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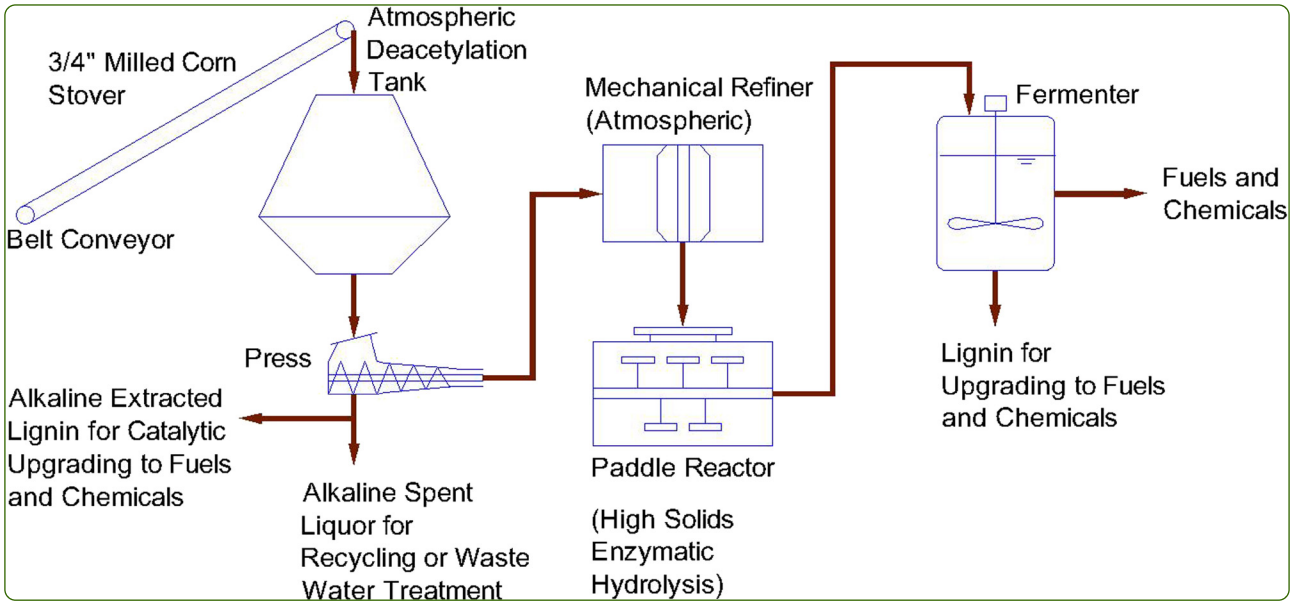

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Chen *et al. Biotechnology for Biofuels* 2014, **7**:98
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A highly efficient dilute alkali deacetylation and mechanical (disc) refining process for the conversion of renewable biomass to lower cost sugars

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Abstract

Background: The deconstruction of renewable biomass feedstocks into soluble sugars at low cost is a critical component of the biochemical conversion of biomass to fuels and chemicals. Providing low cost high concentration sugar syrups with low levels of chemicals and toxic inhibitors, at high process yields is essential for biochemical platform processes using pretreatment and enzymatic hydrolysis. In this work, we utilize a process consisting of deacetylation, followed by mechanical refining in a disc refiner (DDR) for the conversion of renewable biomass to low cost sugars at high yields and at high concentrations without a conventional chemical pretreatment step. The new process features a low temperature dilute alkaline deacetylation step followed by disc refining under modest levels of energy consumption.

Results: The proposed process was demonstrated using a commercial scale Andritz double disc refiner. Disc refined and deacetylated corn stover result in monomeric glucose yields of 78 to 84% and monomeric xylose yields of 71 to 77% after enzymatic hydrolysis at process-relevant solids and enzyme loadings. The glucose and xylose yields of the disc refined substrates in enzymatic hydrolysis are enhanced by 13% and 19%, respectively. Fermentation of the DDR substrates at 20% total solids with *Z.mobilis* utilized almost all sugars in 20hrs indicating the sugar hydrolyzate produced from the DDR process is highly fermentable due to low levels of chemical contaminants. The ethanol titer and ethanol process yield are approximately 70 g/L and 90% respectively.

Conclusions: The proposed new process has been demonstrated using pilot scale deacetylation and disc refiners. The deacetylated and disc refined corn stover was rapidly deconstructed to monomeric sugars at 20% wt solids with enzymatic hydrolysis. High process sugar conversions were achieved, with high concentrations of monomeric sugars that exceeded 150 g/L. The sugar syrups produced were found to have low concentrations of known major fermentation inhibitors: acetic acid, furfural and HMF. The low levels of these fermentation inhibitors lead to high fermentation yields. The results suggest that this process is a very promising development for the nascent cellulosic biofuels industry.

Keywords: Biofuels, Pretreatment, Enzymatic hydrolysis, Deacetylation, Mechanical refining, Disc refining, No acid pretreatment, PFI milling, Clean sugar production

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Introduction

In recent years, significant progress has been made toward the development of a host of sugar upgrading technologies that would enable the production of an array of bio-based fuels and chemicals if low cost sugars were available. However, the development of a viable bioeconomy using these technologies is dependent on the availability of low cost, highly concentrated sugar syrups with minimal biological and catalytic poisons. Thus, the deconstruction of renewable biomass feedstocks into soluble sugars at low cost is critical to the commercial viability of many biological and chemical catalytic conversion processes.

