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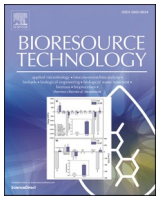

High-efficiency conversion of corn bran to ethanol at 150 L scale

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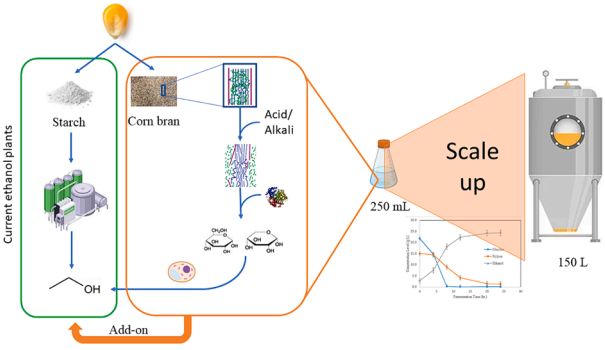
HIGHLIGHTS

- Dilute acid pretreatment of corn bran with low temperature (90 °C for 60 min).
- High sugar yields (95% for starch, 77% for both cellulose and xylan).
- Highly efficient fermentation (100 % glucose and 91 % xylose conversion within 24 h).
- The process was scaled up to 150-L.

GRAPHICAL ABSTRACT



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ABSTRACT

Fractionated corn bran was processed to maximize ethanol production from starch, cellulose, and xylan. After various bench-scale experiments, an optimized process with dilute acid pretreatment (1.5 % w/w H_2SO_4) at 90 °C for 60 min was utilized followed by enzymatic hydrolysis using cellulase and hemicellulase for 48 hr. After simultaneous saccharification (regarding starch) and fermentation at 150 L using an engineered yeast, which consumes both glucose and xylose to make ethanol, the 86 % total sugar conversion yield was achieved, including conversions of 95 % for starch, 77 % for cellulose and 77 % for xylan. Also, an accurate mass balance was formulated for ethanol-producing carbohydrates including starch, cellulose, and xylan from feedstock to final ethanol. A highly efficient process of converting corn fiber to ethanol was successfully scaled up to 150 L.

1. Introduction

Biofuel production plays a critical role in the energy transition, greenhouse gas reduction, and improved national energy security (Khanna et al., 2011). First-generation bioethanol, which is produced from sugar and starch crops, is commercially successful. The United States is the largest corn producer in the world (~15 billion bushels in 2023, USDA). About 34 % of the corn produced was used for bioethanol production in 2023, which makes the US also the largest bioethanol producer (generating ~ 17.7 billion gallons, U.S. Energy Information

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Administration). Corn kernels are 60 % starch, which is converted to glucose for yeast fermentation to make ethanol, However, there is also a fiber-rich portion, mostly from the hard outer shell layer of a corn kernel, which contains about 7 % hemicellulose and 3 % cellulose, and these carbohydrates are potentially available to be converted to monosaccharides (glucose and xylose) for yeast fermentation to make ethanol. Ethanol can be used as a direct fuel and as a building block for higher carbon chain fuels such as sustainable aviation fuels. Presently, the corn bran (or corn fiber) ends up in lower-value distiller's dried grains with solubles (DDGS) and is used for animal feed. Corn fiber accounts for approximately 10 % w/w (dry basis) of the corn kernel (Kurambhatti et al., 2018), and is composed of 15 %–20 % cellulose, 30 %–50 % hemicellulose (primary arabinoxylan), and 10 %–25 % adherent starch (Gáspár et al., 2007; Kim et al., 2016). The ability to use corn fiber for ethanol production will increase ethanol yield per bushel of corn, increase the protein content of the feed co-product DDGS, and generate more revenue for ethanol manufacturers.

Currently, there are two main processes to convert corn fiber to ethanol. The first one is *in-situ* conversion (Li et al., 2018, 2019), which has a low sugar conversion yield because pretreatment is needed to fully expose cellulose and hemicellulose fibers (Mankar et al., 2021) for enzymatic hydrolysis to achieve high-sugar conversion. The second pathway is to fractionate the corn fiber through grinding and size classification operations, pretreat the fractionated corn fiber, and convert the sugars to ethanol. The presence of pretreatment exposes cellulose and hemicellulose fibers (Mankar et al., 2021) for enzymatic hydrolysis to achieve high-sugar conversion. The Fiber Separation Technology ™ (FST ™, ICM, Colwich, KS) is an example of a commercial process that recovers fiber before fermentation (front-end fractionation). The separation technology involves slurry flows through a separation device for liquid and solid separation, the solid is selectively flaked, and a rotary press removes fiber through counterflow washing.

Corn fiber has low lignin (the structure is also not fully developed as in wood) and high hemicellulose contents, therefore mild pretreatment is sufficient to achieve a high sugar yield. Various pretreatment methods have been explored, such as physical extrusion, dilute acid, dilute alkali, ammonia immersion, and hot steam (Kurambhatti et al., 2018; Li et al., 2019; Myat and Ryu, 2014), followed by hydrolysis using cellulase and hemicellulase enzyme cocktails, to convert cellulose and hemicellulose into C6 and C5 monosaccharides (primarily glucose and xylose) from corn fiber.

