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# EXTRUSION AND ALKALI EXTRUSION OF CORN STOVER TO IMPROVE ENZYME SACCHARIFICATION

Shujing Zhang
*University of Nebraska-Lincoln*, yaoyaoflyaway@hotmail.com

# EXTRUSION AND ALKALI EXTRUSION OF CORN STOVER TO IMPROVE ENZYME SACCHARIFICATION

by

Shujing Zhang

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# EXTRUSION AND ALKALI EXTRUSION OF CORN STOVER TO IMPROVE ENZYME SACCHARIFICATION

Shujing Zhang, M.S.

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Advisor: Milford A. Hanna

Biomass pretreatment constitutes an important part in the whole process of bioethanol production. The main goals of pretreatment include improving cellulose accessibility to enzyme, enhancing sugar yields from hemicellulose, achieving low capital and energy costs, as well as low sugar degradation and low chemical requirements. Extrusion, as a continuous and high throughput pretreatment method, requires much less chemical compared to traditional alkali pretreatment. In this research, twin-screw extrusion was evaluated for its effect with regard to promoting sugar yields from corn stover. Also, the underlying factors contributing to this improvement were examined. It was found that extrusion with no chemicals and post-pretreatment steps achieved significantly higher sugar yields than the untreated corn stover. It was shown that crystallinity indices were not different among the extruded and untreated samples. However, for pretreated samples, the specific surface areas estimated from the Langmuir

adsorption model, were significantly different than the untreated corn stover. Extrusion using sodium hydroxide was shown to transform the structure of lignin. The sugar yields were greatly increased over the extrusion only and untreated corn stover. Crystallinity index and pore quantity were both examined. Crystallinity index was not significantly different from the untreated corn stover, however, pore quantities, measured by Congo red adsorption, were significantly amplified due to alkali combined extrusion.

# ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

Page

# INTRODUCTION

Limited reserves of fossil fuels and increasing price of gasoline have spurred a great amount of research on renewable energy sources in the past decades. Conversion of abundant lignocellulosics to ethanol can serve as a reliable transportation fuel and presents a feasible solution to both energy security and greenhouse gas emissions. Apart from the feedstock, the most costly part of converting biomass to ethanol is pretreatment (1). Therefore, achieving high sugar yields through improved pretreatment is of utmost importance for ethanol to be competitive with fossil fuels.

Apart from extractives and other minor constituents, the structure of lignocellulosic biomass is composed mainly of a lignin sheath around the hemicelluloses and cellulose. In order to improve the accessibility of hemicelluloses and cellulose to enzymes or acids, pretreatment is required to disrupt the structure of the biomass to reduce the crystallinity of cellulose, remove the lignin and increase the porosity. A promising pretreatment method should improve the hydrolysis yields with little sugar degradation and formation of byproducts as well as being cost effective (2).

Extrusion, as a manufacturing process is used widely in food and polymer industries. It has the desirable features of low cost, monitoring and control of process parameters such as temperature and screw speed, high shear and high

throughput. Extrusion can cause micro-fibrillation of biomass resulting in a large specific surface area, conducive for enzymatic hydrolysis. It also is a high-solids pretreatment which produces little effluent and results in limited disposal and solid losses. It also is a mild pretreatment that does not cause sugar degradation like dilute acid and alkali. It shows good adaptability to other processing methods such as steam injection.

In the two manuscripts that follow, the effects of extrusion and alkali extrusion as pretreatment methods were examined in terms of sugar yields. For the extrusion only method, the effects of operating parameters and enzyme dosage were examined. The physical properties, specific surface area and crystallinity index, were investigated to show their relation to sugar yields. For the alkali extrusion, effects of alkali loading ratios and post extrusion processing types on sugar yields were investigated. Solid loss was evaluated. Crystallinity indices and pore quantities were estimated for samples with different sugar yields to reveal the mechanism.

# EXTRUSION MICROFIBRILLATION OF CORN STOVER TO ENHANCE ENZYME SACCHARIFICATION

Shujing Zhang[1], Yixiang Xu[2] and Milford A. Hanna[1]

1. Department of Biological Systems Engineering, University of Nebraska, Lincoln, Nebraska 68583
2. Agricultural Research Station, Virginia State University, Petersburg, VA 23806

ABSTRACT

Pretreatment of biomass before subjecting it to enzyme saccharification is crucial with regards to facilitating access of enzyme to biomass. Extrusion, as a continuous and cost-effective pretreatment method, combines heating with high shear and mixing resulting in much greater specific surface areas of biomass. The objective of this study was to examine the effect of extrusion as a pretreatment method and the underlying factors that impact the improvement of sugar yields. The optimum glucose and xylose sugar recoveries were 48.79% and 24.98%, respectively, at 27.5% moisture content and 80 rpm screw speed. These yields were 2.2 and 6.6 times higher than those for untreated corn stover. X-ray diffraction (XRD) analysis showed that crystallinity index was not a good indicator of sugar yield. However, scanning electron microscopy (SEM) showed that cellulose network was exposed due to destruction of the lignin sheath. Langmuir adsorption model was shown to be an effective tool for estimation of specific surface area (SSA) of corn stover. The SSA of pretreated samples were significantly amplified over the control revealing that extrusion can open the cell wall at microscopic scale which was especially favorable on sugar yields.

INTRODUCTION

Renewable energy, including ethanol, was not given enough attention until the eruption of the “oil crisis” in the 1970s. The price advantage of fossil fuel was impaired and spurred multiple explorations such as National Alcohol Program in

Brazil and the “gasohol” program in the USA [1]. Renewable energy is labeled as sustainable and environmentally friendly in terms of green house emissions [2]. In light of the fact that the rising cost of fossil fuels and related products add to the cost of agricultural production, most agree that grain and crop residues can be exploited to produce residue-based biofuel economically [3]. Among all of the high residue crops, corn is receiving the most attention given that it produces 1.7 times more residues than other leading cereals based on current production capacity [4]. It is estimated that the US produces more than 1 billion tons of biomass each year, with the potential to manufacture about 80 billion gallons of liquid fuels replacing roughly 30% of fossil fuel [5].

Biomass is composed of three primary polymers (cellulose, hemicellulose and lignin) together with other minor components such as chlorophyll, inorganic salts, waxes, non-structural polysaccharides (starch), pectin, protein and ash [6]. However, due to the inconsistencies among species and agronomic practices, the compositions of constituents are different, case by case. Corn stover usually has cellulose content between 34%-42%, xylan content between 19%-28%, and total lignin content between 11%-23% [7]. Around 75 million tons of corn stover are available for ethanol production annually. It is estimated that the reserve of corn stover will increase to 232 million tons by the mid-21st century [5]. The heat potential of corn stover can reach roughly 40% of the total energy capacity of US

petroleum market [8]. Therefore, corn stover is regarded as the most promising feedstock for ethanol production.

In spite of the numerous benefits biomass-based ethanol possesses, there are still many obstacles to its commercialization. Besides factors such as high capital costs, investor's fear of taking the first initiative and the low commodity profits of ethanol [9], there are technological challenges that this paper will focus on. As a prerequisite for survival, plants have developed a unique resistance system to prevent microbial degradation. Lignin is the major contributor to the intrinsic recalcitrance of biomass which introduces structural support and a natural barrier against microbial access to cellulose network [10]. Apart from the lignin, it has been proposed that cellulose crystallinity and porosity of biomass also play important roles in hydrolysis of cellulose [11]. Pretreatment is useful in reducing crystallinity, increasing porosity as well as removing lignin and hemicellulose, which lead to enhanced sugar yields. Numerous pretreatment methods have been investigated, including physical, chemical, physicochemical and biological approaches. However, traditional physical pretreatments like comminution are energy intensive and unrealistic to full scale application [12]. The effects of chemical pretreatments like dilute acid and alkali treatment depend on the composition of biomass. Dilute acid is extremely useful in solubilizing hemicellulose and recovering pentoses, primarily xylose and arabinose, which greatly increases the cellulose accessibility in the residual solids [13]. Alkaline

solution is effective in solubilizing lignin, thus exposing cellulose and hemicellulose to enzyme hydrolysis. However, chemical pretreatments usually require high capital inputs and cause sugar loss as well as adding inhibitors to the reaction mixture thus entailing water purging prior to enzyme hydrolysis. Other pretreatments include steam explosion and rot fungi treatment. Steam explosion also produces inhibitors and a degree of sugar loss [11]. Rot fungi pretreatment inherently has a very slow hydrolysis rate.

Single and twin-screw extruders are used widely in the plastics and food industries [14]. Extrusion has the characteristics of low cost, good monitoring and control of temperature and screw speed, high shear and excellent processing ability. It implements efficient micro-fibrillation of biomass resulting in large specific surface area [15]. It also is a high-solids pretreatment which produces little effluent and no disposal and solid losses occur [16]. It also is a mild pretreatment that does not cause sugar degradation like dilute acid and alkali [17]. It shows good adaptability to other processing methods, for example, it can be injected with steam [18] or used with supercritical fluid [19].

The mechanism of extrusion pretreatment has yet to be fully understood. Therefore, this study aimed to identify the most important factors influencing improvement of sugar yields in enzymatic digestion after implementation of

extrusion pretreatment, e.g. the specific surface area and crystallinity index. The effects of extruder operating parameters and enzyme dosage also are discussed.

## MATERIALS AND METHODS

### Raw material

The corn stover was harvested from a local farm operated by the Biological Systems Engineering Department at the University of Nebraska, Lincoln. The stover was ground in a Wiley mill to pass through a 2.0 mm screen. It was air-dried and stored in sealed Ziploc bags at room temperature. Its compositions of structural carbohydrates and lignin were analyzed as outlined by Sluiter et al. [20]. The extractives in biomass were determined using a Soxhlet extraction apparatus [21]. Its ash content was obtained using a muffle furnace [22]. The moisture content of the material was analyzed using an automated infrared moisture analyzer [23]. In order to examine the effect of moisture content on sugar yields, the moisture contents of corn stover were adjusted to 22.5%, 25%, and 27.5% on a wet basis by adding distilled water and letting them equilibrate overnight.

### Extrusion

Corn stover, with the preselected moisture levels, was fed into a twin-screw extruder. The extruder was co-rotating and easily re-arranged into different screw

configurations. The first thirteen 30R segments were placed for continuous conveyance of material. In the next region, one reverse flow (RF) screw was used to achieve exceptionally good pulverizing. Lastly, two 20R screws were used to impose greater pressure on the material. The temperatures of zones 1 and 2 were set at 50 ℃ and 140 ℃, respectively. The six screw speeds used were from 40 to 140 rpm with step increases of 20 rpm. The feed rate was 3.67 g/min. Extrusion was conducted without a die to avoid serious packing and blockage. Therefore, the extrusion investigated did not incorporate explosion. For comparison purposes, pretreatments using a single screw extruder with die installed were carried out at five different screw speeds as outlined by Karunanithy and Muthukumarappan [7].

