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# High-Efficient Production of Cellulosic Ethanol from Corn Fiber Based on the Suitable C5/C6 Co-Fermentation *Saccharomyces cerevisiae* Strain

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**Abstract:** As a potential alternative to fossil-based fuels, cellulosic ethanol has attracted much attention due to its great benefit to energy sustainability and environmental friendliness. However, at present, the industrial competitiveness of cellulosic ethanol production is still insufficient compared with fossil-based fuels because of the higher costs. Expanding the range of lignocellulosic biomass may be a promising measure to promote the economical production of cellulosic ethanol. Corn fiber, a byproduct from the corn deep-processing, is an attractive feedstock for cellulosic ethanol production because of its rich carbohydrate content (generally exceeding 65% of dry weight), almost no transportation cost, and low lignin content allow it to be easily handled. This study first optimized the hydrolysis conditions, including the pretreatment and enzymolysis process based on dilute sulfuric acid, to achieve a high sugar yield. Then, the corn fiber hydrolysates obtained under different hydrolysis conditions were suitably fermented by different C5/C6 co-fermentation *Saccharomyces cerevisiae*, indicating that the hydrolysate at high solid loading (20%) needs to detoxification to a certain extent but not low solid loading (10%) to achieve high ethanol yield. Finally, the fermentation of the 20% solid loading hydrolysates with resin detoxification was performed in a 50 L bioreactor, achieving the sugar (glucose and xylose) metabolic rate of 2.24 g $L^{-1}$ $h^{-1}$ and ethanol yield of 92% of the theoretical value, which are the highest reported levels to date. This study provided a potential process route for cellulosic ethanol production from corn fiber from the perspective of the suitability between the upstream hydrolysis process and the downstream fermentation strain.

**Keywords:** corn fiber; pretreatment; enzymolysis; detoxification; cellulosic ethanol; C5/C6 co-fermentation *Saccharomyces cerevisiae*



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## 1. Introduction

As the most abundant renewable substrate on earth, the conversion of lignocellulosic biomass into biofuels and chemicals provides a very promising route to withstand the depletion of petroleum reserves. Microbial conversion of lignocellulose is an environmentally friendly mild process with low energy consumption. Generally, lignocellulose is hydrolyzed in hydrolysate containing fermentable sugars by the combination of pretreatment and enzymatic hydrolysis, and then utilized by microorganisms. Pretreatment is an indispensable procedure in the hydrolysis process of lignocellulose, which efficiently destroys the tight lignocellulose structure and improves the accessibility of lignocellulose-degrading enzymes [1]. However, the inhibitors generated from the pretreatment process strongly influence the microbial conversion efficiency of lignocellulose hydrolysate [2]. These inhibitors include aliphatic acids (primarily acetic acid, formic acid, and levulinic acid), furan aldehydes 5-hydroxymethylfurfural (HMF) and furfural, phenolic compounds,

and unidentified compounds [3]. The types and contents of inhibitors depending on raw material sources and pretreatment methods show different inhibiting effects on microorganisms. For example, acetic acid is released from the acetylated hemicellulose component, and phenolic compounds are generated from the lignin component in lignocellulose [4]. Therefore, a feedstock with a higher hemicellulose content (such as crop straws) easily generates more acetic acid, as well as more phenolic compounds with higher lignin content (such as cork woods) [5]. After pretreatment, enzymatic hydrolysis is performed on the pretreated feedstock to further release soluble sugars as much as possible, which consumes large amounts of cellulase and hemicellulase enzymes. The enzymatic hydrolysis efficiency was various because of the different surface area, crystallinity, hemicellulose residue, lignin content, and characteristics of the pretreated solids as a result of different pretreatment conditions [6]. Among them, the exposed lignin inhibits enzymatic hydrolysis mainly by absorbing cellulase or reducing the amount of enzyme available for cellulose hydrolysis [7]. To date, straw biomass as a raw material to produce fuel ethanol has been widely studied due to its abundance [8]. Although a significant improvement in the conversion of straw biomass to fuel ethanol has been achieved, the realization of industrial production may still suffer from the following challenges: (1) the inhibitors produced by the pretreatment process have a great influence on microorganisms; (2) fermentation microorganisms, such as *Saccharomyces cerevisiae*, have a0 weak xylose metabolism in lignocellulosic hydrolysate; (3) a mass of cellulase and hemicellulase will be consumed because of the high lignin content of straw biomass decreasing the efficiency of enzymatic hydrolysis, leading to the high cost. In order to enhance xylose metabolism, numerous effective strategies have been performed, including xylose reductase and xylitol dehydrogenase (XR-XDH) or xylose isomerase (XI) pathway engineering, transporter engineering, cofactor engineering, evolutionary engineering, and other combinational metabolic engineering [9]. Additionally, many strategies are developed to counteract the problems of inhibitors, mainly including a variety of different chemical, biological, and physical methods used to detoxify hydrolysates, and breeding microbial species and strains with high resistance to inhibitors [10,11]. Recently, biological pretreatment has been considered as an eco-friendly, efficient, and economical alternative to the digestibility of lignocellulosic biomass by white, brown, and soft-rot fungi with the generation of fewer inhibitors [12]. However, low hydrolysis rate and long pretreatment time are disadvantages compared with other technologies. Therefore, this pretreatment can be used in combination with other pretreatments for efficient hydrolysis.

Although extensive efforts have been made to reduce the production cost of fuel ethanol using renewable lignocellulose feedstocks, the commercial production of cellulosic ethanol has still been limited by high production costs compared to starch- or sugar-containing feedstocks. Expanding the range of raw materials serves as a promising route beneficial for the commercial production of cellulosic ethanol, particularly low lignin-containing lignocellulose feedstocks showing less inhibition of cellulolytic enzymes to improve the efficiency of enzymatic hydrolysis. Corn fiber, as a residue of the corn processing industry, is mainly composed of cellulose, hemicellulose, and residual starch, representing 60–70% of carbohydrates based on dry weight [13]. Corn fiber is regarded as an attractive lignocellulose feedstock for cellulosic ethanol production due to its rich carbohydrates, abundant reserves, easy collection, and almost no transportation cost. It is reported that if corn fiber could be economically converted to ethanol based on existing infrastructure, it would enable a 13% increase in total corn ethanol production while preserving the protein products as animal feed [14,15]. Until now, some progress on fuel ethanol production from corn fiber has been reported. For example, corn fiber was pretreated by steam explosion at various degrees of severity, showing maximum total sugar (glucose and xylose) yields of 81% [16]. To improve sugar yields, Juneja et al. (2021) developed a two-step pretreatment of corn fiber, including liquid hot water pretreatment (LHW) and disk milling, and found that the yields of glucose, xylose, and arabinose after enzymatic hydrolysis reached 96%, 72%, and 66% under optimal conditions, respectively, indicating that degradation of hemicellulose requires further improvement [17]. Many studies have reported that

dilute acid pretreatment is an effective method for the degradation of hemicellulose, but the monosaccharides could be further degraded to form inhibitors, such as furfural and 5-hydroxymethylfurfural (5-HMF) [18]. To decrease the formation of inhibitors, the pretreatment conditions of corn fiber by dilute acid were optimized by Box–Behnken design, and 82% of total glucose and xylose could be recovered together with a low level of inhibitors. Then, the mixture of hydrolysates of corn fiber were fermented without detoxification, and ethanol yield reached 81% of the theoretical value [13]. However, the production of bulk chemicals is yield-dependent, which implies that it is not reasonable to accept a poor sugar yield, and, consequently, a poor overall product yield, due to the use of insufficient pretreatment conditions.

For corn fiber with high hemicellulose content, dilute acid pretreatment is still a priority approach due to its advantage for improving enzymatic digestibility [19]. Although many pretreatment approaches based on dilute acid were developed for lignocellulose stock with high hemicellulose, the pretreatment and enzymatic processes still need to be optimized to further improve sugar yield while minimizing inhibitor formation [20]. In particular, the suitable *S. cerevisiae* strains with the co-utilization capacity of glucose and xylose are the key to achieving efficient conversion of sugar components of feedstock hydrolysate into ethanol [21]. In this study, the dilute sulfuric acid pretreatment and enzymatic conditions of corn fiber at different solid loadings were optimized to achieve a high sugar yield, and then two strains with the co-utilization capacity of glucose and xylose and tolerance to multiple inhibitors, obtained in our previous work [22,23], were tested for fermentation of the hydrolysate with or without the optimized detoxification method. Finally, the fermentation scale was amplified to 50 L from the shake flask, and the ethanol yield reached approximately 92% of the theoretical yield. This study presented an efficient process route for the production of cellulosic ethanol from corn fiber.