In past decades, a major focus of biorefining research has been on increasing its effectiveness and reducing the costs of pretreatment. While there are many competing pretreatment technologies, each with its own set of strengths and weaknesses, dilute acid pretreatment has been recognized as a promising technology for the production of lignocellulosic sugars at relatively low cost and at commercially relevant scales [1]. In recent years, significant efforts have been made to improve dilute acid pretreatment technologies, including testing and modifying reactor designs and screening large arrays of pretreatment conditions. At the National Renewable Energy Laboratory (NREL), an integrated corn stover to ethanol process was successfully demonstrated at a 1-ton per day continuous pilot scale using dilute acid pretreatment. Publications in 2011 indicate that the pilot scale trial should achieve a modeled minimum ethanol selling price (MESP) of $2.15 per gallon when results are applied in an n^{th} plant scenario to a commercial scale biorefinery plant producing 61 MMgal ethanol per year [2].

Effective dilute acid pretreatment with sulfuric acid should hydrolyze most of the hemicellulosic sugars (mainly xylan) in the biomass and decrease the mean particle size, thus leading to improved cellulose digestibility [3-5]. Low cost and widespread availability is one of the advantages of sulfuric acid relative to other available acids and pretreatment processes, including phosphoric acid, nitric acid and ammonia processes [6-11]. However, sulfuric acid pretreatment also suffers from a number of issues. Dilute acid pretreatment requires capital-intensive reactors due to the corrosive nature of sulfuric acid. In addition, pretreatment with acid requires precise control of process parameters, including residence time and temperature, which may be difficult in a large scale continuous pretreatment reactor due to back-mixing and other factors [2]. Dilute acid pretreatment also produces comparatively significantly high concentrations of fermentation inhibitors if severe enough. The acid catalyzed degradation products from xylose and glucose, namely furfural and HMF (Hydroxy-methyl-furfural), are strong inhibitors for ethanol fermentation [12,13]. Another strong inhibitor, acetic acid, is released from the xylan backbone as a byproduct of acid hydrolysis [12]. The inhibitors not only lower the yield of sugars from enzymatic hydrolysis, but also significantly inhibit and reduce the ethanol or other fermentation product yields. Furthermore, dilute acid pretreated biomass slurries need to be neutralized prior to enzymatic hydrolysis; this is typically accomplished using calcium or ammonium hydroxide, which results in some sugar losses [14]. The ammonia and sulfur present in the hydrolyzate liquors increases the need for downstream cleanup unit operations and increases the cost and complexity of wastewater treatment [2]. High temperature dilute acid pretreatment also catalyzes lignin condensation reactions [15]. The products from lignin condensation reactions have lost some of their reactivity and are less likely to be amenable for downstream upgrading to higher-value products. Finally, dilute acid pretreatment of biomass also generates char-like products which will not only lower sugar yields, but also accumulate within the reactor, eventually causing increased and potentially unscheduled maintenance downtime and expenses [16].

Other leading pretreatment technologies that use sulfur bisulfite (SPORL process) [17,18], ammonia [9,11], organic solvents [19,20] or ionic liquids [20,21] to enable high yields of sugars from biomass suffer from a host of other issues, including recovery of catalysts and solvents and other drawbacks similar to those described for dilute sulfuric acid pretreatment.

To mitigate some of these challenges and potentially further reduce the MESP of cellulosic ethanol, investigations were carried out that added deacetylation prior to lower severity dilute acid pretreatment, followed by mechanical refining as a means to improve biomass digestibility and hydrolysate fermentability while maintaining high process yields [22,23]. The deacetylation process, described in greater detail in previous publications [3,22,23], was designed to solubilize and remove acetyl groups prior to the pretreatment step allowing for a more cost-effective separation of acetic acid from the solids. The advantages of removing acetate from biomass structures are described elsewhere [3,22,23]. Deacetylation is reached by dilute alkaline extraction and washing. Unlike conventional alkaline pretreatment which focuses on the removal of lignin by applying higher reaction temperatures (≥100°C) and alkaline loadings (≥100 kg NaOH per ODMT (oven dried metric tonnes) biomass) [24], deacetylation is conducted at very mild temperatures (<100°C) with very low alkaline loadings (≤40 kg NaOH per ODMT biomass). The mild deacetylation conditions significantly reduce hemicellulose sugar losses from peeling reactions relative to conventional alkaline pretreatment processes, wherein 30% or more of hemicellulose is typically lost [24,25]. In addition, deacetylation is conducted at atmospheric pressure, allowing the use of low cost open tanks instead of more expensive

pressure-rated vessels. The residence time of deacetylation is also less than two hours which, compared to lime treatment [26], is much faster and more efficient. The process's low alkaline utilization also significantly reduces the size and cost of a recovery boiler since alkaline recovery is normally expensive and is always the bottleneck in the production rate when higher alkaline loading is used and the alkali must be recovered [24].

Mechanical refining is a proven and scalable technology that historically has been widely used in the pulp and paper industry to improve fiber strength [27]. However, it is also attractive for biomass deconstruction processes as a means to increase the accessibility of cellulose in biomass and thus improve process sugar yields [27,28]. Our previous work has shown that the enzymatic digestibility of low severity dilute acid pretreated corn stover is significantly improved by mechanical refining using PFI milling, twin screw extrusion, disc refining and/or Szego milling [29]. Among those refining techniques, screw extrusion is studied extensively in the literature [30-36]. Although the digestibility of screw-extruded biomass is comparable to other pretreatment technologies, the application of screw extrusion on the commercial scale still remains questionable due to the high level of energy consumption and the capital investment required. Alternatively, disc refining has received comparatively little attention, and limited research is available in the literature exploring its effect on enzymatic digestibility of the biomass.

The present work shows that a new process for the conversion of renewable herbaceous biomass to low cost sugars at high yields and at high sugar concentrations is possible without a severe chemical pretreatment step. This process consists of dilute alkali deacetylation followed by disc refining to achieve up to 84% glucose yields after high solids enzymatic hydrolysis at moderate enzyme loadings. Eliminating the need for acid or higher alkaline usage has the potential to create significant cost savings relative to a traditional chemical pretreatment process because capital and operational costs are reduced. The proposed new process is shown in Figure 1.