**Enzymatic saccharification and HPLC analysis**

Enzymatic saccharification was conducted with a cellulase complex (Cellic Ctec, Novozyme) at a solid ratio of 6.1%. The formulation was modified as outlined by Selig et al. [24]. It contained 0.05 M, pH 5.0 sodium citrate buffer and a Cellic Ctec enzyme complex at a preselected dosage (cellulose basis) to achieve 0.028 g enzyme/g dry corn stover. Low enzyme dosage is commercially viable, while high enzyme dosage is required to determine the maximum accessible cellulose content. Therefore, enzyme dosage of 0.3 g enzyme/g cellulose was selected for the optimum treatment group to compare the sugar yields with low enzyme

dosage. Sodium azide, at 20 mg/mL, was added as an antibiotic to prevent microbial growth. The biomass was corrected for moisture content to equal a dry weight of 0.1 g cellulose. Sodium citrate buffer was prepared by titrating 0.05 M sodium citrate solution with 0.05 M citric acid solution. Digestion mixtures were added in glass tubes with water-proof plastic caps, and were shaken at a speed of 3.5 to ensure the suspension of solids. The temperature of the shaking bath was adjusted to 50 ℃ for a period of 72 h. After saccharification, the enzymes were deactivated in boiling water. Then, the enzyme hydrolysates were centrifuged at 10,000 rpm for 15 min to remove biomass for subsequent HPLC analyses. Two Biorad HPX87H columns were attached in tandem to achieve better resolution, and the temperatures, flow rate, injection volume were determined as by Sluiter et al. [20]. The individual sugar yields were calculated using the following formula:

$$IY_i = \frac{S_{is}}{S_{ic}} \times 100 (1)$$

where $IY_i$= Individual sugar yield (%),

$S_{is}$= Individual sugars released during enzymatic saccharification (%) and

$S_{ic}$= Compositions of individual sugars in raw material (%).

**X-ray analysis**

The crystallinity indices (C.I.) of selected samples were investigated using a Rigaku-D/Max-B diffractometer. The sample and detector rotated at angles of θ and 2θ as to the incident beam. Diffractograms were formed with CuKα radiation (~1.544 Å). Similar to the conditions adopted by Linder and Gatenholm [25], scans were conducted in the range from 5° to 45° (2θ) at a rate of 2°/minute and a step size of 0.05° (2θ). The crystalline indices were calculated with the following equation [26]:

$$C.I. = \frac{(I_{max} - I_{min})}{I_{max}} \quad (2)$$

where $I_{max}$ is the maximum intensity ($I_{002}$) and $I_{min}$ is the minimum intensity between $I_{002}$ and $I_{101}$ peaks. For cellulose I, the characteristic peaks are at 2θ =14.8°, 16.8°, 22.6°; for cellulose II, the peaks are at 2θ =12.1°, 19.8°, 22.0° [27].

**Dye adsorption model and SSA estimation**

Specific surface area (SSA) was estimated with the theoretical maximum amount of Congo red adsorbed on the substrate surface according to the procedures of Inglesby and Zeronian [28] and Linder and Gatenholm [25]. Selected samples, as substrates, were dyed with a liquor ratio of 100:1 at adsorbate concentrations ranging from 0.25% of the oven dry weight of substrates (ows) to 10% ows. NaCl at 20% ows was added as an electrolyte. Moisture content was taken into account

to correct substrate weight. A substrate blank, with exactly the same formulation but without dye, was incubated simultaneously. Dye blank at 2 mg/mL without substrate also was taken through the dyeing process. Dyeing was conducted in a shaking bath at 60 ℃ for 24 h. After dyeing, the samples were centrifuged at 10,000 rpm for 10 min to remove biomass, and the absorbance of supernatant was obtained using UV-visible spectrophotometer at a wavelength of 492 nm. The absorbance of substrate blank was then subtracted from the absorbance of supernatant to get correct residual dye concentration. With residual Congo red concentrations, the adsorbed Congo red can be derived by subtracting the residual concentrations from the initial concentrations. The theoretical maximum amount of adsorbed Congo red was the reciprocal of intercept in Equation 3 obtained from Langmuir theory of adsorption [29].

$$\frac{1}{q} = \frac{1}{q_{mon}} + \frac{1}{K_L q_{mon}} \frac{1}{C} \quad (3)$$

where C (mg/mL) was the free concentration of Congo red after dyeing, q (mg/g biomass sample) was the amount of Congo red adsorbed on the substrate, $K_L$ was the Langmuir constant and $q_{mon}$ (mg/g) was the theoretical maximum amount of Congo red adsorbed on substrate when saturation occurs. The SSA was estimated using Equation 4 [30, 31].

$$SSA = \frac{q_{mon} N_A SA_{CR}}{M_W} \quad (4)$$

where SSA was an estimation of specific surface area for cellulose substrates, $N_A$ was the Avogadro's constant and $SA_{CR}$ was the surface area of one Congo red molecule (1.73 $nm^2$) and $M_W$ was the molecular weight of Congo red (696.7 g/mol) [30].

**Statistical analysis**

The data were analyzed statistically using Proc Glimmix with Dunnett statement to compare pretreated samples with control and Tukey adjustment which enabled multiple comparisons using an experiment-wise error rate (SAS) to get the optimum pretreatment condition. The control is the untreated corn stover. The study was a two-factorial experiment with screw speeds (SS) of 40, 60, 80, 100, 120 and 140 rpm along with moisture contents (MC, w.b.) of 22.5%, 25% and 27.5%. Therefore, 18 treatment combinations were investigated with two replicates for each combination.

## RESULTS AND DISCUSSION

**Characterization of corn stover**

The composition results obtained in this study (Table 1) were in good accordance with the data reported by Kim et al. [32]. Acid insoluble lignin content was

obtained by subtracting ash from acid insoluble residue, and did not consider protein content. It was shown that the maximum potential fermentable sugars accounted for 55.12% of the oven dried corn stover, indicating its strong candidacy as a feedstock for renewable energy.

**Table 1** Chemical compositions of raw corn stover on dry basis (%) (reported values are means with triplicates or more and standard deviations in the parenthesis)

| Component | w/w (%, d.b.) |
|---|---|
| Glucan | 33.15 (2.2) |
| Xylan | 19.16 (1.07) |
| Arabinan | 2.81 (0.17) |
| Lignin(acid soluble+insoluble) | 14.9 (0.6) |
| Extractives(ethanol) | 3.77 (0.5) |
| Extractives(water) | 9.13 (0.9) |
| Ash | 10.91 (2.22) |
| Protein | 4.0 (0.01) |
| Others | 2.2 |

**Individual sugar yields**

This study had the advantages that no delignification was conducted prior to enzyme hydrolysis and no extra processing steps were required after pretreatment. The control had glucose yield of 22.01% and xylose yield of 3.8%. Based on $\alpha$ level of 0.05, the sugar yields of all treatment combinations showed a significant increase over the control, the bracketed values were Dunnett p-values (Table 2). From Tukey test, there was no significant interaction effect of MC×SS. The MC effect under current data scope was not statistically significant for sugar recoveries. However, SS effect approached significance (p-value=0.0571) for

glucose yield. Therefore, the main effect plot of SS was constructed as in Fig 1. The glucose yield decreased from 44.42% to 39.66% with screw speed increased from 40 rpm to 140 rpm. The maximum glucose yield of 47.26% was achieved at screw speed of 80 rpm. The trend for SS effect was similar to what was achieved under optimum enzyme combination and ratio by Karunanithy and Muthukumarappan [7]. It suggested that shear rate developed at screw speed of 80 rpm was enough to soften the lignin. Nevertheless, subsequent increases in screw speed lessened the residence time which possibly led to insufficient destruction of lignin sheath.

**Table 2** Least squares means of sugar yields

| Screw Speed (rpm) | Glucose yield (%) | | | Xylose yield (%) | | |
|---|---|---|---|---|---|---|
| | Moisture Level (w.b.) | | | Moisture Level (w.b.) | | |
| | 22.5% | 25% | 27.5% | 22.5% | 25% | 27.5% |
| 40 | 41.70 (0.0015) | 44.01 (0.0004) | 47.54 (<.0001) | 19.90 (0.0005) | 21.53 (0.0001) | 24.0 (<.0001) |
| 60 | 41.45 (0.0017) | 46.55 (0.0001) | 45.50 (0.0002) | 19.85 (0.0005) | 23.10 (<.0001) | 22.08 (<.0001) |
| 80 | 45.16 (0.0002) | 47.84 (<.0001) | 48.79 (<.0001) | 22.4 (<.0001) | 24.06 (<.0001) | 24.98 (<.0001) |
| 100 | 47.71 (<.0001) | 46.15 (0.0001) | 42.65 (0.0009) | 24.25 (<.0001) | 23.60 (<.0001) | 20.14 (0.0004) |
| 120 | 43.45 (0.0006) | 39.63 (0.0044) | 41.31 (0.0018) | 23.11 (<.0001) | 20.06 (0.0004) | 20.54 (0.0003) |
| 140 | 41.32 (0.0018) | 39.63 (0.0044) | 41.31 (0.0018) | 21.61 (0.0001) | 18.62 (0.0012) | 21.86 (0.0001) |

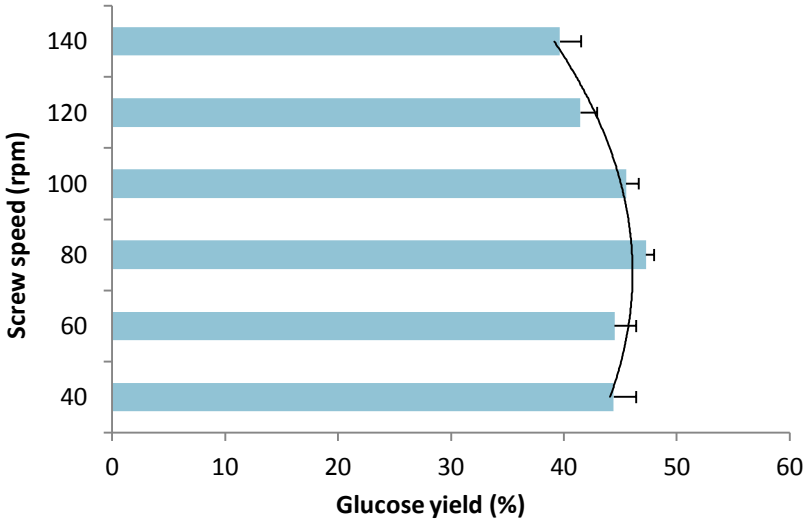


**Fig 1** Effect of screw speed on glucose yield

**Comparison with other study**

For this study, the glucose and xylose sugar yields for pretreated samples were 2.2 and 6.6 times higher than the control. These were greater than those achieved by Karunanithy and Muthukumarappan [7] which declared 2.0 and 1.7 times higher than the control for glucose and xylose sugar yields, respectively. However, the highest sugar yields were much lower compared to the work of Karunanithy and Muthukumarappan [7], so a single-screw extrusion pretreatment was duplicated as outlined by Karunanithy and Muthukumarappan [7]. The treatment groups investigated were extracted from the total combinations in the original study and included the optimum pretreatment condition as summarized to be 75 rpm and 125 ℃. With the same enzymatic saccharification protocol as twin

screw extrusion pretreatment, the highest glucose and xylose sugar yields for single screw extrusion were 42.3% and 28.8% which were comparable to the 48.79% and 24.98% achieved in this study. Therefore, it was suspected that enzyme dosage accounted for most of the variations in sugar yields between the two methods. The enzyme dosage in this study was 0.028 g enzyme/g dry corn stover which was an order of magnitude lower than that used by Karunanithy and Muthukumarappan [7] with optimum enzyme dosage of 0.45 g enzyme/g dry corn stover. Therefore, a higher enzyme dosage was used for the optimum treatment group of 27.5%/80 rpm. It was discovered that glucose and xylose sugar yields were 61.17% and 37.33%, respectively, which were significantly greater ($\alpha$=0.05) than the low enzyme dosage indicating the cellulose content available for conversion into monomeric sugars had not been fully exploited.