## 2. Materials and Methods

### *2.1. Materials, Cellulase, and Reagents*

Corn fiber was obtained from Shandong Shouguang Juneng Golden Corn Co., Ltd. (Weifang, China). It was ground using a high-speed multi-function grinder (MOLing, Wuyi Haina Electric Co., LTD, Jinhua, China) with a 40-mesh sieve, then stored in a sealed polyethylene bag at −20 °C. The sino cellulases, produced from *Penicillium oxalicum*, were presented by Professor Liu Guodong/Professor Yinbo Qu's research group of the State Key Laboratory of Microbiology Technology, Shandong University. This cellulase shows higher β-glucosidase activity [24]. The Youtell cellulase, produced from *Trichoderma reesei*, was provided by Shandong Shouguang Juneng Golden Corn Development Co., Ltd.

Liquid chromatography standards including glucose, xylose, arabinose, acetic acid, furfural, HMF, and vanillin were purchased from Sigma-Aldrich Co., LLC (St. Louis, MO, USA). Reagent-grade sulfuric acid (98%), calcium oxides, sodium hydroxide, sodium sulfite, sodium thiosulfate, and ethanol were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

### *2.2. Strains and Mediums*

*S. cerevisiae* LF1 and 6M-15 are two engineered strains developed by our laboratory. Strain LF1 is an industrial strain of *S. cerevisiae* with efficient co-utilization of glucose and xylose, which was constructed in our previous work. Strain 6M-15 is a mutant strain derived from LF1 and is suitable for the fermentation of corn stover hydrolysate.

YP medium was configured with 1% (*w*/*v*) yeast powder and 2% (*w*/*v*) peptone and maintained at 115 °C for 30 min. The mother liquor of 40% glucose or xylose was configured separately and also kept at 115 °C for 30 min. After sterilization, YPD/X medium was prepared by mixing YP and glucose or xylose mother liquor. The solid medium was prepared by adding 2% (*w*/*v*) agar into the corresponding medium. For the YP hydrolysate medium, it was added directly to the hydrolysate with 1% (*w*/*v*) yeast powder and 2% (*w*/*v*) peptone without sterilization.

### 2.3. Composition Analysis of Corn Fiber

The water content, cellulose, hemicellulose, lignin, fat, and ash components of the corn fiber raw material were determined and calculated according to the methods at the American Renewable Energy Laboratory (National Renewable Energy Laboratory, NREL). The crude protein content was determined using the Kjeldahl method (KN), and the starch content was determined using the BOXBIO starch content kit. The total releasable amounts of glucose, xylose, and arabinose in the raw material were determined using a two-step acid hydrolysis at NREL.

The content of each component of lignocellulose in raw materials is calculated according to the following formula:

$$Content_{Cellulose}(\%) = C_{Glucose} \times V \times 0.9/m_0 \times 100$$
$$Content_{Hemicellulose}(\%) = \left(C_{Xylose} + C_{Arabinose}\right) \times V \times 0.9/m_0 \times 100$$
$$Content_{lignin}(\%) = (m/m_0) \times 100 + (A \times V \times 3/m_0/30) \times 100$$

where $C_{Glucose}$, $C_{Xylose}$, and $C_{Arabinose}$ are the concentrations of glucose, xylose, and arabinose in the liquid after two-step acidolysis, respectively, g/L; $V$ represents the reaction system, L; $m_0$ is the initial weighing volume, g; 0.9 and 0.88 are the coefficients of conversion of cellulose and hemicellulose to six- and five-carbon sugars; $m$ is the mass of the solid left after drying after acid digestion, g; $A$ is the absorbance value of the acid digestion supernatant at 320 mm; 30 is the absorbance coefficient at the measurement wavelength of 320 nm, L/g·cm.

### 2.4. Pretreatment and Enzymolysis of Corn Fiber

Corn fiber powder without pretreatment was directly enzymatically digested by adding different cellulases with a dosage of 10 or 20 FPU/g dry matter (DM) at 50 °C and pH 4.8. Firstly, $H_2SO_4$ concentration, reaction time and solid loading were optimized at 115 °C. The $H_2SO_4$ concentration was set at 0.25%, 0.5%, 1%, 1.5%, and 2% ($w/v$), pretreatment time was set at 90 min, 120 min, and 150 min, and solid loading was set at 10% and 20% ($w/v$). The above condition ordering factors were performed in 100 mL triangular bottles.

After pretreatment, the triangular bottles were cooled down to room temperature. A part of the pretreated corn fiber sludge was collected to analyze the contents of glucose, xylose, arabinose, furfural, HMF, and acetic acid. The sugar yield was calculated by the following formula:

$$Yield_{Sugar}(\%) = \frac{C_{HPLC} \times V}{m \times Content_{Sugar}} \times 100$$

where $C_{HPLC}$ is the concentration of glucose, xylose, or arabinose in the reaction system, g/L; $V$ is the reaction volume, L; $m$ is the total amount of corn fiber material, g; $Content_{Suger}$ is the theoretically percentage of glucose, xylose, and arabinose in corn fiber, %.

### 2.5. Detoxification Methods

For the overliming detoxification, the pH was adjusted to 10.0 using calcium oxide and left at room temperature for 1 h. The pH was then adjusted to 4.8 with sulfuric acid. For the reducing agent detoxification, the agent was detoxicated by in situ hydrolysis in the hydrolysate. During fermentation, either 5 mM or 10 mM $Na_2SO_3$ were added to the hydrolysate at different time intervals. For the resin detoxification, the 732 strong acid styrene cation exchange resin was activated as follows. The resin was washed with deionized water and then immersed into 1 M hydrochloric acid for 12 h. Next, the resin was treated with 1 M sodium hydroxide for another 12 h. After cleaning the resin again, it was continuedly soaked in 1 M hydrochloric acid for 12 h. Finally, the resin was rinsed until reaching a pH of around 7.0. The activated resin was placed in a column with a diameter of 60 mm and a length of 400 mm with a fill volume of 90%. After shaking and removing bubbles, the flow rate of the hydrolysate was controlled at approximately 6 mL/min. The

hydrolysate referred to the detoxified product, and its pH was adjusted to around 4.8 with CaO.

### *2.6. Oxygen-Limited Shake Flask and Batch Fermentation*

The seed culture was prepared by activating cells twice in the YPX medium for all batch fermentations. The hydrolysate was fermented in 120 mL serum bottles with a 40 mL medium, and 3.5 $OD_{600}$ seeds were inoculated into the reaction system. Fermentation occurred in a constant temperature air bath shaker at 30 °C and 200 rpm. Oxygen-limited conditions in serum bottles were maintained using a rubber stopper with a syringe needle to allow carbon dioxide release. Samples were taken at 6-hour intervals to determine glucose, xylose, and ethanol concentrations.

Batch fermentation was completed in a Bailun 50 L fermenter at a controlled temperature of 30 °C and a rotating speed of 200 rpm. The fermenter was filled with approximately 35 L of hydrolysate with a pH of 4.8, and seeds with an $OD_{600}$ of 3.5 were inoculated into the reaction system. The pH was not adjusted during fermentation. The initial aeration rate was 0.6 L/min to maintain cell rapid growth, which was closed after 6 h until fermentation was finished. Samples were collected every 6 h for $OD_{600}$ measurement and metabolite analysis.

Ethanol yield and the ratio of ethanol yield to theoretical value were calculated using the following formulas:

$$Yield_{Ethanol}(g/g) = C_{Ethanol}/C_{Suger}$$
$$Ratio_{Ethanol}(\%) = Yield_{Ethanol}/0.51 \times 100$$

where $C_{Ethanol}$ represents the ethanol concentration at the end of fermentation, g/L; $C_{Suger}$ represents the glucose and xylose concentration at the beginning of fermentation, g/L; and 0.51 represents the theoretical conversion factor of 1 g of glucose or xylose to ethanol.

### *2.7. Analytical Methods*

The filter paper unit enzyme activity (FPU) was determined using the dinitrosalicylic acid method (DNS). A UV spectrophotometer was used to determine the absorbance of glucose at 540 nm, and a standard curve was drawn. The absorbance of enzymatic hydrolysis within a fixed time was then measured at 540 nm to calculate the glucose concentration based on the standard curve. The enzyme activity of the filter paper was subsequently calculated.