In this process, native corn stover milled to pass a ¾-inch round screen is first subjected to a deacetylation step where 70 to 80% of the acetyl groups in the biomass are removed by a dilute alkali solution heated to approximately 80°C. The acetate- and lignin-rich liquor is drained through a screen, leaving a slurry of approximately 20% (w/w) total solids. The deacetylated solids are then rinsed with water followed by dewatering using a screw press to achieve a final solids concentration of between 30 and 40%. The deacetylated corn stover is mechanically refined through a single stage of disc refining. The refined corn stover is then hydrolyzed using cellulase and hemicellulase enzymes. The high concentration sugar syrups can then be biologically or catalytically upgraded to produce biofuels and chemicals.

There are many advantages to replacing dilute acid pretreatment with an effective disc refining process. The elimination of the need to use acid chemistry is critical because it avoids many of the corrosion and degradation issues mentioned previously. Since the whole process is carried out at less than 100°C, atmospheric pressure tanks and process equipment can be used to reduce capital investment relative to the costly pressurized vessels and equipment required for other pretreatment processes. This new process uses low amounts of sodium hydroxide (slightly above stoichiometric of acetate content of biomass) and consumes only a modest amount of electrical energy in disc refining. The hydrolyzate liquors have been found to contain sugar concentrations comparable to those produced in a dilute acid pretreatment process [2]. However, lower concentrations of toxic compounds (furfural, HMF and acetic acid) allow these hydrolyzate liquors to be highly fermentable and more amenable to catalytic upgrading. In addition, the unit operations involved in this process have been commercially applied and are generally proven in pulping processes and first generation bioethanol plants,

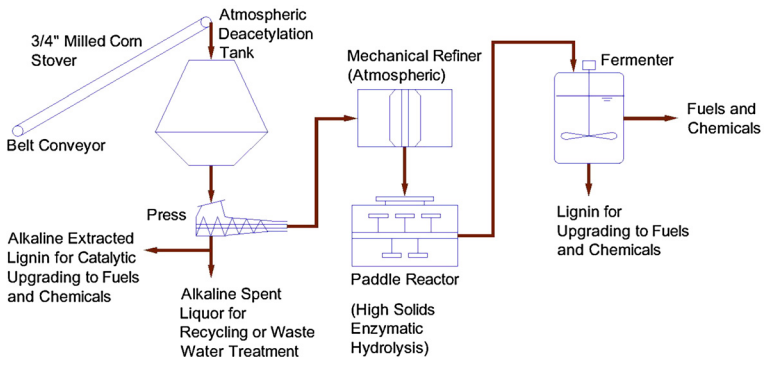

Figure 1 Proposed schematic diagram for new deacetylation/mechanical refining process for production of soluble sugars.

suggesting the process is potentially more readily realized at the commercial scale compared to some other pretreatment processes. Finally, the new process might be installed in an existing corn ethanol plant utilizing already installed equipment performing similar unit operations including wet milling.

In this study, mechanical refining experiments were conducted at various levels of energy consumption using a 36-inch small commercial scale Sprout model 401 atmospheric double disc refiner provided by Andritz (Springfield, Ohio, United States). Following disc refining, high monomeric sugar yields were produced in enzymatic hydrolysis experiments performed at low solids and high enzyme loadings, as well as more process relevant high solids conditions with modest enzyme loadings, and the enzymatic hydrolysis slurries were found to be highly fermentable.

Results and discussion

Deacetylation in pilot scale batch paddle reactor

Deacetylated corn stover (DCS) was produced in a pilot scale batch paddle reactor. The impact of deacetylation on corn stover was described in our previous paper [22]. As shown in Table 1, the native corn stover used in the current study contained approximately 37% glucan and 31% xylan, while acetyl and lignin content were approximately 2.7% and 15%, respectively. After deacetylation, approximately 80% of the acetyl groups, 10% of the xylan and 2% glucan was solubilized along with 30% of the lignin and 80% of the ash. The DCS solids was analyzed and found to contain approximately 43.6% glucan, 33.0% xylan, 12.6% lignin, and 0.3% acetyl groups. The deacetylated biomass was also significantly lower in ash content (approximately 0.6 %wt) compared to native corn stover.

As would be expected of a mild alkaline pretreatment process, deacetylation significantly increases the digestibility of native corn stover. A set of enzymatic hydrolysis experiments carried out at 2% (w/w) total solids with an enzyme loading of 32 mg CTec3/g cellulose and 5 mg HTec3/g cellulose showed that the glucose and xylose yields increase from 29% and 28% using native corn stover to 65% and 54%, respectively, after treatment. This indicates that partial removal of acetate and lignin can significantly reduce the recalcitrance of biomass to enzymatic hydrolysis. The significant improvement in biomass digestibility could also be partly attributed to swelling in the biomass that occurs when alkali is introduced, as observed in other alkaline pretreatment techniques [37].

The spent alkaline liquor from deacetylation is rich in acetate and lignin, valuable components for which there are many potential pathways to produce value-added products [38]. On the other hand, the recovery and recycling of sodium within a deacetylation unit operation is an important environmental aspect of the commercialization of this process. Recovery of the sodium from black liquor is a mature technology in the pulp and paper industry, whereas the recovery of sodium from a dilute alkaline solution remains a challenge to be addressed.

