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Technical Report
NREL/TP-5100-61563
April 2014

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Definitions

CS	Corn stover
CSL	Corn steep liquor
DI	Dynamic impregnator
GHG	Greenhouse gas
H_2SO_4	Sulfuric acid
HPLC	High performance liquid chromatography
HSEHR	High-solids enzymatic hydrolysis reactor
IBRF	Integrated Biorefinery Research Facility
LCA	Life cycle assessment
LCI	Life cycle inventory
LH	Large horizontal (reactor)
MESP	Minimum ethanol selling price
NaOH	Sodium hydroxide
NH_4OH	Ammonium hydroxide
NREL	National Renewable Energy Laboratory
OD	Optical density
PCS	Pretreated corn stover
SOT	State of technology
TS	Total solids
VT	Vertical (reactor)
WWT	Wastewater treatment

Executive Summary

For the U.S. Department of Energy's Bioenergy Technologies Office, the annual State of Technology (SOT) assessment is an essential activity for quantifying the benefits of biochemical platform research. This assessment has historically allowed the impact of research progress achieved through targeted Bioenergy Technologies Office funding to be quantified in terms of economic improvements within the context of a fully integrated cellulosic ethanol production process. As such, progress toward the ultimate 2012 goal of demonstrating cost-competitive cellulosic ethanol technology can be tracked. With an assumed feedstock cost for corn stover of $58.50/ton, this target has historically been set at $1.41/gal ethanol for conversion costs only (exclusive of feedstock) and $2.15/gal total production cost (inclusive of feedstock) or minimum ethanol selling price (MESP). This year, fully integrated cellulosic ethanol production data generated by National Renewable Energy Laboratory (NREL) researchers in their Integrated Biorefinery Research Facility (IBRF) successfully demonstrated performance commensurate with both the FY 2012 SOT MESP target of $2.15/gal (2007$, $58.50/ton feedstock cost) and the conversion target of $1.41/gal through core research and process improvements in pretreatment, enzymatic hydrolysis, and fermentation. Some of the key technical accomplishments in 2012 that allowed for the remaining cost reductions (between the 2011 and 2012 SOTs) to be realized were:

- Incorporation of deacetylation prior to pretreatment as a pretreatment cost savings measure and a downstream inhibitor mitigation strategy.
- Pilot-scale whole-slurry enzymatic hydrolysis (at 20% solids loadings) with an industrial enzyme package capable of converting cellulose to glucose at ~80% with an enzyme loading of <20 mg/g.
- Pilot-scale fermentation with an industrial organism capable of cofermenting 5- and 6-carbon sugars at yields of >90% while achieving a final ethanol titer of >70 g/L.

When these improvements, along with others described in detail in this report, are translated into an estimated n^{th} plant MESP for commercial production using NREL's economic model described in its 2011 design report (Humbird et al. 2011), **the resultant FY 2012 SOT MESP is $2.15/gal ethanol and the resultant conversion cost contribution is $1.32/gal ethanol (Table ES-1).** The actual performance data shown in the far right column of Table 1 are from one particular integrated pilot-scale run (Run 4). Another integrated pilot-scale run achieved slightly higher yields and lower MESP, as did other runs where bench-scale fermentation was conducted in parallel fashion. Key sustainability metrics associated with the 2012 SOT biorefinery model were also evaluated, and were found to improve relative to NREL's 2011 design case ("2012 target") model with respect to greenhouse gas emissions and fossil energy use: net greenhouse gas emissions for the modeled biorefinery decreased from –0.03 to –1.2 kg CO_{2e}/gal ethanol, while fossil energy demand decreased from 0.85 to –13.66 MJ/gal ethanol (design case versus 2012 SOT case, respectively). These improvements were driven largely by increased coproduction of electricity, a key sustainability driver for the process. The improvements are attributed to the displacement of standard grid electricity by the excess coproduct electricity from the biorefinery.

Table ES-1: Biochemical Platform Performance Targets

	2011 Targets	2011 Washed Solids	2011 Whole Slurry	2012 Target	2012 SOT
Minimum Ethanol Selling Price (2007$)	**$2.62**	**$2.56**	**$2.37**	**$2.15**	**$2.15**
Feedstock contribution ($/gal)	$0.76	$0.76	$0.82	$0.74	$0.83
Conversion contribution ($/gal)	$1.86	$1.80	$1.55	$1.41	$1.32
Yield (gal/dry ton)	78	78	71	79	71
Feedstock					
Feedstock cost ($/dry ton)	$59.60	$59.60	$59.60	$58.50	$58.50
Pretreatment					
Solids loading (wt%)	30%	30%	30%	30%	30%
Xylan to xylose (including enzymatic)	88%	88%	78%	90%	81%
Xylan to degradation products	5%	5%	6%	5%	5%
Conditioning					
Ammonia loading (g/L of hydrolysate)	4.8	3.8	4.2	4.8	1.6
Hydrolysate solid-liquid separation	Yes	Yes	No	No	No
Solids loading (wt%)	30%	30%	30%	30%	30%
Xylose sugar loss	1%	1%	1%	1%	0%
Glucose sugar loss	1%	1%	1%	0%	0%
Enzymes					
Enzyme contribution ($/gal)	$0.36	$0.34	$0.38	$0.34	$0.36
Enzymatic hydrolysis and fermentation					
Pretreatment solids loading (wt%)	20%	17.5%	20%	20%	20%
Combined saccharification and fermentation time (d)	5	5	5	5	5
Corn steep liquor loading (wt%)	0.6%	0.25%	0.25%	0.25%	0.25%
Overall cellulose to ethanol	86%	89%	80%	86%	74%
Xylose to ethanol	85%	85%	85%	85%	93%
Arabinose to ethanol	80%	47%	47%	85%	54%

Contents

1 Introduction

In 2007, the U.S. Department of Energy's Office of the Biomass Program (now titled Bioenergy Technologies Office), in collaboration with the National Renewable Energy Laboratory (NREL), established the goal of achieving cost-competitive cellulosic ethanol production from corn stover by 2012. The conversion process consists of dilute-sulfuric-acid pretreatment followed by enzymatic hydrolysis and cofermentation of the resultant biomass sugars to ethanol using a metabolically engineered fermentative strain. The technology improvement strategy was to achieve yearly increases in process conversion yields, along with associated reductions in modeled ethanol production costs. Progress toward this goal was measured using n^{th}-plant techno-economic models to estimate ethanol production cost based on conversion yields obtained during integrated bench- and pilot-scale experiments. The final objective is to demonstrate integrated pilot-scale performance that achieves the 2012 minimum ethanol selling price (MESP) cost target (Humbird et al. 2011) of $2.15/gal.