During the hydrolysis of corn fiber and the fermentation of hydrolysate, the metabolites in samples were filtered through a 0.22 μm Millipore filter (Tianjin China) before undergoing high-performance liquid chromatography (HPLC) using a Waters 2414 refractive index detector (Waters e2695, Milford, MA, USA). The concentrations of glucose, xylose, acetic acid, and ethanol were determined using an Aminex HPX-87H ion exchange column (300 × 7.8 mm; Bio-Rad, Hercules, CA, USA) at 35 °C, and a Waters 2414 refractive index detector. Sulfuric acid (5 mM) was used as the mobile phase at a flow rate of 0.6 mL/min. The concentrations of the inhibitors, including HMF and furfural, were determined using a WondaSil C18 column (4.6 × 250 mm, 5 μm; Shimadzu, Kyoto, Japan) at 40 °C, and a PDA-2998 UV detector (Waters) using 40% methanol as the mobile phase with a flow rate of 0.6 mL min−1. Total phenolics were determined using the Folin phenol method as described in a previous report [23].

The dry cell weight (DCW) was measured using a previously described method based on the relationship between $OD_{600}$ and DCW (DCW g $L^{-1}$ = 0.19 × $OD_{600}$ − 0.0065). The linear regression coefficient of the plot of ln($OD_{600}$) vs. time during the exponential growth phase was taken as the maximum growth rate ($\mu_{max}$). The specific consumption or production rates of glucose, xylose, and ethanol were calculated as previously described [21,22].

## 3. Results and Discussions

### *3.1. Corn Fiber Is Considered an Excellent Feedstock for Cellulosic Ethanol Production*

The chemical compositions of corn fiber are shown in Table 1. The raw corn fiber contains 26.34% cellulose, 37.64% hemicellulose, and 17.28% starch with a high carbohydrate content equivalent to 81.26% sugars based on dry matter (*w*/*w*). Corn fiber has higher hemicellulose compared to the main lignocellulose feedstock, such as corn stover, which means that more pentose (mainly xylose and arabinose) will be released in the hydrolytic process of feedstock. Therefore, this requires the fermentative microorganism with a capacity for pentose utilization. Unlike corn stover, corn fiber also contains a considerable percentage of starch aside from cellulose, resulting in additional glucose. Furthermore, the 10.68% crude protein in corn fiber may provide part of the organic nitrogen source for the better growth of fermentative microorganisms. It is important that only 2.06% of lignin is present in corn fiber. The exposed lignin from the pretreatment process can inhibit the efficiency of enzymatic hydrolysis by absorbing cellulase or by reducing the amount of enzyme available for cellulose hydrolysis, and also be degraded into phenolic compounds which can strongly inhibit the growth of microorganisms [25]. Therefore, the lower lignin content is beneficial to the bioconversion of lignocellulose because of alleviating the inhibition of the efficiency of enzymatic hydrolysis and generating the lesser phenolic compounds from lignin. Overall, corn fiber shows a greater potential as an excellent feedstock for cellulosic ethanol production according to the above analysis of chemical compositions of corn fiber.

**Table 1.** The composition of dried raw corn fiber.

| | **Components** | **Composition Contents (%, *w*/*w*)** |
|---|---|---|
| Raw corn fiber | Cellulose | 26.34 ± 1.29 |
| | Hemicellulose | 37.64 ± 1.01 |
| | Starch | 17.28 ± 1.77 |
| | Crude protein | 10.68 ± 0.32 |
| | Lipids | 2.11 ± 0.36 |
| | Lignin | 2.06 ± 0.11 |
| | Ash | 0.19 ± 0.08 |
| | Others | 3.70 ± 0.22 |
| Main sugars of the completely acid-treated corn fiber * | Glucose | 35.62 ± 0.11 |
| | Xylose | 24.15 ± 0.09 |
| | Arabinose | 14.26 ± 0.21 |

* This is a hydrolysate after two-step acid hydrolysis by the U.S. Department of Energy NREL process.

### *3.2. Optimization of Pretreatment and Enzymatic Hydrolysis Conditions of Corn Fiber*

The native lignocellulose biomass is recalcitrant to bioconversion due to its physical and chemical structure barriers. Pretreatment could clear away these barriers and uncover the cellulose amenable to enzymatic hydrolysis. However, pretreatment often involves side reactions resulting in lignocellulose-derived inhibitors that are inhibitory to subsequent biochemical processes. Considering the low lignin content and loose structure of corn fiber, we directly attempt to perform enzymatic hydrolysis of corn fiber. The enzymatic hydrolysis efficiency of two commercial cellulases, produced from *T. reesei* (Youtell cellulase) and *P. oxalicum* (Sino cellulase), respectively, were evaluated. However, the glucose yield was only 54.79% in spite of adding a higher amount of Sino cellulase (20 FPU/g DM) into the reaction system, when a lower yield of glucose was obtained with Youtell cellulase [26]. With either cellulase, the yields of xylose were less than 10% (Figure A1). Corn fiber has a high content of glucuronoarabinoxylan (GAX), a hemicellulosic polysaccharide, consisting of a backbone of 1,4-linked β-xylose residues that are often substituted with arabinose side chains [27]. The GAX of corn fiber has been speculated to be particularly recalcitrant to enzymatic hydrolysis because of its high degree of substitution as well as the variety and complexity of its substituents.

Considering the high hemicellulose content in corn fiber, we preferentially used acid-based methods to pretreat feedstock. The dilute sulfuric acid ($H_2SO_4$) method has been studied for various lignocellulosic biomass. It had a high recovery on hemicellulosic sugars and enhanced enzymatic convertibility on the cellulose fraction. To achieve a high yield of sugars and reduce side reactions to generate inhibitors, we mainly optimized acid concentration, reaction temperature, and pH adjustment reagents before enzymatic hydrolysis under the solid loading of 10%($w/v$). Firstly, the $H_2SO_4$ concentration range of 0.5% to 2% ($w/v$) was investigated for pretreatment at a lower temperature (115 °C) and 120 min according to the literature reports for lignocellulose pretreatment. As shown in Figure 1, two hemicellulosic sugars, xylose and arabinose, were released with a high-level yield. The yields of xylose and arabinose both were higher than 90% at 0.5% and 1% $H_2SO_4$. On the contrary, the yield of glucose was approximately 45% in the pretreatment stage. These results also demonstrate that sulfuric acid enables easily hemicellulose degradation. Additionally, the cellulose fraction along with partial glucose release will be easily hydrolyzed by cellulase. It was found that fewer inhibitors were generated at 0.5% $H_2SO_4$ (Figure A2). Next, we investigated the effects of the different reaction times on the sugar yields using 0.5% $H_2SO_4$. As shown in Table 2, the main inhibitor contents increased with the incremental reaction time. On the basis of the inhibitor tolerance of the *S. cerevisiae* strain in our previous study, 120 min was considered as the suitable reaction time in this study, as it also had a higher yield of sugars.

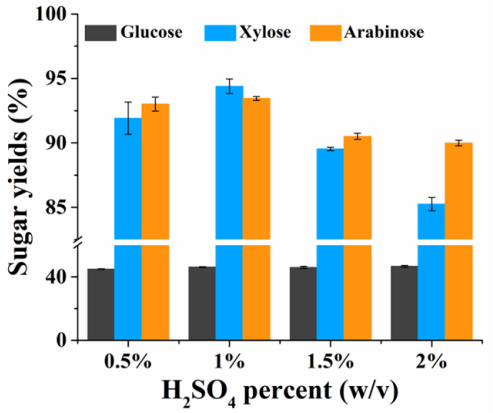


**Figure 1.** Changes in yields of glucose, xylose, and arabinose with different dilute sulfuric acid concentrations under the pretreatment conditions of 115 °C and 120 min. The error bars represent the standard deviation of triplicates.

**Table 2.** Comparison of the main monosaccharide and inhibitor of corn fiber at 0.5% $H_2SO_4$ ($w/v$) at 10% solid loading for different reaction times.

| Components | Contents (g/L) for Different Reaction Times | | |
|---|---|---|---|
| | **90 min** | **120 min** | **150 min** |
| Glucose * | 8.89 ± 0.52 | 13.54 ± 0.21 | 12.03 ± 0.32 |
| Xylose | 18.09 ± 0.64 | 22.20 ± 1.25 | 21.98 ± 0.98 |
| Arabinose | 11.15 ± 0.98 | 12.36 ± 0.54 | 12.27 ± 0.34 |
| Acetic acid | 2.13 ± 0.09 | 2.63 ± 0.21 | 2.72 ± 0.24 |
| Furfural * | 0.45 ± 0.07 | 0.53 ± 0.07 | 0.76 ± 0.04 |
| 5-HMF | 0.06 ± 0.02 | 0.08 ± 0.02 | 0.13 ± 0.03 |

The statistical significance of the difference on glucose contents among different reaction times (* $p < 0.05$).