**Crystallinity Index**

It can be observed from the diffractogram that the control showed an apparent cellulose I crystal structure (Fig 3). For chosen extruded samples (other samples are not shown here), corn stover had a tendency of crystal lattice transition from cellulose I to cellulose II as asserted by the transformation of polymorph patterns. However, the characteristic peaks for cellulose II at around 12.1$^\circ$ 2$\theta$ and a shoulder between 19$^\circ$ and 20$^\circ$ 2$\theta$ did not appear or were ambiguous for 25%/140 rpm and 22.5%/100 rpm (Fig 4 and Fig 5). It can be noticed that extrusion resulted in obvious cellulose II structure under extrusion condition of 27.5%/80

rpm (Fig 6), though certain amount of cellulose I structure still held. For cellulose II structure, the chains were not parallel aligned as in cellulose I, but anti-parallel staggered with each other. Using equation 2, CIs for different treatment combinations were reported as means of 2 replicates with standard deviations in parentheses as shown in Table 3. There were no significant differences among CIs for treatment combinations and the control. It also was observed that a peak appeared within $26.6^{\circ}$-$27^{\circ}$ $2\theta$ for selected extruded samples but not for the control which could have been due to the new crystal structure caused by recrystallization. Extrusion led to partial pulverization of amorphous lignin as indicated in SEM (Fig 2). However, the crystallinity did not change much. Therefore, it can be inferred that extrusion improved enzymatic saccharification by inducing micro- or nano-fibrillation of corn stover without dissipating energy to destroy crystallinity which was similar to the conclusion made by Lee et al. [33].

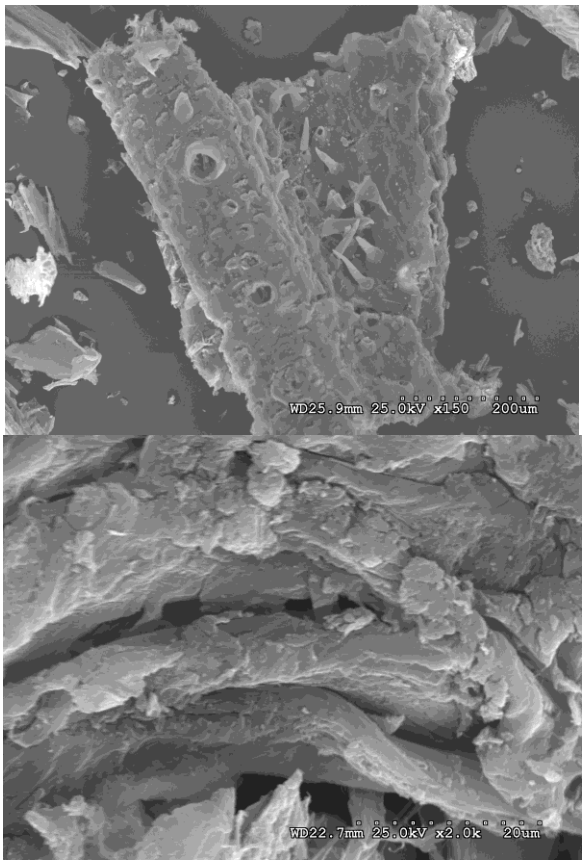


**Fig 2** Intact tracheid cell of raw corn stover (above) versus exposed cellulose surface of extruded corn stover at 27.5% MC and 80 rpm (below)

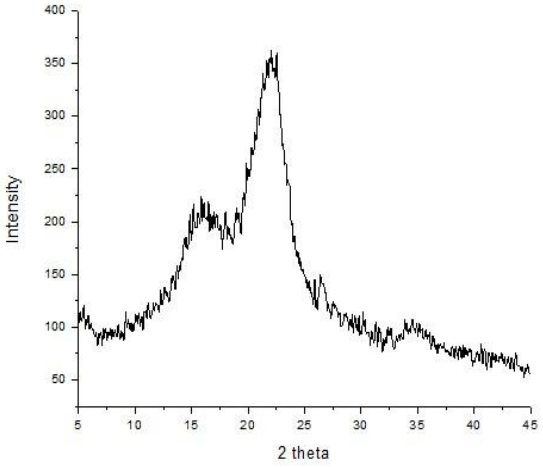


**Fig 3** X-ray diffractogram for the control sample

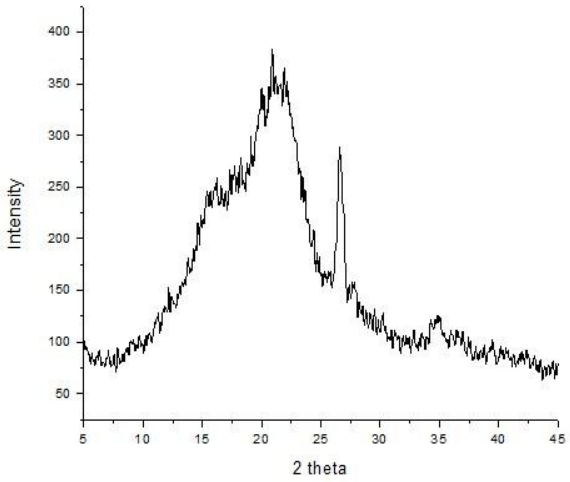


**Fig 4** X-ray diffractogram for 25%/140 rpm

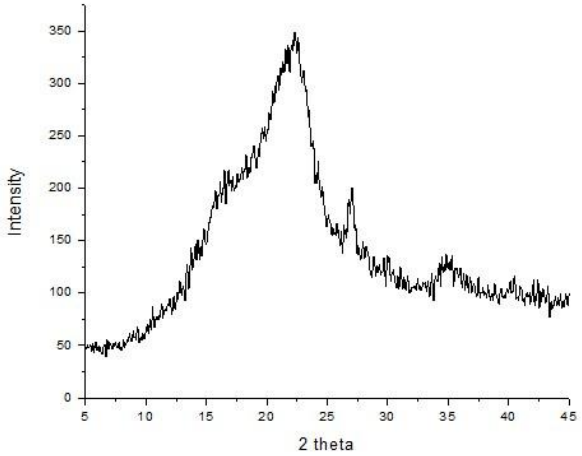


**Fig 5** X-ray diffractogram for 22.5%/100 rpm

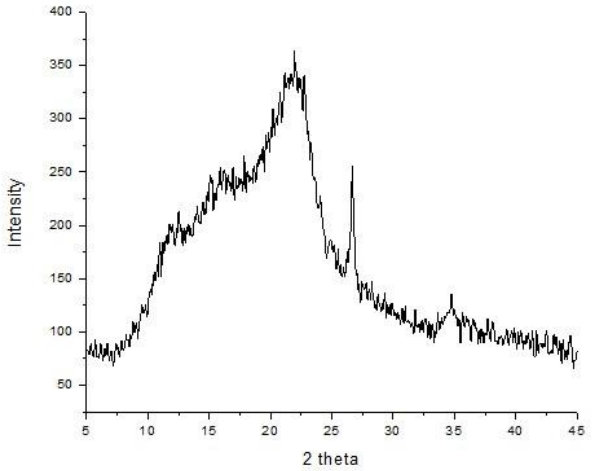


**Fig 6** X-ray diffractogram for 27.5%/80 rpm

**Table 3** Crystallinity of selected samples

| Samples | C.I. |
|---|---|
| Control | 0.5221 (0.0338) |
| 25%/140 rpm | 0.5634 (0.0558) |
| 22.5%/100 rpm | 0.5432 (0.1469) |
| 27.5%/80 rpm | 0.5304 (0.0049) |

**Estimation of specific surface area**

It was found that the concentration of the dye blank did not change over the 24 h dyeing process. Thus, the decrease of Congo red in the dye bath was largely caused by dye adsorption. Congo red is a direct dye. It was proposed that the Langmuir isotherm can model satisfactorily the adsorption of direct dyes onto cellulose substrates at very low concentrations [34]. The prerequisites for Langmuir adsorption include the formation of mono-layer, thus specific surface area can be derived from the theoretical maximum amount of adsorption of the dye [25]. The isotherms (Fig 7) showed an exponential increase of dye adsorbed on substrate (DAOS) initially with increasing free dye concentrations (FC) up to a saturation point. Beyond this point, further increase in dye concentrations would not boost DAOS anymore. This is typical Langmuir behavior represented by the limited numbers of accessible sites in the fiber. Comparison among the isotherms revealed that extrusion resulted in much higher dye adsorption at saturation compared to the control. Also, the initial slopes of isotherms were very close to the ordinate suggesting the strong affinity of Congo red with corn stover. The SSA as shown in Table 4 showed that 25%/140 rpm and 27.5%/80 rpm had 5-6 times larger SSA compared to the control. Therefore, it can be concluded that extrusion resulted in well-fibrillated corn stover with greater specific surface areas which was favorable for enzyme adsorption and, thus, significantly improved

sugar yields which agreed well with the conclusion made by Lee et al. [33] about Douglas fir.

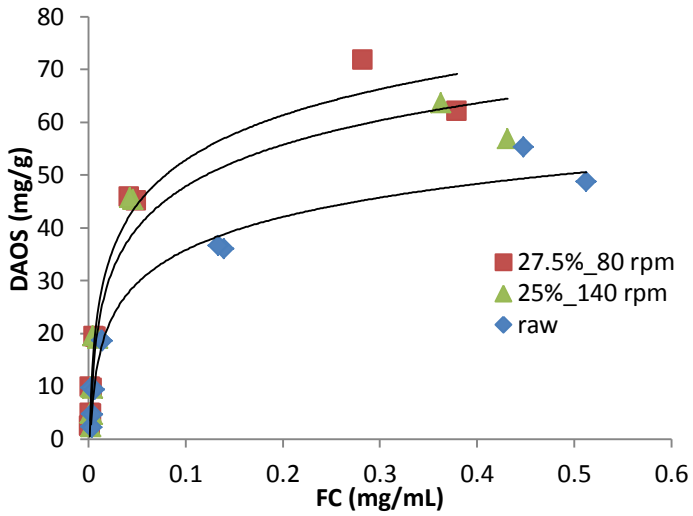


**Fig 7** Langmuir plots of selected samples

**Table 4** Theoretical maximum DAOS and SSA for control and pretreated samples with minimum and optimum sugar yields

| Sample | $q_{mon}$ (mg/g) | SSA ($m^2$/g) |
|---|---|---|
| Control | 48.9 | 73.2 |
| 25%/140 rpm (minimum) | 245.7 | 367.4 |
| 27.5%/80 rpm (optimum) | 293.3 | 438.5 |

## CONCLUSIONS

The costs of lignocelluloses-based ethanol have been challenged by costs of feedstock, pretreatment and enzyme. The consumption of enzyme per grams of dry biomass is not explicitly mentioned in many publications. Therefore, the high

yields obtained may have been at the price of high enzyme input and other harsh operating conditions. Extruders, as readily scalable equipment, have well-established vendor quotes and can be used as a pretreatment unit operation in an integrated biorefinery, offering good process control and excellent adaptability to chemicals (reactive extrusion) as well as high throughput leading to low specific mechanical energy requirement. This study targeted at fibrillation of corn stover with continuous twin-screw extruder to get improved sugar yields and shedding light on the intrinsic factors contributing to the improvement. No chemicals were applied, thus no extra processing were needed prior to enzymatic hydrolysis. The highest glucose and xylose sugar yields were 48.79% and 24.98%, respectively, and were found at 27.5% MC and 80 rpm at an enzyme dosage which was commercially viable (0.028 g enzyme/g dry corn stover). The values were 2.2 and 6.6 times higher than those of untreated corn stover. The crystallinity was not the main property underlying the improvement. Nevertheless, specific surface area was found to be closely related to enhanced sugar yields showing that twin-screw extrusion opened the cell walls of corn stover at the microscopic level which was enough for efficient enzyme adsorption on cellulose.