To assess production costs, NREL maintains a techno-economic model that describes the process and production economics of one conceptual biochemical ethanol conversion process. This is described in detail in the 2011 biochemical design report (Humbird et al. 2011). The overarching process design in this model is dilute-acid pretreatment of corn stover, followed by enzymatic saccharification and pentose/hexose cofermentation using a recombinant fermentative organism. Ethanol is separated from the fermentation broth, water is recycled back to the process, and lignin and other residues are burned to produce steam and electricity. For a given set of biochemical conversion parameters, Aspen Plus process simulation software is used to generate material and energy balance and flow rate information (Aspen Technology, Inc. 2007). These data are used to size and cost process equipment and compute raw material and other operating costs, assuming a feed rate to the biorefinery of 2,205 dry U.S. tons of corn stover per day. Using a discounted cash flow rate of return analysis, the MESP ($/gal) required to obtain a net present value of zero for a 10% internal rate of return (IRR) is determined. The result is a so-called techno-economic model that reasonably estimates an "n^{th}-plant" production cost for this pre-commercial process from the pilot-scale conversion data generated in the Integrated Biorefinery Research Facility (IBRF) at NREL.

This design report incorporates recent progress in the conversion areas (pretreatment, conditioning, saccharification, and fermentation), optimizations in product recovery, and an improved understanding of the ethanol plant's supporting processes (wastewater and utilities) relative to earlier versions. Using 2012 conversion targets and "n^{th}-plant" project costs and financing, and a feedstock cost of $58.50/dry ton, the target MESP is $2.15/gal (in 2007$). The specific FY 2012 MESP target of $2.15/gal can be found in the April 2012 *Biomass Program Multi-Year Program Plan* (MYPP) Table C-3 (DOE 2012). This report will discuss the state of technology (SOT) analysis using this most recent 2011 design report model exclusively.

Table 1 shows the yield and cost performance objectives for the Biochemical Platform. The technical targets for 2012 are listed in the far right column. While clearly significant progress was made toward the final cost and technical targets through 2011, further improvements were needed if we were to capture the final required incremental cost savings. The key yield targets that determine ethanol production and would be instrumental in capturing such cost savings are xylan-to-xylose, cellulose-to-ethanol, xylose-to-ethanol and arabinose-to-ethanol. The 2011 SOT

also established that these key yield targets must be demonstrated in a whole-slurry mode, because the cost associated with separating and washing the solids was too high to overcome and still hit the $2.15/gal target. In 2012 we used a strategy to improve these yields that incorporated a deacetylation step upstream of pretreatment. The rationale was that removing a significant amount of acetic acid from the process upfront would lead to reduced inhibition of both the enzyme package (improving cellulose to glucose and xylan to xylose yields) and the fermentation organism (improving total sugar to ethanol yields and raising the final ethanol titer). This was first tested at bench scale and the resulting yield improvements demonstrated at that point with the deacetylation strategy (even while accounting for the extra capital and operating costs associated with this new step) gave us enough confidence to use this approach in our end-of-year pilot-scale demonstration as well.

Table 1. Biochemical Platform Performance Targets

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Enzyme contribution ($/gal)	$0.36	$0.34	$0.38	$0.34
Enzymatic hydrolysis and fermentation				
Pretreatment solids loading (wt%)	20%	17.5%	20%	20%
Combined saccharification and fermentation time (d)	5	5	5	5
CSL* loading (wt%)	0.6%	0.25%	0.25%	0.25%
Overall cellulose to ethanol	86%	89%	80%	86%
Xylose to ethanol	85%	85%	85%	85%
Arabinose to ethanol	80%	47%	47%	85%

* CSL – Corn steep liquor

The 2012 technical targets were met during bench-scale testing in mid-2012. Deacetylated corn stover was pretreated in NREL's 200 kg/d pilot-scale pretreatment reactor and then enzymatically hydrolyzed at bench scale with a commercial enzyme package. The resulting sugars were fermented to ethanol (again at bench scale) using an organism developed under a DOE cost-shared research solicitation. The feedstock was deacetylated by soaking corn stover in a dilute sodium hydroxide (NaOH) solution (0.4% w/v) at 80°C for 2 h. This procedure removes a large fraction of the acetyl groups from the hemicellulose backbone prior to pretreatment. This process lowers acetic acid concentrations in the pretreated liquor, which improves carbohydrate polymer-to-sugar yields and sugar-to-ethanol yields because acetic acid inhibits both processes. Integrated bench-scale, whole-slurry testing produced a cellulose-to-ethanol yield of 87% and a xylose-to-ethanol yield of 94% at 20% total solids (TS). These yields exceeded the cellulose-to-ethanol target of 86% and xylose-to-ethanol target of 85% (Table 1), and represent a dramatic improvement over performance results demonstrated at the end of FY 2011, which did not employ deacetylated stover (77% cellulose-to-ethanol and 80% xylose-to-ethanol yields, respectively). However, the one remaining caveat (other than bench- versus pilot-scale operation) was that the cellulose enzymatic hydrolysis was performed at a high enzyme loading (40 mg protein/g cellulose) during this work. This was done to ensure that enough sugars were generated to test the hypothesis that higher fermentation yields and ethanol titers could be achieved by removing acetic acid inhibition. In the subsequent pilot-scale runs it was recognized that enzymatic hydrolysis would need to be done at a lower enzyme loading (closer to 20 mg/g) to meet the ultimate 2012 cost target. Nevertheless, this work clearly established the process to use for pilot-scale testing, illustrated in Figure 1.

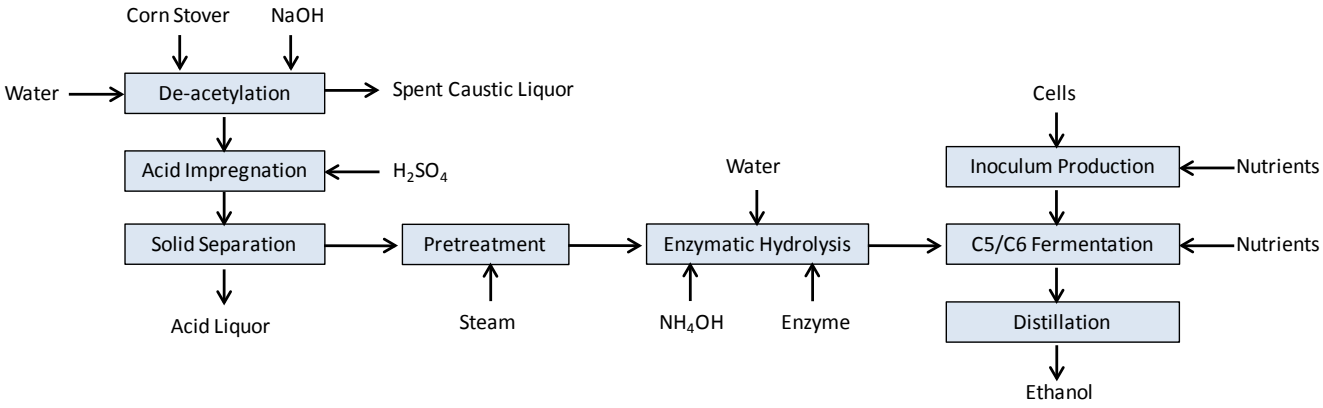

Figure 1. Corn stover-to-ethanol process flow diagram used for integrated pilot-scale testing

The remainder of this report presents results for several integrated pilot plant demonstration runs performed in the latter half of FY 2012 to produce yield data needed to estimate the MESP using the previously described techno-economic model and ultimately successfully demonstrating the $2.15/gal cost target shown in Figure 1. In parallel with the pilot-scale demonstration runs, samples of pre- and post-inoculated enzymatic hydrolysate were taken and fermented in a 500-mL fermentor. The pre-inoculated sample was inoculated with a separate lab-grown inoculum.

