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RESEARCH PAPER

# Optimization of Dilute Sulfuric Acid Pretreatment of Corn Stover for Enhanced Xylose Recovery and Xylitol Production

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**Abstract** Pretreatment steps are necessary for the bioconversion of corn stover (CS) to xylitol. In order to optimize the pretreatment parameters, the sulfuric acid concentration, sulfuric acid residence time, and solid slurry concentration were evaluated, based on the glucose and xylose recovered from CS at the relatively low temperature of 120°C. The optimum conditions were found to be pretreatment with 2.5% (w/v) sulfuric acid for 1.5 h, with a solid slurry concentration of 90 g/L. Under these conditions, the hydrolysis rates of glucan and xylan were approximately 26.0 and 82.8%, respectively. High xylitol production (10.9 g/L) and conversion yield (0.97 g/g) were attained from corn stover hydrolysate (CSH) without detoxification and any nutrient addition. Our results were similar for xylitol production in synthetic medium under the same conditions. The non-necessity of both the hydrolysate detoxification step and nutrient addition to the CSH is undoubtedly promising for scale-up application on an industrial scale, because this medium-based manufacturing process is expected to reduce the production cost of xylitol. The present study demonstrates that value-added xylitol could be effectively produced from CS under optimized pretreatment conditions, especially with CSH as the substrate material.

**Keywords:** corn stover, crude glycerol, xylitol, dilute sulfuric acid pretreatment, *Candida tropicalis* XK12K

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## 1. Introduction

Xylitol ($C_5H_{12}O_5$), selected as one of the potential building block chemicals in both the 2004 and 2010 reports of the United States Department of Energy [1], is a natural five-carbon sugar alcohol that has applications in the pharmaceutical, food, and odontological industries owing to its similar high sweetening power, but fewer calories, relative to sucrose [2,3]. However, current commercial xylitol production processes require high temperature (80 ~ 140°C) and high-pressure conditions (up to 50 atm), as well as the toxic nickel catalyst, rendering the chemical process expensive and environmentally unfriendly [4,5]. Global efforts have been made to reduce the high cost of production and concerns about environment pollution. The alternative biotechnological production of xylitol from hemicellulosic hydrolysates and industrial by-products has been proposed, since the process is relatively easy and the cost of production is cheaper than with chemical methods. Currently, corn is one of the most widely cultivated cereal crops in the world, with global production estimated to expand from 31 billion bushels in 2011 to 39 billion bushels in 2022 [6]. Assuming a harvest index of 0.5, about 35 billion bushels are available in the world each year, a trend that has generated a huge surplus of corn stover (CS) [7]. Moreover, global markets of the biodiesel, oleochemical, bioethanol, and soap industries have enlarged. The production

of crude glycerol (CG) as a major by-product of these industries is rising (*e.g.*, 1 kg of CG is produced per 10 kg of biodiesel) [8]. Such enormous amounts of CG and CS have resulted in increased concern about environmental pollution, since these by-products are considered waste materials owing to the saturated market for glycerol and to CS being embedded in a lignin-hemicellulose network [9]. Conversion of these by-products into value-added products like xylitol presents a viable solution not only for the pollution problem but also as an economical option to reduce xylitol production costs.

Many types of microorganisms, including *Escherichia coli*, *Corynebacterium glutamicum*, *Bacillus subtilis*, and *Trichoderma reesei*, have been studied for the biotechnological production of xylitol from several substrates [10-13]. However, yeasts remain the primary organism of focus, producing xylitol from commercial xylose or several hemicellulose hydrolysates such as corn cob [14], cashew apple bagasse [4], sugarcane bagasse [5], and olive tree pruning [15].

Dilute acid pretreatment is an effective method for dissolving hemicellulose to improve the conversion of lignocellulose to xylose, and is normally performed at 180 ~ 200°C [16,17]. However, during those conditions, inhibitors such as furfural, 5-hydroxymethylfurfural (5-HMF), and acetic acid are produced [14,18]. These inhibitors affect the viability of the microorganisms and often result in a low conversion yield and productivity; therefore, a detoxification step is usually required before hemicellulose hydrolysates fermentation [19]. The detoxification processes include applications of physical (evaporation), chemical (over-liming), adsorption (ion-exchange resins and activated charcoal), and biological (microbial and enzymatic) methods [20]. However, all of these lead to increased loss of the sugar, which decreases the efficiency of the fermentation process. A further problem associated with detoxification is the need for additional facilities, which significantly increases the xylitol production cost [2]. In some previous investigations, a relatively low temperature condition, such as 130°C, could reduce sugar degradation and inhibitor formation [21,22].