Small commercial scale disc refining of deacetylated corn stover

Disc refining is a proven technology that has been used on a commercial scale in the pulp and paper industry for many years [39]. In this study, a small commercial scale 36 inch (91 cm) Sprout Model 401 atmospheric double disc refiner was used to investigate the effect of disc-refining power consumption on the enzymatic digestibility of deacetylated corn stover. The Durametal 36104 plate pattern (Durametal, Irwin, PA) consisting of a fine bar design formulated for fiber strength development in pulping was used to configure the plates in the refiner. A 36-inch disc refiner provides a reliable, accurate and repeatable size for a refiner for both refining effects and specific electrical refining energy that is being applied to the substrate. Furthermore, the 36-inch disc refiner is large enough to measure 'real' energy usage levels (as compared to the small pilot scale machines that have been used for our previous work [29]). Based on pulp and paper work over the last 20 years, a correction factor of approximately 10% was used for correcting from a 36-inch to a 60-inch refiner (as a larger disc refiner uses less refining energy for the same effect). Five energy levels were investigated in this study. Table 2 shows the operating parameters during refining including energy consumption with corresponding plate gap and throughput. Refining energy is controlled mainly by adjusting refining plate gap and throughput. Decreasing the throughput from 32.0 ODMT/d to 17.3 ODMT/d as well as reducing the plate gap from 1.78 to 0.00 mm, the refining energy increased from 128 kWh/ODMT to 468 kWh/ODMT for DCS.

Disc refining significantly reduced the particle size of DCS. Table 2 shows that the volume-weighted mean particle size for disc-refined deacetylated corn stover (DRDCS decreased from 300 μm to approximately 200 μm by increasing the disc refining energy from 128 to 468 kWh/ODMT. Specific surface area is believed to be the most

Table 1 Compositional analysis (weight percent dry basis) of native and deacetylated corn stover

	Ash	Lignin	Glucan	Xylan	Galactan	Arabinan	Acetyl
Native corn stover	2.3(0.1)*	14.9(0.0)	36.4(0.0)	30.8(0.4)	1.8(0.0)	3.4(0.0)	2.7(0.2)
Deacetylated corn stover	0.6(0.2)	12.6(0.4)	43.6(0.1)	33.1(0.1)	1.4(0.0)	2.5(0.1)	0.3(0.2)

* ± one standard deviation

Table 2 Operating conditions[a] of disc refining

Plate gap (mm)	Throughput, ODMT/d	Energy consumption (kWh/ODMT)	Volume weighted mean particle size (μm)
1.78	32.0	128	302.9
1.02	31.3	212	260.4
0.56	26.2	317	220.6
0.25	22.8	408	245.8
0.00	17.3	468	198.1

[a]Feed consistency and bulk density are 33.4% (w/w) and 104.7 dry kg/m^3, respectively. The disc refiner was operated at 1200 rpm (revolutions per minute).

critical aspect affecting the enzymatic digestibility of biomass [5,29]. Disc refining provides significant external fibrillation and results in increased specific surface area, thus increasing the digestibility of corn stover [29]. The investigation of specific surface area of disc-refined corn stover is a subject of future work.

Screening enzymatic hydrolysis experiments under low total solids and high enzyme loadings

The monomeric glucose and xylose yields from low solids (2% (w/w) total solids, 1% cellulose (w/w) loaded in the hydrolysis slurry) enzymatic hydrolysis of DCS and DRDCS substrates are depicted in Figure 2. The enzyme loading used in this experiment for all substrates was 32 mg CTec3 and 5 mg HTec3/g cellulose. While not economically viable, the high enzyme loadings used during low solids hydrolysis were intended to determine the maximum potential glucose and xylose yield for each substrate. Glucose yields increased from 69% for DCS to 79% for DRDCS refined at the lowest energy input (130 kWh/ODMT). Increasing the energy input improved monomeric glucose yield to 85% when 317 kWh/ODMT of energy was applied. Above 317 kWh/ODMT there was no significant increase in glucose yield. Xylose yields increased from 54% for DCS to near 78% for DRDCS at an energy input level of 317 kWh/ODMT. Increasing energy input to 400 kWh/ODMT did not produce a significant increase in xylose yield, again suggesting that there is little value in exceeding a refining energy input level of 317 kWh/ODMT.

Analysis of time course data indicates that enzymatic digestions are complete in approximately three days under low solids hydrolysis experiments, as shown in Figure 3. The results show that DCS and DRDCS are very digestible, with most of the digestion occurring within the first 24 hours. While the disc refining significantly improved the hydrolysis rate in the first 24 hours, it showed no impact on the hydrolysis kinetics afterwards. The short digestion times may reduce sugar losses due to possible bacterial contamination during saccharification.

Enzymatic hydrolysis at high solids loadings

While the results shown in Figure 2 are encouraging, additional enzymatic hydrolysis results were generated at higher total solids concentrations and lower enzyme loadings. These process conditions are more favorable for the commercial production of biofuels, especially corn stover derived ethanol, because these conditions significantly reduce operational costs (enzyme and water usage) as well as capital costs due to the decreased size requirements for enzymatic hydrolysis reactors and downstream fermenters [40]. Therefore, following the previously discussed low

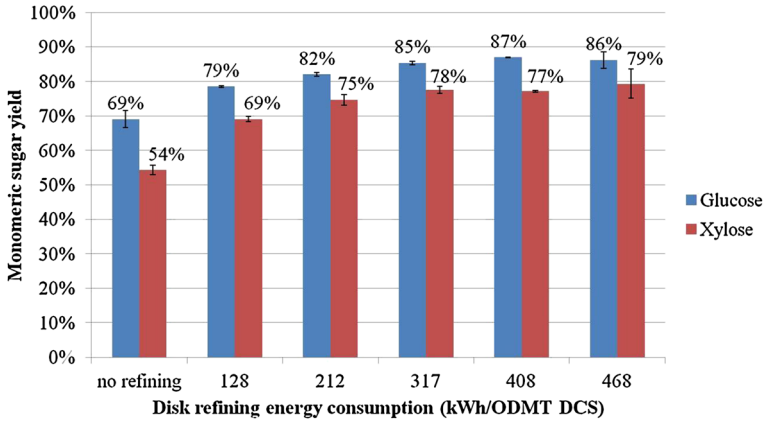

Figure 2 Yield of monomeric glucose and xylose from low solids enzymatic hydrolysis of DCS and DRDCS. The digestions were carried out at 1% (w/w) cellulose loadings with an enzyme loading of 32 mg/g cellulose CTec3 and 5 mg/g cellulose HTec3. Error bars represent ± one standard deviation.