The purpose of this parallel fermentation work was to acquire fermentation data in case the pilot-scale fermentation failed due to equipment problems or contamination and to demonstrate correlations between the bench- and pilot-scale operations. Finally, the techno-economic model was updated to the current process configuration being demonstrated in the pilot plant, new capital and operating cost information developed over the last year was incorporated into the model, and several economic sensitivity analyses were performed using performance results from the demonstration runs.

2 Methods

2.1 Demonstration Runs

2.1.1 Feedstock

The demonstration runs used Pioneer 33B51 corn stover received in 2003 from Wray, Colorado (Kramer farm). The stover was received tub ground and was further knifed milled (Jordan Reduction Solutions, Birmingham, Alabama) through a ¾-in. rejection screen in the NREL IBRF.

2.1.2 Description of Pilot Plant Activities

Figure 2 illustrates the sequence of operations and associated pilot plant equipment used for the 2012 demonstration runs. This figure shows the equipment used for each unit operation performed in the pilot plant along with associated chemical flows and mode of operation (batch, semi-batch, continuous). Plant operating conditions are given in Table 2. A description of each unit operation follows.

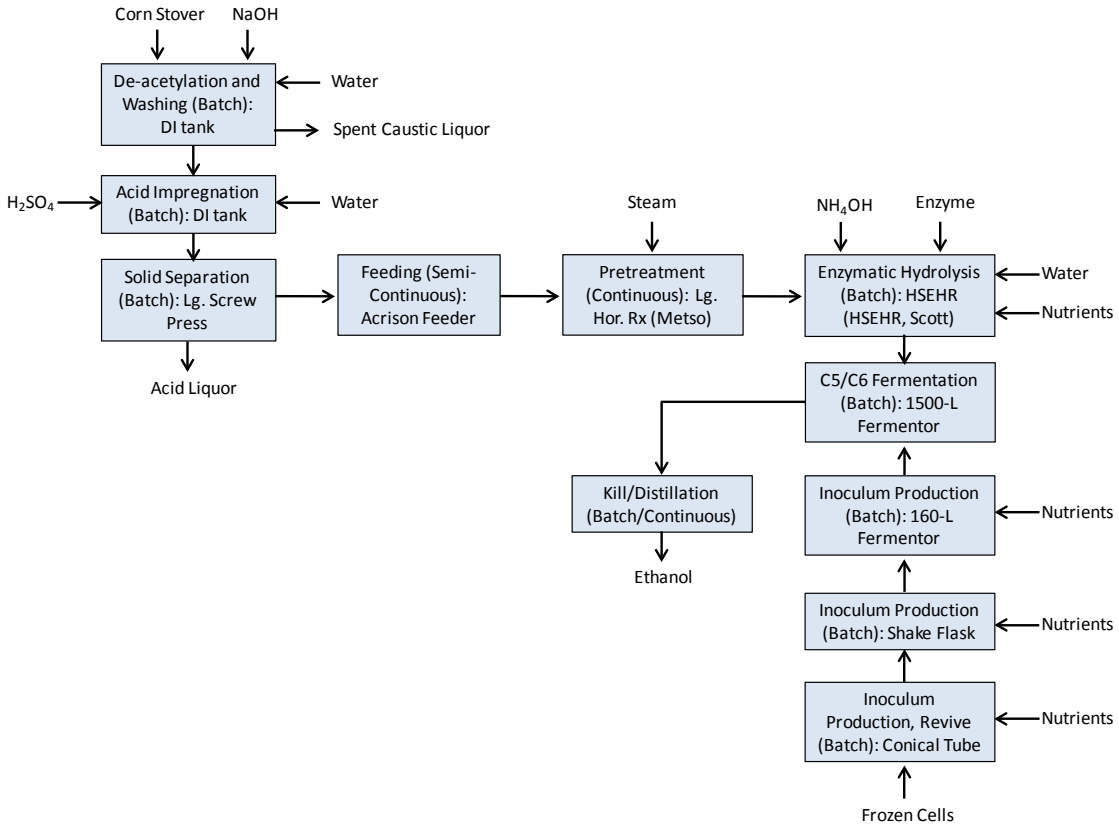

Figure 2. Pilot plant sequence of operations and associated equipment

Table 2. Plant Operating Conditions for Each Unit Operation

Unit Operation/Vessel	Temp (°C)	Catalyst/ Loading	Residence Time	Solids Loading (%,w/w)	pH	Agitation Speed (rpm)	Working Volume (L)
Deacetylation/DI[a]	80	NaOH/0.4 % (w/w) in liquid	2 h	~8	n/a	15	n/a
Acid impregnation/DI	Amb.	H_2SO_4/0.8 % (w/w) in liquid	2 h	~8	n/a	15	n/a
Pretreatment/VT,LH[b]	160–190	n/a	1–10	~30	n/a	n/a	n/a
Enzymatic hydrolysis/ HSEHR[c]	50	Variable	1–4 d	20	5.0	[d]	1200
Enyzmatic hydrolysis/ V-450A	50	n/a	1–3 d	20	5.0	[d]	900
Fermentation/V-450A	33	Cells/ 1.0 OD[e]	2–4 d[f]	n/a	5.8	[d]	1000
Inoculum/V-445A	33	n/a	18–22 h[g]	n/a	5.8	50	110
Inoculum/shake flask	33	n/a	~11 h	n/a	5.8	n/a	0.750
Inoculum/tube	33	n/a	~9 h	n/a	5.8	n/a	0.010

[a] Dynamic impregnator. DI tank loaded with 100–120 dry kg corn stover per batch
[b] VT vertical reactor, LH-large horizontal reactor
[c] High-solids enzymatic hydrolysis reactor
[d] As needed to achieve sufficient mixing and temperature control, may have been reduced as run proceeded
[e] Optical density
[f] Until fermentation is complete
[g] Until culture glucose concentration drops to ~50 g/L

2.1.2.1 Deacetylation and Acid Impregnation

Corn stover deacetylation and sulfuric acid impregnation were performed in the 1900-L dynamic impregnator (DI) tank (American Process Systems, Gurnee, Illinois). Dry corn stover was added to the tank along with a dilute sodium hydroxide solution. The slurry was heated to 80°C and held for 2 h, then the liquor was allowed to drain overnight. Water was added to the tank to rinse the solids and the rinse water was then discharged. A dilute sulfuric acid solution was added to achieve the desired feedstock acid concentration (see Table 2). After thoroughly mixing the acid-impregnated solids at room temperature for 2 h, the solids were pumped to a continuous screw press (Vincent Corporation Model CP10, Tampa, Florida) for dewatering (see Section 2.1.2.2). Approximately 250–300 kg (dry basis) of impregnated corn stover was required per run. The largest batch size that can be processed in the DI tank is 100–120 dry kg of corn stover, so at least three batches of material were prepared for each run. For some batches, data were collected for mass balance and yield calculations for the deacetylation process.

