Results And Discussion

Effect of bioaugmentation on the composition of corn stalks

It is believed that lignin is the main obstacle of lignocellulosic biomass saccharification for cellulose and hemicellulose were bundled with lignin[20], while the removal of lignin not only depolymerized the hash structure of the corn stalks but also facilitated the contact of cellulase with holocellulose[21]. It is reported that the high solid loading culture white rote fungi commonly used to decomposed the lignin of lignocellulosic feedstocks, which consumes low energy and environmental friendly[22]. Compared with high solid loading culture, low solid loading improved the biological reaction efficiency and shorten reaction time for more contact of ligninase with lignin[23]. In this study, as shown in Fig. 1(a), in the first 30 days of high solid loading culture, the degradation ratio of cellulose and hemicellulose were similar with lignin. At this time, the lignin degradation ratio was 26.43%, the cellulose and hemicellulose degradation ratio were 28.78% and 25.45%, respectively. 30 days later, the lignin degradation ratio was higher than that of cellulose and hemicellulose. 60 days later, the lignin degradation ratio was 52.22%, while the degradation ratio of cellulose and hemicellulose were 44.78% and 33.86%, respectively. Compared with high solid loading culture, the corn

stalks degradation ratio increased rapidly under low solid loading culture. As shown in Fig. 1(b), 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 ratio of cellulose and hemicellulose was 7.48% and 6.93%, respectively. 20 days later, the lignin degradation ratio reached 50.5%. Meanwhile, hemicellulose and cellulose degradation ratio reached 27.95% and 29% respectively. While under high solid loading culture, the degradation ratio of lignin, cellulose, and hemicellulose were only 18.08%, 20.17%, and 21.41% in 20 days. The results indicate that *A. fumigatus* CLL able to degrade lignin under high/low loading culture effectively, compare with high solid loading culture, less holocellulose consumed under low solid loading culture which is more conducive to subsequent saccharification and utilization for *A. fumigatus* CLL. In previous studies, some lignocellulosic fungi, especially white rot fungi or brown rot fungi, were conducted to pretreat cellulosic feedstocks and some progress were obtained[24, 25]. In contrast, *Aspergillus* sp. has been reported for high β-glucosidase production[26, 27]. More importantly, the product of degrading lignin is fatty acids rather than aromatic monomers[14], was clearly brought about substantial demethoxylation and dehydroxylation, whereas white rot fungi degraded lignin closely resembled undegraded kraft lignin[28], Compared with white rot fungi, enhanced the hydrophobicity typically enables stronger hydrophobic interactions between cellulase and lignin, reduced inhibitory effect of lignin and its derivatives on cellulase[29, 30].

The performance of lignin degradation is closely related to the ligninase. Therefore, the trend of ligninase activity in high/low solid loading culture 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). As shown in Fig. 1(c) and Fig. 1(d). During the low solid loading culture process, the laccase (Lac) activity and increased in the first 10 days and the maximum Lac activity (15.6 U/mL) was obtained at 10 days, 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 at 10 days, 20 days later the activity of Lip was just 1.1 U/mL. Different from Lac and Lip, the peak of Manganese peroxidase (Mnp) activity obtained at 8 days, the maximum Mnp was 16.3 U/mL, 12 days later, the Mnp activity was only 10% of the peak.

Different from the low solid loading culture, the lignase activity of high solid loading culture peaked at 25 days and decreased quickly. Overall, the peak lignase activity of high solid loading culture similar with low solid loading culture, but the peak time for lignase has been doubled, which indicates that low solid loading culture is more conducive for *A. fumigatus* CLL to induce ligninase, and the better lignin degradation performance might be attributed to the increase of the contact of ligninase with corn stalks under low solid loading culture, and speed up the synthesis rate of ligninase. It is reported that the presence of proteins induced a high production of the ligninase [31]. In the low solid loading culture, peptone dissolved in the liquid medium, which increases the contact of the protein with *A. fumigatus* CLL which enhanced the activity of ligninase, what's more, a certain level of readily available carbon sources is necessary to induce and maintain the activities of ligninase[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. On the other hand, It has been suggested that the extracellular glucan plays a role in the degradation of lignin as an indirect source of hydrogen peroxide[33, 34]. Another participation of the extracellular glucan in the fungus metabolism, and in particular in wood degradation is that they function as a supporting network on which some of the excreted ligninase adsorb[35]. They may also contribute in maintaining an optimal pH for ligninase[36].

