Accounting for all Sugars Produced during Integrated Production of Ethanol from Lignocellulosic Biomass

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Abstract

Accurate mass balance and conversion data from integrated operation is needed to fully elucidate the economics of biofuel production processes. This study explored integrated conversion of corn stover to ethanol and highlights techniques for accurate yield calculations. Acid pretreated corn stover (PCS) produced in a pilot-scale reactor was enzymatically hydrolyzed and the resulting sugars were fermented to ethanol by the glucose-xylose fermenting bacteria, *Zymomonas mobilis* 8b. The calculations presented here account for high solids operation and oligomeric sugars produced during pretreatment, enzymatic hydrolysis, and fermentation, which, if not accounted for, leads to overestimating ethanol yields. The calculations are illustrated for enzymatic hydrolysis and fermentation of PCS at 17.5% and 20.0% total solids achieving 80.1% and 77.9% conversion of cellulose and xylan to ethanol and ethanol titers of 63 g/L and 69 g/L, respectively. These procedures will be employed in the future and the resulting information used for techno-economic analysis.

Keywords: pretreatment, enzymatic hydrolysis, fermentation, ethanol, corn stover, yields, mass balance

1. Introduction

Cellulosic biomass-to-ethanol process technology has matured to the point where several companies built demonstration and commercial-scale plants with capacities up to 25 million gallons of ethanol per year (Brown and Brown, 2013). Nevertheless, work continues on this technology with many studies exploring different feedstocks, pretreatment technologies, and enzymatic hydrolysis/fermentation processes with the goal of defining performance of potentially lower cost process options for producing fuel-grade ethanol. With a staggering number of possible process combinations that can be explored, ultimately techno-economic analysis (TEA) must be used to understand the relative cost tradeoffs. The challenge is producing performance data (e.g., yields) to estimate ethanol production cost for TEA using process-relevant operating conditions. That is, at conditions (e.g., total biomass solids loadings, etc.) and with equipment (pilot scale equipment must sometimes suffice) that as near as possible simulate commercial operation. The results are more relevant if the data is produced during integrated operation, which is usually easier to achieve in pilot scale or demonstration plants. Integrated operation in this paper refers to the process of understanding and optimizing overall process performance instead of optimizing performance of individual unit operations. Some integration can be achieved during bench-scale processing; however, there is usually a finite time interval between processing steps that does not occur during continuous pilot-scale operations. This delay may or may not affect the outcome, but is usually assumed not to. For example, pretreated slurries are likely cooled and stored before enzymatic hydrolysis, and solids precipitation is often seen during this cooling process.

Besides the multitude of pretreatment options, there are various routes for enzymatic hydrolysis and fermentation including separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and variations thereof (e.g., fed-batch operation, partial hydrolysis, etc.). While the advantages and disadvantages of these routes are well known (Ask et al., 2012), the selection of which

option works best often depends on the characteristics of the fermenting microorganism. For example, in our work with a glucose-xylose fermenting bacterium, *Zymomonas mobilis* 8b, SHF works best because that microorganism doesn't perform well at the low glucose concentrations typically seen during SSF. Other researchers report similar or better performance in SSF compared to SHF (Saha and Cotta, 2007; Manzanares et al., 2011; Cotana et al., 2015). Additionally, enzymatic hydrolysis is performed on whole pretreated slurries, the dewatered pretreated solids, or the washed solids. Any of these options may be commercially viable, particularly if the recovered liquor stream is used to produce other high-value products. For dilute-acid pretreatment, the liquor stream contains primarily hemicellulosic sugars (xylose for many biomass feedstocks) that could be used to produce a by-product.

In this study, we explored one of many possible biomass-to-ethanol options using a corn stover feedstock. The process used here consisted of feedstock deacetylation (mild caustic treatment of the raw corn stover prior to dilute acid pretreatment, Chen et al. 2012), dilute acid pretreatment, and enzymatic hydrolysis of the whole pretreated slurry followed by fermentation of the sugars to ethanol. The process steps are illustrated in Figure 1 and described in more detail in the Methods section. Performance results for this integrated biomass-to-ethanol process were generated using both laboratory- and pilot-scale equipment. Deacetylation, dilute sulfuric acid pretreatment, and pressing were performed using pilot-scale equipment. The pretreated whole slurry was then enzymatically hydrolyzed and the resulting sugars fermented to ethanol using *Z.mobilis* 8b in laboratory-scale vessels. Yield data used to calculate ethanol production and needed to perform TEA was collected for each operation (Humbird et al., 2011). However, our goal was not to use the data to perform TEA, but to demonstrate a process and the associated techniques needed to acquire integrated yield data. In particular, we wanted to highlight calculation techniques accounting for high solids operations, oligomer conversion during enzymatic hydrolysis and fermentation, and using enzyme solutions containing glucose. The techniques are