2.1.2.2 Solid Separation

The acid-impregnated solids were pumped to a large screw press and dewatered to approximately 45%–50% TS. The dewatered solids (feed for pretreatment) were collected into drums and stored for later use.

2.1.2.3 Feeding

Dewatered, acid-impregnated corn stover (feedstock) was manually fed to the Acrison feeder (Acrison, Inc., Moonachie, New Jersey) installed upstream of the weigh belt. The feedstock was added to the Acrison's hopper as needed to maintain a consistent flow of feedstock to the pretreatment reactor. The feedstock flow rate was varied between runs to achieve optimal performance out of the pretreatment reactor pressurized feed system, but was typically 25–35 dry kg/h.

2.1.2.4 Pretreatment

The pretreatment reactor was operated at conditions given in Table 2. Except for Run 2, all pretreatment runs were conducted in the IBRF large horizontal (LH) pretreatment reactor (Metso Inc., Norcross, Georgia) configured for two-tube operation (Shekiro et al. 2014). Run 2 was conducted in the original Sunds vertical (Vermont) pretreatment reactor (Sunds Defribrator (now Metso Inc., Norcross, Georgia) located in the north wing of the IBRF. Pretreatment was performed for approximately 10–12 h at steady-state conditions until 220–260 kg (dry basis) of feedstock was pretreated. Pretreated corn stover (PCS) was discharged to the flash tank and then fed continuously via gravity to the high-solids enzymatic hydrolysis reactor (HSEHR, Scott Equipment Company, New Prague, Minnesota). Approximately 1000–1400 kg of PCS slurry was produced after dilution to 20% (w/w) TS. When pretreatment was performed in the vertical reactor, the PCS slurry was collected into drums that were then emptied into the HSEHR.

2.1.2.5 Enzymatic Hydrolysis

After the HSEHR was filled with PCS, the material was sampled and held in the reactor until a TS content value was available (2–3 days). The TS content is needed to accurately calculate feed additions (water, rich media, enzyme, etc.) and adjust the TS of the slurry to the target value of 20% TS. To begin enzymatic hydrolysis, the reactor was heated to 50°C and then 10X rich media (rich media, yeast extract and potassium phosphate at concentrations given below) and a partial addition of water were added to the reactor. The sterile rich media solution was prepared in a 160-L fermentor (Associated Bio-Engineers and Consultants, Bethlehem, Pennsylvania) and pneumatically transferred to the reactor. The pH was then adjusted to 5.0–5.4 using 15% ammonium hydroxide (NH_4OH). Enzyme (using commercially available enzyme packages) was pumped into the reactor and then a known amount of chase water followed to rinse enzyme solution into the reactor. The amount of chase water was adjusted to achieve the target 20% TS. As the cellulose content of the PCS solid could only be estimated, the actual enzyme loading was calculated after the run using the measured cellulose content of the PCS. After 3 days, approximately 750 kg of enzymatic hydrolysis slurry was transferred to a 1500-L fermentor (Associated Bio-Engineers and Consultants, Bethlehem, Pennsylvania).

2.1.2.6 Enzymatic Hydrolysis and Fermentation

Enzymatic hydrolysis was continued in the 1500-L fermentor for an additional day by maintaining the fermentor temperature at 50°C. To begin fermentation, the fermentor temperature and pH (using 15% NH_4OH) were adjusted to the values required for fermentation (see Table 2). Then seed culture (10% v/v) was added to the fermentor. Ammonium hydroxide (15%) was used to control fermentor pH. The fermentation was continued until ethanol concentration no longer increased.

2.1.2.7 Seed Culture Preparation

Approximately 40 hours before the 1500-L fermentor was inoculated, ethanologen seed culture was revived in a 50-mL conical tube. After approximately 11 h, this culture was transferred to a 1-L shake flask. After approximately another 9 h, 750–1000 mL of this seed culture was transferred to the 160-L fermentor, which contained approximately 120 L of fermentation media. The final seed culture was grown for about 18–22 h or until the glucose concentration dropped to approximately 30–50 g/L with a target optical density of 8–10 absorbance units (@ 600 nm). Table 3 shows the media components and their concentrations in all seed culture fermentations.

Table 3. Fermentation Media Concentrations at All Scales

Component	Concentration in Seed Fermentations (160-L and smaller) (g/L)	Concentration in Main Fermentation (1500-L) (g/L)
Yeast extract	10	5
KH_2PO_4	2	1
Sorbitol	2*	–
Glucose	150	–

* Sorbitol was not used in shake flasks and conical tubes.

2.1.3 Sample Plans and Data Analysis

2.1.3.1 Sampling and Analysis Plan

Sample analysis and scheduling for each run is presented in Table 4. All enzymatic hydrolysis and fermentation samples that could not be immediately processed were frozen. Because enzymatic hydrolysis was occurring prior to completing enzyme addition, time-zero hydrolysate samples were not taken because it would not provide the good sample for measurement of TS and fraction insoluble solids (FIS). The initial enzymatic hydrolysis time-zero slurry properties (TS and insoluble solids and component concentration) were calculated by mass balance from measurement of feed, caustic, water, and enzyme additions to the HSEHR. In later runs, a sample of slurry was taken prior to enzyme addition to check the calculations.

Table 4. Sample Analysis Plan and Schedule

Sample	Measurement	Technique	Schedule
Corn stover	Total solids Solids composition[a]	105°C oven Wet chemistry and near infrared (NIR)	One sample from each CS lot place in DI tank
Spent caustic liquor	Total solids Liquor composition[b,c,d] Soluble lignin Liquor density	40°C vacuum oven HPLC[e]-Shodex, Fast Acid Spectroscopic Densitometer	One sample from each deacetylated CS lot prepared in DI tank
Acid-impregnated feed	Total solids Total solids Solids composition[a]	40°C vacuum oven Infrared balance Wet chemistry	One sample from each deacetylated CS lot prepared in DI tank
Pretreated slurry	Total solids Insoluble solids Liquor composition concentration[b,c,d] Solids composition concentration[e] Soluble lignin Liquor density	40°C vacuum oven Best available method HPLC-Shodex, Fast Acid Wet chemistry Spectroscopic Densitometer	3 random samples from HSEHR after completely filling the tank and then extensively mixing the slurry
Flash tank stream	Component concentration[d]	HPLC-Fast Acid	4 times during pretreatment operation
Vent stream	Component concentration[d]	HPLC-Fast Acid	4 times during pretreatment operation
Enzymatically hydrolyzed slurry	pH Glucose concentration Liquor composition concentration[b,d]* Liquor composition concentration[c] Solids composition concentration[f] Insoluble solids Liquor density	pH meter YSI HPLC-Shodex, Fast Acid HPLC-Shodex Wet chemistry Best available method Densitometer	At 3, 6, 12, 24 h and every 24 h thereafter
Fermentation broth	pH Liquor composition concentration[bd]* Liquor composition concentration[c] Insoluble solids Liquor density	pH meter HPLC-Shodex, Fast Acid HPLC-Shodex Best available method Densitometer	At 0, 3, 6, 12, 24 h and every 24 h thereafter
Seed culture	Glucose concentration OD Composition concentration[b,d] Dry cell weight (DCW)	YSI Spectrometer HPLC-Shodex, Fast Acid Oven drying	At 0 h, final time point, and as needed to track glucose concentration and OD during cell growth

[a] Glucan, xylan, galactan, mannan, arabinan, sucrose, extractives, lignin, ash, acetyl
[b] Monomeric glucose, cellobiose, xylose, arabinose, galactose; data required 2 days after run completion
[c] Oligomeric sugars (total sugars)
[d] Furfural, hydroxymethylfurfural (HMF), acetic acid, ethanol
[e] High performance liquid chromatography
[f] Glucan, xylan, galactan, mannan, arabinan, lignin, ash, acetyl
*Monomeric sugar and ethanol/inhibitor data required within 24 h of sampling

The composition of pretreated solids was determined by a two-stage acid digestion procedure (Sluiter et al. 2008). Soluble oligomeric sugars were determined by dilute acid hydrolysis of liquor samples followed by quantifying sugars by high performance liquid chromatography (HPLC) as described by Sluiter et al. 2006.