The pretreated corn fiber under the pretreatment conditions (solid loading of 10%, $H_2SO_4$ concentration of 0.5%, and a reaction time of 120 min) were used for the subsequent enzymatic hydrolysis. *P. oxalicum* cellulase was used for enzymatic hydrolysis due to its higher enzymatic efficiency on the cellulose component (Figure A1). The pH adjustment to the pretreated lignocellulose is a necessary step before enzymatic hydrolysis. Here,

we investigated the effects on enzymatic efficiency using NaOH and CaO as neutralization reagents. The results showed that glucose concentration had a remarkable increase within 24 h in the enzymatic hydrolysis stage, reaching 28.03 g/L from 12.08 g/L in the pretreatment stage (Figure 2A). However, xylose and arabinose concentrations had a slight increase (Figure 2B,C). These results also indicated that glucose release mainly occurred in the enzymatic hydrolysis stage, while xylose and arabinose release mainly occurred in the pretreatment stage. As shown in Figure 2A,D, pH adjustment with CaO slightly facilitated glucose release compared with NaOH, resulting in a 5% increase in glucose yield. Additionally, two pH adjustment methods did not display a positive effect on xylose and arabinose yields, possibly owing to the higher yields of two sugars in the pretreatment stage. It has been reported that CaO had a certain degree of absorption of the unknown inhibitors generated during the pretreatment of lignocellulose, which is beneficial for microbial fermentation. Taken together, CaO was chosen as a pH adjustment reagent for enzymatic hydrolysis of the pretreated corn fiber.

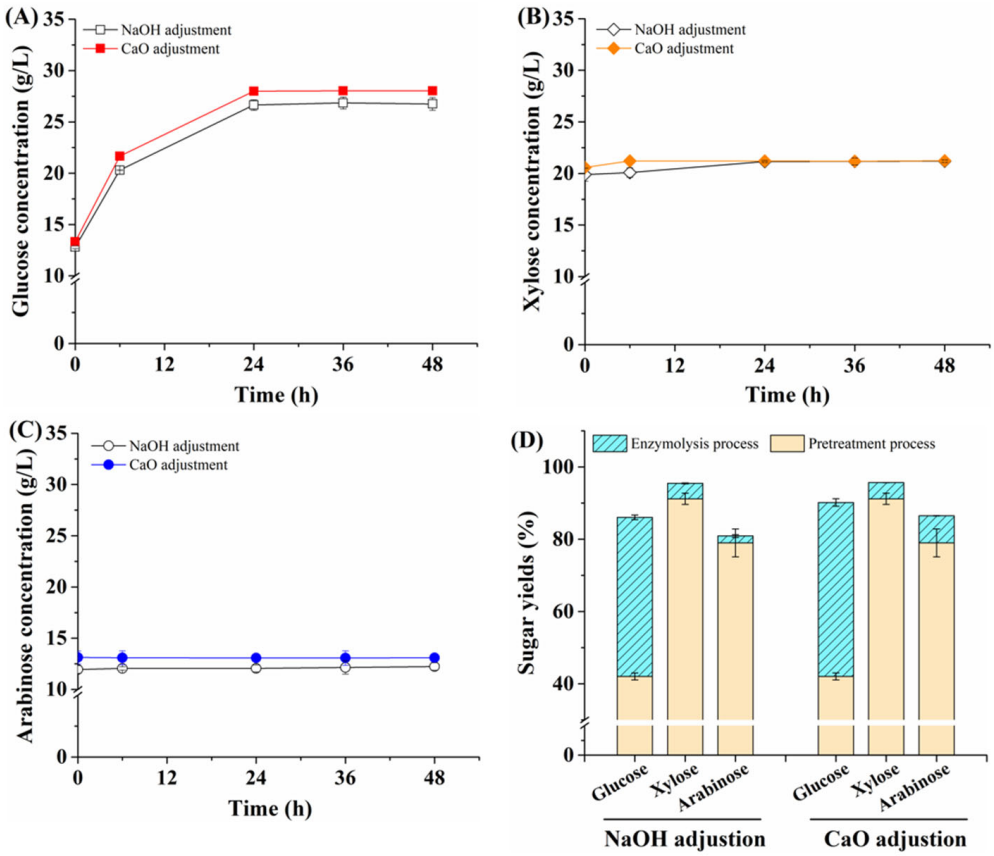


**Figure 2.** Effect on the concentrations (**A**–**C**) and yields (**D**) of glucose, xylose, and arabinose using NaOH or CaO as a pH regulator for the pretreated corn fiber during enzymatic hydrolysis. The error bars represent the standard deviation of triplicates.

### *3.3. Ethanol Production from the Corn Fiber Hydrolysate by the C6/C5 Co-Fermenting S. Cerevisiae*

Corn fiber hydrolysate was prepared by acid pretreatment and enzymatic hydrolysis under the above-mentioned optimized hydrolysis conditions and the solid loading of 10% (*w*/*v*), yielding a main monosaccharide content of 26.64 g/L glucose, 20.02 g/L xylose, and 13.12 g/L arabinose, and a main inhibitor content of 2.65 g/L acetic acid, 0.53 g/L furfural, 0.08 g/L 5-HMF, and 2.01 g/L phenolic compounds. To evaluate the conversion of corn fiber hydrolysate to ethanol, *S. cerevisiae* LF1 and 6M-15 with the capacity of co-fermenting glucose and xylose, as obtained in our previous work, was used for microbial fermentation. The distinction between LF1 and 6M-15 is that the former has a higher xylose metabolism

and lower inhibitor tolerance, and the latter is the opposite [22]. As shown in Figure 3, the glucose was completely exhausted at 12 h; at the same time, 50% and 25% of xylose were only exhausted by LF1 and 6M-15, respectively. Also, the xylose consumption rate of 6M-15 was slower than that of LF1. Finally, 6.23 g/L of xylose was not utilized in 6M-15 compared to LF1, with a residual 2.62 g/L of xylose. Therefore, higher ethanol production was obtained, achieving the highest ethanol yield of 0.46 g/g by LF1 at 36 h. Judging from these results, the consumption characteristics of sugar (glucose and xylose) in corn fiber hydrolysate from a 10% solid loading were consistent with the inherent metabolic characteristics of the two strains. It is speculated that the inherent metabolic capacity of the two strains is a key factor to influence the consumption rate of the hydrolyzed sugars owing to the lower inhibitor content with a lesser inhibition on strain growth under a 10% solid loading. Compared to the YPDX medium, the xylose consumption rate was inhibited to a certain extent in corn fiber hydrolysate, probably due to the presence of multiple inhibitors which showed a minor impact on glucose consumption for two strains [22,23]. These results indicate that LF1 showed better fermentability on corn fiber hydrolysate at a 10% solid loading than 6M-15.

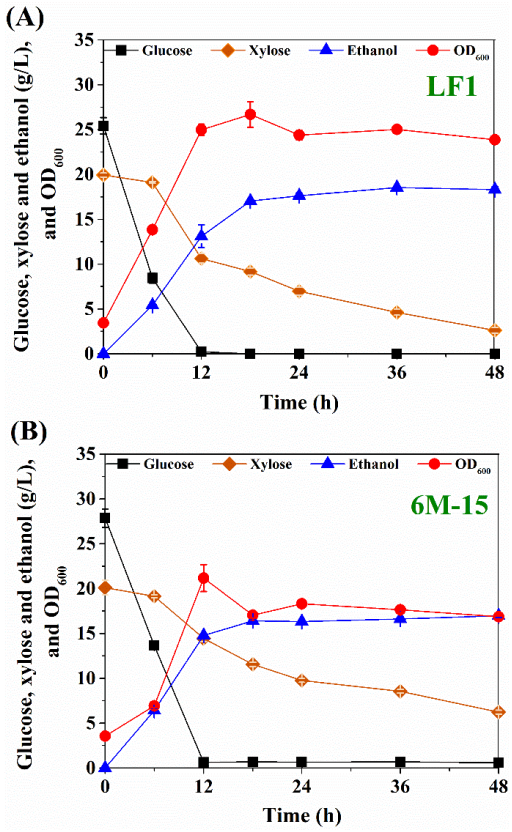


**Figure 3.** Ethanol production from corn fiber hydrolysate by strain LF1 (**A**) or 6M-15 (**B**). The corn fiber hydrolysate was prepared through the pretreatment at 0.5% $H_2SO_4$, 115 °C, and 120 min, and the enzymatic hydrolysis with Sino cellulase at 10% solid loading. The strains were cultivated in oxygen-limited serum bottles with an initial $OD_{600}$ of 3.5 at 200 rpm, 30 °C. The error bars represent the standard deviation of biological triplicates.