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# ALKALI COMBINED EXTRUSION PRETREATMENT OF CORN STOVER TO ENHANCE ENZYME SACCHARIFICATION

Shujing Zhang[1], Deepak R. Keshwani[1], Yixiang Xu[2] and Milford A. Hanna[1]

1. Department of Biological Systems Engineering, University of Nebraska, Lincoln, Nebraska 68583
2. Agricultural Research Station, Virginia State University, Petersburg, VA 23806

ABSTRACT

In previous studies, twin-screw extrusion with good adaptability, high throughput and continuous feature was shown to be a promising pretreatment method for corn stover to open the cell wall at the microscopic scale and largely increase the specific surface area for enzyme adsorption regardless of crystallinity. The objective of this study was to further improve the effect of extrusion by combining alkali with extrusion and identify the properties which were important to optimizing sugar yields. The optimum glucose and xylose sugar yields for extrusion with alkali solutions were 86.8% and 50.5%, respectively, at an alkali loading of 0.03 g/g biomass, at a screw speed of 80 rpm and with washing which were 3.9 and 13.3 times higher than those of the raw material. XRD analysis (X-ray diffraction) showed apparent cellulose structure conversion from cellulose I to cellulose II and crystallinity index was not found to be related with hydrolysis yields**.** Congo red dye adsorption experiments indicated that alkali-assisted extrusion led to significantly more pores at the meso and large scales compared to the untreated material, which tremendously improved the efficiency of enzyme adsorption and, thus, the sugar yields.

INTRODUCTION

The irreversible depletion of fossil fuel reserves, along with environmental concerns has driven more and more researchers to explore alternative energy

sources. Biofuels derived from lignocelluloses, as a transportation fuel, shows promising features with regards to sustainability and green house emissions (Wyman 1999) and is projected to have low-cost and significantly larger reserves of feedstock as compared to starch-based ethanol (Dalgaard et al., 2006).

However, in order to protect themselves against microbial attack, plants have developed a unique defense barrier in which lignin is the main contributor. For utilization of biomass to produce ethanol, the lignin barrier must be destroyed to expose the main polysaccharides (cellulose and hemicellulose) to either chemical reagents or enzymes to break the polymers down into mono-sugars what then can be fermented into ethanol. Therefore, pretreatment serves as the most crucial part to unlocking affordable conversion of biomass into ethanol. To date, various pretreatment methods of corn stover have been investigated; namely dilute acid, alkali, ammonium fiber explosion and microwave or combinations of these (Zhu et al., 2004; Millet et al., 1976; Lau et al., 2008; Keshwani and Cheng 2010; Hu and Wen 2008). Dilute acid pretreatment is very effective in converting hemicellulose into pentose sugars and has been demonstrated commercially. However, the low solids loading ratio for a continuous flow process requires a large amount of acid per unit of biomass and more water to wash out inhibitors after pretreatment. All these add to a high cost of product recovery and energy consumption. Moreover, it was proposed that corn stover pretreated at acidic pH

and T > 150 ℃ resulted in deposition of droplets on the surface which might be composed of lignin and lignin-carbohydrate complexes having negative effect on enzymatic hydrolysis (Selig et al., 2007). However, alkali pretreatment does not cause as much sugar degradation and requires much lower pressure and temperature compared to other chemical pretreatment methods (Moiser et al., 2005). Of the common alkalis investigated, sodium hydroxide has been shown to have a significant effect on straws with relatively low lignin contents of 10%-18% (Bjerre et al., 1996). The corn stover in this study had lignin content within the "effective range" and therefore, sodium hydroxide was adopted in this study to further enhance the sugar yields of corn stover over just extrusion.

Extruders, as readily scalable equipment, have been used widely in the food and polymer industries. One of the advantages is that they can be easily adapted to different process modifications. Addition of solvent into samples prior to extrusion is not an unfamiliar approach to industries currently using this technology. Many researchers have used reactive extrusion to produce various products and make modifications to a wide variety of polymers. It can greatly lower the demand of chemicals and improve the properties of the end-product. In our previous work, twin-screw extrusion, without addition of chemicals showed significant enhancement of sugar yields and amplification of specific surface area over the untreated material. However, there is still much room for improving

sugar yields. As inspired by the production of microcrystalline cellulose (MCC) from lignocellulosics through reactive extrusion (Hanna et al., 2001), basic solutions of corn stover were extruded to achieve much greater sugar yields. The impacts of different conditions and various properties on sugar yields were investigated deduce reaction mechanism.

## MATERIALS AND METHODS

### Raw material

The raw material was corn stover obtained from a local farm. The stover was milled in a Wiley mill to pass through a 2.0 mm screen. It was then air dried and stored in ziploc bags at ambient conditions. Its composition of structural carbohydrates, lignin, ash and extractives were analyzed as outlined in NREL standard procedures (Sluiter et al., 2008a; Sluiter et al., 2005a; Sluiter et al., 2005b). The weight of material was corrected for moisture content each time prior to experiments (Sluiter et al., 2008b).

### Alkali treatment

Reagent grade sodium hydroxide powder was dissolved in distilled water to prepare aqueous solutions at four different concentrations (w/w). The basic solutions were made with the amounts of water required to adjust the MC of 100 g corn stover to 50% (dry basis). The resulting sodium hydroxide loadings were 0.0039, 0.0119, 0.01 and 0.03 g/g biomass. Then the alkaline solutions were

thoroughly mixed with corn stover in a mechanical mixer and the biomass was equilibrated for 8 h at ambient conditions. Raw material and corn stover soaked in alkali at four different loading ratios without extrusion were used as controls.

**Twin-screw extrusion**

After alkali treatment, corn stover was fed into the twin screw extruder. The configuration was composed of 16 segments of which the first 13 enabled continuous movement of material. Then a RF segment with similar function as a kneading block was placed to achieve good pulverizing. The last two segments were 20R screws to induce more pressure on the material. The temperatures of zone 1 and 2 were set at 50 ℃ and 140 ℃, respectively. Screw speeds of 40, 60, 80 and 100 rpm were adopted. The feed rate was 3.67 g/min.

**Post-extrusion processing**

After alkali-assisted extrusion, samples were washed with 44 mL of distilled water/g corn stover through vacuum filtration and dried under ambient conditions for 3 days. Then the washed samples were put in sealed plastic bags and stored in a refrigerator for subsequent enzyme hydrolysis. Alkali treatment controls also were washed through using the same protocol. In order to examine the resulting inhibitory effect to enzymatic saccharification introduced by alkali-based extrusion, samples without washing were digested using the same procedure.

**Estimation of solid loss**

The most severe solid loss usually occurs in the separation of liquid and solid after aqueous processing. To reveal the extent of solid loss, alkali treatment at the highest loading ratio was examined, and also the solid loss caused by washing before enzyme digestion was examined for optimum pretreatment condition (0.03 g alkali/g biomass and 80 rpm).

**Enzymatic saccharification and HPLC analyses**

As modified from the standard procedure (Selig et al., 2008), the enzyme digestion mixture contained 0.05 M, PH 5.0 sodium citrate buffer and a Cellic Ctec enzyme complex at a preselected dosage (cellulose basis) to equal 0.028 g enzyme/g dry corn stover. Sodium azide at 20 mg/mL was used as an alternative antibiotic. The biomass was corrected for moisture content to equal a dry weight comprising 0.1 g cellulose. The temperature of shaking bath was adjusted to 50 ℃ for a period of 72 h. After saccharification, enzymes were deactivated in boiling water and stored in a refrigerator until HPLC analyses. Two HPX87H columns were attached to quantify sugar concentrations in the aliquots and the operating conditions were based on the standard procedure by Sluiter et al. (2008). Based on composition analyses, the individual sugar yields were determined as follows:

$$IY_i = \frac{S_{is}}{S_{ic}} \times 100 \quad (1)$$

Where $IY_i$= individual sugar yield (%),

$S_{is}$= individual sugars released during enzymatic saccharification (%), and

$S_{ic}$= compositions of individual sugars in raw material (%).

**X-ray diffraction**

The crystallinity indices (C.I.) of samples, pretreated with four alkali loading ratios nested within 80 rpm and washing, were examined using a Rigaku-D diffractometer. The sample and detector rotated at angles of θ and 2θ as to the incident beam, respectively. Diffractograms were formed with CuKα radiation (~1.544 Å). Similar to the conditions adopted by Linder and Gatenholm (2004), scans were conducted in the range from 5° to 45° (2θ) at a rate of 2°/minute and a step size of 0.05° (2θ). The relative crystallinity index was calculated with the following equation (Buschle-Diller and Zeronian 1992):

$$C.I. = \frac{(I_{max} - I_{min})}{I_{max}} \quad (2)$$

where $I_{max}$ is the maximum intensity ($I_{002}$) and $I_{min}$ is the minimum intensity between $I_{002}$ and $I_{101}$ peaks. For cellulose I, the characteristic peaks appeared at 2θ =14.8°, 16.8°, 22.6°; for cellulose II, the peaks appeared at 2θ =12.1°, 19.8°, 22.0° (Ago et al., 2004).