Sugar concentrations in liquor samples were measured by HPLC using an Agilent 1100 series HPLC (Agilent Technologies, Inc., Santa Clara, California) with a Shodex SP0810 carbohydrate column (Showa Denko K.K., Kawasaki, Japan) and a de-ashing guard cartridge (Bio-Rad Laboratories, Hercules, California). The column temperature was 85°C and the mobile phase was ultra-pure water at a flow rate of 0.6 mL/min. Ethanol, acetic acid, hydroxymethylfurfural (HMF), and furfural were measured by HPLC using a Phenomenex Rezex RFQ Fast Fruit H+ organic acid column and Cation H+ guard cartridge (Bio-Rad Laboratories), also following NREL standard laboratory analytical procedures with the column operating at 55°C. The mobile phase was dilute sulfuric acid (0.01 N) at a flow rate of 0.6 mL/min. A refractive index detector was used for compound detection. Mixed component standards were periodically run with the HPLC samples to verify calibration accuracy. The density of liquid samples was measured using an Anton-Paar model DMA-500 density meter (Anton-Paar USA, Inc., Ashland, Virginia).

Slurry TS concentrations were determined 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 (Schell et al. 2003). Triplicate measurements were performed on each sample.

2.1.3.2 Data Recording

The pilot plant's data acquisition and control (DACS) system monitored and recorded all sensor data for the duration of equipment operation. The flow rate data were used to calculate conversion yields and mass balances. Some data (specifically weight of additions) were manually collected and recorded.

3 Results

3.1 Demonstration Run Results

Five demonstration runs were performed in the summer of 2012. Actual operating conditions are given in Table 5. The best pretreatment operating conditions were determined in a study performed prior to the demonstration runs. Enzymatic hydrolysis was performed at a target 20% TS loading with a commercial enzyme package at enzyme loadings varying from 33 to 19 mg protein/g cellulose. The first run used a high enzyme loading consistent with previous bench-scale work. In subsequent runs, the enzyme loading was reduced to understand the economic impact of lower enzyme loadings. pH was controlled at 4.8–5.4 with 15% (w/w) NH_4OH. The final two runs used an enzyme loading of 19 mg/g as reported below, which is a slight improvement beyond the 2012 target. Fermentation was performed with a glucose-xylose-arabinose fermenting bacteria. Fermentation operating conditions were fixed at 33°C and pH was controlled at 5.8 with 15% NH_4OH.

Table 5. Actual Operating Conditions for Each Demonstration Run

Operating Condition	Demonstration Run #				
	1	2	3	4	5
Pretreatment					
Reactor	LH	VT	LH	LH	LH
Solids loading[a] (%)	29.0	28.3	28.1	27.5	30.3
Temperature (°C)	160	190	160	160	160
Residence time (min)	10	1	10	10	10
Acid loadings (mg/g) effective acid	9	9	9	9	9
Concentration[b] (%)	0.35	0.35	0.33	0.31	0.38
Enzymatic hydrolysis					
TS loading (%)	20.1	19.6	20.0	20. 0	20.0
Enzyme loading (mg/g)[c]	33	33	26	19	19

[a] Solids loading was not controlled, but actual value was determined by steam heating requirements

[b] Actual acid concentration in the reactor

[c] Based on protein content of 175 mg protein/g solution

3.1.1 Deacetylation

During preparation of deacetylated stover, sufficient data were collected to calculate mass and carbohydrate loss during the deacetylation process. In addition to acetate removal, the deacetylation process removes a portion or all of the ash, sucrose, lignin, xylan, galactan, and arabinan. In a previous bench study, approximately 2%–4% of the original xylan content of the corn stover was removed (Chen et al. 2012). In the demonstration runs approximately 6% of the original xylan was removed during the deacetylation process. The stover was likely treated more severely in the demonstration runs than needed; that is, more acetate was removed than is necessary to achieve good fermentation performance. Unfortunately, the deacetylation process has not been optimized in the short time we have been performing this process. It is possible to reduce both NaOH use and decrease xylan losses to further reduce processing cost without sacrificing fermentation performance. Because of the unusual nature of the stover used in the study, and because the deacetylation process has not been optimized, a xylan loss of 2% as is currently used in the techno-economic model is a reasonable assumption. The impact of higher xylan losses in the deacetylation step was modeled as a sensitivity case and is presented later in this report.

3.1.2 Pretreatment

The pretreatment yield results are given in Table 6. The third column is the adjusted xylan-to-xylose yield where the yield value has been increased because of additional xylose produced from enzymatic hydrolysis of xylan and xylo-oligomers during whole-slurry enzymatic hydrolysis and fermentation. The other columns are self-explanatory. The xylan carbon mass balance closures were in the range of 90%–99% (data not shown), which is typical of continuous pretreatment systems. Runs 1, 3, 4, and 5 were performed in the horizontal reactor and xylan-to-xylose yields were consistent, except for Run 1 results. Xylan mass balance closure for Run 1 was high at 103%, so there is less confidence in this result. Even though the Run 3 and Run 4 xylan-to-xylose yields were consistent, the Run 3 adjusted xylan-to-xylose yield was higher than Run 4, likely because the higher enzyme loading used in Run 3 improved xylo-oligomer conversion compared to Run 4.

Table 6. Pretreatment Performance Results

Demonstration Run #	Xylan-to-Xylose Yield (%)	Adjusted Xylan-to-Xylose Yield* (%)	Xylan-to-Furfural Yield (%)	Residual Xylan Yield (%)	Arabinan-to-Arabinose Yield (%)	Cellulose-to-Glucose Yield (%)
1	78.4	92.7	4.4	20.4	96.1	5.6
2	70.0	81.2	7.0	12.9	85.6	6.8
3	74.6	88.0	5.4	15.3	92.2	6.5
4	73.1	81.2	5.2	17.9	110.8	6.6
5	71.9	81.7	5.4	16.8	90.7	5.9

* Monomeric xylose yield which includes xylose produced during enzymatic hydrolysis and fermentation

Another result of note is the high arabinan-to-arabinose yields, but the small quantity of arabinan in the biomass causes more variability in these results. Nevertheless, an average arabinan-to-arabinose yield of 95% seemed to be a reasonable assumption. The pretreatment results for the demonstration runs were similar to results achieved in early 2012 on a small horizontal reactor. Overall monomeric xylose yields were somewhat lower than the 90% target value, but this loss was nearly compensated for by achieving higher-than-targeted xylose-to-ethanol yields in the fermentation step (see Table 8) by virtue of more complete sugar utilization and higher ethanol titers. The deacetylation step also enables less sulfuric acid usage in pretreatment, resulting in lower furfural formation and less salt formation by requiring less NH_4OH for neutralization, in addition to dramatically lowering acetic acid concentration. All these effects are believed to improve the ability of the fermentation organism to more completely utilize available sugars and achieve high ethanol titers.