Effect of bioaugmentation on the structural features of corn stalks

The degradation of lignin not only released holocellulose from the lignin package, at the same time, loosen the structure of lignocellulose raw materials for subsequent use [37]. As shown in Fig. 2(a), the raw corn stalks show a dense layer of lignin structure, 1 day later, some breakage was obtained on the corn stalks surface(b), which suggest that the corn stalks start to degrade. 2 days later, the corn stalks surface has been destroyed more obviously, the cellulose and hemicellulose exposed from lignin while the structure is relatively complete(c).10 days later, as shown in Fig. 2(d), the surface structure of the corn stalks has been completely destroyed.

The FTIR result was shown in Fig. 2(e). The functional groups of corn stalks showed obvious changes during treated by *A. fumigatus* CLL. In the first two days, compared with untreated raw materials, the 1512cm^{-1} band showed obvious absorption, which corresponds to the aromatic skeleton of lignin vibration C=C[16]. In addition, the characteristic peaks near the 1266cm^{-1} waveband appear to be significantly weakened, where it is the C-O bond[38]. Obvious absorption appeared at 2919-2922cm^{-1} and 3400cm^{-1} indicates that non-cellulose species such as lignin were degraded and the holocellulose were exposed and preserved to some extent[39] .

Effect of bioaugmentation on the saccharification of corn stalks

A key factor affecting the efficiency of cellulase hydrolysis was the availability of holocellulose. It is believed that the lignin content closely related to the lignocellulosic feedstocks saccharification performance[40], removal the lignin and loosen the hard structure facilitated the lignocellulosic saccharification, therefore, removing lignin from lignocellulose and destroying the structure of lignin is a crucial step in the saccharification of lignocellulose feedstocks. To evaluate the bioaugmentation on the saccharification of pretreated/unpretreated corn stalks, commercial cellulase and *T. reesei* cellulase, which play a key role in the saccharification process was conducted to hydrolysis corn stalks which treated by for 1 day. As shown in Fig. 3(a), the saccharification ratio increased as the commercial cellulase concentration increased from

0.6 g/L to 4.8 g/L, increased the commercial cellulase to 6.0 g/L, the saccharification was barely increased. The maximum saccharification ratio (42.8%) was obtained as the 4.8g/L commercial cellulase.

The result of samples hydrolysis by *T. reesei* cellulase was shown in Fig. 3(b). The peak saccharification ratio also obtained at *T. reesei* cellulase 4.8g/L (45.6%). It is worth noting that both the peak saccharification ratio of *T. reesei* cellulase and commercial cellulase were both obtained at 21 hours then maintained stability. It is reported that cellulase is more susceptible to end-product inhibition caused by glucose, once glucose is accumulated in the medium in a higher amount, high concentration glucose can either block the active site for the substrate or prevent the hydrolyzed substrate from leaving[41]. Feedback inhibition exhibits inhibiting effect on the cellulase hydrolysis of lignocellulosic biomass.

Previous researchers found that the low β-glucosidase activity of *T. reesei* reduced the efficiency of lignocellulosic hydrolysis[42], and the catalytic efficiency of *T. reesei* cellulase was lower than that of commercial cellulase composed of multiple fungi cellulase cocktail[43, 44]. Most of the cellulase producer filamentous fungi are characterized by low secretion of β-glucosidase which advocates the activity to be insufficient to convert cellobiose (an intermediate product in cellulose hydrolysis) to glucose[45]. The less abundance of β-glucosidase even under conditions of cellulase induction and the product inhibition to which it is susceptible, limits the use of native cellulase preparations in lignocellulosic biomass saccharification [46]. It is worth noting that the saccharification performance of *T. reesei* cellulase was comparable with commercial cellulase in this study, which suggest that external β-glucosidase was added in the *T. reesei* cellulase system. In previous studies, some species of have been reported to have the ability of producing β-glucosidase with high activity [26, 47]. 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.