In this study, the corn fiber prepared with a front-end fractionation process was evaluated. A low-severity dilute-acid pretreatment process that operated at only 90 °C was optimized for enzymatic hydrolysis. The hydrolysate was successfully fermented to ethanol using an engineered yeast that can consume both glucose and xylose to make ethanol (C5C6 yeast). Next, the process was scaled up to 150 L fermentation and the pilot results were used to calculate sugar conversion yields for starch, cellulose, and xylan, and the total ethanol yield. Finally, a complete mass balance was presented for all the corn fiber components, including non-converted material that ends up in the DDGS.

2. Materials and methods

2.1. Yeast strain, cultivation, and material

Corn bran was obtained from Shockwave, LLC (Des Moines, Iowa). Commercially available cellulases (Ctec3), hemicellulases (Htec3), and glucoamylase (Spirizyme Fuel HS) were provided by Novozymes. Chemicals were purchased from MilliporeSigma (St. Louis, MO). The xylose-utilizing strain, an engineered *Saccharomyces cerevisiae* (Novozymes Strain 22273), was maintained in a 25 % (w/v) glycerol stock solution at − 80 °C. The seed culture was grown in 250 mL baffled flasks, which contain 100 mL of yeast extract − peptone – dextrose − xylose (YPDX) medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, and 10 g/L xylose) inoculated by 10 μL defrosted cell suspension from the glycerol stock, and the flask was maintained at 32 °C and 200 rpm for ~ 16 h. In general, the inoculum volume was 10 % (v/v) of the corn bran hydrolysate volume at either lab or 150 L scale.

2.2. Corn fiber pretreatment, hydrolysis and fermentation

In the bench scale study, about 23 g of ground corn bran (~10 % moisture) was mixed with 1.5 % w/w KOH or 1–3 % w/w H_2SO_4 aqueous solution (about 110 g) at 15 % w/w solids loading and pretreated at 90 °C or 105 °C for 60 min in a rotary mixer (Labomat, Germany). After cooling, the bran mash was adjusted to a pH of ~ 5.5 by adding 10 N H_2SO_4 or 10 N KOH, and tap water was added to dilute to 10 % total solids loading. Cellulase and hemicellulase were dosed to start the enzymatic hydrolysis (Ctec3 0.04 g/g cellulose, Htec3 0.03 g/g xylan), which was operated at 50 °C and 200 rpm for ~ 48 h. Next, to start the fermentation, Spirizyme Fuel HS (0.03 % w/w of starch in corn bran), urea (500 ppm nitrogen level), Lactrol (4 ppm), and the yeast inoculum (about 10 % v/v of the hydrolysate) were added to the hydrolysate; the fermentation was performed at 32 °C and 200 rpm for ~ 72 h.

In the 150 L scale-up study, multiple 5 L and 3 L vessels were used for the pretreatment and enzymatic hydrolysis trials to prepare a total volume of hydrolysate of 85 L. Sugar concentrations were measured for each batch of hydrolysate to ensure acceptable and repeatable sugar conversion. Hydrolysates were stored at −20 °C.

The propagation train for the 150 L fermentation trial was conducted in three steps: 1) 10 μL glycerol stock was inoculated into 100 mL YPDX medium and grown at 32 °C and 200 rpm for ~ 16 h; 2) the 100 ml medium obtained from Step 1 was inoculated into 900 mL new YPDX medium (10 % v/v inoculum) and grown at 32 °C and 200 rpm for ~ 24 h; 3) 10 L 50 % v/v hydrolysate medium (5 L YPDX medium from Step 2 and 5 L filtered hydrolysate from the multiple 5 L trials) was incubated at 32 °C, 200 rpm, and 2 L/min aeration for ~ 24 h (30 L bioreactor, Applikon, USA). Finally, 9 L of yeast inoculum (from step 3) was used to inoculate a 150 L bioreactor (ABEC, Springfield, MO) filled with 80 kg corn bran hydrolysate, Lactrol (4 ppm), urea (500 ppm nitrogen), and Spirizyme Fuel HS (0.03 % w/w of starch in corn bran). The fermentation was sampled periodically for sugars and ethanol. After 24 h, when the sugars, including glucose, xylose, and small oligosaccharides were almost consumed (lower than 2 %), the total mass of the beer (~90 L) was weighed and transferred to a vacuum oven (Model #IWT-560-DSP, United McGill, Columbus, OH) for drying at 55 °C for 2 days. The dry solids (e.g., DDGS) were weighed and stored at −20 °C. The weight of ethanol produced in the 150 L scale was determined using the following equation:

$$M_{\mathrm{EtOH}} = M_{final} - M_{initial} = \frac{\left(M_{\mathrm{beer,\,f}}\right)\left(\frac{100\ -\ S_{\mathrm{ss,\,beer}}}{100}\right)\left(\frac{C_{\mathrm{final}}}{100}\right)}{\rho_{\mathrm{beer,\,liq}}} - \frac{\left(M_{\mathrm{mash}}\right)\left(\frac{100\ -\ S_{\mathrm{ss,\,mash}}}{100}\right)\left(\frac{C_{\mathrm{inital}}}{100}\right)}{\rho_{\mathrm{mash,\,liq}}} \quad (1)$$