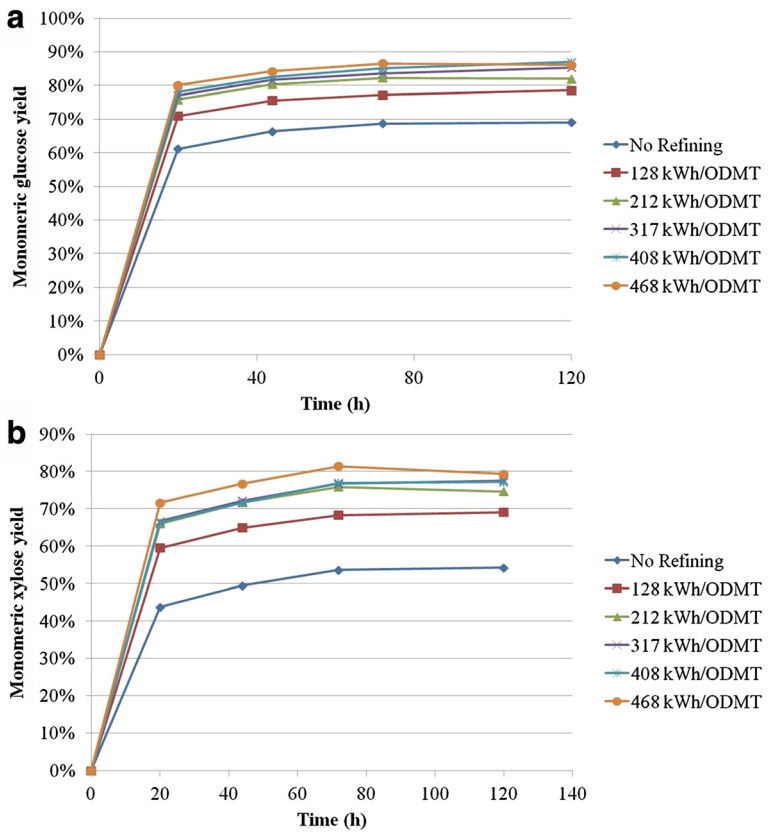

Figure 3 Glucose (a) and xylose (b) yield during enzymatic hydrolysis of DCS and DRDCS. The digestions were carried out at 1% (w/w) cellulose loadings with enzyme loadings of 32 mg/g cellulose CTec3 and 5 mg/g cellulose HTec3. Panel **a**: monomeric glucose yield; panel **b**: monomeric xylose yield.

solids enzymatic hydrolysis screening experiments, the DCS and DRDCS substrates were subjected to enzymatic hydrolysis at 15% (w/w) total solids using lower enzyme loadings (20 mg CTec3 (15 FPU) and 2.5 mg HTec3/g cellulose). Figure 4 shows the monomeric glucose and xylose yields achieved at 15% total solids enzymatic hydrolysis. The monomeric glucose and xylose yields ranged from 79 to 84% and 71 to 77%, respectively, only 2 to 3% lower than those obtained in the previous low-solids, high-enzyme loading experiments. These yields, demonstrated at high solids with more process relevant enzyme loadings, are very promising when compared to the yields achieved

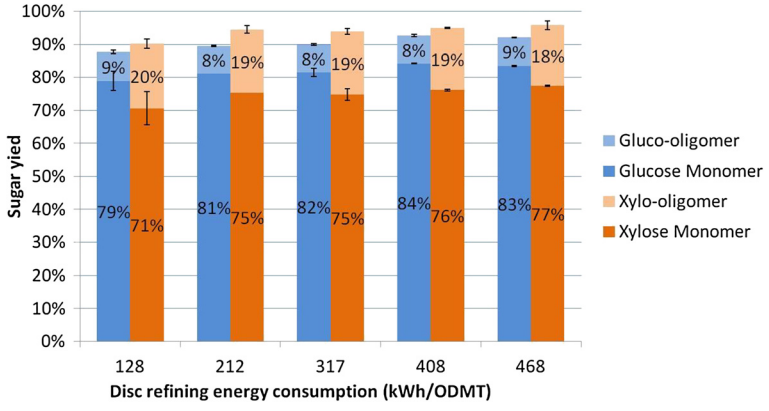

Figure 4 Enzymatic hydrolysis yields of monomeric glucose and xylose for DRDCS. The digestions were carried out at 15% (w/w) total solids with enzyme loadings of 20 mg/g cellulose CTec3 and 2.5 mg/g cellulose HTec3.

in our previous work (55 to 65% glucose yields and <10% xylose yields for acid only pretreated corn stover; 60 to 70% glucose yields and <10% xylose yields for deacetylated acid pretreated corn stover) [22]. In addition to monomeric sugars, 8 to 9% of glucan and 18 to 20% of xylan were solubilized as oligomeric glucose and xylose during enzymatic hydrolysis. At an energy application of 212 kWh/ODMT, the total yield of hydrolyzed glucan and xylan approaches 90% and 95%, respectively, for the DRDCS with a monomeric glucose and xylose yield of 81% and 75%, respectively.