illustrated for a limited set of runs, but at conditions producing high ethanol yields and titers. In the future, these techniques, including TEA results, will be applied to fully integrated pilot-scale runs.

2. Materials and Methods

2.1 Biomass and materials

The corn stover used in this study was Pioneer variety 34M95 harvested in the fall of 2003 from a farm in northeastern Colorado. The stover was knife-milled (Model # G1420, Jordan, Birmingham, AL, USA) through a 1.9 cm rejection screen prior to further processing.

Cellulase (Cellic® CTec 2) was acquired from Novozymes (Franklinton, NC, USA). Sulfuric acid (93%) and sodium hydroxide (50%) were purchased in 208-L drum quantities from Univar (Denver, CO, USA). Medium components KH_2PO_4 , D-xylose, and sorbitol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bacto yeast extract was purchased from Becton, Dickinson, and Company (Sparks, MD, USA) and dextrose was purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA).

2.2 Deacetylation and acid impregnation

Corn stover was deacetylated in a 1900-L hot-water heated, paddle reactor (American Process Systems, Gurnee, IL, USA). The deacetylation process was previously described by Chen et al. (2012). Forty (40) dry kg of stover, sufficient water, and concentrated NaOH solution were loaded into the reactor to achieve a 7.5% (w/w) total solids (TS) loading and 0.4% (w/w) NaOH concentration. All TS loading reported throughout this paper will be on weigh percent basis. The slurry was heated to 80°C

and the reactor was agitated at a moderate level of 12 rpm sufficient to achieve good mixing. After two hours, the solids were dewatered by draining the black liquor from the reactor, increasing the slurry TS content to approximately 17%. The reactor was filled with additional water multiple times and allowed to drain until the pH of the drained liquid was about 8.5. Then enough water and H₂SO₄ were added to adjust the slurry to an 8% TS loading and 2.0% (w/w) liquid phase H₂SO₄ concentration. The acidified slurry was agitated at 30 rpm and held at 45°C for one hour. The excess liquor was drained and the solids were further dewatered to about 45%–50% TS using a screw press (Model CP10, Vincent Corp., Tampa, FL, USA). A sample of the material was collected and sealed in plastic bags and stored for several days at room temperature prior to compositional analysis.

2.3 Pretreatment

The acidified solids were pretreated in a 200 dry kg/d continuous horizontal screw reactor (Valmet, Norcross, GA, USA) described by Wang et al. (2014) at 158°C for 5.0 min. The pretreated corn stover (PCS) was sampled for compositional analysis and stored in 208-L plastic drums at 4°C. The compositions of the raw, acid-impregnated, and PCS are shown in Table 1, and the composition of the liquor fraction of the PCS slurry is shown in Table 2. The TS and the insoluble solids (composed primarily of cellulose and insoluble lignin) concentration of the materials are also shown. Xylan-to-monomeric xylose yield was calculated as discussed in a previous publication (Shekiri et al., 2014) and then adjusted as described in section 3.0 and the supplemental information.

2.4 Enzymatic hydrolysis

The pH of the PCS slurry was adjusted to 5.0 at room temperature using approximately 0.005 g NH_4OH /g slurry. Approximately 500 or 550 g of pH-adjusted PCS slurry, enzyme (loaded at 40 mg protein/g cellulose), nutrients, and sufficient water were combined in 2-L capped bottles (1000 g total working weight) to a target of 17.5% or 20.0% TS loading, respectively. The nutrients were added as a 10X-strength solution to achieve a final concentration of 5 g/L yeast extract and 1 g/L KH_2PO_4 . The nutrients were added during this step to avoid further diluting the enzymatic hydrolysate prior to fermentation. The bottles were placed in a shaking incubator (48°C, 150 rpm) for 120 hours and only sampled at the final time point. After enzymatic hydrolysis was complete, 320 g of the enzymatic hydrolysate was transferred to a 500-mL bioreactor (Biostat-Q Plus, Sartorius, Goettingen, Germany). The monomeric glucose yield from cellulose was calculated based on previously published methods for high solids enzymatic hydrolysis with further modifications as described in section 3.0 (Hodge et al. 2009, Zhu et al. 2011).