where M_{final} is the final mass of ethanol produced, $M_{initial}$ is the initial mass of ethanol at the beginning of the fermentation carried over from inoculation. $M_{beer,\,f}$ is the final mass of beer, M_{mash} is the initial mass of the mash (corn bran hydrolysates, yeast inoculation and nutrient additions). $S_{ss,\,beer}$ is the % (w/w) suspended solids in the final beer (which is calculated by taking the difference between the measured % (w/w) total solids and % (w/w) dissolved solids), $S_{ss,\,mash}$ is the % (w/w) suspended solids in the initial mash. C_{final} and $C_{initial}$ are the final and initial concentrations of ethanol measured by HPLC. $\rho_{beer,\,liq}$ is the density of the filtered beer supernatant measured by the Anton Paar densitometer. $\rho_{mash,\,liq}$ is the density of the filtered mash supernatant.

2.3. Analysis methods and compositional analysis

A method developed by the National Corn to Ethanol Research

Center (NCERC) specifically for corn matrix samples was used to determine total starch, cellulose, xylan, and other ethanol-producing carbohydrates (Kukielski et al., 2023). Also, AOAC official method 996.1 for total starch (KOH format) was modified to ensure that all the forms of starch in the corn matrix were converted to glucose at the end of the enzymatic hydrolysis. The NREL LAP method (Sluiter et al., 2012) with modification was used to measure cellulose and xylan. Acid hydrolysis at 121 °C helped to convert various carbohydrates to monomers, afterwards, specific enzymes (Ctec3 and Htec3) were added to ensure maximal conversion for both cellulose and xylan, and the method was validated for accuracy and precision.

The total solid percentage (% w/w) of the post-fermentation beer was measured by drying samples at 105 °C for 3 h. After fermentation, the dissolved percent solids of the beer sample were measured as follows: filtrate the beer samples through a 0.2 μm filter, and dry a small amount of clear filtrate at 105 °C for 3 h. Moreover, the density of the clear filtrate was measured using an Anton Paar densitometer (St. Louis, MO).

The glucose, xylose, and ethanol concentrations in the beer sample were measured using a Shimadzu 20 series (Shimadzu America, Columbia, MD) HPLC system equipped with a Shimadzu Series 10 refractive index detector. ~ 1 mL sample was filtered through a 0.2 μm filter and transferred to a 2 mL glass vial. The column used was a 30-cm × 7.8-mm I.D. Supelcogel C610H (Sigma Aldrich, St. Louis, MO) with a guard column (Supelcogel H Guard Column, 9 μm particle size, 5 cm × 4.6 mm, Sigma Aldrich, St. Louis, MO) maintained at 60 °C. The system was operated with a 0.01 N H_2SO_4 isocratic mobile phase pumped at 0.6 mL/min. The concentrations of 5-Hydroxymethylfurfural (HMF) and furfural were measured by HPLC using the same conditions but with a UV/UIS detector (SPD-20AV, Shimadzu America, Columbia, MD) at 280 nm. External standards were used to quantify their concentrations.

Sugar yields were calculated by the following equations:

$$Yield_{glu} = \frac{M_{actual,glu}}{M_{theoretical,glu}} \times 100\% = \frac{C_{glu}V}{M_{bran} \times Cel\% \times \frac{180}{162}} \times 100\% \quad (2)$$

$$Yield_{xyl} = \frac{M_{actual,xyl}}{M_{theoretical,xyl}} \times 100\% = \frac{C_{xyl}V}{M_{bran} \times HCel\% \times \frac{150}{132}} \times 100\% \quad (3)$$

$$V = \frac{(M_{\text{mash}})\left(\frac{100 - S_{ss,\ \text{mash}}}{100}\right)}{\rho_{\text{mash, liq}}} \quad (4)$$

where C_{glu} and C_{xyl} are the glucose and xylose concentration obtained from HPLC. M_{bran} is the initial dry weight of corn bran. Cel% and HCel% are the measured cellulose and hemicellulose (xylan) percentages in corn bran. Other definitions are the same as in Equation (1).

2.4. Statistical analysis

All experiments were at least duplicated for each condition studied, and the means with standard errors are reported. Student's *t*-test analysis with JMP software was performed to determine the significant difference ($p < 0.05$).