To verify the inference that *A. fumigatus* CLL completed the cellulase system of *T. reesei*, the cellulase activities of untreated sample saccharified by *T. reesei* cellulase (group I), pretreated sample saccharified by commercial cellulase (group II), and pretreated sample without external cellulase(group III), pretreated sample saccharified by *T. reesei* cellulase (groupIV), were investigated at 5 g/L feedstocks, 55 ℃, 130 rpm for 24 hours, the activities of endo-glucanohydrolase (shorted for EG), exo-glucanohydrolase (shorted for CBH) and β-glucosidase (shorted for BG) were determined every 3 hours. As shown in Table 2, EG, CBH and EB were observed in the untreated sample saccharified by *T. reesei* cellulase, while the peak activity of EG (0.229IU/mL) and CNH (0.216IU/mL) were much higher than that of EG (0.087IU/mL). It is commonly believed that *T. reesei* has poor ability to produce β-glucosidase, the saccharification of lignocellulose feedstocks was accomplished by the synergy of EG, CBH and BG, the lack of BG reduced the hydrolysis efficiency of lignocellulose, since the external β-glucosidase was indispensable for the saccharification by *T. reesei* cellulase. Compared with the untreated sample saccharified by *T. reesei* cellulase, pretreated sample saccharified by *T. reesei* cellulase demonstrated a high BG activity (0.318IU/mL) which similar with the sample saccharified by commercial cellulase (0.242IU/mL for EG, 0.203IU/mL for CBH and 0.287IU/mL for EG), suggest the addition of strain CLL not only enhanced the BG activity, but also completed the cellulase system. Meanwhile, what's important is that the cellulase(EG, CBH and BG) activity obtained a obviously drop after 21 hours. For the untreated sample saccharified by *T. reesei* cellulase, the activities of EG, CBH and BG were about 54.1%, 50.4%, 28.7% of the peak, respectively. For the treated sample saccharified by *T. reesei* cellulase, the activities of EG, CBH and BG were about 61.9%, 60.9%, 58.8% of the peak, respectively, similar results were obtained from the sample saccharide by commercial cellulase which consistent with the results of saccharification ratio

Table 2
The cellulase activity during the corn stalks saccharification.