The furfural concentration (5.2 g/L) in the PCS liquor was much higher than previously seen in pretreated liquors (1.0-1.5 g/L) produced in a larger 900 dry kg/d reactor (Shekiri et al. 2014). This finding was attributed to an inefficient flash vent system on the smaller reactor and so was deemed to not accurately represent expected furfural concentration in PCS. Because furfural is a potent fermentation inhibitor and unexpectedly high value is detrimental to microorganism performance, it was removed from the enzymatic hydrolysate by nitrogen sparging to a concentration consistent with values found in PCS produced in the larger reactor. This procedure was performed prior to transferring the enzymatic hydrolysate to the fermentation vessel. Filtered (0.2 μm) nitrogen was introduced into the 2-L bottle containing enzymatic hydrolysate at approximately 300 mL/min-L. The bottle remained in the rotary shaker operating at 48°C and 150 rpm. The effluent gas was vented and liquid mass lost due to

evaporation was measured and replaced with sterile deionized water. The bottle was sparged until the liquor furfural concentration was approximately 3 g/L.

2.5 Microorganism and seed culture

The microorganism used in the study was the glucose-xylose fermenting bacterium *Z.mobilis* 8b (Mohagheghi et al., 2004). A 15-mL tube with 9.0 mL of growth media containing 10 g/L yeast extract, 2 g/L KH_2PO_4 , and 100 g/L glucose was inoculated with 1.0 mL of a frozen stock culture. This pre-seed culture was incubated at 33°C for 8 hours.

Seed culture was prepared in a 500-mL Biostat Q-plus bioreactor using 300 mL of growth media containing 150 g/L glucose, 20 g/L xylose, and 1 g/L sorbitol. After 8 hours of incubation, the glucose concentration in the pre-seed culture drops to 30 g/L. At this time sufficient cells were transferred to the bioreactor to achieve an initial optical density ($\text{OD}_{@600\text{ nm}}$) of 0.01. The bioreactor temperature and agitation were set at 33°C and 300 rpm, respectively, and pH was controlled at 5.8 by adding 4N KOH. The seed culture was grown for about 18 hours, reaching an $\text{OD}_{@600\text{ nm}}$ of approximately 10, and having a residual glucose concentration of 30 g/L or higher. Glucose was measured with a 7100 Multi-parameter Bioanalytical System Analyzer (YSI, Yellow Springs, OH, USA), and OD was measured with a Spectronic 601 spectrophotometer (Milton Roy, Ivyland, PA, USA).

2.6 Fermentation

Fermentations were performed in the 500-mL BioStat-Q Plus bioreactors using 300 g of enzymatic hydrolysate. Each bioreactor was inoculated with enough cells from the same seed culture to achieve an

initial OD_{@600 nm} of approximately 1.0 (typically 10% v/v). The temperature and agitation were set to 33 °C and 300 rpm, respectively, and pH was controlled at 5.8 by adding 4N KOH. The fermentations were conducted for 96 hours and each vessel was sampled daily. Each condition was duplicated and average values are reported.

Ethanol and sugar-to-ethanol yield calculations, as described in section 3.0 and with more details provided in the supplemental information, were based on measurements of initial monomeric and oligomeric glucose and xylose concentrations. Xylose-to-ethanol yields were calculated by subtracting the ethanol produced from glucose assuming a 95% theoretical metabolic yield from glucose (yield based on consumed sugars) from the total ethanol produced.