3. Results and discussion

3.1. Feedstock

Two batches of corn fiber materials (low and high fiber brans) produced with slightly different mechanical separation settings in 2020 and 2021 using Shockwave's proprietary technology were used: materials produced in 2020 were used for the lab bench study and materials produced in 2021 were used for the pilot study. As shown in Table 1, the levels of individual carbohydrates varied in the two types; in general, the High Fiber Bran (HFB) had lower starch (~21 %), higher cellulose (~11 %) and xylan (~25 %) contents while the Low Fiber Bran (LFB) had higher starch (~30 %), lower cellulose (~7%) and xylan (~16 %) contents. All corn bran had more than 50 % carbohydrates available, including starch, cellulose, and xylan. The goal of this study was to convert these carbohydrates to glucose and xylose and ferment the sugar mixture to ethanol using an engineered C5C6 yeast. This front-end fractionation technology can't do a complete separation between corn starch and brans. Significant starch is still contained in the fiber (20–30 %). Another characteristic of corn bran is it has a high hemicellulose (xylan) fraction. The cellulose and hemicellulose ratio is ~ 1:2. This differentiates corn bran from other lignocellulosic biomass, in which the cellulose and hemicellulose ratio is ~ 3:1. Like other lignocellulosic biomass, the composition of corn bran varied between different batches (Table 1).

Table 1
Ethanol-producing carbohydrates in two corn fiber materials (%, dry weight basis).

Produced in	Corn fiber type	Starch	Cellulose	Xylan
2020	High Fiber I	19.8 ± 3.2	12.2 ± 2.6	26.7 ± 2.9
	Low Fiber I	32.0 ± 2.6	6.2 ± 2.5	16.5 ± 1.7
2021	High Fiber II	22.9 ± 0.5	10.6 ± 0.7	24.2 ± 1.2
	Low Fiber II	26.8 ± 1.6	7.7 ± 0.8	16.1 ± 0.8
	DDGS *	2.5 ± 0.0	3.0 ± 0.4	7.0 ± 0.7

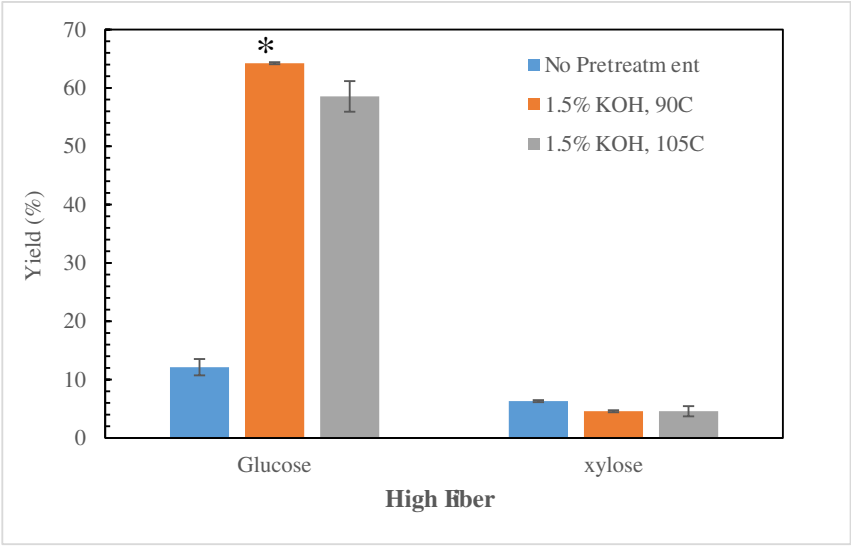
* From 150 L trial (used Low Fiber II as feedstock).

3.2. Alkali pretreatment

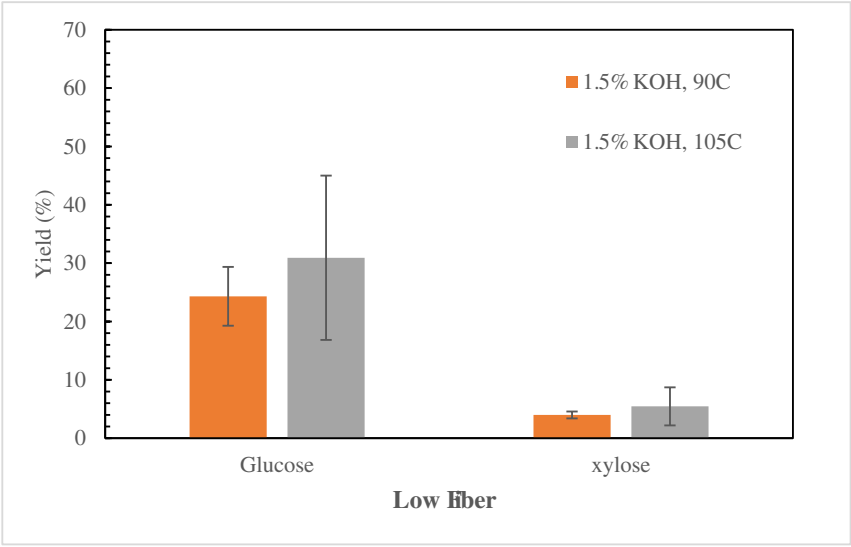
First, alkaline pretreatment was applied to prepare the corn bran for enzymatic conversion to glucose and xylose. Corn bran was treated with 1.5 % w/w KOH at 90 °C and 105 °C for 60 min, followed by enzymatic hydrolysis using Ctec3 and Htec3. The glucose and xylose concentrations in the hydrolysates from multiple shake flasks were measured, and then they were used to calculate the sugar conversion yield. The theoretical 100 % yield assumes all the starch, cellulose, and xylan in the feedstock were converted to glucose and xylose. The average glucose and xylose conversion yields with 10 % w/w total solid loading at both 90 °C and 105 °C are shown in Fig. 1-a for the HFB I and Fig. 1-b for LFB I. As a control, the low yields of glucose (12 %) and xylose (7 %) were obtained from untreated HBF I, which confirms that pretreatment is unavoidable for increasing sugar conversion yield. Although alkali pretreatment increased the glucose yield (64 % at 90 °C & 58 % at 105 °C), it did not increase the xylose yield (~5%). For LFB I, similar to HBF I, alkaline pretreatment increased glucose yield up to 24 % and 31 % at temperatures of 90 and 105 °C, respectively, but did not significantly increase the xylose yield (9 % at 90 °C & 12 % at 105 °C). Overall, alkali pretreatment followed by enzymatic hydrolysis delivered less than 10 % xylan conversion for both bran materials, which led to the evaluation of dilute-acid pretreatment.