**Measurement of porosity**

From previous studies, the adsorption isotherms for Congo red onto raw and extruded corn stover were shown to represent Langmuir behavior where the dye adsorbed on the substrate leveled off at a dye concentration particular of each sample. The equilibrium dye adsorptions of extruded samples were much higher than the untreated material and plateaued at higher concentrations. Congo red has a molecular size of 2.6 nm (Datta et al., 2011) which is over two times bigger than that of direct blue (1 nm); however, it is much smaller than direct orange which ranges from 5 to 7 nm (Esteghlalian et al., 2001; Yu et al., 1995; Yu and Atalla 1998). Therefore, the selective sorption of different dyes by cellulosic substrates revealed the presence of pores of different sizes. Congo red can penetrate meso and large scale pores and, thus, can be used as a molecular sensor to semi-quantify the number of pores larger than 2.6 nm **(**Datta et al., 2011**)**. It was noticed that the dye concentration used should be as high as possible in order to get close to the maximum adsorption capacity of the sample possessing the greatest number of mesoscale and large pores. Based on previous studies, dye concentration was chosen to be 20% ows (oven dry weight of substrate). Samples pretreated with four alkali loading ratios nested within 80 rpm and washing, were selected to clarify the variations in pore quantities. The dyeing conditions were similar to those outlined by Inglesby and Zeronian (1996) except that the temperature was changed to 70 ℃. Substrate blanks were conducted as to correct

the background absorbance. It was shown in previous studies that Congo red concentration did not change over the 24 h dyeing process. After dyeing, the samples were centrifuged at 10,000 rpm for 10 min to remove the cellulose substrates.

**Statistical analysis**

The data were analyzed statistically using Proc Glimmix with Tukey adjustment which enabled multiple comparisons using an experiment-wise error rate (SAS) to get the optimum pretreatment condition. The whole dataset was treated as a three factorial experiment, four screw speeds (SS), four alkali concentrations (Conc) and two post-extrusion processing types (T) which referred to washed (w) or unwashed (uw). Therefore, 32 treatment combinations were investigated and two replicates were conducted for each combination. Two-way interaction effect of Conc×T was examined using the slicediff=Conc and slicediff=T statements in Proc Glimmix.

RESULTS AND DISCUSSION

**Characterization of corn stover**

The compositional data were summarized in Table 1 and agree with the published data (Yang and Wyman 2004; Zhu et al., 2004). It was noted that the maximum

potential fermentable sugars constituted 55.12% of the dry corn stover, implying its great potential as a feedstock for producing ethanol.

**Table 1** Chemical compositions of raw corn stover on dry basis (%) (reported values are means with triplicates or more and standard deviations in the parenthesis)

| Component | w/w (%, d.b.) |
|---|---|
| Glucan | 33.15 (2.2) |
| Xylan | 19.16 (1.07) |
| Arabinan | 2.81 (0.17) |
| Lignin(soluble+insoluble) | 14.9 (0.6) |
| Extractives(ethanol) | 3.77 (0.5) |
| Extractives(water) | 9.13 (0.9) |
| Ash | 10.91 (2.22) |
| Protein | 4.0 (0.01) |
| Others | 2.2 |

**Solid loss**

The solid loss due to alkali treatment was negligible **(**0.7%) which was largely caused by handling and incomplete transfer. This is expected as the solids contents for alkali treatment are above 60%. Washing before enzyme digestion just incurred solid loss of 1.5%. This can be well explained since the filter paper used has coarse porosity and fast flow rate, thus the samples were actually rinsed very quickly by water and only the inorganic material and lignin fragments right on the surface were removed. Therefore, the total solid loss as a result of the pretreatment was 2.2%. Furthermore, the small solid loss indicates that delignification may not be the main cause of enhanced sugar yields.

**Influences of different conditions on digestibility of corn stover**

As shown in Table 2, the SS effect on sugar yields were not significant based on α level equal to 0.05 and it was not involved in any significant interaction effects. However, a two-way interaction of Conc×T was substantiated, so instead of examining the main effects of Conc and T, the simple effect of Conc×T should be examined at a given level of the other.

**Table 2** P values of various effects on individual sugar yields from ANOVA

| Effect | Glucose | Xylose |
|---|---|---|
| Conc | <0.0001 | <0.0001 |
| SS | 0.7295 | 0.6274 |
| Conc×SS | 0.2088 | 0.1035 |
| T | 0.0188 | 0.0045 |
| Conc×T | <0.0001 | <0.0001 |
| SS×T | 0.7338 | 0.7860 |
| Conc×SS×T | 0.6217 | 0.6055 |

As a simple notation, four Conc levels were ranked in increasing order as Conc1 to Conc4. Fig 1 shows the relation of sugar yields with alkali Conc after washing. Positive linear relationships of sugar yields with alkali concentration were substantiated. Linear least squares fitting for sugar yields against alkali concentrations yielded R-squares from 91% to 95%. For sugar yields, Conc2 was slightly greater than Conc1 but not statistically significant; the adjusted p-values for the difference between Conc1 and Con2 were 0.4384 for glucose yield and 0.4365 for xylose yield. However, for any other comparisons made between two

Conc levels, sugar yields increased significantly as alkali concentration increased with adjusted P-values less than 0.0001. With washing, from Conc1 to Conc4, the glucose and xylose yields increased from 38.2% to 78.4% and 20.4% to 43.5%, respectively. The results were in accordance with expectation, that is, the higher the concentration of alkali solutions, the higher the sugar yields. Sodium hydroxide, as a strong alkali, was found to cause swelling resulting in increased internal pore volume and transformation of lignin structure as well as separation of structural linkages between lignin and carbohydrates (Fan et al., 1987). The effects imposed by sodium hydroxide removed nonproductive adsorption sites and enhanced significantly the access of enzyme to cellulose and hemicelluloses (Lee and Fan 1982).

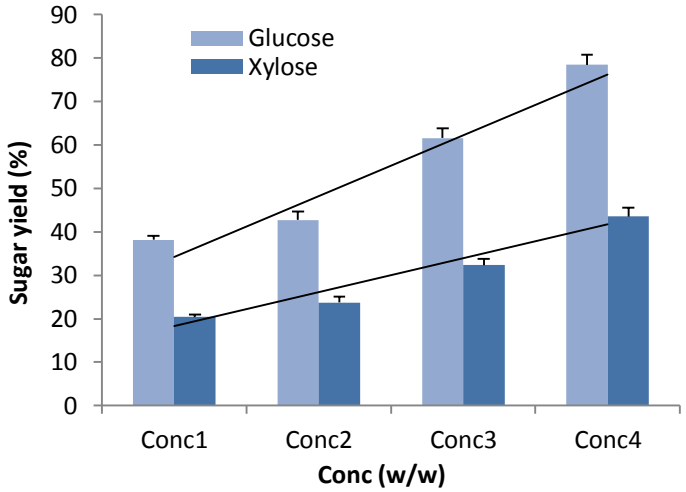


**Fig 1** Effect of alkali concentrations on sugar yields with washing

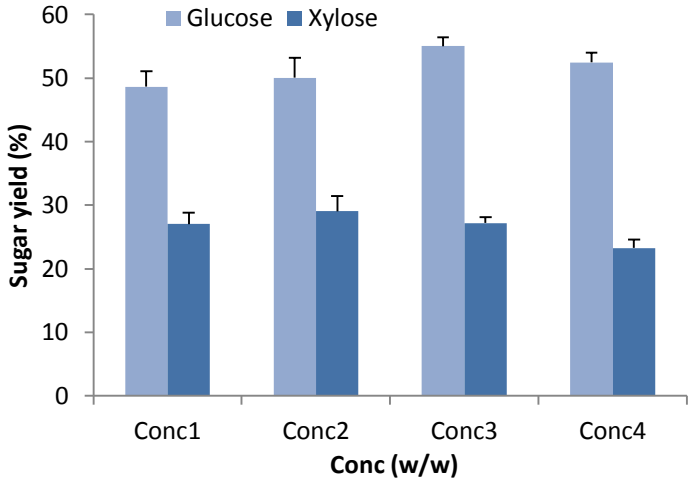


**Fig 2** Effect of alkali concentrations on sugar yields without washing

However, unlike the outstanding positive linear relation of alkali concentrations against sugar yields after washing, Fig 2 shows that the unwashed samples had no significant trend for sugar yields against alkali concentrations. All comparisons made between any two concentration levels had adjusted P-values much greater than 0.05. The trends for sugars yields were similar though not statistically significant where sugar yields increased to a medium concentration level which was either Conc2 or Conc3 and then decreased when Conc4 was applied. The relatively apparent decrease in Conc4 was due to the fact that Conc4 resulted in a much higher pH, which suppressed the activity of enzymes in the following hydrolysis.

From the Conc×SS×T least squares means (data are not shown here), the highest glucose and xylose yields for unwashed samples were found to be 56.3%

and 32.8%, which were much less than the washed counterparts which were 86.8% and 50.5%. However, the differences between washed and unwashed samples were not unidirectional at different concentration levels. The trends for sugar yields were the same; only glucose yield (GY) was shown here. It can be seen from Fig 3 that at lower concentrations, the unwashed samples had higher glucose yields than the washed samples. At Conc1, the glucose yield decreased from 48.7% to 38.2% with an adjusted P-value of 0.0013 when switching the processing type from unwashed to washed; also at Conc2, the glucose yield decreased from 50.1% to 42.7% with an adjusted P-value of 0.0188 from unwashed to washed. Nevertheless, at comparatively high concentration levels, the relation was converse. At Conc3, when switching from unwashed to washed, the glucose yield rose from 55% to 61.6% with an adjusted P-value of 0.0344; at Conc4, the glucose yield increased the most from unwashed to washed which rose by 49% with an adjusted P-value less than 0.0001. In general, the advantage of washed over unwashed was more pronounced at high concentrations, but at low concentrations, washing produced a negative impact. One possible reason could be the air drying mode adopted. It was shown in other studies that different drying modes resulted in variations of pore distributions (Esteghlalian et al., 2001). It was also declared that pore collapsed during drying as a result of surface-tension which led to irreversible changes of cellulose networks and was believed to be adverse toward cellulose conversion (Laivins and Scallan 1993). Therefore, it can

be inferred that the negative effect of pore collapse outweighed the positive impact of washing on cellulose conversion under circumstances of low alkali concentrations. However, as shown by Chandra et al. (2008), air drying at high relative humidity was effective in preserving the accessibility of pretreated samples compared to other drying methods such as oven drying. Moreover, higher alkali concentrations brought in higher pH environment for enzymes, making washing necessary to neutralize the samples. Thus, it was assumed that the disadvantageous pore collapse on cellulose conversion due to air drying was counteracted by the favorable effect of washing at high concentrations.