2.7 Analytical methods

Composition of the raw, acid impregnated, and PCS was determined by a two-stage acid digestion procedure (Sluiter et al., 2012). Soluble monomeric and total (sum of monomeric and soluble oligomeric reported on a monomeric sugar basis) sugars were quantified by high performance liquid chromatography (HPLC) using an Agilent 1100 series HPLC (Santa Clara, CA, USA) with a Shodex SP0810 carbohydrate column (Shawa Denko K.K., Kawasaki, Japan) and a de-ashing guard cartridge (BioRad Laboratories, Hercules, CA, USA) using the procedure of Sluiter et al. (2008a). The column temperature was 85 °C and the mobile phase was ultra-pure water set at a flow rate of 0.6 mL/min. Ethanol, acetic acid, hydroxymethylfurfural (HMF), and furfural concentrations were measured by HPLC (Agilent 1100 series) using a BioRad HPX-87H organic acid column and Cation H⁺ guard cartridge (BioRad Laboratories, Hercules, CA, USA) with the column operating at 55 °C (Sluiter et al., 2008a). The mobile phase was dilute sulfuric acid (0.01 N) set to a flow rate of 0.6 mL/min. A refractive

index detector was used for compound detection for all HPLC measurements. Mixed component standards were periodically run with the HPLC samples to verify calibration accuracy.

The density of liquid samples was measured using an Anton-Parr model DMA-500 density meter (Anton Paar USA, Inc., Ashland, VA, USA).

TS measurements were done by drying samples at 45 °C in a vacuum oven (0.6 bar) until repeated weight measurements were constant. Slurry insoluble solid concentrations were determined by a six-step washing and centrifugation procedure (Sluiter et al., 2008b). Triplicate measurements were performed on each sample.

Enzyme protein concentration was measured using a Pierce BCA protein assay kit (Sigma-Aldrich, St. Louis, MO, USA) with bovine serum albumin as the protein standard. The enzyme preparation was desalted prior to measuring the protein concentration to remove salts, low-molecular-weight peptides, media components, co-factors, inhibitors, and additives that might interfere with the BCA assay (Adney et al., 2012).

2.8 Calculation modifications

Process yield results by unit operation are used to evaluate economics using models and techniques previously developed (Humbird et al. 2011). While there are many process variables tracked in these models, only three yields have a significant impact on ethanol production cost for a dilute sulfuric acid pretreatment/enzymatic hydrolysis process. These variables are monomeric xylose yield from pretreatment, monomeric glucose yield from enzymatic hydrolysis, and ethanol yield from glucose and xylose during fermentation. While conceptually these yields are easy to calculate, it is necessary to correctly account for production and conversion of oligomeric sugars occurring during the various

conversion steps. Not accounting for these sugars can lead to overestimating conversion yields. The need for this correction is likely being driven by better enzymes and more efficient conversion of sugars to ethanol, promoting further conversion of oligomeric sugars to monomeric form.

Since the economic models are not set up to account for oligomer conversion during enzymatic hydrolysis or fermentation, specific modifications were done to the yield calculations. For example, the monomeric xylose yield from pretreatment is increased to account for additional monomeric xylose produced from soluble xylo-oligomers during enzymatic hydrolysis. This occurs because monomeric xylose consumed during fermentation reduces its concentration to a value that is no longer inhibitory to hemicellulose enzymatic hydrolysis. The calculation modification increases the amount of monomeric xylose available from pretreatment for conversion to ethanol during fermentation. A modification to the enzymatic hydrolysis glucose yield calculation is required to account for conversion of residual cellulose and soluble gluco-oligomers contained in the enzymatic hydrolysate to monomeric glucose during fermentation. A final adjustment is necessary to account for glucose contained in many enzyme solutions, which is used as a stabilizer.

The fermentation metabolic ethanol yield (yield based on consumed sugars) and ethanol process yield (yield based on initial sugar available at start of fermentation) are often useful for understanding overall ethanol production efficiency during fermentation, but these yields are not directly used in our TEA model. The model calculates ethanol by summing the individual sugars (glucose, xylose, etc.) produced from each unit operation using the associated polymer-to-sugar yield, and then multiplies the sum by the associated sugar-to-ethanol yield. For example, ethanol from xylose is calculated from the monomeric xylose yield during pretreatment and the xylose-to-ethanol yield during fermentation, with adjustment in the former yield value as discussed above. In section 4.0, we will present both fermentation ethanol yields and individual sugar yields for a set of enzymatic hydrolysis/fermentations runs. All the equations

and methodology used in the calculations just discussed are presented in the supplemental information and for brevity will not be repeated here.