The process sugar yields are shown in Table 3 including monomeric, oligomeric and total (monomeric and oligomeric) glucose and xylose yields. The process sugar yields are calculated based on the composition of the deacetylated biomass, but include a 2% loss of glucan and 10% loss of xylan during deacetylation treatment. The 2% glucan and 10% xylan solubilized during deacetylation stage are currently counted as sugar losses although most of them are still oligosaccharides in the deacetylated spent liquor. Hence, as shown in Table 3, the process monomeric glucose and xylose yields are in the range of 77 to 82% and 64 to 70%, respectively. The process oligomeric glucose and xylose yields are approximately 8% and 17%, respectively. The total glucose and xylose yields in the DDR (deacetylation and disc refining) process approach 90% and 86%, respectively, as refining energy increases.

Effect of enzyme loading on enzymatic hydrolysis yields at high total solids

Figure 5 shows the effect of enzyme loadings on the monomeric glucose and xylose yield during enzymatic hydrolysis of DRDCS at 20% (w/w) total solids and 212 kWh/ODMT refining energy input. The enzyme loadings were varied from a total protein loading of between 16 and 20 mg protein/g cellulose (12–15 FPU Novozymes CTec3 cellulase) and a 4:1 ratio of CTec3 to HTec3. The glucose and xylose yields were found to be 82% and 77%, respectively, at an enzyme loading of 20 mg/g comparable to the yields achieved at 15% total solids using 22.5 mg/g. When enzyme loading decreased to 16 mg/g, the glucose and xylose yields decreased to 78% and 73%, respectively. Future work will be carried out to optimize refining energy input, enzyme loading, cellulase to hemicellulase ratios, and starting hydrolysis solids loadings to achieve further reductions in sugar and fuel production costs in the proposed DDR process.

Table 4 shows the monomeric and oligomeric sugar concentrations, as well as the concentration of acetic acid, furfural, and HMF in the sugar syrup produced after enzymatic hydrolysis at 20% (w/w) total solids. At enzyme loadings of 20 mg protein/g cellulose, the syrups contained over 148 g/L of monomeric sugars with 84 g/L of glucose and 61 g/L of xylose. An additional 23 g/L of oligomeric glucose and xylose were produced. Acetic acid concentrations were below 0.3 g/L, while no significant amount of furfural or HMF was produced because an acid hydrolysis was not performed. The production of sugar syrups with high sugar concentrations and low levels of fermentation inhibitors and catalyst poisons are very attractive for downstream processing to produce fuels and value added products.

Fermentation

Following enzymatic hydrolysis, fermentation experiments were carried out on the DRDCS at a 20% total solids loading using *Zymomonas mobilis* 13-H-9-2. The results of fermentation after 20 hours are shown in Table 5. Glucose and xylose are almost completely utilized, while approximately 95% of the arabinose is also consumed. The final ethanol titers range from 70 to 72 g/L, with corresponding ethanol process yields found between 90 and 92%. The near-complete utilization of both C5 and C6 sugars and high ethanol process yields indicate the sugar hydrolysates produced from the DDR process are highly fermentable, ostensibly due to the low levels of inhibitors in the solutions.

Conclusions

A promising new process for the conversion of renewable biomass to low cost sugars that does not rely on a conventional severe pretreatment step is proposed and validated by both bench and pilot scale experiments. This process instead treats corn stover using a mild, dilute (0.4% w/w) alkaline extraction stage at 80°C (deacetylation) and washing prior to further processing using a high consistency disc refiner. The deacetylated and mechanically refined corn stover was demonstrated to achieve monomeric glucose yields

Table 3 Process sugar yields

Refining energy (kWh/ODMT)	Monomeric glucose yield (%)	Monomeric xylose yield (%)	Oligomeric glucose yield (%)	Oligomeric xylose yield (%)	Total glucose yield (%)	Total xylose yield (%)
128	77.4%	63.5%	8.5%	17.6%	85.9%	81.1%
212	79.6%	67.8%	8.1%	17.3%	87.7%	85.0%
317	79.9%	67.3%	8.1%	17.2%	88.0%	84.4%
408	82.5%	68.5%	8.2%	16.9%	90.7%	85.4%
468	81.8%	69.7%	8.5%	16.5%	90.2%	86.2%

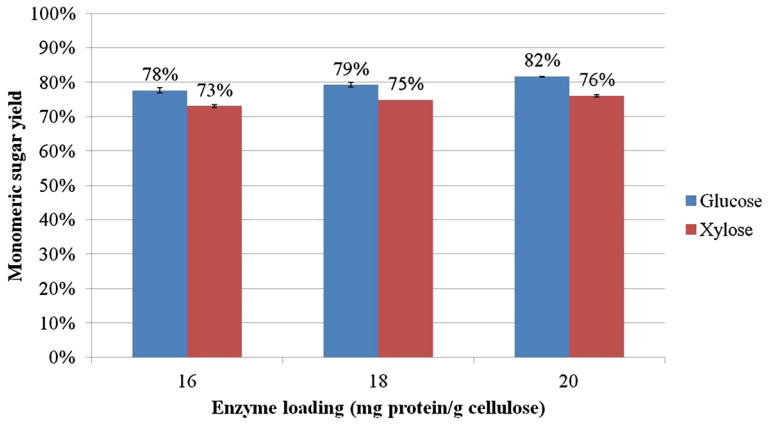

Figure 5 Effect of enzyme loadings on monomeric sugar yields during enzymatic hydrolysis of DRDCS. The digestions were carried out at 20% (w/w) total solids. The substrate was refined at 212 kWh/ODMT. Error bars represent ± one standard deviation.

approaching 85% and monomeric xylose yields approaching 80% after enzymatic hydrolysis was carried out at 20% (w/w) total solids using a total enzyme loading of 22.5 mg protein/g cellulose for four days. The resultant sugar syrup was demonstrated to be rich in glucose and xylose (glucose > 80 g/L, xylose > 60 g/L), and possessed very low concentrations of toxic fermentation inhibitors (furfural and HMF below the analytical detection limit, acetic acid approximately 0.3 g/L). While the economics of the DDR process requires rigorous techno-economic evaluation, the initial results indicate that the DDR process is a promising development and has the potential to significantly decrease the cost and complexity of biomass deconstruction processes while also improving reliability and scalability.