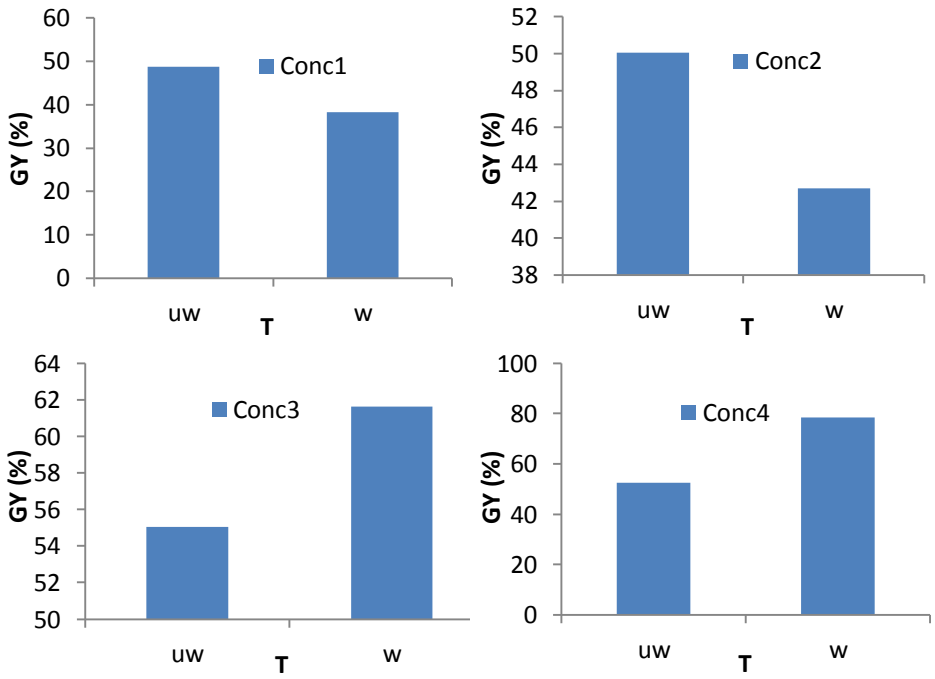


**Fig 3** Effect of post extrusion processing types (uw: without washing; w: with washing) on glucose yields for four alkali concentrations

**Comments on the results**

The optimum treatment combination, 0.03 g/g biomass and 80 rpm combined with washing, achieved a glucose yield of 86.8% and xylose yield of 50.5%. The sugar yields for alkali controls were found to be significantly smaller than alkali-based extrusion. With NREL's theoretical ethanol yield calculator, the potential ethanol production from glucose was 49.7 gallons per dry ton of corn stover. Based on the total of glucose and xylose, the theoretical ethanol production was 66.8 gallons per dry ton of corn stover. The present optimum total sugar yield was 25 g/l which was greater than what was reported by Karunanithy and Muthukumarappan (2010) as 17.39 g/l using single screw extruder and a much higher enzyme dosage. Also, the result was comparable to the maximum monomeric sugars of 23.6 g/l obtained for miscanthus by de Vrije et al. (2002). It should be noted that this pretreatment was a high solid content method (above 60%), so the demand for alkali was reduced tremendously compared to the usual consumption of 0.05-0.3 g NaOH per gram of biomass (Hu and Wen 2008) and, also, it was believed that little solid loss was induced compared to aqueous processes. Another advantage worth mentioning is that the enzyme dosage adopted, 0.028 g enzyme/g dry corn stover, was quite commercially feasible. It was shown by Liz Moore (2010) that enzyme devoted significantly to the cost of ethanol and the targeted enzyme contribution to ethanol selling price for 2012 was set to be 8% of a single dollar's worth of ethanol. Therefore, a low enzyme

loading ratio is of utmost necessity to achieve a commercially applicable cellulose conversion process.

**Crystallinity Index**

As shown in Fig 4, the untreated material presented cellulose I structure with characteristic peaks around 14.8° and 16.8° and also a maximum peak around 22.6°. The alkali combined with extrusion led to an obvious crystal lattice conversion from cellulose I toward cellulose II which was substantiated by the reflections peaking around 2θ =12.1° and ambiguous shoulders at 19.8° (Fig 5-Fig 8). However, the existence of certain amount of cellulose I was observed in the retention of reflections peaking between 14° to 17° for the samples pretreated with alkali combined extrusion. Though the cellulose polymorph was changed due to alkali extrusion, yet the CIs were not significantly different among the extruded samples and the control. As shown in Table 3, the CIs were reported as means with standard deviations in parentheses. This could have been due to the low inputs of sodium hydroxide which were 0.0039, 0.0119, 0.01 and 0.03 g/g biomass, respectively. Furthermore, it was concluded by Chang and Holtzapple (2000) that the impact of crystallization was more pronounced on initial enzyme hydrolysis rate but less on final sugar yields, thus, this study could be credited as an efficient method to improve ultimate hydrolysis yields of corn stover without spending excessive energy to destroy the crystallinity.

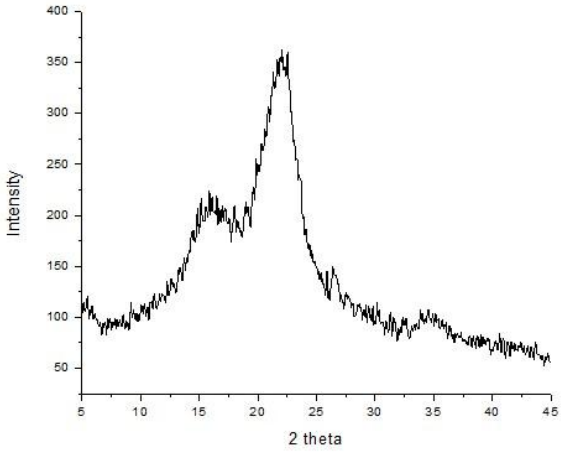


**Fig 4** X-ray diffractogram for raw material

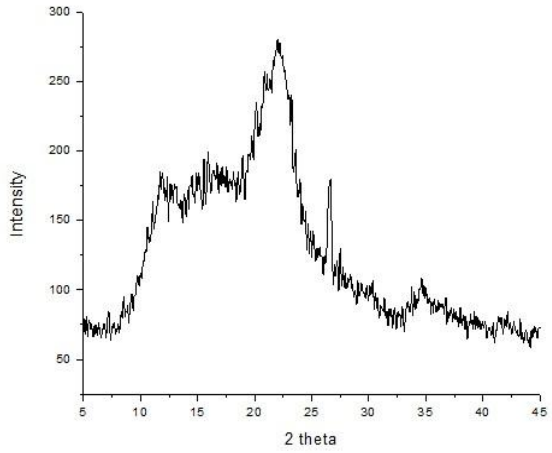


**Fig 5** X-ray diffractogram for sample treated with Conc1 nested within 80 rpm and washing

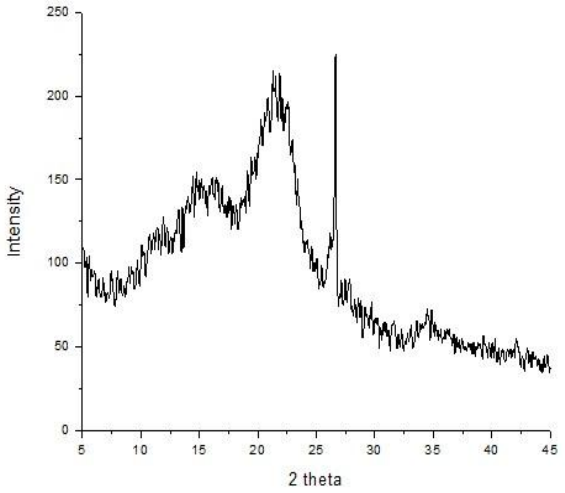


**Fig 6** X-ray diffractogram for sample treated with Conc2 nested within 80 rpm and washing

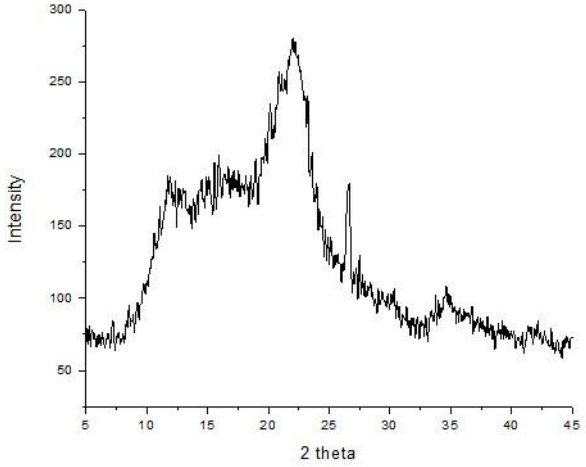


**Fig 7** X-ray diffractogram for sample treated with Conc3 nested within 80 rpm and washing

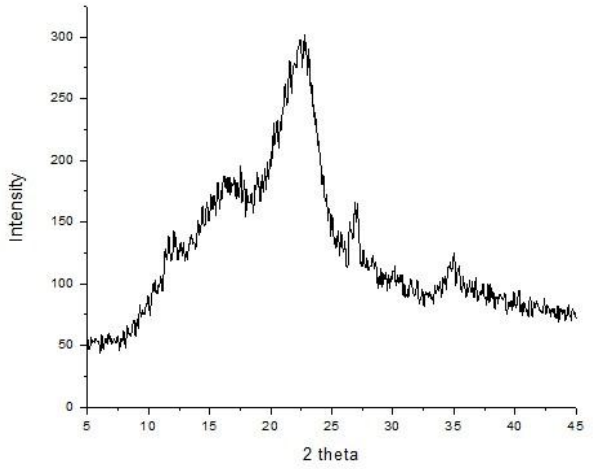


**Fig 8** X-ray diffractogram for sample treated with Conc4 nested within 80 rpm and washing

**Table 3** Relative crystallinity of selected samples

| Samples | C.I. |
|---|---|
| Control | 0.5221 (0.0338) |
| Conc1/80 rpm/w | 0.4805 (0.0245) |
| Conc2/80 rpm/w | 0.5250 (0.1205) |
| Conc3/80 rpm/w | 0.5046 (0.004) |
| Conc4/80 rpm/w | 0.5791 (0.0811) |

**Correlation of pore quantity with glucose yield**

It was proposed by Keshwani and Cheng (2010) that a strong alkali like NaOH was prone to produce larger pores within the range of the molecular size for orange dye (5-7 nm) and also the number of larger pores could more precisely reflect the accessibility of biomass. Congo red can penetrate pores larger than 2.6 nm, thus providing a means to semi-quantify the meso and large scale pores. The samples pretreated with 80 rpm and washing at four different alkali loading ratios

were selected to examine the correlation of pore quantity and glucose yield. Though the DAOS (dye adsorbed on substrate) for pretreated samples were significantly higher than that of the untreated material, the correlation of dye adsorption with glucose yield (xylose is the same), as shown in Fig 9, was not in full agreement with the proposition of Keshwani and Cheng (2010). As seen in Fig 9, while concentration level increased from Conc1 to Conc3, the DAOS increased from 72.6 mg/g to 147.9 mg/g with an adjusted P-value of 0.0003 which had the same trend as glucose yield which increased from 39% to 66% with an adjusted P-value of 0.0004. However, when Conc4 was implemented, the DAOS fell dramatically to 79 mg/g which was totally the reverse of what happened with glucose yield increasing from 66% to 86.8% with an adjusted P-value of 0.0071. The reason could have been that Conc4 led to transformation of lignin structure or removed a large number of acetyl groups from hemicellulose (Kong et al., 1992) which had more impacts on glucose yield than pore quantities. Also, it was assumed that higher alkali concentrations would not necessarily result in more large pores within the scope of this study.