Time	*T□reesei* cellulase (Group I)			Commercial cellulase(Group II)			*A. fumigatus* CLL (Group III)			*T. reesei* cellulase and *A. fumigatus* CLL (Group IV)		
	EG (IU/mL)	CBH (IU/mL)	BG (IU/mL)	EG (IU/mL)	CBH (IU/mL)	BG (IU/mL)	EG (IU/mL)	CBH (IU/mL)	BG (IU/mL)	EG (IU/mL)	CBH (IU/mL)	BG (IU/mL)
0 h	0.229± 0.016	0.216± 0.0094	0.087± 0.0067	0.242± 0.014	0.203± 0.011	0.287± 0.0087	0.198± 0.011	0.122± 0.013	0.279± 0.019	0.265± 0.01	0.233± 0.0093	0.318± 0.0089
3 h	0.217± 0.0088	0.206± 0.0097	0.083± 0.0059	0.221± 0.0087	0.189± 0.012	0.248± 0.0095	0.191± 0.0189	0.116± 0.010	0.265± 0.021	0.257± 0.0087	0.221± 0.0079	0.307± 0.0086
6 h	0.208± 0.0095	0.197± 0.0078	0.078± 0.0067	0.216± 0.0086	0.174± 0.0098	0.225± 0.012	0.188± 0.0095	0.107± 0.0097	0.257± 0.023	0.246± 0.0096	0.218± 0.011	0.287± 0.01
9 h	0.197± 0.0097	0.182± 0.012	0.083± 0.0051	0.211± 0.0079	0.143± 0.0091	0.203± 0.0086	0.183± 0.016	0.099± 0.0094	0.248± 0.018	0.237± 0.0079	0.201± 0.012	0.249± 0.0096
12 h	0.174± 0.0087	0.156± 0.013	0.079± 0.0045	0.187± 0.011	0.135± 0.0087	0.187± 0.0095	0.174± 0.0096	0.095± 0.0089	0.241± 0.011	0.207± 0.0088	0.176± 0.0094	0.233± 0.01
15 h	0.165± 0.0093	0.121± 0.0084	0.061± 0.0029	0.163± 0.0088	0.113± 0.0089	0.154± 0.0079	0.165± 0.013	0.089± 0.0086	0.235± 0.013	0.186± 0.0079	0.165± 0.012	0.212± 0.0092
18 h	0.147± 0.0087	0.112± 0.0077	0.043± 0.0038	0.155± 0.0076	0.102± 0.0082	0.158± 0.0076	0.153± 0.016	0.076± 0.0077	0.226± 0.014	0.182± 0.0086	0.157± 0.0079	0.201± 0.011
21 h	0.124± 0.0089	0.109± 0.012	0.025± 0.0036	0.127± 0.0097	0.086± 0.0073	0.146± 0.0069	0.147± 0.0094	0.071± 0.0069	0.214± 0.0097	0.164± 0.0082	0.142± 0.0071	0.187± 0.0083
24 h	0.081± 0.0067	0.065± 0.0083	0.013± 0.0025	0.077± 0.0067	0.067± 0.0065	0.104± 0.0056	0.134± 0.013	0.058± 0.0075	0.196± 0.017	0.128± 0.0066	0.101± 0.0056	0.143± 0.0045

The pretreatment of lignocellulose was double-edged for the lignin removal companied with the loss of holocellulose, since appropriate pretreatment time is a key factor to improve the saccharification ratio of the lignocellulosic feedstocks. In this study, the corn stalks degrade by strain CLL under high/low solid loading culture were saccharified by *T. reesei* cellulase/commercial cellulase to investigate the effect of pretreatment duration on the feedstocks saccharification performance. As shown in Fig. 4(a), under low solid loading culture, the saccharification ratio of untreated sample was just 20.18%, with the extension of the pretreatment duration, the saccharification ratio gradually increased and the peaked at 2 d, the maximum saccharification was ratio up to 68.4%. 2 days later, extended the pretreatment time reduced the saccharification ratio of corn stalks, the saccharification ratio of *A. fumigatus* CLL treatment for 10 d and 16 d were just 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 10 d (Fig. 4(b)), extend the duration to 30 d and 50d, the corn stalks saccharification ratio were 49.4% (cultured for 30 d under high solid loading) and 33.1%(cultured for 50 d under high solid loading) respectively. Compared with feed stocks saccharification by *T. reesei* cellulase, the feed stocks saccharification by commercial cellulase demonstrated a similar result suggest that the corn stalks treated by promoted the efficiency of *T. reesei* cellulase, and can be a potential client in the lignocellulose biomass energy refining.

Conclusions

The *A. fumigatus* CLL degrade the lignin of corn stalks and decomposed the structure of corn stalks effectively. At the same time, strain CLL supplements β-glucosidase for the cellulase system of *T. reesei* to improve the saccharification efficiency of cellulase. *A. fumigatus* CLL promoted the corn stalks saccharification under low/high solid loading culture. Compared with high solid loading culture, low solid loading culture was more conducive to corn stalks saccharification. *A. fumigatus* CLL was a potential individual on the lignocellulosic biomass energy refining and feasible to reduce the cost of the saccharification downstream process.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Zhiwei Song, Xuechen Wen and Tao Sheng. 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

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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Figures