### *3.4. Different Detoxification Strategies Employed to Improve Fermentation Efficiency of Corn Fiber Hydrolysate*

Although glucose and xylose in corn fiber hydrolysate at a 10% solid loading were completely consumed by LF1, the xylose consumption rate could not reach the level of that in the YPDX medium. Several alternative measures can be applied to avoid problems caused by inhibitors. There are a variety of different chemical, biological, and physical methods that can be used to detoxify lignocellulosic hydrolysates. In this study, three

user-friendly approaches were employed to alleviate the toxicity of inhibitors to improve xylose consumption rate, including overliming with CaO, resin detoxification, and reasonable addition of $Na_2SO_3$. Among these detoxification methods, the highest ethanol yield (0.48 g $g^{-1}$ consumed sugar at 30 h, approximately 94% of the theoretical yield) was obtained when adding 5 mM $Na_2SO_3$ at 12 h into the fermentation process (Figure 4D). Although the CaO overliming detoxification resulted in a much higher specific xylose consumption rate compared with that of non-detoxification (0.62 g $g^{-1}$ DCW $h^{-1}$ vs. 0.42 g $g^{-1}$ DCW $h^{-1}$, representing a 48% increase), the highest ethanol yield was only 0.37 g $g^{-1}$ consumed sugar (approximately 73% of the theoretical yield) at nearly the end of fermentation (Figure 4B). Under the condition of the resin detoxification, all glucose and xylose were completely consumed within 24 h with the higher sugar consumption rate, and the final ethanol yield reached 0.46 g $g^{-1}$ total glucose and xylose (approximately 90% of the theoretical yield). It is worth mentioning that the fermentation period of corn fiber hydrolysate by resin detoxification was shortened by 1-fold compared with non-detoxification treatment. Among the inhibitory compounds produced from biomass degradation, the inhibitory effect of aldehydes and acids was stronger than that of alcohols [28]. It was reported that a reductant, such as sodium hydrosulfite, sodium sulfite, and so on, enabled the reduction reaction of phenolic aldehydes and phenolic acids to phenolic alcohols to decrease the toxicity of phenolic compounds [29,30]. Our results also demonstrated the positive effect of the reasonable addition of $Na_2SO_3$ on the improvement of fermentation efficiency. Additionally, the overliming of hydrolysates produced by pretreatment of lignocellulose with sulfuric acid results in the precipitation of calcium sulfate which could adsorb toxic compounds, and this is an effective method for detoxification according to some reports. However, opposite results were achieved in this study. Although the sugar consumption rate was increased because of the possible adsorption of toxic compounds by calcium sulfate, the ethanol yield showed an obvious decrease. It is possible that xylose was slightly more easily degraded than the other monosaccharides during alkaline treatment by overliming, or that excess calcium affects the synthesis of ethanol, resulting in the decrease in ethanol yield [31]. At present, the detoxifying mechanism of overliming has not yet been fully elucidated. For the resin detoxification method, the aldehydes and phenols compounds with stronger inhibition are more easily removed than acid compounds through analyzing the changes in inhibitor concentration, resulting in an improvement in fermentation rate (Figure A3) [32]. Furthermore, Zhang et al. recently reported a biodetoxification method using *Paecilomyces variotii* FN89 to improve the ethanol fermentability of corn fiber hydrolysate; however, the treatment period of 18 h will increase the time cost [33]. Taken together, a suitable detoxification method is selected according to the varieties and structural characteristics of lignocellulosic materials, which achieves a satisfactory detoxification effect.

### *3.5. Increasing Solid Loading Leads to Higher Toxicity and Its Counteraction Strategies*

It is well known that the production of bulk chemicals, such as fuel ethanol, is yield-dependent, which implies that it is desirable to seek to achieve a high sugar yield and product yield. To improve the content of fermentable sugars, the solid loading of corn fiber was increased to 20% from 10%, leading to an 82% increase in sugar concentration. At the same time, the main inhibitor content almost doubled, including 4.85 g/L of acetic acid, 0.88 g/L of furfural, 0.67 g/L of 5-HMF, and 3.13 g/L of phenolic compounds (Figure A3). Furthermore, increased solid loading also resulted in a decrease in sugar yield; therefore, the hydrolysis condition may need to be optimized to obtain a high sugar yield at high solid loading in future work. As shown in Figure 5A, in shake-flask fermentation of non-detoxification hydrolysate by LF1, the glucose was completely consumed within 12 h. However, the xylose utilization was obviously inhibited, with a maximum ethanol yield of 0.39 g/g consumed sugars, which was only approximately 77% of the theoretical yield. We speculate that the increased inhibitors affected the strain growth and xylose metabolism due to the increment in solid loading. To further facilitate xylose utilization, we first attempted to add $Na_2SO_3$ to alleviate inhibitor toxicity, which showed a positive effect in

the fermentation of corn fiber hydrolysate with 10% solid loading. However, the results showed a slight improvement in xylose metabolism, indicating that the simple addition of $Na_2SO_3$ is possibly beyond the range of regulation under feedstock pretreatment with high solid loading. Subsequently, the resin detoxification method was applied in 20% solid loading to improve the fermentability of hydrolysate. As shown in Figure 5B, total glucose and xylose were completely consumed within 30 h, and the ethanol yield increased from 0.39 to 0.45 g/g, representing a 15% increase compared with the non-detoxification condition. The final ethanol concentration reached 33.35 g/L with a 63% increase compared with that of 10% solid loading (20.51 g/L), representing 88% of the theoretical yield.

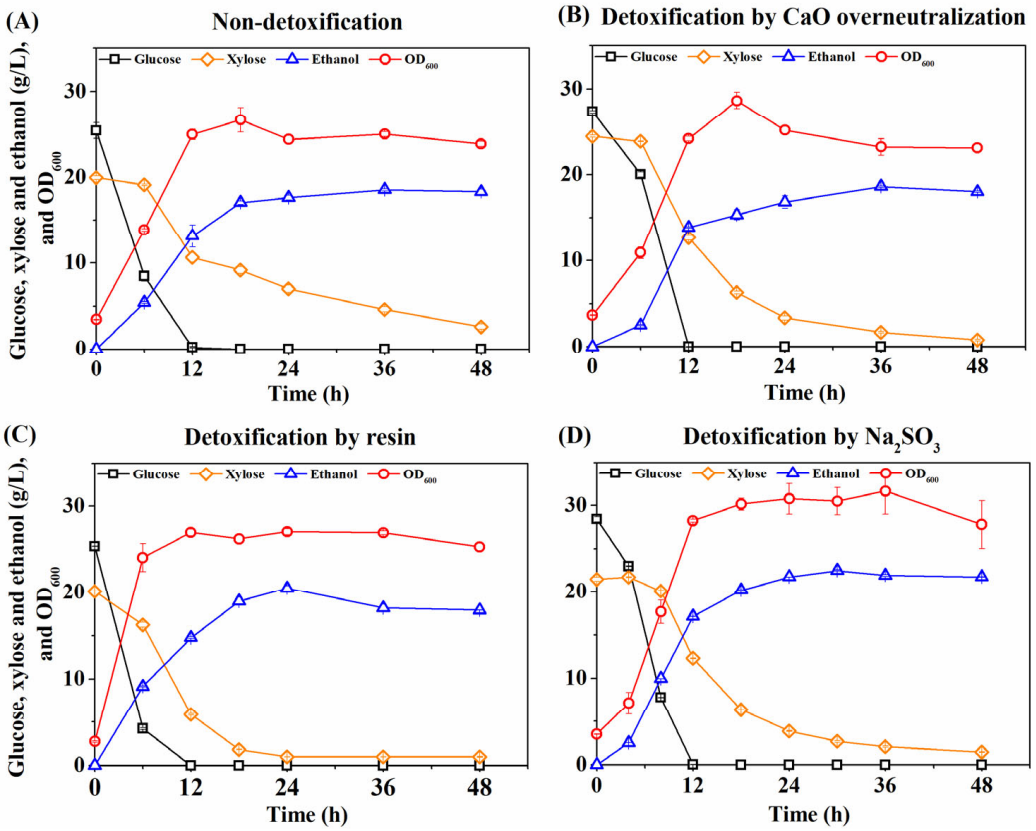


**Figure 4.** Evaluation of the effects on fermentation of corn fiber hydrolysate with different detoxification methods. (**A**) Non-detoxification (control); (**B**) detoxification by CaO over-neutralization; (**C**) detoxification by resin; (**D**) detoxification by adding $Na_2SO_3$ at 12 h. The strains were cultivated in oxygen-limited serum bottles with an initial $OD_{600}$ of 3.5 at 200 rpm, 30 °C. The error bars represent the standard deviation of biological triplicates. The statistical significances of the difference on specific consumption of total glucose and xylose between non-detoxification and detoxification ($p < 0.05$).