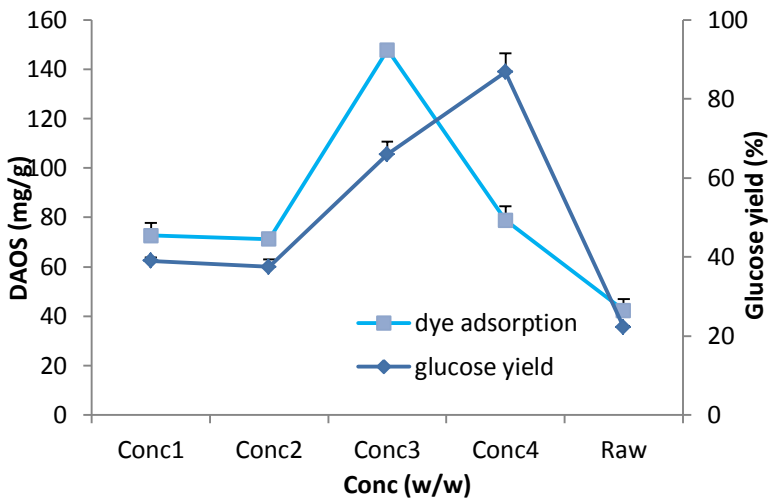


**Fig 9** Comparison of dye adsorption with glucose yield

CONCLUSIONS

This study examined the effects of different pretreatment conditions on final sugar yields for alkali-combined extrusion and also clarified the factors contributing to the improvement of sugar yields. The optimum glucose and xylose sugar yields were 86.8% and 50.5%, respectively, at alkali loading ratio of 0.03 g/g biomass, screw speed of 80 rpm and washing which was a great improvement over the untreated corn stover. And the theoretical ethanol production was 66.8 gallons per dry ton of corn stover based on total sugar yield. The small amount of sodium hydroxide led to a significantly greater number of large pores than the untreated, which appeared to be favorable on ultimate sugar yields. Alkali combined with extrusion shows promise to be a commercially feasible pretreatment method for corn stover with low enzyme dosage, high solids content, high throughput and less chemical demand compared to other approaches.

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# OVERALL CONCLUSIONS

The cost of cellulosic ethanol has been challenged by costs of feedstock, pretreatment and enzyme. The consumption of enzyme per grams of dry biomass is not explicitly mentioned. Therefore, the high yields obtained may be at the price of high enzyme input and other harsh operating conditions. Extruders, as readily scalable equipment, have well-established vendor quotes and can be used as a pretreatment unit operation in a biorefinery, offering good process control and excellent adaptability to chemicals (reactive extrusion) as well as high throughput leading to low specific mechanical energy requirement.

The first manuscript targeted fibrillation of corn stover with continuous twin-screw extruder to get improved sugar yields and shedding light on the intrinsic factors contributing to the improvement. No chemicals were applied, thus no extra processing was needed prior to enzymatic hydrolysis. The highest glucose and xylose sugar yields were 48.79% and 24.98%, respectively, and were found at 27.5% moisture content (w.b.) and 80 rpm at an enzyme dosage which was commercially viable (0.028 g enzyme/g dry corn stover). The values were 2.2 and 6.6 times higher than those of untreated corn stover. It was observed that crystallinity was not the main property underlying the improvement. Nevertheless, specific surface area was found to be closely related to enhanced sugar yields showing that twin-screw extrusion opened the cell walls of corn

stover at the microscopic level which was enough for efficient enzyme adsorption on cellulose.

The second manuscript examined the effects of different conditions on final sugar yields for alkali combined extrusion and also clarified the factors contributing to the improvement of sugar yields. The optimum glucose and xylose sugar yields were 86.8% and 50.5%, at alkali loading ratio of 0.03 g/g biomass, screw speed of 80 rpm and washing which was a significant improvement over the untreated corn stover and alkali controls. And the theoretical ethanol production was 66.8 gallons per dry ton of corn stover based on total sugar yield. Pore quantity was found to be immediately related to ultimate sugar yields. Alkali combined with extrusion shows promise to be a commercially feasible pretreatment method for corn stover with low enzyme dosage, high solids content, high throughput and less chemical demand compared to other approaches.

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# RECOMMENDATIONS FOR FUTURE RESEARCH

In the first manuscript, twin-screw extrusion was shown to produce significantly higher sugar yields than the untreated material. In order to further boost the sugar yields, strong alkali of sodium hydroxide was combined with twin-screw extrusion. At the low enzyme dosage of 8.4% (cellulose basis) and solids ratio of 6.1% (w/w), the sugar yields achieved were greatly amplified over the extrusion-only and untreated corn stover. However, there is still room for improvement. The drying mode adopted in the second manuscript was air drying, and it was proposed that air drying had an adverse impact on pore preservation and led to impaired enzymatic hydrolysis compared to never-dried state and freeze drying. Therefore, further efforts should be devoted to optimization of the drying mode. In addition, the time of alkali treatment could be further optimized to shorten the whole process as much as possible.

Moreover, the enzyme dosage and solids ratio for enzyme saccharification can be tested over a range of values to get corresponding sugar yields. The results can be applied in design of an enzymatic hydrolysis reactor.

Finally, twin-screw extrusion can be investigated over a wide range of temperatures, screw speeds and feed rates to optimize the operating parameters. Also the screw segments can be rearranged to achieve different shear rates. After locating the optimum solid-to-liquid ratio, enzyme dosage and operating

parameters, the whole biomass to sugar process can be made continuous by adding various unit operations. The process economics of the whole process can be potentially evaluated using simulation software such as Aspen Plus.

# APPENDIX A - NOTES ON COMPOSITIONAL ANALYSES

1. As preparation of biomass for subsequent compositional analyses, air-dry the corn stover and then feed it to the mill until the entire sample passes through the 2 mm screen at the bottom of the mill.

2. Make sure to take a representative sample for analyses. Also determine the total solids in corn stover prior to any compositional analysis.

3. Determination of extractives should be performed prior to determination of structural carbohydrates and lignin in biomass to improve the precision of analysis. Failure to remove extractable materials may cause an error in structural sugar values because hydrophobic extractives inhibit penetration of the sulfuric acid into the sample leading to incomplete hydrolysis and also may give rise to biased high lignin content due to the fact that unhydrolyzed carbohydrates condense with acid insoluble lignin.

4. Samples with ash content above 10 wt% may cause interference with proper acid concentrations and may catalyze side reactions; Samples with a moisture content above 10 wt% will cause perturbation to appropriate acid concentrations, therefore, samples need to be air dried or oven dried below 40 centigrade because high temperature will lead to irreversible change of biomass structure; for quantification on HPLC, some guard columns may cause artifact peaks which can be identified through running individual carbohydrates on columns and guard columns.

5. All compositions are reported on the “as received” basis, the “extractives free” basis can be converted to “as received” basis through extractives contents.

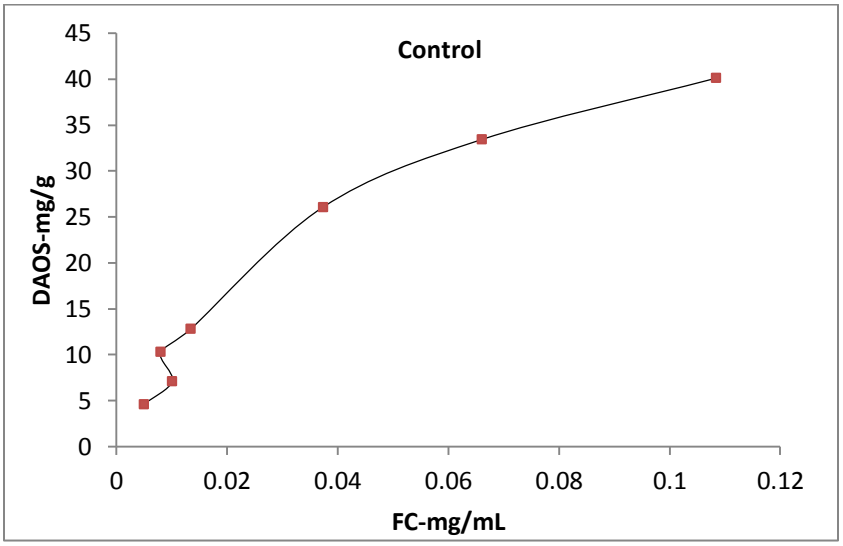
# APPENDIX B - PRELIMINARY STUDIES ON ENZYME HYDROLYSIS AND DYE ADSORPTION

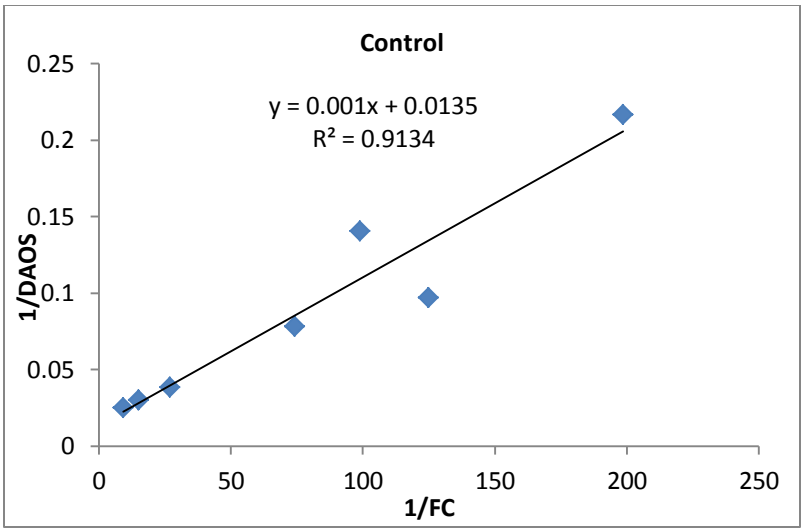
Enzyme Hydrolysis using Cellic Ctec2 in 1 M citrate buffer:

| Treatment Combination | Glucose yield (%) | Xylose yield (%) | Combined yield (%) |
|---|---|---|---|
| 22.5% MC (w.b.)/ 40 rpm | 23.03676 | 14.10669 | 19.76657 |
| 22.5% MC (w.b.)/ 60 rpm | 28.36579 | 16.52835 | 24.03092 |
| 22.5% MC (w.b.)/ 80 rpm | 24.16532 | 15.42709 | 20.96538 |
| 22.5% MC (w.b.)/ 100 rpm | 21.44228 | 12.99458 | 18.34873 |
| 25% MC (w.b.)/ 40 rpm | 26.82618 | 16.67772 | 23.10981 |
| 25% MC (w.b.)/ 60 rpm | 25.90706 | 16.62348 | 22.50741 |
| 25% MC (w.b.)/ 80 rpm | 27.52107 | 18.03041 | 24.04558 |
| 25% MC (w.b.)/ 100 rpm | 28.35399 | 20.42629 | 25.45087 |
| 27.5% MC (w.b.)/ 40 rpm | 22.871 | 15.68973 | 20.24122 |
| 27.5% MC (w.b.)/ 60 rpm | 27.60336 | 18.93939 | 24.43061 |
| 27.5% MC (w.b.)/ 80 rpm | 25.32032 | 18.37905 | 22.77842 |
| 27.5% MC (w.b.)/ 100 rpm | 24.50703 | 17.28491 | 21.86229 |
| Control | 17.78185 | 7.97324 | 14.18993 |