Despite many efforts to improve the production of value-added chemicals, few studies have investigated the potential of non-detoxified hemicellulose hydrolysates for xylitol production [2,23, 24]. The present study focused on the capability of *Candida tropicalis* to produce xylitol from cheap and renewable sources; namely, non-detoxified corn stover hydrolysate (CSH) and CG, a by-product of biodiesel production. Optimization of the pretreatment parameters (*i.e.*, sulfuric acid concentration and residence time, and solid slurry concentration) was evaluated on the basis of the glucose and xylose recovered from raw CS biomass at a relatively low temperature condition, 120°C. In addition, comparisons of the fermentation characteristic were performed, using CSH and pure glucose and xylose with several concentrations of CG.

## 2. Materials and Methods

### 2.1. Raw materials

Raw CS (*Zea mays* L. var. *ceratina*) was obtained from a local farm in Suwon, Gyeonggi-do, South Korea (longitude 127° 0′ 32″ E; latitude 37° 17′ 28″ N) in July 2014. The climate in this area is of continental type, with average winter and summer temperatures of -5 and 31°C, respectively. Total rainfall is up to 1,541 mm, being concentrated mainly in July. The CS was washed to remove adhering impurities, chopped into 20 ~ 30 cm pieces, and then transported to the laboratory where it was dried to < 5% moisture by weight in a drying oven at 45 ~ 50°C and then ground and sieved to a <1 mm particle size. The milled CS was stored in a refrigerator at 4°C until further use. The raw CS was composed mainly of 30.6% cellulose, 24.7% hemicellulose, and 16.8% lignin on a dry weight basis. The CG was kindly provided by JC Chemical Corporation (Ulsan, South Korea) and was 82 ± 2.7% purity, with a pH (in 10% (w/v) aquatic solution) of 9.8 ± 0.7.

### 2.2. Dilute acid hydrolysis

In order to extract the maximum amount of xylose from the CSH, the milled CS particles were pretreated with 0 ~ 4.5% (w/v) sulfuric acid solutions in an autoclave at 120°C (15 psi) with a residence time of 0 ~ 1.5 h. The solid slurry concentration (30 ~ 150 g/L) was also comprehensively investigated in this work. After acid hydrolysis, the solid residue was separated by centrifugation (10,000 *g*, 30 min) and vacuum filtration (pore size, 0.45 μm). The pH of the CSH was adjusted to 6.0 ± 0.2 with the addition of NaOH, KOH, $NH_4OH$, or $CaCO_3$, and stored at 4°C until further use. The non-detoxified CSH was used directly for fermentation.

### 2.3. Microorganisms and culture conditions

Genetically engineered *C. tropicalis* XK12K, a xylitol-producing yeast strain with the xylulose kinase and xylitol dehydrogenase genes deleted was obtained from the Korea Advanced Institute of Science & Technology [25]. The yeast was grown in 30 mL capped tubes, containing 10 mL of Yeast Malt medium (3 g/L yeast extract, 3 g/L peptone, 3 g/L malt extract, and 20 g/L glucose) on a shaking incubator at 30°C and 200 rpm for 24 h, before being transferred into the fermentation medium (inoculation ratio, 5% (v/v)). The components of fermentation medium

(using pure glucose and xylose) were as follows: 7.5 g/L glucose, 17.5 g/L xylose, 10 g/L yeast extract, 5 g/L $KH_2PO_4$, and 0.2 g/L $MgSO_4$, with an initial pH of 6.0 ± 0.2. The fermentation medium (50 mL) was prepared in 250 mL Erlenmeyer flasks together with the pH-adjusted CSH without any further supplementation of nutrients. CG at 1, 3, 5, and 10 g/L concentration, respectively, was added to the CSH. All fermentation experiments were performed at 30°C and 200 rpm for 36 h, in triplicates.

