

# One-Pot NaOH/Urea Pretreatment and Saccharification of Corn Stover for Fermentable Sugar Production

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Conversion of lignocellulose into fermentable sugars and other chemicals usually requires multi-step unit operations, such as pretreatment, filtration/washing, and enzymatic saccharification and fermentation, which are the core steps responsible for increased operating expenses. A low-temperature NaOH/urea solution was shown to be an efficient cellulose solvent for overcoming the recalcitrance of lignocellulose by partially or fully converting rigid cellulose I crystallite into the more easily digestible cellulose II structure and by extracting a majority portion of the lignin and xylan from the lignocellulose. Higher yields of fermentable sugars were produced directly from corn stover in one vessel. This one-pot production of fermentable sugars was achieved *via* a combination process, including pretreatment with low-temperature NaOH/urea solution, pH adjustment, and enzymatic saccharification in a single reactor. This one-pot process liberated 86.3% of glucose and 71.3% of xylose in 24 h at an enzyme loading of 10 FPU/g and solid loading of 5%. Surfactant addition further enhanced enzymatic saccharification. The combination of low-temperature NaOH/urea pretreatment and enzymatic saccharification into a one-pot process is an efficient method for the conversion of lignocellulose into fermentable sugars suitable for conversion into fuels and other chemicals. Further studies related to lignin recovery and economical evaluation will be conducted.

*Keywords:* Corn stover; Low temperature NaOH/urea pretreatment; Enzymatic saccharification

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## INTRODUCTION

Conversion of lignocellulose to biofuel usually requires multi-step unit operations, *i.e.*, pretreatment and enzymatic saccharification and fermentation in different reactors under different conditions (Shi *et al.* 2013; Jung *et al.* 2014; Xu *et al.* 2016). The key advantage of multi-step unit operations is that the steps can be optimized independently. Cost-intensive conversion unit operations make the overall strategies less economical, which is one of the major impediments for economic production of fermentable sugars from lignocellulosic biomass. In order to increase efficiency and minimize operating costs for biofuel production, process intensification methods that combine multiple operations into fewer devices or into a single apparatus, such as simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF), non-isothermal simultaneous saccharification and fermentation (NSSF), and consolidated

biomass processing (CBP), have been developed and optimized (Cardona and Sánchez 2007; Ojeda *et al.* 2011). Comparative studies with conventional multi-step unit operation processes (*i.e.*, separate hydrolysis and fermentation, SHF) were also conducted. The main advantages of process intensification methods such as SSF, SSCF, and CBP include: (1) lower inhibitory effects of monosaccharides and oligosaccharides on enzyme activity, substantially lowering enzyme requirements; (2) higher conversion rates of polysaccharide to fermentable sugars or other target products; (3) shorter processing time, lower water consumption, and lower reactor volume (Charpentier 2005). However, despite so many advantages over multi-step unit operation processes, process intensification methods also face some challenges (Xu *et al.* 2016).

The major challenge of process intensification lies in determining compatible conditions for each unit operation with high product yields (Shi *et al.* 2013; Xu *et al.* 2016). Both SSF and SSCF are generally conducted under conditions that are not optimized for either enzymatic activity or yeast fermentation. Instead, SSF and SSCF take advantage of the applicable conditions for saccharification and fermentation. By converting the freshly hydrolyzed monosaccharides into ethanol, yeast fermentation promotes enzymatic saccharification by reducing the product inhibition effect. Non-isothermal simultaneous saccharification and fermentation is a modified SSF or SSCF process, which uses separate enzymatic and fermentation reactors connected by recirculation of a cell-free stream. Although the complexity of the process and cost were increased to a certain degree, NSSF solved the problem of reaction condition mismatch.

Consolidated biomass processing is an alternative process that utilizes microorganisms to ferment biomass into fuels in one step, without added enzymes (Wargacki *et al.* 2012). The largest obstacle for CBP is the lack of a robust organism with high yield and high titer fuel production from biomass. All of these process intensification methods improve biofuel production to some degree. A recent study showed the feasibility of using process intensification methods for pretreatment and saccharification in ionic liquid, with a self-developed thermophilic and ionic-liquid-tolerant “JTherm” cellulase cocktail (Shi *et al.* 2013).