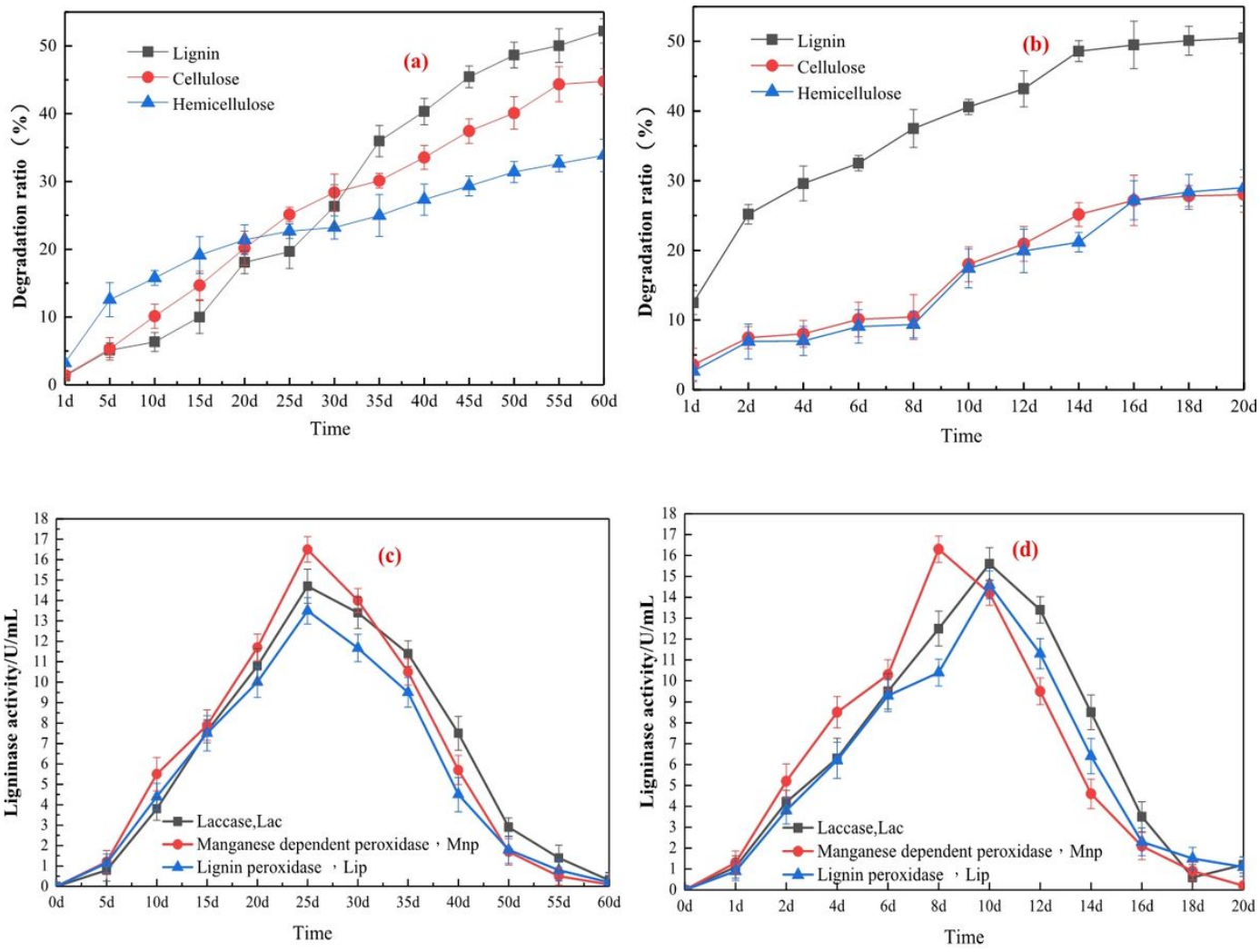

Figure 1

The effect of solid loading on the degradation of corn stalks (a) The composition of corn stalks under high solid content culture (b) The composition of corn stalks under low solid content culture; (c) Ligninase activity under high solid loading culture (d) Ligninase activity under low solid loading culture