3. Results and discussion

3.1 Deacetylation and pretreatment

Previous work demonstrated that deacetylation removes a large fraction of the acetyl groups from the hemicellulose backbone and solubilizes up to 20% of the total corn stover mass by removing most of the extractives, acetyl (60%–70%), and lignin (10%–20%) fractions. One result of this process is slightly higher enzymatic cellulose conversion yields during whole slurry enzymatic hydrolysis, particularly at lower pretreatment severities (Chen et al., 2012). One possible explanation for improved enzymatic cellulose conversion yields is removal of some of the lignin that may be limiting cellulose accessibility. In addition, cellulose conversion is improved since acetic acid concentrations are lower in the pretreated slurry compared to the untreated (non-deacetylated) slurry, and this compound has been shown to be an inhibitor of enzyme activity (Hodge et al., 2008). Acetic acid is also a potent inhibitor to many microorganisms and lowering its concentration during fermentation improves sugar utilization and ethanol yields (Franden et al., 2013). This work provided the rationale for using deacetylated stover for this study.

Table 1 shows the feedstock composition differences between raw and deacetylated corn stover. The mass fraction of cellulose and xylan increase in deacetylated stover compared to raw stover after removal of the soluble compounds. But the mass fraction of the lignin in the deacetylated stover also increased to a value suggesting that little of the lignin was removed during deacetylation. While this

finding is discouraging, it is not the intent of this paper to investigate this phenomenon nor does it affect the purpose of this study to define a new methodology for yield calculations. The much larger processing equipment used in the work may be responsible for the difference in lignin extraction. But more importantly, a large fraction of the stover's acetyl groups were removed, reducing its acetate content to 1.0%, which is consistent with results reported by Chen et al. (2012). After dilute-acid pretreatment, the corn stover is enriched in cellulose and lignin and its composition is consistent with previous work (Shekiro et al. 2014).

The monomeric xylose, oligomeric xylose, xylan, and furfural yields from pretreatment calculated using the normal methodology presented in Shekiro et al. (2014), were 70.2%, 7.9%, 6.0%, and 7.1%, respectively. After enzymatic hydrolysis at 17.5% and 20.0% TS, the adjusted monomeric xylose yields are 76.9% and 75.9%, respectively, a nearly 10% increase in yield due to conversion of xylo-oligomers and residual xylan to monomers during enzymatic hydrolysis and fermentation. As expected, the results suggest that more xylo-oligomers are converted at the lower TS level because of lower concentration of inhibitory background sugars. However, there is insufficient experimental replication to rigorously support this conclusion.

3.2 Enzymatic hydrolysis

Mixed sugar solutions for fermentation were produced by enzymatic hydrolysis of whole PCS slurries diluted to 17.5% and 20.0% TS. The corresponding insoluble solids content for these slurries were 11.3% and 13.0%, respectively. The monomeric glucose yield from enzymatic cellulose hydrolysis, when not accounting for oligomer conversion during fermentation, was 82.8% and 84.2%. When the correction was made (as described in the supplemental file), the monomeric glucose yields were 88.1%

and 88.7% at 17.5% and 20.0% TS, respectively. While a higher yield is expected at the lower TS, the yields are statistically indistinguishable from each other (based on previous experimental work, not shown), particularly for the corrected values. We have seen better performance with the newer enzyme preparations (i.e., CTec 2) at higher TS contents producing nearly identical cellulose conversion yields at 20%–25% TS (data not shown). Finally, it is worth noting that we clearly report the monomeric glucose yield and not cellulose conversion (initial cellulose minus final cellulose divided by initial cellulose) because this latter value does not necessarily reflect the amount of monomeric glucose available for conversion to ethanol.

There are important performance and cost differences when using washed pretreated solids instead of whole slurries as employed in this study, but washed solids are used in many studies investigating dilute acid or autohydrolysis pretreatments (Lu et al. 2010; Manzanares et al. 2011). Removing the hemicellulose-sugar-rich stream and other soluble inhibitor compounds (acetic acid) from the solids prior to enzymatic hydrolysis improves cellulose conversion yields and generates a soluble sugar stream that can be used for producing other products. But there is additional cost for solid-liquid separation equipment. Nevertheless, it is difficult to compare data and the relative merits of both processes without a TEA. Results from an earlier study found that ethanol production cost was cheaper for the washed solids process (Dutta et al. 2009). In the last few years, better enzymes are available that are less inhibited by sugars and they have changed the relative ranking of these processes. We now favor the route presented in this paper for bioethanol production using *Z. mobilis* and its associated economic evaluation presented elsewhere (Humbird et al. 2011).