A low-temperature NaOH/urea solution, as an effective cellulose solution, can disrupt the crystalline structure of cellulose and remove a large amount of lignin and hemicellulose during lignocellulose pretreatment (Wang *et al.* 2015, 2016). No cellulose degradation products, such as furfural and hydroxymethylfurfural are produced under such low temperature. In this study, one-pot, low-temperature NaOH/urea pretreatment and saccharification of corn stover was developed. The pretreatment slurry was directly hydrolyzed using an enzyme cocktail after the pH adjustment. The objectives of this study were to test the concept of one-pot NaOH/urea pretreatment and saccharification of corn stover, and to investigate the effect of substrate loading, enzyme loading, and surfactant supplementation on sugar yield.

## EXPERIMENTAL

### Materials and Chemicals

Corn stover, obtained from a local farmer, was air-dried and manually chopped into 1- to 2-cm lengths and stored in a sealed plastic bag at room temperature. A commercial cellulase complex, Cellic CTec2, was obtained from Novozymes North America

(Franklinton, NC). Sodium hydroxide, urea, and other chemicals were all purchased from a commercial source.

## Methods

### *One-pot pretreatment and saccharification by NaOH/Urea*

Size-reduced corn stover was mixed with a NaOH/urea solution (NaOH: urea: H<sub>2</sub>O 7:12:81 by weight) at a liquid-to-solid ratio of 5. The mixtures were stored in a freezer at -20 °C for 12 h. The suspension was then mixed at 200 rpm, and acetic acid was used to adjust the suspension to a pH of 4.8. Different amounts of acetic acid (sodium) buffer (pH=4.8) were added to the suspensions to ensure that the substrate concentration is diluted to 8%, 5%, and 2% during enzymatic saccharification, respectively. Enzymatic saccharification of the substrates was carried out at various substrate loadings (8%, 5%, and 2%), enzyme loadings (15, 10, and 5 FPU/g), and surfactant concentrations (Tween 20 at 0.5%, 0.2%, and 0.1%) in a shaker at 200 rpm and 50 °C. Separate pretreatment and saccharification was also carried out for comparison. After neutralization, the resulting pretreatment slurry was filtered, washed, and hydrolyzed.

### *Analytical methods*

Sugar and its degradation products were measured according to NREL protocols (Sluiter *et al.* 2011), using a Shimadzu HPLC system (Kyoto, Japan) equipped with a Biorad Aminex HPX-87H sugar column (Hercules, CA), a refractive index detector (RID10A), and a UV detector (SPD-20A). The column was operated at 50 °C with 5 mmol/L H<sub>2</sub>SO<sub>4</sub> at a flow rate of 0.4 mL/min.

## RESULTS AND DISCUSSION

### Substrate Loadings

After pretreatment, the pH of the slurry was adjusted to 4.8 using acetic acid and water to obtain a final substrate loading of either 8%, 5%, or 2% (w/v). No sugar degradation products such as furfural and hydroxymethylfurfural are detected in the pretreatment liquid due to low temperature.