Congo red dye adsorption isotherm for the untreated corn stover:

| Dye Concentration | Abs | FC-mg/mL | DAOS-mg/g | 1/FC | 1/DAOS |
|---|---|---|---|---|---|
| 0.5% ows | 0.049666667 | 0.005037468 | 4.6162532 | 198.512422 | 0.2166259 |
| 0.8% ows | 0.127 | 0.01011874 | 7.128126 | 98.8265306 | 0.14028933 |
| 1.1% ows | 0.095 | 0.008016145 | 10.298386 | 124.748244 | 0.0971026 |
| 1.4% ows | 0.178 | 0.013469752 | 12.813025 | 74.2404181 | 0.07804558 |
| 2.8% ows | 0.417 | 0.037342094 | 26.065791 | 26.7794301 | 0.03836446 |
| 4.0% ows | 0.759 | 0.066105599 | 33.44944 | 15.1273117 | 0.02989587 |
| 5.0% ows | 1.261 | 0.108325715 | 40.107429 | 9.23141845 | 0.02493304 |




Control
y = 0.001x + 0.0135
R² = 0.9134
1/DAOS
1/FC
0.25
0.2
0.15
0.1
0.05
0
0
50
100
150
200
250

# APPENDIX C - DATASETS FOR SUGAR HYDROLYSIS YIELDS, SPECIFIC SURFACE AREA, PORE QUANTITIES AND CRYSTALLINITY

1. Sugar hydrolysis yields:

| | | Extrusion only | | | |
|---|---|---|---|---|---|
| Rep#1 | GY(%) | XY(%) | Rep#2 | GY(%) | XY(%) |
| Raw | 22.41 | 4.57 | Raw | 21.61 | 3.04 |
| 22.5%/40 | 38.89 | 16.56 | 22.5%/40 | 44.51 | 23.25 |
| 22.5%/60 | 37.13 | 15.61 | 22.5%/60 | 45.77 | 24.09 |
| 22.5%/80 | 44.22 | 20.47 | 22.5%/80 | 46.10 | 24.60 |
| 22.5%/100 | 50.05 | 24.18 | 22.5%/100 | 45.38 | 24.31 |
| 25%/40 | 41.54 | 17.23 | 25%/40 | 46.48 | 25.82 |
| 25%/60 | 51.1 | 23.95 | 25%/60 | 41.99 | 22.25 |
| 25%/80 | 48.1 | 22.51 | 25%/80 | 47.57 | 25.61 |
| 25%/100 | 46.02 | 21.86 | 25%/100 | 46.28 | 25.33 |
| 27.5%/40 | 52.85 | 25.32 | 27.5%/40 | 42.22 | 22.69 |
| 27.5%/60 | 46.32 | 20.55 | 27.5%/60 | 44.69 | 23.61 |
| 27.5%/80 | 49.45 | 23.45 | 27.5%/80 | 48.14 | 26.52 |
| 27.5%/100 | 43.56 | 18.78 | 27.5%/100 | 41.74 | 21.50 |
| 22.5%/120 | 38.46 | 20.13 | 22.5%/120 | 48.44 | 26.08 |
| 22.5%/140 | 39.99 | 20.84 | 22.5%/140 | 42.65 | 22.38 |
| 25%/120 | 40.35 | 20.57 | 25%/120 | 38.92 | 19.55 |
| 25%/140 | 32.66 | 16.27 | 25%/140 | 40.84 | 20.97 |
| 27.5%/120 | 40.18 | 20.31 | 27.5%/120 | 42.45 | 20.77 |
| 27.5%/140 | 45.45 | 23.44 | 27.5%/140 | 36.35 | 20.28 |

Alkali extrusion

| Rep#1 | GY(%) | XY(%) | Rep#2 | GY(%) | XY(%) |
|---|---|---|---|---|---|
| 1%/40/w | 38.65 | 20.98 | 1%/40/w | 39.22 | 21.09 |
| 1%/40/uw | 40.45 | 20.90 | 1%/40/uw | 51.39 | 28.73 |
| 1%/60/w | 38.53 | 21.15 | 1%/60/w | 36.09 | 18.70 |
| 1%/60/uw | 42.21 | 22.43 | 1%/60/uw | 49.02 | 27.63 |
| 1%/80/w | 38.15 | 20.72 | 1%/80/w | 39.93 | 21.03 |
| 1%/80/uw | 47.53 | 26.42 | 1%/80/uw | 50.88 | 28.83 |
| 1%/100/w | 41.36 | 22.55 | 1%/100/w | 33.61 | 16.75 |
| 1%/100/uw | 45.07 | 24.24 | 1 %/100/uw | 62.81 | 37.13 |
| 3%/40/w | 42.40 | 24.25 | 3%/40/w | 46.17 | 26.09 |
| 3%/40/uw | 40.28 | 22.34 | 3%/40/uw | 67.79 | 43.18 |
| 3%/60/w | 50.43 | 29.36 | 3%/60/w | 48.54 | 27.37 |
| 3%/60/uw | 46.62 | 26.28 | 3%/60/uw | 55.95 | 33.05 |
| 3%/80/w | 35.65 | 19.18 | 3%/80/w | 39.41 | 21.08 |
| 3%/80/uw | 42.26 | 23.28 | 3%/80/uw | 51.68 | 29.71 |
| 3%/100/w | 36.60 | 19.66 | 3%/100/w | 42.46 | 22.66 |
| 3%/100/uw | 44.30 | 24.14 | 3%/100/uw | 51.66 | 30.46 |
| 5.3%/40/w | 60.51 | 27.93 | 5.3%/40/w | 50.07 | 25.44 |
| 5.3%/40/uw | 56.70 | 26.45 | 5.3%/40/uw | 50.41 | 24.50 |
| 5.3%/60/w | 63.23 | 33.55 | 5.3%/60/w | 57.13 | 30.53 |
| 5.3%/60/uw | 49.47 | 23.50 | 5.3%/60/uw | 61.55 | 31.64 |
| 5.3%/80/w | 69.22 | 37.88 | 5.3%/80/w | 62.70 | 33.45 |
| 5.3%/80/uw | 56.08 | 27.67 | 5.3%/80/uw | 56.56 | 29.99 |
| 5.3%/100/w | 69.27 | 36.71 | 5.3%/100/w | 60.85 | 33.33 |
| 5.3%/100/uw | 55.39 | 26.84 | 5.3%/100/uw | 54.24 | 26.99 |
| 14.4%/40/w | 78.07 | 43.83 | 14.4%/40/w | 80.17 | 45.78 |
| 14.4%/40/uw | 46.42 | 20.26 | 14.4%/40/uw | 47.50 | 19.54 |
| 14.4%/60/w | 75.24 | 40.07 | 14.4%/60/w | 71.21 | 37.60 |
| 14.4%/60/uw | 48.19 | 16.96 | 14.4%/60/uw | 55.66 | 27.74 |
| 14.4%/80/w | 82.03 | 46.0 | 14.4%/80/w | 91.62 | 55.05 |
| 14.4%/80/uw | 53.56 | 26.25 | 14.4%/80/uw | 55.99 | 25.72 |
| 14.4%/100/w | 77.09 | 41.41 | 14.4%/100/w | 72.1 | 38.35 |
| 14.4%/100/uw | 56.73 | 23.36 | 14.4%/100/uw | 55.64 | 26.29 |

2. Dye adsorption experiments for estimation of specific surface areas of samples pretreated with only extrusion:

| 27.5%/80rpm (maximum) | | 25%/140rpm (minimum) | | Raw | |
|---|---|---|---|---|---|
| FC-mg/mL | DAOS-mg/g | FC-mg/mL | DAOS-mg/g | FC-mg/mL | DAOS-mg/g |
| 0.002018116 | 2.29818839 | 0.00239358 | 2.26064204 | 0.0026126 | 2.23874001 |
| 0.002862909 | 4.71370911 | 0.002018116 | 4.79818839 | 0.002909842 | 4.70901582 |
| 0.002088516 | 9.79114845 | 0.00239358 | 9.76064204 | 0.002581311 | 9.74186887 |
| 0.007650068 | 19.2349932 | 0.009527385 | 19.0472615 | 0.013798282 | 18.6201718 |
| 0.049091848 | 45.0908152 | 0.04619765 | 45.380235 | 0.139625475 | 36.0374525 |
| 0.379218097 | 62.0781903 | 0.431782982 | 56.8217018 | 0.512507627 | 48.7492373 |
| 0.001361055 | 2.36389449 | 0.002252781 | 2.27472192 | 0.00351997 | 2.148003 |
| 0.001548787 | 4.84512132 | 0.004583783 | 4.54162169 | 0.004130098 | 4.58699019 |
| 0.004020588 | 9.59794121 | 0.004552494 | 9.54475055 | 0.006382879 | 9.36171211 |
| 0.005631952 | 19.4368048 | 0.004693293 | 19.5306707 | 0.01440841 | 18.559159 |
| 0.04177031 | 45.822969 | 0.042755902 | 45.7244098 | 0.133758859 | 36.6241141 |
| 0.282536256 | 71.7463744 | 0.3632609 | 63.67391 | 0.447740179 | 55.2259821 |

3. Quantification of pores for alkali combined extrusion:

| S.Blank | Abs |
|---|---|
| 1% alk/80/w | 0.011 |
| 3% alk/80/w | 0.029 |
| 5% alk/80/w | 0.004 |
| 14% alk/80/w | 0.015 |
| Raw | 0.053 |

| Treatment | Abs/Rep1 | Abs/Rep2 | FC (mg/mL)/Rep1 | FC (mg/mL)/Rep2 | DAOS (mg/g)/Rep1 | DAOS (mg/g)/Rep2 |
|---|---|---|---|---|---|---|
| 1%/80/w | 1.276 | 1.38367 | 1.22307223 | 1.324134478 | 77.69277702 | 67.58655215 |
| 3%/80/w | 1.333 | 1.356 | 1.276575773 | 1.298164922 | 72.34242268 | 70.18350777 |
| 5%/80/w | 0.515 | 0.541 | 0.508752992 | 0.533158117 | 149.1247008 | 146.6841883 |
| 14%/80/w | 1.202 | 1.323 | 1.153611489 | 1.267189187 | 84.63885108 | 73.28108133 |
| Raw | 0.838 | 0.788 | 1.623879476 | 1.530013611 | 37.61205238 | 46.99863894 |

4. Crystallinity indices

| Extrusion only | | | | |
|---|---|---|---|---|
| C.I. | Rep#1 | Rep#2 | Mean | S.D. |
| Raw | 0.87402 | 0.87273 | 0.873375 | 0.000912 |
| 27.5%/80 rpm | 0.834688 | 0.869231 | 0.85196 | 0.024425 |
| 25%/140 rpm | 0.87154 | 0.8507 | 0.86112 | 0.014736 |
| 22.5%/100 rpm | 0.90107 | 0.861925 | 0.881497 | 0.02768 |

| Alkali combined extrusion | | | | |
|---|---|---|---|---|
| C.I. | Rep#1 | Rep#2 | Mean | S.D. |
| Raw | 0.87402 | 0.87273 | 0.873375 | 0.000912 |
| 1%/80/w | 0.845614 | 0.795556 | 0.820585 | 0.035397 |
| 3%/80/w | 0.874016 | 0.837963 | 0.855989 | 0.025493 |
| 5%/80/w | 0.875622 | 0.804878 | 0.84025 | 0.050023 |
| 14%/80/w | 0.84399 | 0.866883 | 0.855436 | 0.016188 |