3.3. Acid pretreatment

Three H_2SO_4 concentrations (1 %, 2 %, 3 % w/w) were used with multiple shake flasks at 90 °C followed by enzymatic hydrolysis for HFB I (Fig. 2-a) and LFB I (Fig. 2-b). The 1 % w/w H_2SO_4 pretreatment of HFB I gave glucose yield of 74 %, 66 % from 2 % w/w and 68 % from 3 % w/w. Furthermore, the xylose yield was 44 %, 72 %, and 77 % at 1 % w/w, 2 % w/w, and 3 % w/w, respectively. For LFB I, acid pretreatment also gave a higher sugar yield, especially a higher xylose yield. Glucose yield was 45 %, 39 %, and 46 % after pretreating with 1 % w/w, 2 % w/w, and 3 % w/w H_2SO_4 respectively. Similarly, the xylose yield from LFB I increased to 43 %, 73 %, and 83 % after pretreating with 1 % w/w, 2 % w/w, and 3 % w/w H_2SO_4 respectively. The glucose yield from LFB I was low (~40 %) because the starch was incompletely digested into



(a)



(b)

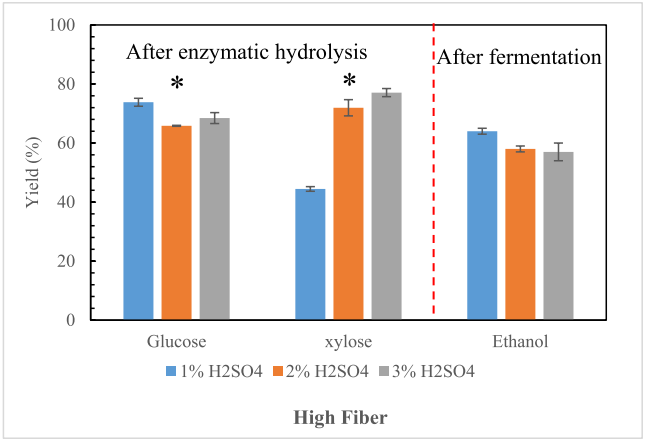
Fig. 1. Average sugar conversion yields from alkali pretreatment and enzymatic hydrolysis: (a) HFB I; (b) LFB I (* Significant differences ($p < 0.05$) between three sets of data).

oligosaccharides, so glucose was not obtained. During fermentation, when glucoamylase was added, these oligosaccharides were further converted into glucose.