3.3 Fermentation

Average monomeric glucose and xylose and ethanol concentrations produced during fermentation of 17.5% and 20.0% TS enzymatic hydrolysates are shown in Figure 2. At 17.5% TS, all of the glucose and nearly all of the xylose is consumed within 48 hours, producing a maximum ethanol titer near 63 g/L. Based on the initial combined glucose and xylose concentration of 121 g/L and a residual xylose concentration of 3 g/L at the end of the fermentation, the calculated metabolic ethanol yield is 105% ($= 63 / [(121 - 3) \cdot 0.51] \cdot 100$) and the ethanol process yield is 102%. Many times such an unexpected result (over or near theoretical) might be attributed to experimental error; however, we believe the continuing conversion of oligomers, and perhaps even some of the insoluble carbohydrates to monomeric sugars, produce more sugars for conversion to ethanol. Using the methodology for calculating conversion yields presented in section 3.0, the ethanol metabolic and process yields are 98% and 94%, respectively. Clearly, not accounting for oligomer sugar conversion during fermentation can lead to a sufficient overestimation of ethanol yield.

The results at 20.0% TS follow a similar trend for sugar consumption, except the sugar uptake rate was slower, requiring nearly four days before ethanol production ceased, and more xylose was left unconverted (approximately 13.5 g/L). As noted by other researchers (Manzanares et al. 2011; Zhu et al. 2014), the higher inhibitor concentrations (e.g., ethanol, acetic acid, furfural, and others) in the 20% TS slurry compared to the 17.5% TS slurry are likely responsible for poorer performance. The simple ethanol metabolic and process yield calculation give 108% and 97%, respectively, compared to 103% and 92% from the more accurate calculation. While the ethanol process yield is reasonable and consistent with the greater amount of xylose left unconverted, the metabolic ethanol is slightly greater than 100%. *Z. mobilis* is known to efficiently convert sugars to ethanol, so metabolic yields near 100% are expected. The variance around 100% is likely due to experimental error caused by difficulties in

accurately mixing and weighing biomass slurries, which can easily dewater, thus posing measurement challenges.

While the ethanol process yield is useful for understanding overall performance, mass balance calculation and TEA often rely on tracking the disposition of the individual sugars and, in fact, our mass balancing for TEA uses this strategy. So it is necessary to determine ethanol yields from the individual sugars. But accurately calculating these yields is difficult because all the sugars produce the same product (ethanol). In this study, and as discussed in the supplemental information, we made the simplifying assumption that the glucose metabolic yield is 95%, which is the same as the glucose-to-ethanol yield (i.e., fraction of available glucose converted to ethanol) since all of the glucose is consumed by the microorganism. The ethanol derived from glucose is then subtracted from the total ethanol produced and the remaining ethanol amount is assumed to come from xylose, allowing easy calculation of the xylose-to-ethanol yield. While neither of these individual sugar yield values may exactly represent the actual value, together they accurately account for all ethanol produced. This technique can easily be extended to a three-sugar system (glucose, xylose, and arabinose) by also assuming a value for the arabinose-to-ethanol yield and then using the subtraction technique.

Using this methodology (method 1), the xylose-to-ethanol yields at 17.5% and 20.0% TS were 96.3% and 88.4%, respectively. An alternative strategy (method 2) to calculate individual sugar to ethanol process yields is to multiply sugar utilization (fraction of initial sugar consumed) by the metabolic ethanol yield. The metabolic ethanol yield is based on all sugars consumed and so represents an average yield. Sugar utilization and the associated ethanol yield values calculated by both methods are shown in Table 3. While each method gives a different value for the ethanol yield from the individual sugars, it is not obvious that any method is more accurate than the other.