3.1.3 Enzymatic Hydrolysis

The first and second demonstration runs were performed with a high enzyme loading (~33 mg/g) to match previous experimental conditions, even though lower enzyme loadings are needed to meet the cost target. The third run used an intermediate loading of ~26 mg/g, while the final two runs were loaded at 19 mg/g. For Runs 1–4, the entire enzyme amount was added to the HSEHR all at once. Periodic NH_4OH additions were required to maintain the pH in a range of 4.8–5.2. However, the fifth run used a staged addition policy in an attempt to achieve better conversion. The concept of staged addition is to initially use a small amount of enzyme to partially liquefy the slurry. Once the slurry is liquefied, pH and temperature control will improve, which will lead to less enzyme damage and presumably better conversion yields. Approximately 20% of the

enzyme amount was added initially, at 3.5 h the pH was readjusted to about 5.4, and then the remaining enzyme was added.

Figure 3 shows monomeric glucose production profiles for each runs. As expected, both final glucose titers and yields are higher at higher enzyme loadings. For all loadings, maximum glucose production is achieved in 3–4 days. There is insufficient information to know if the alternative enzyme addition policy employed in Run 5 was beneficial.

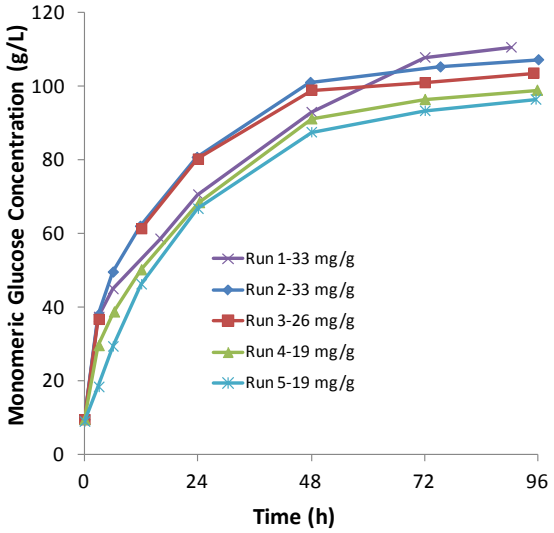

Figure 3. Monomeric glucose production during enzymatic hydrolysis of pretreated slurries from Runs 1–5

Table 7 shows enzymatic hydrolysis performance results for each run. The NH_4OH usage is provided to check the prediction of NH_4OH requirements from the techno-economic model (calculated based on stoichiometric neutralization demand). Note that NH_4OH use for Run 1 was higher than the other runs because the target pH value was overshot during base addition. Because of the difficulties in adequately mixing high solids slurries, there was a significant lag between NH_4OH addition and a response from the pH probe. More careful addition of NH_4OH avoided this problem during ensuing runs.

Table 7. Enzymatic Hydrolysis Performance Results

Demonstration Run #	Enzyme Loading (mg/g)	NH_4OH Use[a] (g/kg)	Cellulose-to-Glucose Yield (%)	Adjusted Cellulose-to-Glucose Yield[b] (%)
1	33	11.4	86.5	91.8
2	33	7.1	85.8	88.4
3	26	7.6	83.7	88.9
4	19	6.4	77.4	78.3
5	19[c]	7.7	80.3	82.5

[a] 15% (w/w) NH_4OH solution per kg of hydrolysate slurry on a 20% TS basis

[b] Adjusted for additional glucose produced during fermentation (from conversion of gluco-oligomers)

[c] Value calculated assuming a protein content of 175 mg protein/g solution.

Table 7 presents both monomeric glucose yield following enzymatic hydrolysis and the higher yields achieved with subsequent conversion of gluco-oligomers-to-glucose during fermentation. The rapid decrease in glucose concentration during fermentation relieves glucose inhibition of the enzyme promoting conversion of gluco-oligomers to glucose. The adjusted yield value was used for techno-economic modeling. As expected, cellulose-to-glucose yield decreases at lower enzyme loadings, so economic modeling is necessary to understand the cost tradeoffs. Good cellulose conversion was achieved in Run 5 and the staged enzyme addition policy may have produced a higher yield at the same enzyme loading used in Run 4.

3.1.4 Fermentation

A typical component concentration profile during enzymatic hydrolysis and fermentation for a successful run (Run 4) is shown in Figure 4. The plot shows 4 days of enzymatic hydrolysis prior to inoculation of the slurry at time zero. The instantaneous drop in component concentration is from dilution of the hydrolyzed slurry by the 10% (v/v) seed culture. Glucose is utilized very quickly (as expected) while xylose and arabinose are converted at slightly slower—but still acceptable rates—producing an ethanol titer of >70 g/L in as little as 24 h.

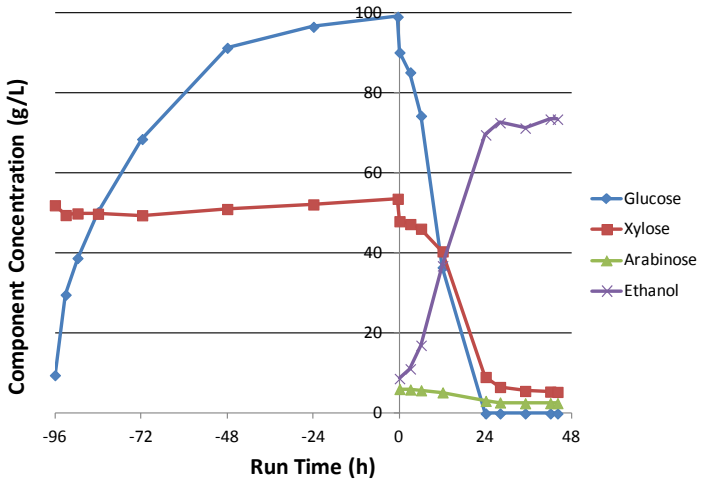

Figure 4. Typical (Run 4 illustrated) component concentration profiles during enzymatic hydrolysis and fermentation (time zero is at the start of fermentation)

Figure 5 plots ethanol and lactic acid concentration profiles for each demonstration run. Runs 2, 4, and 5 all performed well, achieving final ethanol titers of 71–76 g/L in less than 2 days. But lactic acid-producing bacteria were clearly present in Runs 1 and 3. Run 1 was most impacted by the contamination as the ethanol concentration reached only 45 g/L with over 30 g/L of lactic acid being produced by the contaminating bacteria. Also, more than 30 g/L of xylose remained in solution. Run 3 was slightly affected (7 g/L lactic acid), but 70 g/L of ethanol was still produced. Loss of sugars to lactic acid production, usually from a *Lactobacillus* bacterium (a common contaminant in ethanol fermentations), reduced the amount of ethanol produced.