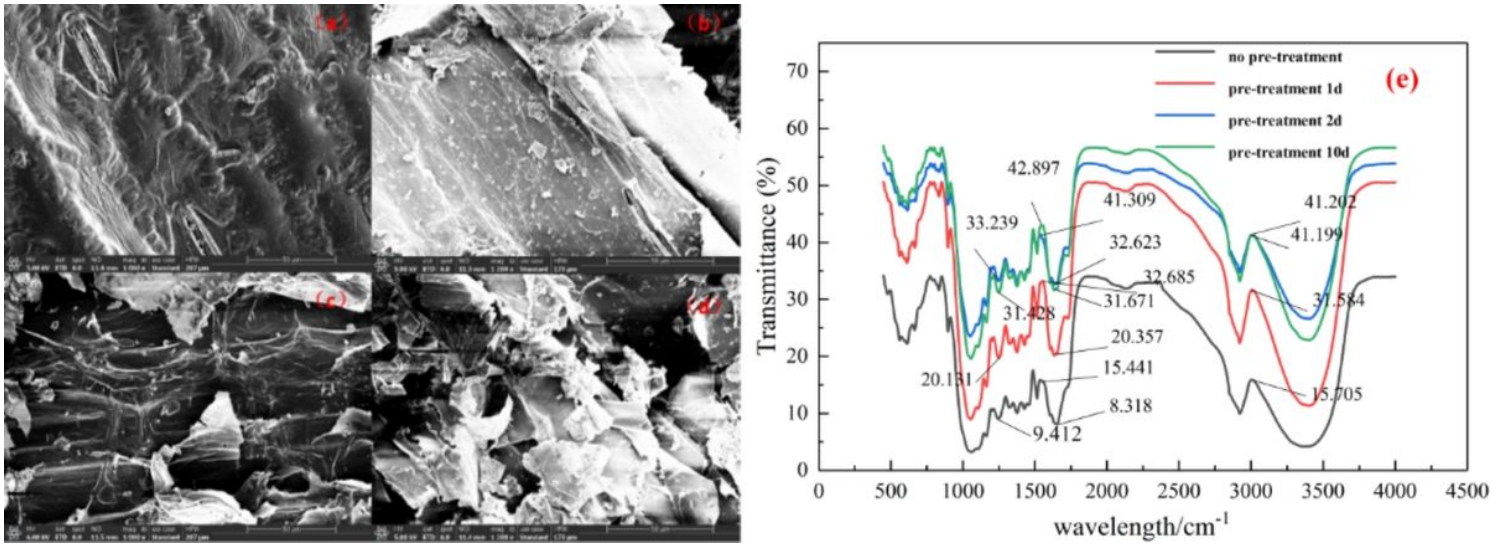

Figure 2

The SEM and FTIR characteristics of corn stalks (a) Raw corn stalks (b) Corn stalks under low solid loading culture pretreated for 1 d (c) Corn stalks under low solid loading culture pretreated for 2 d (d) Corn stalks under low solid loading culture pretreated for 10 d (e) The FTIR spectrum of pretreated/untreated corn stalks