To determine how these values compare, the overall ethanol yield (based on cellulose and xylan in the raw feedstock) is shown in the last row of Table 3. This value is calculated by applying pretreatment (adjusted monomeric xylose yield) and enzymatic hydrolysis conversion yields to a representative corn stover feedstock with an assumed cellulose and xylan content of 35% and 20% (w/w dry basis), respectively, to determine the monomeric glucose and xylose available for fermentation. While the feedstock cellulose and xylan content are assumed values, the actual number has little impact on the calculated yield. The ethanol produced is calculated by applying the glucose/xylose-to-ethanol yields presented in Table 3 to the sugar generated from pretreatment and enzymatic hydrolysis and then dividing by the theoretical amount of the ethanol available from cellulose and xylan. As shown in Table 3, there is reasonable agreement between the values calculated by the two methods. However, we will continue to use method 1 to report sugar-to-ethanol yields used in TEA, as this method more accurately reflects overall ethanol production. The second method provides better insight into how effectively the microorganism is converting the individual sugars to ethanol.

4. Conclusions

The overarching purpose of this paper was not to focus on the experimental performance results presented here but, instead, use the results to highlight improved procedures and methodologies for data analysis and interpretation. A rigorous methodology to calculate conversion yields is presented and illustrated for integrated dilute sulfuric acid pretreatment, enzymatic hydrolysis, and fermentation of sugars from corn stover to ethanol; however, the methods are not necessarily specific to this conversion process. More discussion of performance results, particularly the impact of total solids loading, will be presented on a more extensive data set in the future using this methodology.

Acknowledgments

The authors wish to thank Xiaowen Chen, Eric Kuhn, and Nick Nagle for preparing and pretreating corn stover. We would also like to thank the U.S. Department of Energy's Bioenergy Technologies Office for funding this research.

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Table Captions:

Table 1. Composition (weight percent dry basis) and solid content (w/w) of the raw, acid impregnated, and pretreated corn stover

Table 2. Composition of the pretreated slurry liquor fraction (g/L)

Table 3. Sugar-to-ethanol yields and overall ethanol yield based on the two different calculation methods

Figure Captions:

Figure 1. Flow diagram for processing corn stover to ethanol.

Figure 2. Average monomeric glucose (solid line), monomeric xylose (dotted line), and ethanol (dashed line) concentrations during fermentation of enzymatic hydrolysate produced at 17.5% (▲) and 20.0% (■) total solids.

Figure 1

Figure 2

Table 1

Component	Raw	Acid Impregnated	Pretreated
Cellulose	32.2	43.4	61.7
Xylan	19.3	27.4	2.8
Arabinan	3.4	3.4	0.7
Galactan	1.4	2.3	0.0
Lignin	11.9	17.0	25.8
Extractives ^a	14.1	-	-
Protein ^b	1.5	-	-
Ash	5.5	3.4	5.9
Acetate	2.6	1.0	0.3
Total Solids	94.0	49.9	29.0
Insoluble Solids	-	-	18.8

^a Water and ethanol soluble extractives, assumed to be removed from acid impregnated and pretreated material

^b Protein not measured in acid impregnated and pretreated material

Table 2

Component	Monomeric Sugars	Total ^a Sugars	Other Compounds
Glucose	14.5	14.5 ^b	
Xylose	84.1	90.9	
Arabinose	10.6	11.1	
Galactose	6.9	7.0 ^b	
Acetic Acid			5.9
Furfural			5.2
5-Hydroxymethylfurfural			0.2

^a Sum of monomeric and oligomeric sugars

^b All of the glucose and galactose appears only in the monomeric form

Table 3

Calculated Parameter	Total Solids (%)			
	17.5		20.0	
	Method 1 ^a	Method 2	Method 1 ^a	Method 2
Glucose utilization (%)		98.5		98.4
Xylose utilization (%)		91.7		70.9
Metabolic ethanol yield (%)		98.3		103.1
Glucose-to-ethanol yield (%)	95.0	96.8 ^b	95.0	101.5 ^b
Xylose-to-ethanol yield (%)	96.3	90.1 ^c	88.4	73.1 ^c
Overall ethanol yield (%) ^d	80.1	82.6	77.9	77.5

^aGlucose and xylose utilization and metabolic ethanol yield are not needed for this method

^bGlucose utilization times metabolic ethanol yield

^cXylose utilization times metabolic ethanol yield

^dBased on an assumed cellulose and xylan content in the raw corn stover calculated from the associated sugar yields from pretreatment, enzymatic hydrolysis and fermentation