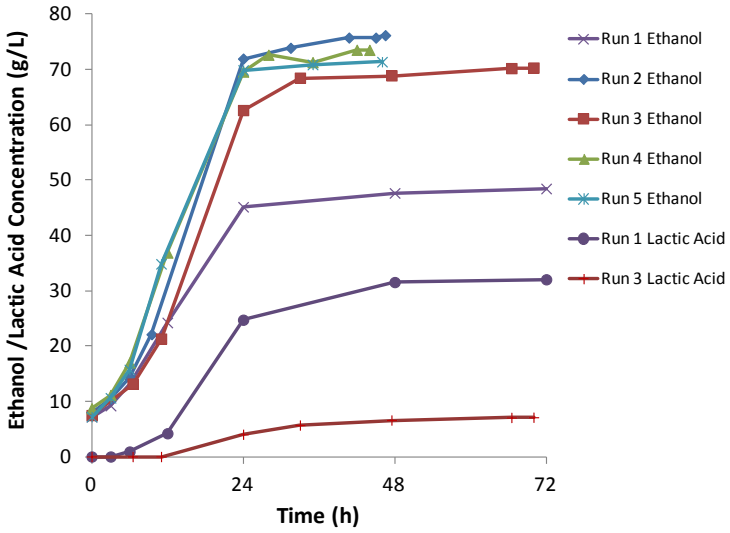

Figure 5. Ethanol and lactic acid (Runs 1 and 3 only) concentration profiles for each demonstration run

We believe the health of the cells used to inoculate the main fermentor may have played a role in the success of the fermentation (success is defined as no appreciable production of by-products such as lactic acid). After we detected contamination in the main fermentor during Run 1, we began plating enzymatic hydrolysate and fermentation broth samples. This work identified other bacteria in the enzymatic hydrolysate. Since the high temperature (50°C) conditions during enzymatic hydrolysis are not favorable to growth, no proliferation of bacteria was seen during enzymatic hydrolysis. However, once temperature and pH are changed to values required for fermentation, the bacteria would be able to grow. If a healthy inoculum of seed culture is added to the fermentor, these cells (because of their much higher cell density and rapid growth rate) will quickly convert most of the sugars to ethanol before the contaminating bacteria can utilize much of the sugars. But if the cells in the inoculum are not growing rapidly or are otherwise unhealthy, then contaminating bacteria would be able to compete and produce by-products such as lactic acid, as seen in Runs 1 and 3.

Figure 6a shows the cell density (as measured by OD) and the corresponding glucose consumption curves (Figure 6b) for inoculum grown for each demonstration run, which is the best indicator of cell health that was employed for these runs. High cell densities (9–10) were achieved in Run 4 and 5 inoculums, in which no significant lactic acid was produced during the main fermentation. The high cell densities are not necessarily required (as illustrated by the success of Run 2), which was transferred at an OD of 7.5. While the OD of Run 1's inoculum was 8, this run had the highest lactic acid production. Nevertheless, the inoculum cell density of Run 3 was somewhat low (~7), so these results still suggest that high cell densities are more likely to lead to a successful fermentation.

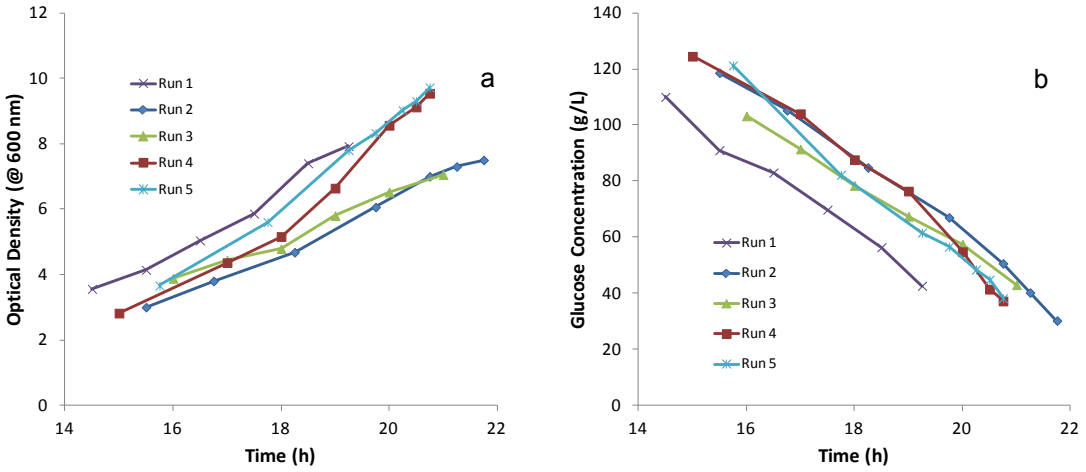

Figure 6. Cell density (a) and corresponding glucose concentration (b) during inoculum growth in the 160-L fermentor

As shown in Figure 4, glucose is rapidly utilized and was entirely consumed within 24 h. However, differences in ethanol yields between runs depends primarily on the amount of xylose consumed, ignoring the small amount of arabinose that also contributes to ethanol production but at relatively constant yields. Figure 7 shows the xylose concentration profiles for each run. For the runs contaminated by the lactic acid-producing bacteria, xylose consumption is clearly impacted as higher xylose concentrations remain at the end of fermentation. For the successful runs (2, 4, and 5), the residual xylose concentration is directly related to the initial sugar concentrations (sum of glucose and xylose). Higher initial sugars concentrations at the start of fermentation lead to higher residual xylose concentrations, which may be due to ethanol inhibition.

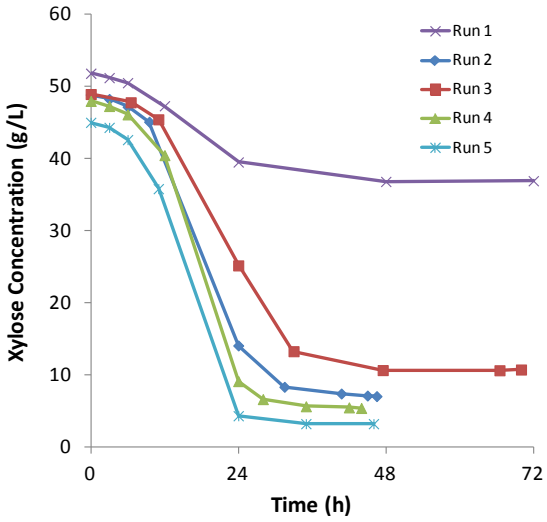

Figure 7. Xylose concentration profiles for each demonstration run

Table 8 provides calculated fermentation yields for each pilot-scale demonstration run. Yields for Run 1 are not reported because the fermentation was contaminated. Run 3's xylose-to-ethanol yield is also low because of contamination as previously discussed. Arabinose-to-ethanol yields show little variability. Cellulose-to-ethanol yields exactly mirror enzymatic hydrolysis yields. Xylose-to-ethanol yields follow the trend of residual xylose concentrations as discussed above.