### 2.4. Analytical methods

The glucan and xylan contents of the raw CS and pretreated CS were analyzed using the method of the Laboratory Analytical Protocol-002, developed by the National Renewable Energy Laboratory (NREL Golden, CO, USA). In briefly, raw CS samples were immersed in 72% (w/w) sulfuric acid and reacted at 30°C and 110 rpm for 2 h. Then, they were diluted by adding distilled water to the reaction bottle and autoclaved at 120°C for 1 h. The liquid samples were centrifuged at 21,500 *g* for 20 min and filtered through a 0.2 μm membrane filter for high-performance liquid chromatography (HPLC; Waters, Milford, MA, USA) analysis.

Organic compounds and sugars, including glucose, xylose, and xylitol were measured using HPLC (600S; Waters) in combination with an autosampler (717; Waters Chromatography Division, Millipore Corp., Milford, MA, USA). An Aminex HPX-87H column (300 mm × 7.8 mm, 9 μm; Bio-Rad Co., Richmond, CA, USA) was used for the separation, and a refractive index detector (410 Differential Refractometer; Waters) was used for the detection. The column was eluted with 0.4 mM sulfuric acid at a flow rate of 0.4 mL/min at 30°C.

### 2.5. Calculation of hydrolysis rate

The glucan and xylan hydrolysis rates (%) were calculated on the basis of their respective contents in the dry weights of untreated and treated CS. The hydrolysis rate of glucan was calculated with equation (1):

$$\text{Glucan hydrolysis rate (\%)} = \left(1-\frac{\text{Glucan in treated solid (g)}}{\text{Glucan in untreated solid (g)}}\right)\times 100$$

That of xylan was calculated with equation (2):

$$\text{Xylan hydrolysis rate (\%)} = \left(1-\frac{\text{Xylan in treated solid (g)}}{\text{Xylan in untreated solid (g)}}\right)\times 100$$

Error bars in the graphs show the standard deviation of triplicate measurements.

## 3. Results and Discussion

### 3.1. Study of sulfuric acid concentrations and residence times for enhancement of sugar recovery

The effective sulfuric acid pretreatment concentration was evaluated from the sugar concentrations and hydrolysis rate of carbohydrates in 30 g/L solid raw CS biomass slurry (Fig. 1). Generally, increasing the acid concentration increased the concentrations of glucose and xylose in the CSH. When the sulfuric acid concentration was increased from 0 to 2.5%, the glucose concentration increased from 1.46 to 2.41 g/L, respectively. Further increases of the sulfuric acid concentration beyond 2.5% resulted in similar glucose concentrations and glucan hydrolysis rates, which showed that these diluted sulfuric acid solutions were unable to hydrolyze the glucan in CS to glucose [26,27]. Xylan hydrolysis was more sensitive to the lower sulfuric acid concentrations than was glucan hydrolysis. When the sulfuric acid concentration was increased from 0 to 2.5%, the xylose concentration increased from 1.26 to 6.09 g/L,