Although the detoxification treatments, such as water washing, overliming, resin adsorption, and biodetoxification, could obviously improve fermentation efficiency, these processes usually result in sugar loss and a long production period which will influence the economy of industrial manufacture. Recently, Guo et al. reported a detoxification-free process for enhancing ethanol production from corn fiber using semi-simultaneous saccharification and fermentation, and the final ethanol concentration reached 40.14 g/L in shake-flask fermentation, representing approximately 81% of the theoretical yield [13]. However, the total fermentation time was long (144 h), and the xylose utilization rate was still low. In this study, the fermentation ended at 30 h, and the total (glucose and xylose) sugar consumption rate reached 2.56 g/L/h (Figure 5B). Therefore, whether to adopt a detoxification process may be considered according to overall cost accounting during the industrial production of cellulosic ethanol.

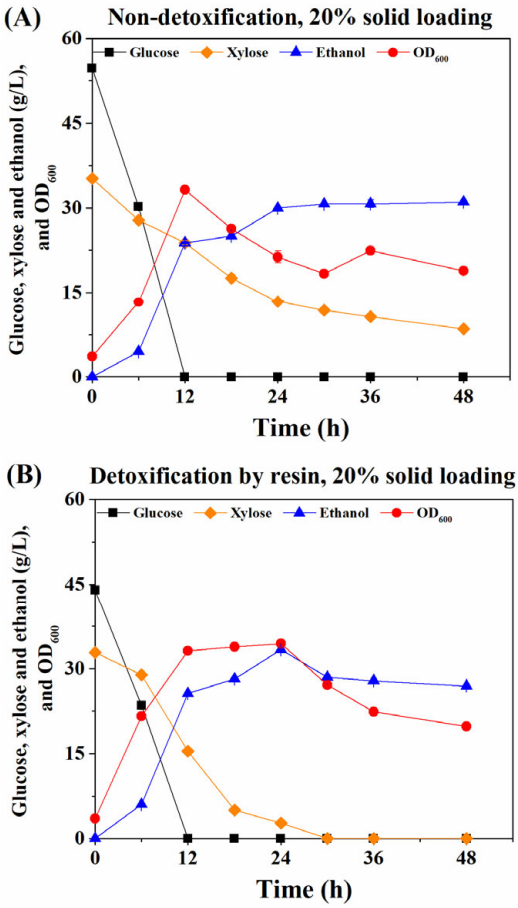


**Figure 5.** Improving the fermentation efficiency of corn fiber hydrolysate with 20% solid loading detoxified by resin. The strains were cultivated in oxygen-limited serum bottles with an initial $OD_{600}$ of 3.5 at 200 rpm, 30 °C. (**A**) shows the fermentation results of hydrolysate without detoxification, and (**B**) shows the fermentation results of hydrolysate after resin detoxification. The error bars represent the standard deviation of biological triplicates. The statistical significances of the difference on specific consumption of total glucose and xylose between non-detoxification and resin detoxification ($p < 0.05$).

### *3.6. 50 L Batch Fermentation of Corn Fiber Hydrolysate*

To further evaluate the industrial production potential of the process route in this study, the 50 L batch fermentation was conducted for the 20% solid loading corn fiber hydrolysate with detoxification treatment in strain LF1. Compared to the shake-flask fermentation, batch fermentation showed a sugar (glucose and xylose) metabolic rate of 2.24 g/L/h and the highest reported ethanol yield of 0.47 g/g fermentable sugars, which was approximately 92% of the theoretical yield, probably due to the better control of the fermentation conditions (Figure 6). The final ethanol titer reached 37.20 g/L, resulting in a 11.54% increase compared with shake-flask fermentation. These results also indicated that the ethanol production route from corn fiber was stable during the magnifying fermentation scale. To our knowledge, 50 L was the highest fermentation scale for cellulosic ethanol production from corn fiber. Additionally, the 50 L fermentation data will provide a technical reference for industrial process magnification of ethanol production from corn fiber. In the future, the fermentation conditions need to be optimized in terms of pH, stirring speed, aeration, and medium nutrition to improve fermentation efficiency. It is also possible to design the fermentation process to avoid problems with inhibition, for example by using simultaneous saccharification and fermentation (SSF) to avoid the inhibition of cellulolytic enzymes by sugars or inhibition of cell growth by inhibitors, or by using fed-batch or continuous cultivation rather than batch cultivation. Other possibilities that target

microorganisms include selection or adaptation of the microbial strains that exhibit strong resistance to inhibitors to remove detoxification steps, which is beneficial to reducing the production cost.

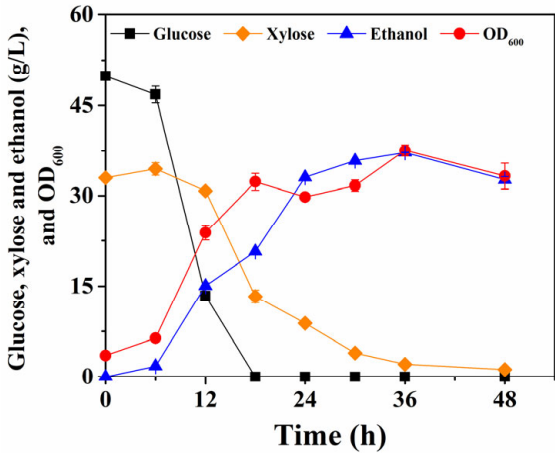


**Figure 6.** 50 L fermentation amplification for corn fiber hydrolysate with 20% solid loading detoxified by resin. Batch fermentation was conducted in a 50 L fermentation reactor with an initial loading volume of 35 L. The starting fermentation conditions were 30 °C, 200 rpm, an initial pH of 4.8, $OD_{600}$ of 3.5, and without pH regulation during fermentation. The aeration was initially 0.6 L/min and was then closed after 6 h until the end of fermentation. The samples were taken at 6 h intervals for $OD_{600}$ measurement and metabolite analysis. The error bars represent the standard deviation of biological duplicates.

## 4. Conclusions

The research findings about cellulosic ethanol production using corn fiber as a feedstock through different process routes are summarized in Table 3. Although the ethanol concentration and yield have gradually improved over the past few years, the productivity and metabolic rate of fermentable sugars remain unsatisfying, which are important factors to reduce the production cost of cellulosic ethanol. In this study, the pretreatment and enzymatic hydrolysis process of corn fiber were optimized to achieve a high sugar yield (93% and 84% at 10% and 20% solid loading, respectively), and the obtained corn fiber hydrolysate (20% solid loading) with resin detoxification was efficiently fermented to produce cellulosic ethanol by a suitable *S. cerevisiae* LF1 at 50 L-scale though process control, resulting in an ethanol yield of 92% of the theoretical value, which is the highest reported level to date. In summary, from the perspective of the suitability between the upstream hydrolysis process and downstream fermentation strain, this study provided a potential process route for cellulosic ethanol production from corn fiber.