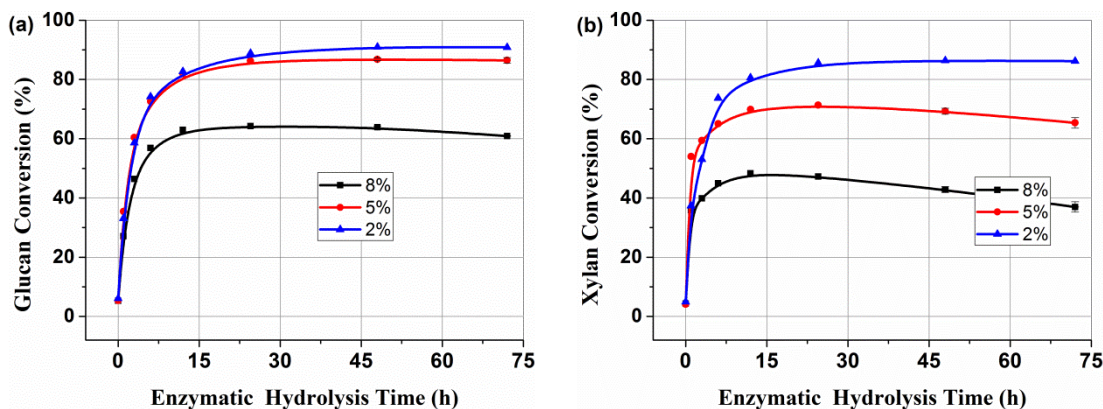


Fig. 1. Glucan and xylan conversions with various substrate loadings

The enzyme cocktail was then applied directly to the diluted pretreatment slurry at an enzyme loading of 10 FPU per g of starting biomass. The fermentable sugar production from corn stover was investigated during the enzymatic saccharification, and the results are shown in Fig. 1.

As shown in Fig. 1a, the glucan conversion increased rapidly for 24 h and then plateaued. A slightly lower enzymatic saccharification efficiency was observed for corn stover substrate pretreated in NaOH/urea solvent with a substrate loading of 5% compared to saccharification at a 2% solid loading. A theoretical glucose yield of 88.9% was obtained within 24 h with a substrate loading of 2%, compared to only 64.3% in a substrate loading of 8%. Similar trends were found for xylan conversion. The conversion rates of xylan for substrate loadings of 8%, 5%, and 2% were 47.3%, 71.4%, and 85.6%, respectively. The fermentable sugar conversion was highest with a substrate loading of 2%. These results indicated that the solid-liquid ratio had a greater effect on the xylan conversion than the glucan conversion. One explanation for this finding could be that the enzymes were able to achieve full contact with the lignocelluloses at the lower concentration, resulting in a higher conversion percentage.

## Enzyme Loadings

Enzyme cost poses one of the main economic challenges in the production of lignocellulosic biofuels. In order to achieve an economically viable technology, the enzyme loading must be reduced while maintaining high saccharification efficiency.

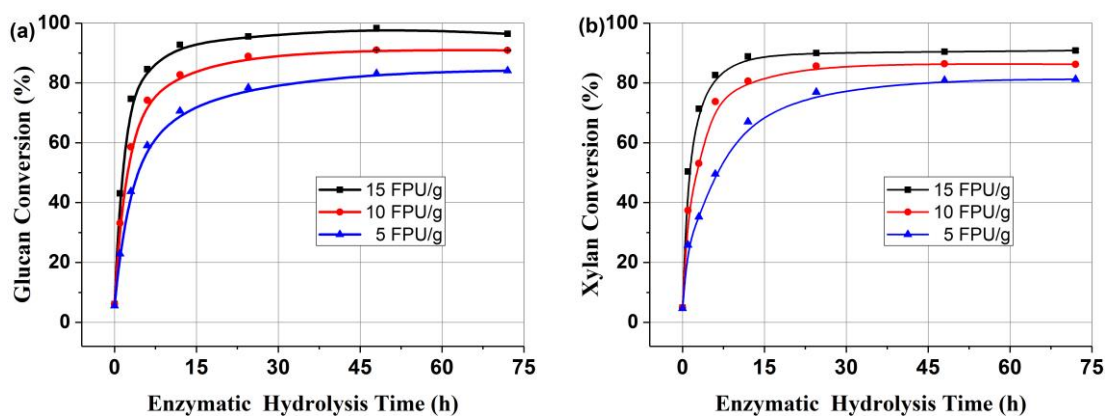
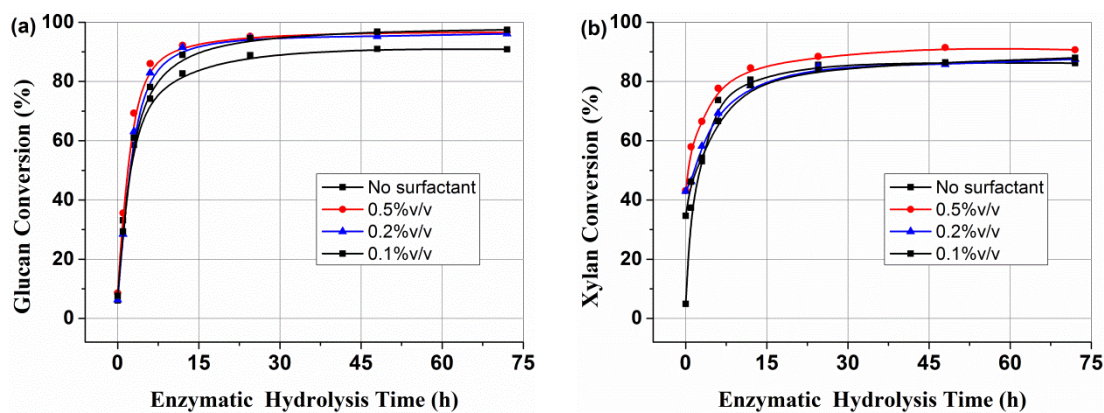