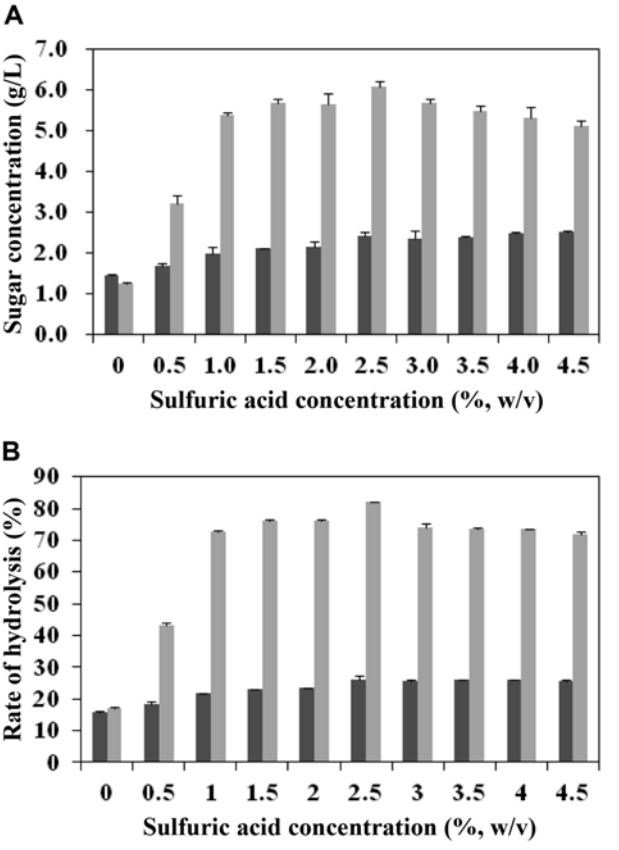


**Fig. 1.** Concentrations of glucose (dark grey bar) and xylose (light grey bar) in corn stover hydrolysate at different sulfuric acid concentrations for 1.5 h (A) and hydrolysis rate of glucan (dark grey bar) and xylan (light grey bar) under the same conditions (B), with a solid slurry concentration of 30 g/L.

respectively (Fig. 1). The easier sulfuric-acid-catalyzed hydrolysis of xylan in CS, compared with that of glucan, was likely due to the higher accessibility and lower degree of polymerization of hemicellulose compared with the solubility of crystalline cellulose [28,29]. The maximum xylose concentration of 6.09 g/L was observed at the sulfuric acid concentration of 2.5%. However, the xylose concentration and xylan hydrolysis rate decreased to 5.12 g/L and 72% at 4.5% sulfuric acid, respectively, which may be due to the degradation of xylose by the severe acid conditions. Overall, these results indicate that higher acidity could have a negative effect on xylose recovery from CS [27].

Based on the sugar concentrations and xylan hydrolysis rate, a 2.5% sulfuric acid concentration was selected as the optimal acid concentration. The sugar concentrations and carbohydrate hydrolysis rates were then estimated after pretreating the CS with 2.5% sulfuric acid for 0, 0.5, 1, and 1.5 h, respectively, at 120°C. With the increase in residence time from 0 to 0.5 h, the xylose concentrations increased from 3.55 to 5.28 g/L, respectively (Fig. 2). The highest concentration of xylose and hydrolysis rate of xylan obtained after a 1.5 h residence time was about two-fold higher than the values obtained from 0 h residence time. The results showed that the acid residence time at the relatively low temperature of 120°C played an important role in improving glucan and xylan hydrolysis from CS into glucose and xylose. We have demonstrated that pretreatment under 2.5% sulfuric acid for a 1.5 h residence time was sufficient for efficient hydrolysis.

### 3.2. Effect of solid slurry concentration and neutralizing agent on sugar recovery

To reduce the processing costs of acid hydrolysis, minimizing the reactor size by increasing the solid load is necessary [30]. Solid slurry concentrations from 30 to 150 g/L of CS, were tested to evaluate the resulting carbohydrate hydrolysis rates and sugar concentrations. At a solid slurry concentration increased from 30 to 90 g/L, the hydrolysis rate of glucan and xylan were approximately 24 ~ 26 and 81 ~ 83%, respectively (Fig. 3A). However, further increase of the solid slurry concentration beyond 90 g/L, decreased the glucan hydrolysis rate slightly, to 21 and 18% at the solid slurry concentrations of 120 and 150 g/L, respectively. A similar trend was observed for xylan, which showed a