Materials and methods

Corn stover feedstock

Corn stover was harvested in 2009 in harvested in Hurley County (South Dakota, United States) and transported to the Idaho National Laboratory where it was stored indoors. It was shipped to NREL in January 2013. Upon receipt at NREL, the corn stover was knife milled (Jordan Reduction, Birmingham, Alabama, United States) to pass through a 19 mm (0.75 inch) round screen and stored in 200 kg lots in supersacks.

Pilot scale deacetylation

Corn stover deacetylation was performed in a 1900-L paddle mixer (American Process Systems, Gurnee, Illinois, United States). Dry corn stover (100 to 120 dry kg) was added to the paddle mixer along with a dilute 0.1 M sodium hydroxide solution for an 8% (w/w) total solids slurry. The slurry was heated to 80°C and held for 2 hours, and then the liquor was allowed to drain overnight through screens (2mm openings) located in the bottom ports of the paddle mixer. Water was added to the mixer and the solids mixed. The rinse water was then drained from the mixer through the screens in the ports. The solids were pumped to a continuous screw press (Vincent Corp. Model CP10, Tampa, Florida, United States) for dewatering to between 45 and 50% (w/w) total solids. Nine batches of deacetylated corn stover (1000 kg total) were prepared in this manner, sealed in plastic bags, loaded into 55-gallon drums and shipped to the Andritz R & D facility in Springfield (Ohio, United States) for mechanical refining in their Sprout Model 401 36-inch (91cm) commercial scale disc refiner. The composition of the native and deacetylated corn stover (DCS) feedstock is listed in Table 1.

Commercial scale 36-inch disc refiner

Commercial scale disc refining was carried out at the Andritz pilot plant and R & D laboratory in Springfield (Ohio, United States) using the Sprout model 401 36-inch disc refiner. The atmospheric refiner trials were conducted at five different feed mass flow rates. The Sprout model 401 refiner has two counter rotating discs, each driven by 225 kW (300 hp) motors. For the studies reported here, the refiner rotational speed was maintained at 1200 rpm.

Table 4 Compositional analysis of enzymatically hydrolyzed DRDCS slurry liquor

Enzyme loading	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Gluco- oligomers (g/L)	Xylo-oligomers (g/L)	Acetic acid (g/L)	Furfural (g/L)	HMF (g/L)
16 mg/g	78.6	57.6	3.5	8.2	13.8	0.2	0.0	0.0
18 mg/g	81.5	60.0	3.6	8.0	13.4	0.3	0.0	0.0
20 mg/g	84.4	61.4	3.6	9.3	14.0	0.3	0.0	0.0

Table 5 Fermentation yield

Refining energy (kWh/ODMT)	Enzyme loading (mg/g of cellulose	Glucose utilization (%)	Xylose utilization (%)	Arabinose utilization (%)	Ethanol process yield (%)	Ethanol titer (g/L)
212	20	99.6	98.7	95.3	90.5	69.5
212	26	99.6	98.4	93.4	89.8	70.6
317	20	99.6	97.7	96.2	91.5	70.7
317	26	99.6	98.8	94.7	90.9	72.3

A Durametal 36104 plate pattern consisting of a fine-bar design formulated for fiber strength development in pulping was used to configure the rotating plates in the Sprout 401 refiner. The feed material was weighed onto a conveyor feeding the refiner. The target for each refining series was to maximize refiner motor load for the given feed mass flow rate. The energy consumptions varied from 128 to 468 kWh/ODMT.

Particle size analysis

Particle size analysis of DDR samples were measured using laser diffraction by a Mastersizer 2000 with the Hydro 2000 G module (Malvern Instruments, Worcestershire, United Kingdom). The instrument measures particle sizes over a range of 0.02 to 2000 μm in a recirculating liquid suspension. For the analysis, 0.05 to 0.2 g of each DDR sample was dispersed in water in a 15-mL centrifuge tube. Thereafter, individual dispersed samples were vortex mixed and transferred to the Hydro 2000 G module that contained 0.8 to 1.0 L of deionized water ($n_r = 1.33$ at 20°C), with a stir setting of 600 rpm and a pump setting of 1250 rpm. After a 30 second delay, three 15 second readings of the circulating samples taken 30 seconds apart were acquired and averaged. The volume-weighted mean value was used to represent the mean particle diameter (MPD). Each sample was run in triplicate and MPD is presented as the average of the triplicates.

Low solids enzymatic hydrolysis

Enzymatic digestions of washed refined residues from the deacetyalation/mechanical refining experiments were performed in 125 mL Erlenmeyer shake flasks in a shaking incubator at 1% (w/w) cellulose loading (approximately 2% solids loadings), 50°C, and 130 rpm according to NREL's LAP (laboratory analytical procedure) [41]. Novozymes (Franklinton, North Carolina, United States) Cellic® CTec3 and HTec3 cellulase and hemicellulase enzyme preparation were added at the designated levels. The total slurry volumes of saccharification assay slurries after adding enzymes and buffer was 50 mL. Slurry samples were taken at 24, 48, 96, and 168 hours and sugar concentrations were measured by HPLC (high performance liquid chromatography).