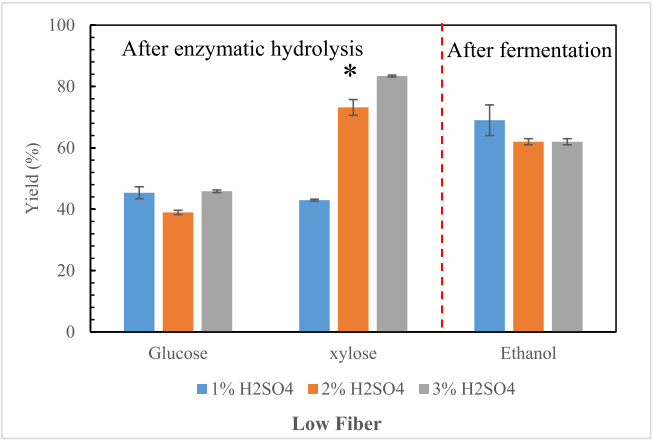
3.4. Fermentation

The hydrolysates from acid pretreatment were converted to ethanol in shake flask fermentations. The fermentation results at three acid concentrations demonstrated high ethanol titers (Fig. 2) and high sugar conversion (Table 2). The residue sugars were low, <1.2 g/L for glucose and < 3.3 g/L for xylose (except at 3 % w/w H_2SO_4 for HFB I) for both corn bran types. The highest byproduct was glycerol with < 2.1 g/L. The highest ethanol titers and yields (percentage compared to the theoretical conversion of cellulose, xylan, and starch to ethanol) were achieved with 1 % w/w H_2SO_4 pretreatment for both bran materials, 28.0 g/L & 64 % for HFB I and 27.5 g/L and 69 % for LFB I. Glucose and xylose in hydrolysates were converted at near theoretical efficiency to ethanol (1 mol glucose produces 2 mol ethanol, 1 mol xylose produces 1.667 mol ethanol). Increasing the H_2SO_4 concentration decreased the ethanol titers and increased residual xylose. One possible reason was the high toxicity due to a high acid concentration pretreatment (Table 2). The degradation of glucose and xylose generates inhibitory compounds which inhibit cell growth and ethanol production (Vanmarcke et al., 2021). The most common two inhibitors for *S. cerevisiae* are furfural and HMF. However, their concentrations were < 0.1 g/L after enzymatic hydrolysis. Therefore, there must be some other inhibitors causing this toxicity. Due to greater toxicity to yeast cells, the increased xylose yield at 2 % or 3 % w/w H_2SO_4 did not improve ethanol titers. It only led to higher xylose concentrations in the final beer as residual sugar for both corn bran materials (Table 2).

Based on the obtained results, the acid level was kept at 1.5 % w/w H_2SO_4 for pretreatment at 90 °C during the 150 L scale-up. This pretreatment condition keeps the xylose yield close to 70 % and achieves a high glucose yield. The produced hydrolysates were also not toxic to



(a)



(b)

Fig. 2. Sugar yield and ethanol yield from hydrolysates using acid pretreatment and enzymatic hydrolysis: (a) HFB I; (b) LFB I. LFB and HFB were pretreated with 1–3 % w/w H_2SO_4 solutions. (* Significant differences ($p < 0.05$) between three sets of data).

yeast and won't influence ethanol yields.

3.5. Pilot scale

Eleven batches of pretreatment and enzymatic hydrolysis were run to accumulate 85 L hydrolysate for the fermentation. The sugar concentrations from the 11 batches were consistent (25.0 ± 1.8 g/L glucose and 17.8 ± 0.5 g/L xylose, see e-supplementary materials), which demonstrated the reproducibility of the pretreatment and enzymatic hydrolysis.

The C5C6 yeast strain was propagated from 100 ml to 1 L to 10 L. For the 10 L size propagation, a mixture of YPDX medium with filtered hydrolysates (50:50) was used to adapt the strain to the hydrolysates (Barcelos et al., 2021) for enhanced sugar consumption and ethanol production. The sugar and ethanol concentrations were monitored during the fermentation. As illustrated in Fig. 3, the yeast consumed sugars rapidly within 24 hr. This fast sugar consumption was probably due to the adaptation during the last step of propagation. Glucose and xylose were consumed simultaneously. All glucose was depleted within 8 h (<0.2 g/L residual glucose). Within 24 h, 91 % of the xylose was fermented (<1.4 g/L residual xylose). At the end of fermentation, the final beer was weighed and transferred to an industrial vacuum oven. The beer was dried at 50 °C for 2 days to dry the final DDGS to a moisture level of < 5 %.

The final ethanol titer was low (24.3 g/L±1.2 g/L) because of the low sugar concentrations (25.0 ± 1.8 g/L glucose and 17.8 ± 0.5 g/L xylose) obtained from the hydrolysis. Separation of low-titer ethanol will cost an extensive amount of energy, especially by distillation. Therefore, it is necessary to further increase ethanol titer to make the whole process economically viable. Several directions for this future research: First, increase solid loading to > 15 %, which will bring challenge to the mixing during pretreatment; Second, concentrate the sugar stream after hydrolysis by evaporation or membrane separation, which will add cost to the process; Third, seek alternate less energy intensive ethanol separation technology, such as membrane separation.

Mass balances were constructed to determine the conversion of the carbohydrate to ethanol (Fig. 4). By comparing the individual ethanol-producing carbohydrates in the feedstock and DDGS, the overall sugar conversions were: 95 % for starch, 77 % for cellulose, and 77 % for xylan, with an overall sugar yield of 86 %. The theoretical ethanol yields were 0.57 g/g glucan (1 mol glucose to 2 mol ethanol, also including the water added during saccharification) and 0.58 g/g xylan (1 mol xylose to 1.667 mol ethanol). The starch, cellulose, and xylan in the DDGS were also measured and their potential ethanol yields were calculated.

According to Fig. 4, the total theoretical ethanol production was 1773 g while the total actual ethanol production was 1818 g which indicates the mass balance efficiency on ethanol is close to 102 %. The factors contributing to the 2 % difference are the error bars related to all the analytical testing on carbohydrate concentration in the feedstock and DDGS and the actual ethanol production quantitation, which relies on collecting representative samples and the accuracy of the tests on total solid, dissolved solid and density of the supernatant. At the pilot scale, the starting feedstock and residual sugar amounts indicate a potential for total theoretical ethanol production of 641.7 L per metric ton of corn fiber (153.8 Gal per short ton), including a cellulosic yield of 561.5 L per metric ton (134.5 Gal/short ton) for the cellulose and xylose fractions. By 102 % efficiency of ethanol production, the actual ethanol production of 658 L per metric ton (157.7 Gal/short ton) was measured.