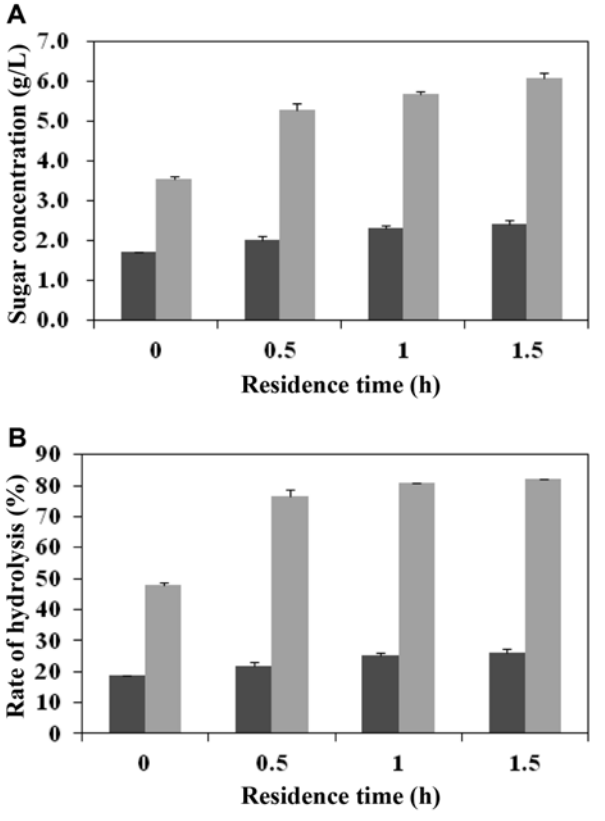


**Fig. 2.** Concentrations of glucose (dark grey bar) and xylose (light grey bar) in corn stover hydrolysate at various residence times (A) and hydrolysis rates of glucan (dark grey bar) and xylan (light grey bar) under the same conditions (B), with a solid slurry concentration of 30 g/L.

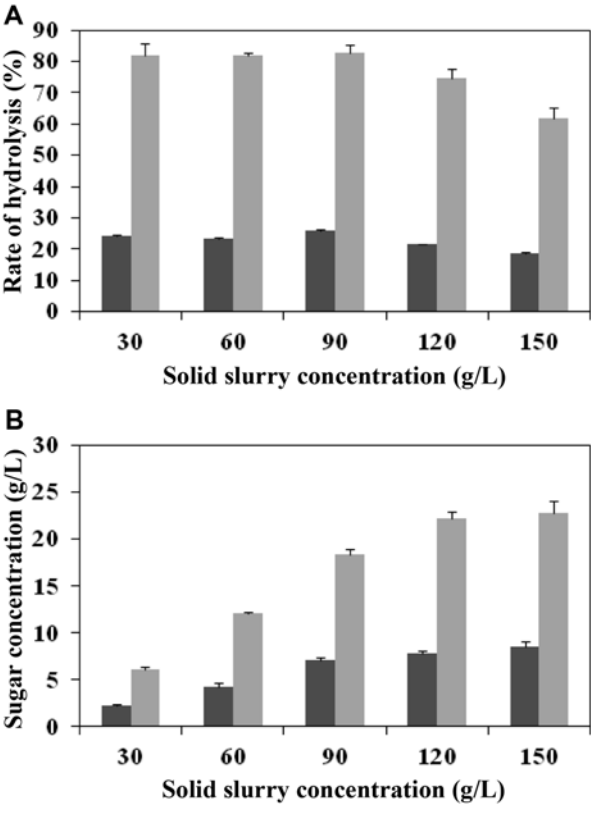


**Fig. 3.** Effect of solid slurry concentration on glucan (dark grey bar) and xylan (light grey bar) hydrolysis (A) and reducing glucose (dark grey bar) and xylose (light grey bar) concentration (B). The corn stover biomass was pretreated at 120°C for 1.5 h with 2.5% (w/v) sulfuric acid.

**Table 1.** Effect of pH neutralization by base agents NaOH, KOH, $NH_4OH$, and $CaCO_3$ on the reducing sugar concentration

| | pH | Sugar concentration (g/L) | | Sugar loss (%) | |
|---|---|---|---|---|---|
| | | Glucose | Xylose | Glucose | Xylose |
| No-treatment | ND | 7.37 ± 0.11 | 18.66 ± 0.47 | - | - |
| NaOH | 4.0 | 6.57 ± 0.06 | 16.87 ± 0.10 | 9.44 ± 0.78 | 11.84 ± 0.50 |
| | 5.0 | 6.33 ± 0.16 | 16.13 ± 0.04 | 12.81 ± 2.22 | 15.73 ± 0.21 |
| | 6.0 | 6.18 ± 0.17 | 15.69 ± 0.03 | 14.88 ± 2.28 | 18.03 ± 0.17 |
| KOH | 4.0 | 6.56 ± 0.07 | 16.49 ± 0.27 | 9.55 ± 0.90 | 13.81 ± 1.43 |
| | 5.0 | 6.29 ± 0.05 | 16.08 ± 0.04 | 13.33 ± 0.66 | 15.96 ± 0.21 |
| | 6.0 | 5.98 ± 0.09 | 15.56 ± 0.19 | 17.61 ± 1.18 | 18.67 ± 0.98 |
| $NH_4OH$ | 4.0 | 6.49 ± 0.02 | 16.52 ± 0.16 | 10.52 ± 0.23 | 13.69 ± 0.84 |
| | 5.0 | 6.23 ± 0.06 | 16.18 ± 0.05 | 14.18 ± 0.83 | 15.47 ± 0.28 |
| | 6.0 | 6.02 ± 0.07 | 15.80 ± 0.06 | 17.00 ± 0.93 | 17.45 ± 0.33 |
| $CaCO_3$ | 4.0 | 7.13 ± 0.02 | 18.90 ± 0.18 | 1.74 ± 0.29 | 1.21 ± 0.96 |
| | 5.0 | 7.11 ± 0.02 | 18.85 ± 0.03 | 2.08 ± 0.28 | 1.50 ± 0.15 |
| | 6.0 | 7.08 ± 0.05 | 18.77 ± 0.22 | 2.37 ± 0.88 | 1.92 ± 1.63 |