Fig. 2. Glucan and xylan conversions with various enzyme loadings

Enzymatic conversion of the substrates was carried out at a 2% substrate loading, and an enzyme loading of either 15 FPU/g, 10 FPU/g, or 5 FPU/g. Figure 2 shows the effect of enzyme loading on glucose and xylose yields during the one-pot NaOH/urea pretreatment and saccharification process. Glucan conversion values were increased from 78.4% to 95.5% when enzyme loading was increased from 5 FPU/g to 15 FPU/g starting biomass, while a similar amount of improvement (from 76.9% to 90.0%) was observed for xylan conversion within 24 h. The fermentable sugar conversion was slightly lower than 80% with an enzyme loading of 5 FPU/g, indicating that the lower enzyme dose works well within 24 h, and that NaOH/urea solvent pretreatment is an effective approach in converting corn stover into hydrolysable substrates.

### Surfactant Loadings

Surfactants, especially non-ionic surfactants, have been shown to improve enzymatic saccharification, or reduce the amount of enzyme needed to achieve a given conversion. This phenomenon is caused by a few possible mechanisms, such as alteration of the substrate structure for improved accessibility to the enzymes, stabilization of the enzymes and relief of deactivation during hydrolysis, and reduced nonproductive adsorption of enzymes to lignin. The effect of surfactant supplementation (Tween 20 at 0.1, 0.2, or 0.5%, v/v) on sugar conversions with a substrate loading of 2% and an enzyme loading of 10 FPU/g during the one-pot process was tested. The glucan and xylan conversions are shown in Fig. 3. Supplementation with Tween 20 resulted in notable improvement in glucan conversion (Fig. 3a).



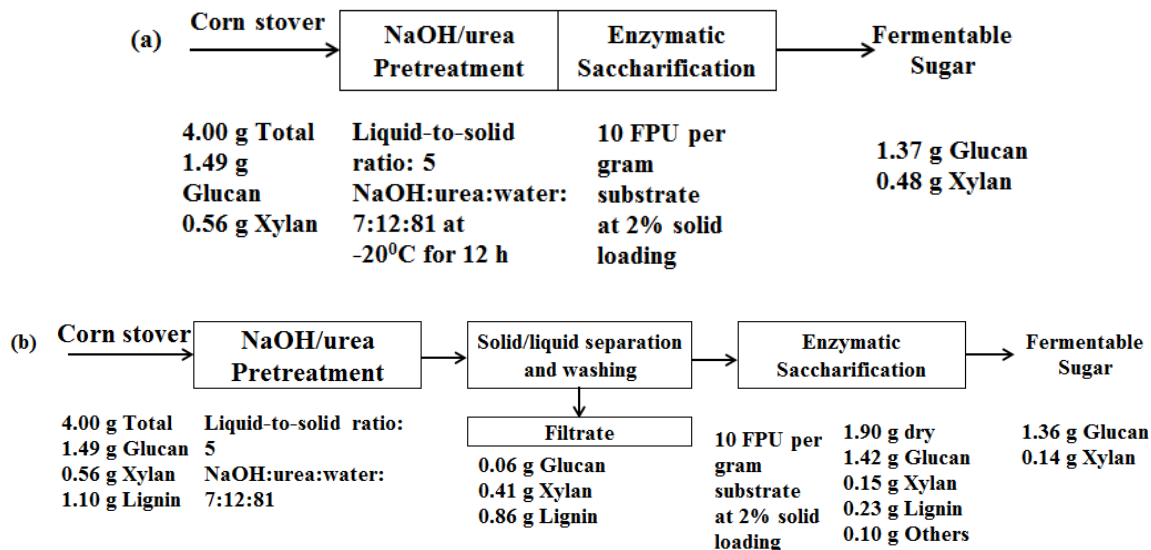
**Fig. 3.** Glucan and xylan conversions with various surfactant loadings