The mass balance also includes non-fermented material, which includes corn protein, oil, arabinan, ash, etc., from the feedstock to DDGS. The inert material in the feedstock and DDGS was calculated by the total mass minus starch, cellulose, and xylan respectively. The non-fermentable fractions were 3.619 kg in the feedstock and 3.459 kg in the DDGS. A small portion of the inert material was lost (0.482 kg) due to the propagation needs (50:50 YPDX medium: hydrolysate). The overall mass balance showed a recovery of 110 % for inert material.

Table 2
Fermentation results for both bran materials pretreated with 1–3 % w/w H_2SO_4 solutions.

Fiber sample	Pretreatment H_2SO_4 levels (w/w)	Ethanol (g/L)	Ethanol yield	Glucose (g/L)	Xylose (g/L)	Glycerol (g/L)
HFB I	1 %	28.0 ± 1.0	64 %	1.2 ± 0.1	2.1 ± 0.1	1.3 ± 0.0
	2 %	25.4 ± 1.2	58 %	0.8 ± 0.0	3.3 ± 1.0	1.5 ± 0.1
	3 %	25.0 ± 0.8	57 %	1.1 ± 0.1	5.9 ± 1.0	1.8 ± 0.1
LFB I	1 %	27.5 ± 0.4	69 %	0.7 ± 0.1	0.8 ± 0.0	1.2 ± 0.0
	2 %	25.6 ± 0.9	62 %	0.6 ± 0.0	1.8 ± 0.1	1.9 ± 0.0
	3 %	25.7 ± 0.5	62 %	0.7 ± 0.0	2.3 ± 0.0	2.1 ± 0.1

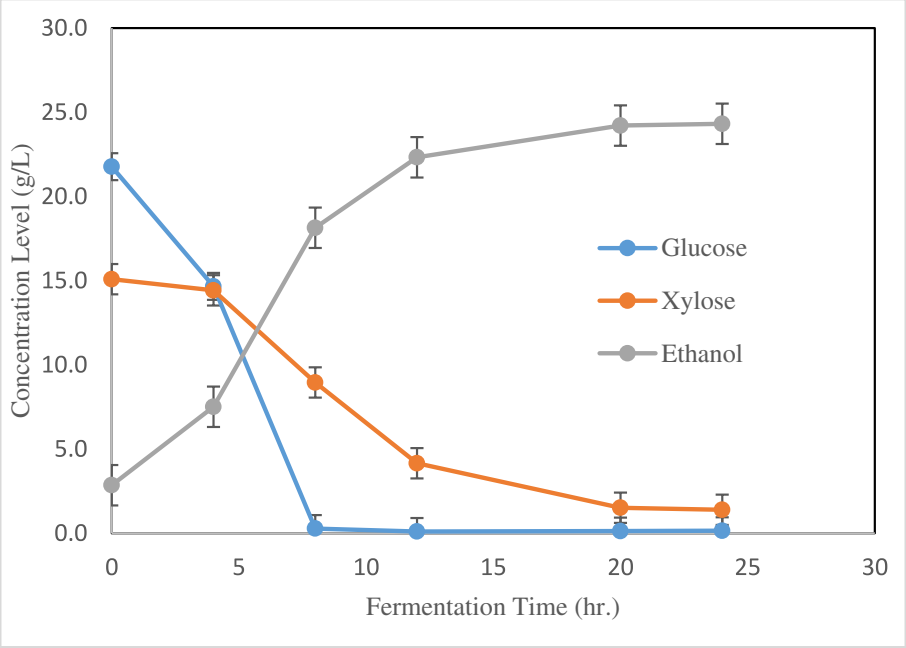


Fig. 3. Scale-up fermentation kinetics using corn fiber hydrolysate at 150 L.