sharp decrease in the rate of hydrolysis with increasing solid slurry concentration. These results may be due to the higher solid content (upper 90 g/L) creating a higher viscosity, causing a lower hydrolysis rate by decreasing the amount of acid used per unit weight of CS. Similar results for the effect of solid slurry concentration on hydrolysis have been reported [31-33]. In light of the results obtained in this study (Fig. 3B), the solid slurry concentration of 90 g/L was determined to be optimal for the efficient hydrolysis of glucan and xylan in CS.

After the acid hydrolysis, the pH values of the separated CSHs were increased to target values (pH 4, 5, and 6), using several base agents (NaOH, KOH, $NH_4OH$, and $CaCO_3$) for optimal microbial activity. Table 1 shows that the sugar (glucose and xylose) concentrations decreased after the neutralization process and the loss of sugars increased with increasing pH values. The glucose and xylose yields decreased from 7.37 g/L to 5.98 ~ 6.18 g/L and from 18.66 g/L to 15.56 ~ 15.8 g/L, respectively, at neutralization to pH 6 by all the bases except $CaCO_3$. The neutralization using $CaCO_3$ resulted in a relatively lower loss of glucose and xylose, approximately 1.74 ~ 2.37% and 1.21 ~ 1.92%, respectively. The loss of sugars using the base agents was mostly caused by the decomposition of sugars during the stage in the process where the pH was significantly elevated [21]. Our results demonstrate that $CaCO_3$ was the optimal base reagent for adjusting the solution acidity of the hydrolysate to pH 6.

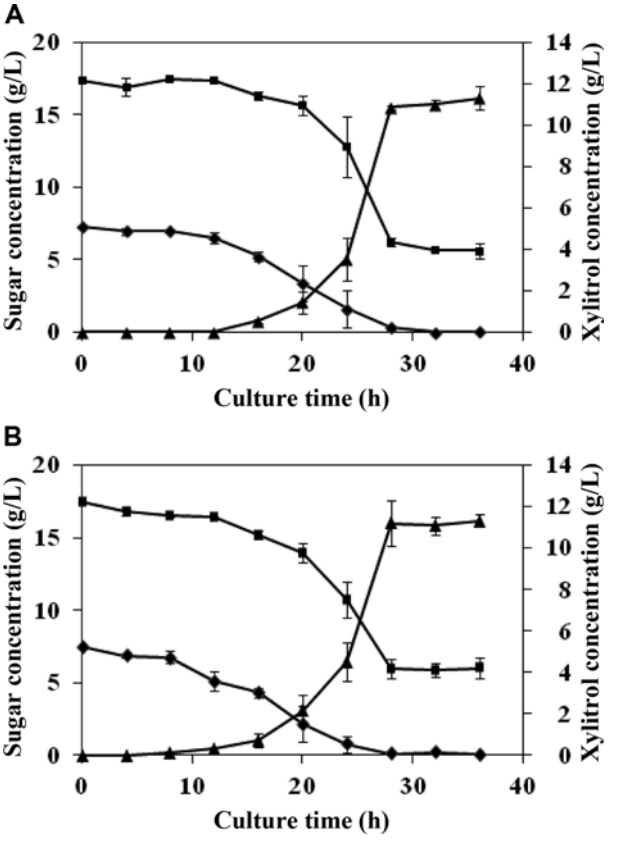


**Fig. 4.** Time-course profile of xylose concentration (closed square), glucose concentration (closed diamond), and xylitol concentration (closed triangle) in corn stover hydrolysate medium (A) and synthetic medium (B) at 30°C for 36 h.