A 94.6% theoretical glucose yield was obtained within 24 h, compared to 88.9% without surfactant. No obvious difference in glucan conversion was detected for corn stover substrate enzymatic saccharification with various levels of surfactant supplementation. Xylan conversions with various levels of surfactant supplementation were also investigated. The xylan conversions demonstrated no obvious differences with surfactant supplementation of 0.1%, 0.2% (v/v), and without surfactant supplementation. A maximum 88.5% theoretical xylan yield was obtained within 24 h, which was achieved with a surfactant supplementation of 0.5% (v/v). It is interesting to note that the addition of surfactant greatly increased the xylan conversion at 0 h. One possible mechanism for this finding could be that the surfactant released substrate-attached xylose to the solution quickly, or the surfactant could have resulted in faster reactions between the degraded xylan and the enzyme than the reaction without supplemental surfactant.

### Mass Balance of One-Pot Pretreatment and Saccharification and Separated Pretreatment and Saccharification

Pretreatment and enzymatic saccharification were conducted under the conditions listed in Fig. 4a. The vast majority of glucan was recovered after low-temperature NaOH/urea pretreatment, as shown in Fig. 4b. Only trace amounts of glucan were lost in the filtrate. For both the one-pot process and the separate processes, more than 90% of glucan in corn stover was converted to glucose. The one-pot process greatly increased the conversion of xylan, from 25.0% to 85.7%, as compared to the separate processes; 91.9% glucan and 85.7% xylan conversion was achieved in this one-pot study. For comparison,

81.9% glucose and 85.4% xylose in the liquid stream was obtained by one-pot ionic liquid pretreatment and saccharification of switchgrass using 23 mg JTherm per substrate for 72 h (Shi *et al.* 2013). Jung reported that 70.7% theoretical ethanol yield was achieved by one-pot pretreatment, saccharification and fermentation of biomass (Jung *et al.* 2014).



**Fig. 4.** Glucan and xylan balance of (a): one-pot processes, and (b): separated pretreatment and saccharification processes

A comparison was made between this one-pot process and other promising pretreatments, such as diluted acid, ammonia fibre expansion (AFEX), ammonia recycled percolation process (ARP), and controlled pH, for glucose and xylose conversion based on the original material. The data from Wyman *et al.* (2005) are shown in Table 1. The cellulose solvent-based pretreatment used in this study resulted in higher glucose recovery compared with the other promising pretreatment methods. Separate processes resulted in the lowest xylose conversion, while the one-pot process resulted in the highest xylose conversion. In conclusion, the one-pot process provides the highest total sugar yield.

**Table 1.** Comparison of Glucose and Xylose Conversions

Pretreatment Method	Glucose Conversion (%)	Xylose Conversion (%)	Reference
One-Pot NaOH/Urea	91.9	85.7	Current Study
Separate NaOH/Urea	91.3	25.0	
Dilute Acid	57.2	35.3	Wyman <i>et al.</i> (2005)
AFEX	59.8	34.6	
ARP	56.1	33.3	
Controlled pH	52.9	30.8	

## CONCLUSIONS

1. The results indicate that low-temperature NaOH/urea pretreatment and saccharification can be combined into a one-pot process. This one-pot process shows advantages in efficient monosaccharide production; 86.3% glucose and 71.3% xylose was liberated in 24 h at an enzyme loading of 10 FPU/g and solid loading of 5%.
2. The effects of solid loading and enzyme dosage were studied. The fermentable sugar conversion was highest at the lowest substrate loading and highest enzyme loading, as expected. It was also noted that the glucan and xylan conversion were 64.3% for glucan and 47.3% for xylan at the highest substrate loading (8%) in this one-pot process.
3. Supplementation of Tween 20 was associated with improvement of glucan conversion, while xylan conversion showed no obvious difference. This one-pot process liberated 94.6% of glucose and 88.5% of xylose in 24 h with a substrate loading of 2%, enzyme loading of 10 FPU/g, and surfactant supplementation of 0.5% v/v.

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