High solids enzymatic hydrolysis

Enzymatic cellulose digestibility of DCS treated by the disc refiner was also measured at high solids conditions. Hydrolysis was conducted with 100 g of slurry in 250-mL capped Schott media bottles. The bottles were autoclaved empty, then the pH-adjusted disc-refined substrates were manually introduced into the bottles using a small funnel to reach the target total solids concentration of 15 or 20% (w/w). Two mL of citrate buffer (pH 5.1, 1.0 M) was added to each flask to help maintain pH at approximately 5.0 throughout the experiment. Enzymatic hydrolysis began by adding enzyme to achieve the target enzyme dosages of 16.5, 18.5, 20.5 and 22.5 mg protein/g cellulose, then placing the fully loaded and capped bottles in a shaking incubator operating at 150 rpm and 48°C. A NIST (National Institute of Standards and Technology) certified thermometer (Thermo Scientific, Waltham, Massachusetts, United States) was used to verify shaker incubator temperature. Duplicate flasks were performed at all enzyme loadings. The experiments were run for four days, with time course samples taken once daily throughout the four-day run time. Time zero concentration values were calculated based on composition of the pretreated slurry and then adjusted based on the weight additions of water, citrate buffer and enzyme. Final samples were taken at day four and analyzed for density and total and insoluble solids, as well as monomeric and oligomeric total sugar concentrations. Cellulose conversion yield during enzymatic saccharification was calculated from the net amount of monomeric glucose produced, which also used measurements of liquid density and liquid volume [42].

Microorganism and revive/pre-seed culture

Zymomonas mobilis strain 13-H-9-2 was used in this evaluation. The strain was taken from cell stock stored at −70°C. The pre-seed medium consisted of 10 g/L yeast extract and 2 g/L potassium phosphate monobasic (1X RM (rich media)), supplemented with 100 g/L glucose and 20 g/L xylose. The reviving culture was started by transferring 1 mL of *Z. mobilis* strain 13-H-9-2 cell stock into 9 mL of pre-seed medium in a 15 mL tube. The culture was incubated at 33°C with no agitation. The culture was sampled at 8 hours for an optical density reading at 600 nm. The pre-seed culture was used to inoculate the batch seed fermenter with media composition of RM (1X), 150 g/L

glucose, 20 g/L xylose and 1 g/L sorbitol. pH and temperature were controlled at 5.8 by Potassium Hydroxide (4 N) and at 33°C.

Fermentation

Fermentation experiments to evaluate the neutralized saccharified whole slurry were performed in BioStat-Q Plus fermenters (Sartorius, Biotech, Germany) at a 300 mL working volume using recombinant *Z. mobilis* strain 13-H-9-2. Rich media consisting of 10 g/L yeast extract and 2 g/L KH_2PO_4 was added to enzymatically-hydrolyzed whole slurry. The fermenters were inoculated at an optical density (600 nm) of approximately 1.0 absorbance units using a direct transfer procedure (10% v/v). The fermentation was conducted at a temperature of 33°C, a pH of 5.8 (controlled with 4 M KOH) and an agitation speed of 300 rpm. The fermentation was typically finished in 72 hours. Ethanol yield calculations were based on initial glucose, xylose and fructose concentrations as well as differences between initial and final ethanol concentrations.

Analytical methods

The composition of the milled solids was determined by a two-stage acid digestion procedure based on NREL standard laboratory analysis procedure (LAP Number NREL/TP-510-42627) [43]. Soluble sugars, acetic acid and degradation products were determined by NREL LAP Number NREL/TP-510-42623 [44]. The density of liquid samples was measured using an Anton-Parr model DMA-500 density meter (Anton Paar USA, Inc., Ashland, Virginia, United States).

Abbreviations

DCS: deacetylated corn stover; DDR: deacetylation and disc refining; DRDCS: disc-refined deacetylated corn stover; FPU: filter paper unit; HMF: hydroxylmethylfurfural; MESP: minimum ethanol selling price; NREL: National Renewable Energy Laboratory; ODMT: oven dried metric tone; rpm: revolutions per minute; RM: rich media; HPLC: high performance liquid chromatography; LAP: laboratory analytical procedure; NIST: National Institute of Standards and Technology; BETO: Bioenergy Technologies Office; DOE: Department of Energy; EERE: Office of Energy Efficiency and Renewable Energy.

Competing interests

The authors declare that they have no competing interests.

Author's information

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Authors' contributions

XC designed the process and conducted the experimental work including deacetylation, disc refining, and enzymatic hydrolysis as well as analyzed the data and drafted the manuscript. JS co-conducted the deacetylation experiments, reviewed results and help draft and revised the manuscript. LT, RE, MH, and DJ reviewed results and revised the manuscript. SP conducted PFI refining experiments. MS and TP helped design and conduct the disc refining experiments. EJ and RN conducted high solids enzymatic hydrolysis and analyzed the data. KF conducted twin screw extrusion experiments. OT helped to designed the Szego mill experiment. WW conducted particle size distribution analysis. MT is the principle investigator of this project, helped designed the whole process, helped conduct deacetylation, and arrange commercial scale disc refining, and helped draft and revised the manuscript. All authors have read and approved the final manuscript.

Acknowledgements

We would like to acknowledge the funding support from the BETO (Bioenergy Technologies Office) program in DOE (Department of Energy) EERE (Office of Energy Efficiency and Renewable Energy). We also want to thank the biomass analytical team for their technical assistance and analysis.

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Received: 28 February 2014 Accepted: 10 June 2014
Published: 25 June 2014

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doi:10.1186/1754-6834-7-98
Cite this article as: Chen *et al.*: **A highly efficient dilute alkali deacetylation and mechanical (disc) refining process for the conversion of renewable biomass to lower cost sugars.** *Biotechnology for Biofuels* 2014 **7**:98.