**Table 3.** Comparison of cellulosic ethanol production from corn fiber under different pretreatment and enzymatic conditions reported in the literature and in this study.

| Pretreatment Stage | | | | Enzymolysis Stage | | | Fermentation Stage | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Pretreatment Conditions** | **Solid Loading (%)** | **Sugar Concentration (g/L)** | **Sugar Yield (%, *w*/*w*)** | **Dosage of Enzymes (per Gram Dry Matter)** | **Sugar Concentration (g/L)** | **Sugar yield (%, *w*/*w*)** | **Detoxification** | **Sugar Metabolic Rate (g $L^{-1}$ $h^{-1}$)** | **Ethanol Yield (g/g)** | **References** |
| 8% Citric acid (*w*/*w*); 165 °C; 2 min | 25 | Glucose ~5.00, xylose ~12.75 | Glucose 6.40, xylose 68.90 | 10 FPU CTec 2.0 | Glucose 94.10, xylose 30.90 | | Biodetoxification | 1.60 | ~0.44 | [33] |
| Extrusion; melt temperature 140 °C | ~7 | Glucose 10.04, xylose 15.10 | Glucose 12.76, xylose 68.90 | ~5.9 FPU celluclast; 38 CBU β-glucosidase; 1 FBG viscozyme L | | | None | ~0.88 | 0.45 | [34] |
| 0.5% $H_2SO_4$ (*w*/*v*); 105 °C; 43 min | 20 | Glucose 4.60, xylose 3.92 | Glucose 10.5, xylose 38.3 | 10 FPU MCAX | Glucose ~54.00, xylose ~22.00 | Glucose 95.50, xylose 72.40 | None | ~0.70 | 0.41 | [13] |
| 2% NaOH (*w*/*w*); 30 °C; 2 h; | 20 | Glucose ~10.20, xylose ~1.60 | Glucose ~16.41, xylose ~3.61 | ~15 FPU spezyme CP | Glucose ~23.40, xylose ~2.36 | Glucose ~37.64, xylose ~5.32 | Water washing | ~0.18 | ~0.21 | [35] |
| Hot water; 180 °C; 10 min; wet disk milling | 20 | Glucose ~7.00, xylose ~12.00 | Glucose 88.50, xylose 41.00 | 0.05 g cellulase | Glucose ~46.44, xylose ~27.08 | Glucose 94.90, xylose 74.20 | None | | ~0.37 | [17] |
| 0.5% $H_2SO_4$ (*w*/*v*); 115 °C; 120 min | 10 | Glucose 13.54, xylose 20.72 | Glucose 43.56, xylose 93.38 | 10 FPU Youtell | Glucose 28.03, xylose 21.07 | Glucose 90.19 xylose 94.95 | 5 mM $Na_2SO_3$ | 1.57 | 0.48 | This study |
| 0.5% $H_2SO_4$ (*w*/*v*); 115 °C; 120 min | 20 | Glucose 18.15, xylose 35.88 | Glucose 29.20, xylose 80.85 | 10 FPU Youtell | Glucose 52.13, xylose 37.19 | Glucose 83.86, xylose 83.80 | Ion exchange resin | 2.24 | 0.47 | This study |

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**Conflicts of Interest:** The authors declare no conflict of interest with the publication of this paper.

## Appendix A

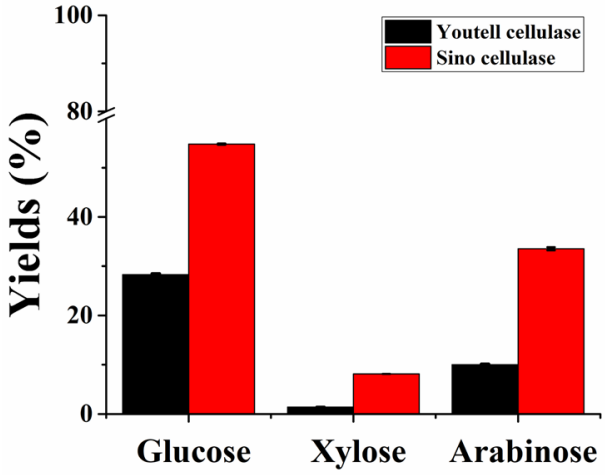


**Figure A1.** The yields of glucose, xylose, and arabinose during direct enzymatic hydrolysis of corn fiber. Enzymatic hydrolysis was performed at 10% (*w*/*v*) solid loading and 50 °C for 72 h using Youtell cellulase or Sino cellulase with a dosage of 10 FPU/g DCW. The error bars represent the standard deviation of triplicates.

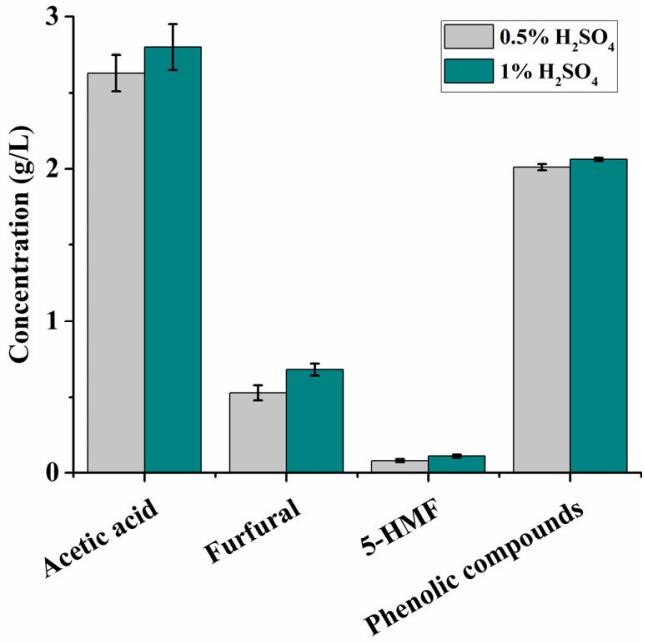


**Figure A2.** The concentrations of acetic acid, furfural, 5-HMF, and phenolic compounds in the corn fiber pretreatment hydrolysate process under 0.5% or 1% (*w*/*v*) $H_2SO_4$. Other pretreatment conditions are 115 °C, 120 min, and 10% solid loading. The error bars represent the standard deviation of triplicates.

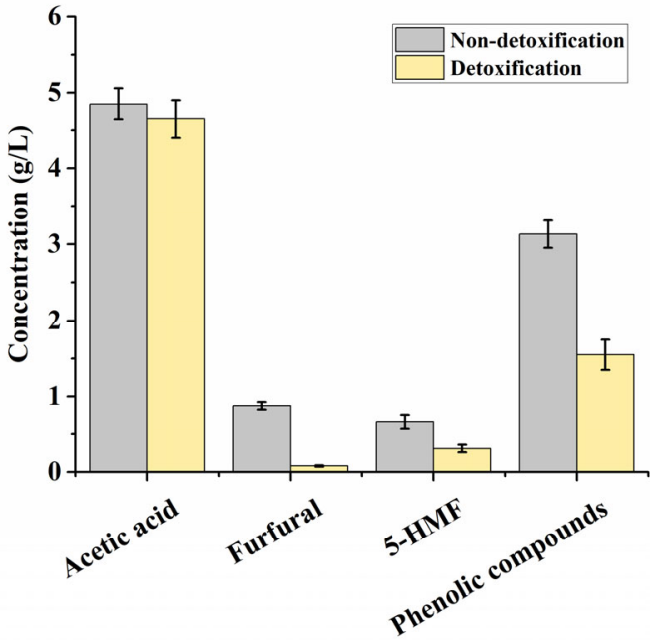


**Figure A3.** Changes in concentrations of acetic acid, furfural, 5-HMF, and phenolic compounds in corn fiber hydrolysate at 20% solid loading through resin detoxification. The error bars represent the standard deviation of triplicates.

## References

1. Yildirim, O.; Ozkaya, B.; Altinbas, M.; Demir, A. Statistical optimization of dilute acid pretreatment of lignocellulosic biomass by response surface methodology to obtain fermentable sugars for bioethanol production. *Int. J. Energy Res.* **2021**, *45*, 8882–8899. [CrossRef]
2. Ujor, V.C.; Okonkwo, C.C. Microbial detoxification of lignocellulosic biomass hydrolysates: Biochemical and molecular aspects, challenges, exploits and future perspectives. *Front. Bioeng. Biotechnol.* **2022**, *10*, 1061667. [CrossRef]