### 3.3. Xylitol production using non-detoxified corn stover hydrolysate

Because *C. tropicalis* XK12K has not been studied for its ability to produce xylitol from any biomass hydrolysate, the bioconversion of xylose to xylitol was carried out at

**Table 2.** Effect of different concentrations of pure glycerol (PG) and crude glycerol (CG) on xylose and glycerol consumption and xylitol yield by *C. tropicalis* XK12K at 28 h

| Carbon sources | Xylose consumption (g/L) | Glycerol consumption (g/L) | Xylitol titer (g/L) | Xylitol yield (g/g) |
|---|---|---|---|---|
| CSH | 11.16 ± 0.46 | - | 10.86 ± 0.10 | 0.97 ± 0.03 |
| CSH + 1 g/L PG | 12.84 ± 0.67 | 0.90 ± 0.06 | 12.12 ± 0.96 | 0.94 ± 0.04 |
| CSH + 3 g/L PG | 14.96 ± 0.20 | 2.00 ± 0.19 | 13.66 ± 0.58 | 0.91 ± 0.04 |
| CSH + 5 g/L PG | 16.63 ± 0.31 | 2.59 ± 0.29 | 15.58 ± 0.60 | 0.94 ± 0.04 |
| CSH + 10 g/L PG | 16.51 ± 0.50 | 5.36 ± 0.77 | 16.11 ± 0.53 | 0.98 ± 0.01 |
| CSH + 1 g/L CG | 13.11 ± 1.05 | 0.93 ± 0.03 | 12.24 ± 1.18 | 0.93 ± 0.03 |
| CSH + 3 g/L CG | 14.66 ± 0.59 | 1.89 ± 0.12 | 14.09 ± 0.28 | 0.96 ± 0.03 |
| CSH + 5 g/L CG | 16.38 ± 1.08 | 2.70 ± 0.06 | 16.06 ± 0.70 | 0.98 ± 0.06 |
| CSH + 10 g/L CG | 16.98 ± 0.43 | 4.74 ± 0.65 | 16.15 ± 0.75 | 0.95 ± 0.07 |

30°C with an initial pH of 6, without detoxification and any nutrient addition. The time-course data (Fig. 4A) showed that *C. tropicalis* XK12K was able to produce xylitol from the fermentable sugars in a non-detoxified hydrolysate. *C. tropicalis* XK12K could convert 11.2 g/L xylose to 10.9 g/L xylitol, which was equivalent to a yield of 0.97 g of xylitol per 1 g of xylose. Experiments with pure glucose and xylose were also performed to compare the behavior of the yeast in a synthetic medium (Fig. 4B). Overall, 11.2 g/L of xylitol was produced from the synthetic medium, with a conversion yield of 0.97 g/g, which was comparable to that with CSH. This indicates that CS is an efficient and economical substrate material for xylitol production, and *C. tropicalis* XK12K can produce xylitol efficiently using CSH without the need for detoxification and any nutrient addition.

### 3.4. Xylitol production using crude glycerol for NADPH regeneration

Because of the lack of NADPH, *C. tropicalis* XK12K stopped growing and xylose metabolism ceased after glucose depletion [34]. Therefore, we added pure glycerol (PG) and CG as the co-substrate to regenerate NADPH during xylitol production from CSH. To determine the most effective glycerol concentration for optimal xylitol production, xylitol fermentation was performed in pH-adjusted CSH with various PG or CG concentrations (1, 3, 5, and 10 g/L) (Table 2). After glucose depletion, glycerol began to be assimilated and xylitol was simultaneously accumulated again. During the 28 h of fermentation, most