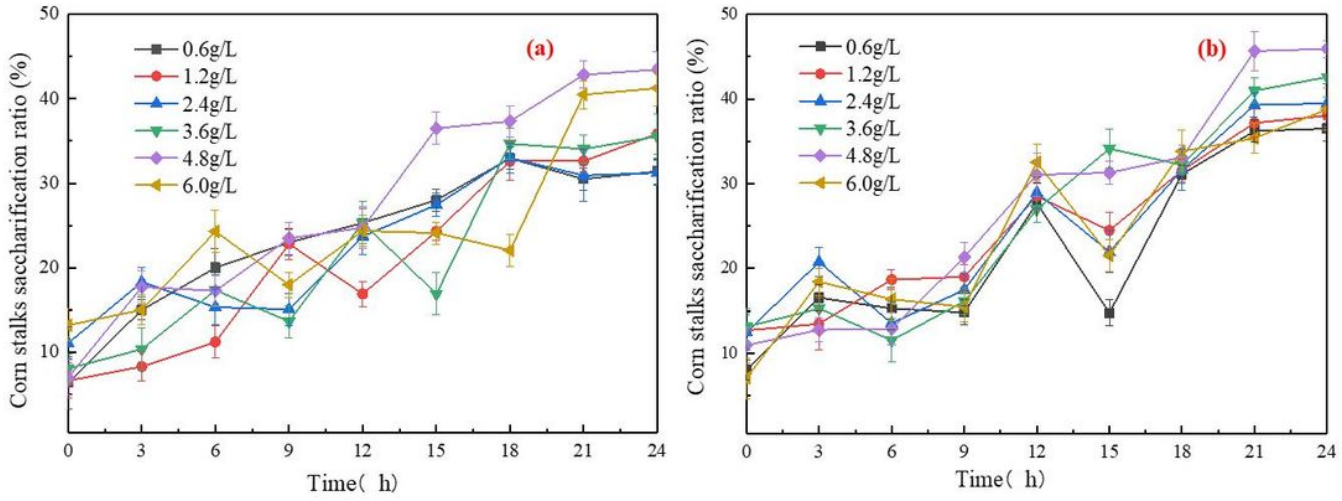

Figure 3

Effect of cellulase concentration on the saccharification of pretreated corn stalks (a) samples saccharified by commercial cellulase (b) samples saccharified by Trichoderma reesei cellulase

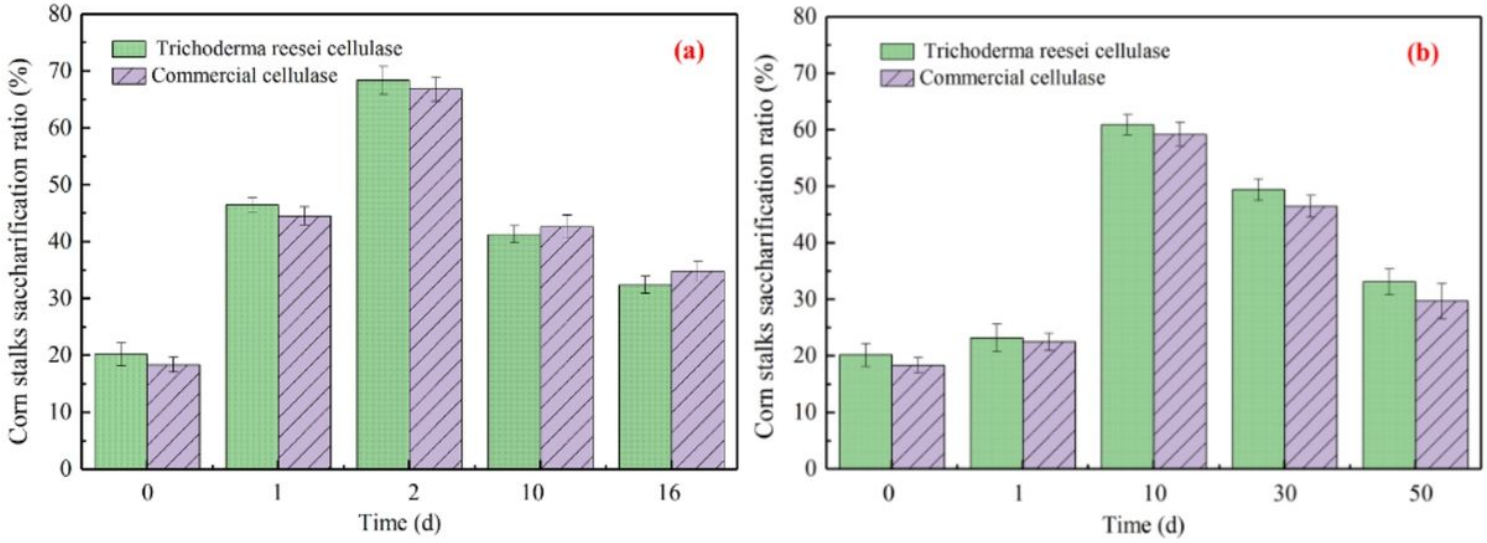

Figure 4

Effect of pretreatment duration on the saccharification of pretreated corn stalks (a) samples under low solid loading culture (b) samples under high solid loading culture