3. Deshavath, N.N.; Mohan, M.; Veeranki, V.D.; Goud, V.V.; Pinnamaneni, S.R.; Benarjee, T. Dilute acid pretreatment of sorghum biomass to maximize the hemicellulose hydrolysis with minimized levels of fermentative inhibitors for bioethanol production. *3 Biotech.* **2017**, *7*, 139. [CrossRef] [PubMed]
4. Bukhari, N.A.; Luthfi, A.A.I.; Rahim, N.A.; Nasrin, A.B.; Sukiran, M.A.; Loh, S. Biomass Deacetylation at Moderate Solid Loading Improves Sugar Recovery and Succinic Acid Production. *Fermentation* **2023**, *9*, 235.
5. Iqbal, Z.; Siddiqua, A.; Anwar, Z.; Munir, M. Valorization of Delonix regia Pods for Bioethanol Production. *Fermentation* **2023**, *9*, 289.
6. Saini, J.K.; Himanshu; Hemansi; Kaur, A.; Mathur, A. Strategies to enhance enzymatic hydrolysis of lignocellulosic biomass for biorefinery applications: A review. *Bioresour. Technol.* **2022**, *360*, 127517. [CrossRef] [PubMed]
7. Cui, P.; Ye, Z.; Chai, M.; Yuan, J.; Xiong, Y.; Yang, H.; Yao, L. Effective fractionation of lignocellulose components and lignin valorization by combination of deep eutectic solvent with ethanol. *Front. Bioeng. Biotechnol.* **2023**, *10*, 1115469. [CrossRef] [PubMed]
8. Li, J.; Chen, Y.; Gao, Y.; Mo, Y.; Long, T.; Yao, B.; Li, Y. Engineering Trichoderma reesei for the hyperproduction of cellulose induced protein 1 (Cip1) on a sophorose-containing inducer to efficiently saccharify alkali-pretreated corn stover. *Prep. Biochem. Biotechnol.* **2022**, *53*, 880–890. [CrossRef]
9. Liu, T.; Huang, S.; Geng, A. Recombinant Diploid Saccharomyces cerevisiae Strain Development for Rapid Glucose and Xylose Co-Fermentation. *Fermentation* **2018**, *4*, 59. [CrossRef]
10. Jönsson, L.J.; Alriksson, B.; Nilvebrant, N.-O. Bioconversion of lignocellulose: Inhibitors and detoxification. *Biotechnol. Biofuels* **2013**, *6*, 16. [CrossRef]
11. Stovicek, V.; Dato, L.; Almqvist, H.; Schöpping, M.; Chekina, K.; Pedersen, L.E.; Koza, A.; Figueira, D.; Tjosås, F.; Ferreira, B.S.; et al. Rational and evolutionary engineering of Saccharomyces cerevisiae for production of dicarboxylic acids from lignocellulosic biomass and exploring genetic mechanisms of the yeast tolerance to the biomass hydrolysate. *Biotechnol. Biofuels Bioprod.* **2022**, *15*, 22. [CrossRef]
12. Anil, K.; Alexander, R.; Gotthard, K.; Sanjeev, K.; Davender, S.; Bijender, S. Multifarious pretreatment strategies for the lignocellulosic substrates for the generation of renewable and sustainable biofuels: A review. *Renew. Energy* **2020**, *160*, 1228–1252.
13. Guo, Y.; Huang, J.; Xu, N.; Jia, H.; Li, X.; Zhao, J.; Qu, Y. A Detoxification-Free Process for Enhanced Ethanol Production from Corn Fiber Under Semi-Simultaneous Saccharification and Fermentation. *Front. Microbiol.* **2022**, *13*, 861918. [CrossRef]
14. Manish, G.; Karen, K.; Michael, R.L.; Robert, H.; Rodney, J.B. Assessment of ethanol production options for corn products. *Bioresour. Technol.* **1996**, *58*, 253–264.
15. Bothast, R.J.; Schlicher, M.A. Biotechnological processes for conversion of corn into ethanol. *Appl. Microbiol. Biotechnol.* **2005**, *67*, 19–25. [CrossRef]
16. Bura, R.; Mansfield, S.D.; Saddler, J.N.; Bothast, R.J. $SO_2$-catalyzed steam explosion of corn fiber for ethanol production. *Appl. Biochem. Biotechnol.* **2002**, *98–100*, 59–72. [CrossRef]
17. Juneja, A.; Noordam, B.; Pel, H.; Basu, R.; Appeldoorn, M.; Singh, V. Optimization of two-stage pretreatment for maximizing ethanol production in 1.5G technology. *Bioresour. Technol.* **2021**, *320*, 124380. [CrossRef] [PubMed]
18. Buruiană, C.T.; Georgescu, L.; Isticioaia, S.F.; Constantin, O.E.; Vizireanu, C.; Dinică, R.M.; Furdui, B. Insights on Monosaccharides and Bioethanol Production from Sweet Sorghum Stalks Using Dilute Acid Pretreatment. *Processes* **2020**, *8*, 1486. [CrossRef]
19. Liu, S.; Yu, Y.; Xu, Z.; Chen, S.; Shen, G.; Yuan, X.; Deng, Q.; Shen, W.; Yang, S.; Zhang, C.; et al. Efficient Corncob Biorefinery for Ethanol Initiated by a Novel Pretreatment of Densifying Lignocellulosic Biomass with Sulfuric Acid. *Fermentation* **2022**, *8*, 661. [CrossRef]
20. Du, J.; Liang, J.; Gao, X.; Liu, G.; Qu, Y. Optimization of an artificial cellulase cocktail for high-solids enzymatic hydrolysis of cellulosic materials with different pretreatment methods. *Bioresour. Technol.* **2020**, *295*, 122272. [CrossRef]
21. Peng, B.; Shen, Y.; Li, X.; Chen, X.; Hou, J.; Bao, X. Improvement of xylose fermentation in respiratory-deficient xylose-fermenting Saccharomyces cerevisiae. *Metab. Eng.* **2011**, *14*, 9–18. [PubMed]
22. Wei, F.; Li, M.; Wang, M.; Li, H.; Li, Z.; Qin, W.; Wei, T.; Zhao, J.; Bao, X. A C6/C5 co-fermenting Saccharomyces cerevisiae strain with the alleviation of antagonism between xylose utilization and robustness. *GCB Bioenergy* **2020**, *13*, 83–97.
23. Li, H.; Shen, Y.U.; Wu, M.; Hou, J.; Jiao, C.; Li, Z.; Liu, X.; Bao, X. Engineering a wild-type diploid Saccharomyces cerevisiaestrain for second-generation bioethanol production. *Bioresour. Bioprocess.* **2016**, *3*, 51. [PubMed]
24. Du, J.; Cao, Y.; Liu, G.; Zhao, J.; Li, X.; Qu, Y. Identifying and overcoming the effect of mass transfer limitation on decreased yield in enzymatic hydrolysis of lignocellulose at high solid concentrations. *Bioresour. Technol.* **2017**, *229*, 88–95.
25. Liu, G.; Qin, Y.; Li, Z.; Qu, Y. Improving lignocellulolytic enzyme production with Penicillium: From strain screening to systems biology. *Biofuels* **2013**, *4*, 523–534.
26. Leif, J.J.; Carlos, M. Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. *Bioresour. Technol.* **2016**, *199*, 103–112.
27. Beri, D.; York, W.S.; Lynd, L.R.; Peña, M.J.; Herring, C.D. Development of a thermophilic coculture for corn fiber conversion to ethanol. *Nat. Commun.* **2020**, *11*, 1937.
28. Cámara, E.; Olsson, L.; Zrimec, J.; Zelezniak, A.; Geijer, C.; Nygård, Y. Data mining of Saccharomyces cerevisiae mutants engineered for increased tolerance towards inhibitors in lignocellulosic hydrolysates. *Biotechnol. Adv.* **2022**, *57*, 107947.

29. Björn, A.; Adnan, C.; Leif, J.J. Improving the fermentability of enzymatic hydrolysates of lignocellulose through chemical in-situ detoxification with reducing agents. *Bioresour. Technol.* **2010**, *102*, 1254–1263.
30. Adnan, C.; Leif, J.J. Detoxification of lignocellulosic hydrolysates using sodium borohydride. *Bioresour. Technol.* **2013**, *136*, 368–376.
31. Nilvebrant, N.-O.; Persson, P.; Reimann, A.; De, S.F.; Gorton, L.; Jönsson, L.J. Limits for alkaline detoxification of dilute-acid lignocellulose hydrolysates. *Appl. Biochem. Biotechnol.* **2003**, *105–108*, 615–628. [CrossRef]
32. Yang, Y.; Lew, P.C. Detoxification of hemicellulose-rich poplar hydrolysate by polymeric resins for improved ethanol fermentability. *Fuel* **2017**, *203*, 187–196.
33. Zhang, B.; Zhan, B.; Bao, J. Reframing biorefinery processing chain of corn fiber for cellulosic ethanol production. *Ind. Crop. Prod.* **2021**, *170*, 113791. [CrossRef]
34. Myat, L.; Ryu, G.-H. Characteristics of destarched corn fiber extrudates for ethanol production. *J. Cereal Sci.* **2014**, *60*, 289–296.
35. Shrestha, P.; Khanal, S.K.; Pometto, A.L., III; Hans van Leeuwen, J. Ethanolproduction via in situ fungal saccharification and fermentation of mild alkali andsteam pretreated corn fiber. *Bioresour. Technol.* **2010**, *101*, 8698–8705. [CrossRef] [PubMed]