**Table 3.** Comparison of xylitol production by various yeast strains reported in the literature

| Microorganisms | Feedstocks/methods of detoxification | Xylitol production | | | References |
|---|---|---|---|---|---|
| | | Concentration (g/L) | Yield (g/g) | Productivity (g/L/h) | |
| *Candida tropicalis* BCRC 20520 | Hard wood hydrolysate /activated charcoal and ion exchange | 32.3 | 0.73 | 0.54 | 38 |
| *Candida magnoliae* FERMP-16522 | Corn cob hydrolysate /activated charcoal | 18.7 | 0.75 | 0.52 | 37 |
| *Candida guilliermondii* FTI 20037 | Sugarcane bagasse hydrolysate /over-liming and activated charcoal | 26.9 | 0.59 | 0.53 | 36 |
| *Candida guilliermondii* FTI 20037 | Eucalyptus hydrolysate /activated charcoal and ion exchange | 32.7 | 0.57 | 0.68 | 39 |
| *Kluyveromyces marxianus* CCA510 | Cashew apple bagasse hydrolysate /activated charcoal | 12.7 | 0.36 | 0.11 | 4 |
| *Pichia stipitis* YS-30 | Corn stover hydrolysate /over-liming | 12.5 | 0.61 | 0.18 | 35 |
| *Debaryomyces hansenii* NRRL Y-7426 | Corn cob hydrolysate /without detoxification | 12.9 | 0.53 | 0.23 | 24 |
| *Candida tropicalis* JH030 | Rice straw hydrolysate /without detoxification | 31.1 | 0.71 | 0.44 | 23 |
| *Candida tropicalis* CCTCC M2012462 | Corn cob hydrolysate /without detoxification | 38.8 | 0.7 | 0.46 | 2 |
| *Candida tropicalis* XK12K | Corn stover hydrolysate /without detoxification | 16.1 | 0.98 | 0.57 | This study |

of the xylose was used, resulting in the production of 15.58 and 16.11 g/L of xylitol from CSH containing 5 and 10 g/L PG, with a yield of 0.94 and 0.98 g/g, respectively. Similar titer, yield, and productivity of xylitol were obtained when replacing PG with CG, demonstrating the feasibility of using CG as a co-substrate for xylitol production by *C. tropicalis* XK12K without the need to pretreat the CG to a pure form.

Previous studies have used various raw material hydrolysates for xylitol production; a comparison of these is provided in Table 3. The hydrolysates investigated were mostly detoxified by over-liming [35,36], activated charcoal [4,36-39], or ion exchange [38,39] to remove inhibitors such as furfural and 5-HMF and to improve the fermentation ability. However, detoxification resulted into 5 ~ 10% sugar losses and increased the process cost. The maximum xylitol concentration and yield of these studies differed widely, from 12.5 to 32.7 g/L and 0.36 to 0.75 g/g, respectively, depending on the removal efficiency of inhibitors and the hydrolysate composition. Furthermore, although many studies have reported on xylitol production from hydrolysates of hard wood, corn cob, corn stover, sugarcane bagasse, eucalyptus, cashew apple bagasse, and rice straw, only a few studies have been reported on production from non-detoxified hydrolysates [2,23,24]. To the best of our knowledge, the 98% yield obtained in our study is the best reported so far among xylitol production processes using non-detoxified hydrolysates. Although the xylitol concentration of 16.11 g/L was similar to or lower than other results, it can be easily improved by fed-batch fermentation using a bioreactor.

## 4. Conclusion

Although many acid pretreatment methods for lignocellulosic biomass have been reported for the production of xylitol, their effectiveness is very much dependent on the substrate. For this reason, pretreatment conditions must be optimized for each substrate used. The pretreatment of CS with dilute sulfuric acid (2.5%) at a relatively low reaction temperature (120°C) is a suitable strategy to maximize xylitol production. Nevertheless, to overcome the lack of NADPH observed in the culture with only CSH, a mixed carbon source of CSH with the addition of CG is suggested for optimal xylitol production. Through fermentation, 16.1 g/L of xylitol was produced from the CSH and CG by *C. tropicalis* XK12K, with a 0.98 g/g conversion yield and 0.57 g/L/h productivity. It is concluded that the production of xylitol from CSH and CG using *C. tropicalis* XK12K is undoubtedly promising for scale-up application to the industrial level, because it does not require any nutrient supplementation and detoxification steps.

## Acknowledgement

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## Nomenclature

CSH : Corn stover hydrolysate
CS : Corn stover
CG : Crude glycerol
PG : Pure glycerol

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