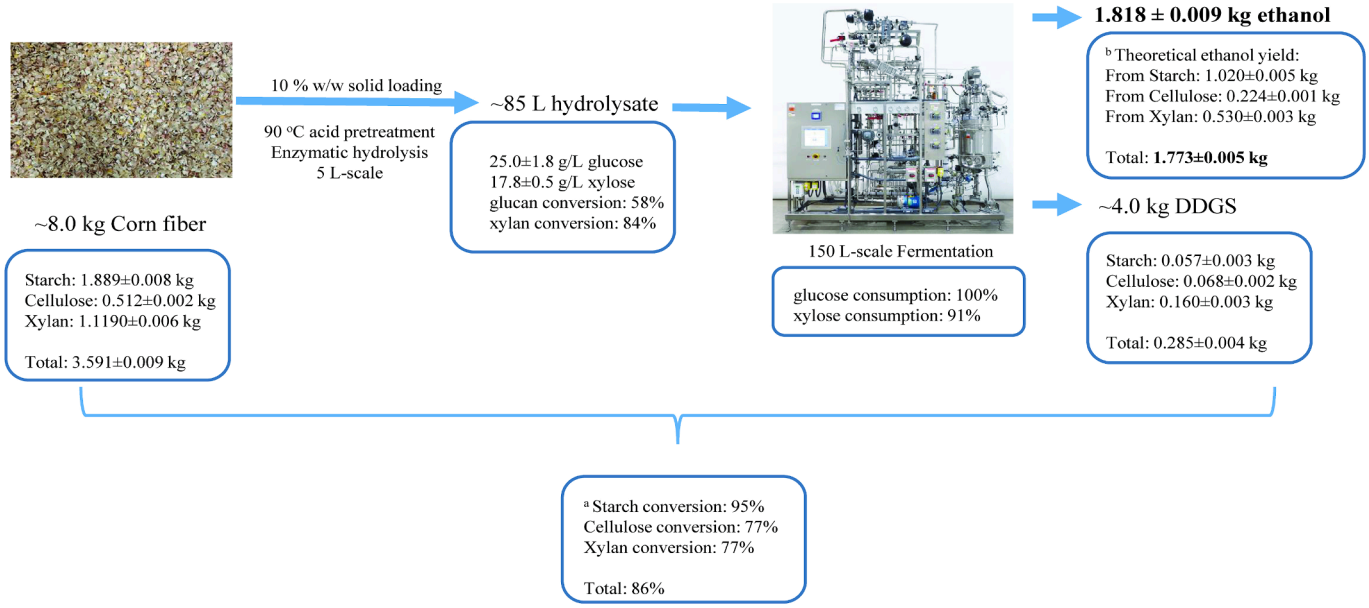


Fig. 4. Mass balance of carbohydrates from feedstock to final ethanol production. ([a] Compares the difference in total carbohydrates between feedstock and DDGS. Difference includes simultaneous starch saccharification during fermentation. [b] Assume 1 mol of glucose produces 2 mol of ethanol, 1 mol of xylose produces 1.667 mol of ethanol).

Given 10 % yeast biomass growth through the fermentation, the recovery on the inert material was close to 100 %.

Overall, a highly efficient conversion process was developed for converting corn bran to ethanol and scaled up to 150 L. A low temperature (90 °C) dilute acid pretreatment method and enzymatic hydrolysis were used to efficiently convert the carbohydrates (starch, cellulose, and hemicellulose) in corn bran to sugars. The fermentation process was also efficient, because the glucose and xylose consumptions reached 100 % and 91 %, respectively, within 24 h fermentation time. The final ethanol titer was 24.3 g/L. By comparing the carbohydrate contents of the DDGS and corn bran, carbohydrate conversions were 95 % for starch, 77 % for cellulose, and 77 % for xylan.

Prior studies for the conversion of corn bran to ethanol primarily used hot water or dilute acid. For hot water pretreatments (at > 160 °C), >70 % sugar recoveries were achieved (Mosier et al., 2005, Dien et al., 2006, Jiang et al., 2018, Kurambhatti et al., 2018). For dilute acid pretreatments, previous studies used various acids at high temperatures (>121 °C) and pressures to achieve 35 %–90 % sugar recoveries (Dien et al., 2004, Noureddino and Byun, 2010, Chen et al., 2010, Guo et al., 2013, Li et al., 2019, Zhang et al., 2021). High-temperature acid pretreatments are problematic because of the inhibitors generated from sugar degradation (Prasad et al., 2018). Therefore, detoxification methods are used to remove these inhibitors. In both hot water and dilute acid pretreatments, the high temperature (>121 °C) and high-

pressure requirements make the pretreatment process cost intensive from capital expenditure (pressure and corrosion-resistant equipment) and operational expenditure (steam). This process gives high glucose (91 %) and xylose (77 %) conversions without incurring toxicity problems for the yeast fermentation. The low temperature (90 °C) pretreatment in this process will significantly reduce pretreatment costs and enable process scale-up.

4. Conclusions

In this study, the high-efficiency conversion of starch, cellulose, and xylan to ethanol from fractionated corn bran at 150 L was investigated. Corn bran was pretreated by 1.5 % w/w H_2SO_4 at 90 °C for 60 min, followed by enzymatic hydrolysis for 48 h. The total sugar yield was 86 % obtained after simultaneous saccharification and fermentation using an engineered C5C6 yeast. By applying accurate analytical methods for the determination of carbohydrates and ethanol, the ethanol production efficiency achieved 102 % which demonstrates the optimized conditions for bioethanol production at a large scale.

CRediT authorship contribution statement

Jie Dong: Writing – original draft, Methodology, Conceptualization. **Mohamadali Fakhari:** Writing – review & editing, Data curation. **Lilia Ban:** Methodology, Investigation, Formal analysis. **Krystin Polhemus:** Investigation, Formal analysis. **Muhammed Roji Shehu:** Investigation, Formal analysis. **Forough Doustkhahvajari:** Writing – review & editing. **Philip Kukielski:** Investigation. **Ajay Venigalla:** Investigation. **Terry Lash:** Investigation. **Noppadon Sathitsuksanoh:** Writing – review & editing. **Yanhong Zhang:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jie Dong reports financial support was provided by Shockwave, LLC. Co-author currently employed by Elemental Enzyme - L. B. Co-author currently employed by University of Pittsburgh - F. D. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2024.131216.

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