Table 8. Fermentation Performance Results

Demonstration Run #	Xylose-to-Ethanol Yield[a] (%)	Arabinose-to-Ethanol Yield[b] (%)	Cellulose-to-Ethanol Yield[c] (%)
1[d]	–	–	87.2
2	86.2	61.6	84.0
3[e]	66.1	55.9	82.7
4	92.6	54.1	74.4
5	91.3	50.9	78.3

[a] Yield based on ethanol remaining after accounting for ethanol from glucose and arabinose
[b] Arabinose utilization times an assumed metabolic yield of 95%
[c] Yield calculated assuming a constant glucose-to-ethanol yield of 95%
[d] Fermentation yield not reported because run was contaminated
[e] Run had a low level of contamination that reduced fermentation yields compared to other runs

3.2 Techno-Economic Modeling Results

3.2.1 Feedstock Composition

The corn stover composition described in the 2011 design report is used in the current FY 2012 state of technology model to be consistent with the information presented in the above-referenced *Biomass Program Multi-Year Program Plan* and FY 2011 SOT documents. The major stover components are 35% glucan, 20% xylan, and 16% lignin.

3.2.2 Demonstration Run Data Selection

Pilot-scale demonstration Run 4 was selected as the basis for inputs to the FY 2012 SOT model. Corresponding results are shown in Table 9 through Table 11.

3.2.3 Pretreatment Process Design

In the FY 2011 SOT effort, primary pretreatment was performed in the continuous horizontal reactor, with a subsequent secondary thermal oligomer conversion step to convert additional oligomers to monomers. Beginning in late 2011, we started using new strategies (e.g., deacetylation, post-pretreatment milling) to produce pretreated material to improve hemicellulose and enzymatic conversion yields and to reduce inhibitor concentrations to improve fermentation yields (Chen et al. 2012). Demonstrated yield improvement in overall ethanol production (especially in fermentation) proved the addition of deacetylation (followed by acid pretreatment) to be a more appealing solution. Therefore, in the FY 2012 pretreatment process design, the alkaline deacetylation step was added prior to the subsequent pretreatment operations, as described above. The secondary thermal oligomer conversion was deemed to be unnecessary.

Reduced pretreatment acid loadings were demonstrated in the continuous pilot-scale pretreatment reactors, and the modeled acid loadings and other pretreatment conditions for the FY 2012 SOT case are shown in Table 9. Additional acid is used to pre-impregnate the

deacetylated biomass (the cost of that usage is included in the purchased sulfuric acid cost in the techno-economic model), but much of this acid is removed when the impregnated corn stover is dewatered and does not enter the pretreatment reactor. The direct cost benefit of lower acid loading in pretreatment is that the neutralization ammonia usage is reduced, as is the formation of inhibitors (such as HMF and furfural), while effective pretreatment performance is maintained. The effective sulfuric acid concentration in the pretreatment reactor (estimated at 0.3–0.4 wt% after dilution by condensing steam is accounted for) may allow for the use of lower cost metallurgies in the reaction zone (such as 904L or other duplex stainless alloys) instead of Incoloy-clad carbon steel, which could reduce pretreatment equipment costs. However, relevant corrosion data under these conditions are not available, so in the FY 2012 SOT model, Incoloy-825 cladding is still conservatively assumed for the material of construction (consistent with the 2011 design report). The potential impacts of lower cost metallurgy and other pretreatment reactor cost savings measures are addressed in the sensitivity analysis.

Table 9. Pretreatment Conditions Applied in the 2011 and 2012 SOT Models

Metric	2011 SOT	2012 SOT
Sulfuric acid loading	22 mg/g dry biomass	9 mg/g dry biomass
Residence time	5 min	10 min
Temperature	158°C	160°C
Pressure	5.5 atm	5.5 atm
TS loading	30 wt%	30 wt%

The deacetylation step uses milled corn stover soaked in a NaOH solution at 80°C for 2 h, with a NaOH loading of 0.009 g/g dry biomass. The deacetylated corn stover is dewatered by draining through screens at the bottom of the deacetylation reactor. The drained liquor, often referred to as *black liquor*, contains 20%–25% of the original dry biomass constituent material, including water extractives, soluble ash constituents, and 33% of lignin, 2% of xylan, and 88% of acetate that was originally present in the feedstock (dry basis); this black liquor stream is sent to wastewater treatment in the model. The deacetylated feedstock (containing the remaining 75%–80% of the dry biomass) is fed to the continuous pretreatment reactor upon acid impregnation and dewatering to achieve an actual sulfuric acid loading of 9 mg/g dry feedstock. The reaction proceeds at 160°C for 10 min. The pretreated slurry is cooled and conveyed to enzymatic saccharification and fermentation. Note that in this design (and as demonstrated in the pilot-scale runs), the secondary thermal oligomer conversion step is no longer needed.

The FY 2012 pretreatment target yield of monomeric xylose from xylan is 90%. The FY 2012 SOT demonstrated an overall 82% yield of monomeric xylose from all combined thermochemical and enzymatic processes, and demonstrated monomer arabinose yield higher than 95%. Although the monomeric xylose yield is below the technical target of 90%, subsequent xylose-to-ethanol yields achieved are substantially higher than their technical target, meaning that the effective conversion of xylan to ethanol is only slightly lower than the overall target.

3.2.4 Enzymatic Hydrolysis Process Design

Because deacetylation significantly reduced acetate inhibition, hydrolysate solid-liquid separation and subsequent washed-solid processing are also no longer required. The whole-slurry hydrolysate is conditioned with ammonia for subsequent enzymatic hydrolysis. Consistent with the experimental data discussed above, the enzyme loading was 19 mg protein/g cellulose for Run 4, but varied from 19–33 mg protein/g cellulose for the other runs. Neutralized PCS, enzyme, media, and sufficient water are combined to achieve 20% TS loading in the saccharification tank, for a total of 84 h (3.5 days) and maintained at 48°C. Although most of the enzymatic hydrolysis runtime during the pilot-scale demonstration occurred in the HSEHR, the hydrolysate liquefied significantly in the first 24 h, indicating that the 24-h residence time in a high solids continuous reactor assumed in the techno-economic model prior to transfer to a lower cost stirred tank batch reactor is a valid—and perhaps conservative—assumption. On-site enzyme production is assumed using parameters consistent with the 2011 design report. After enzymatic hydrolysis is complete, the hydrolyzed material is cooled prior to fermentation. Cellulose conversion results for the past several SOT cases are summarized in Table 10. FY 2009 and FY 2010 SOTs were based on washed solids and 40 mg/g enzyme loading and thus achieved good cellulose conversion. Yield decreased in FY 2011 when whole slurry enzymatic hydrolysis was employed even at an enzyme loading of 40 mg/g, since the background sugars present in whole slurries inhibit enzyme hydrolysis. In the FY 2012 SOT, the removal of acetate before enzymatic hydrolysis and use of better enzyme preparations improved cellulose conversion. The economic impact of the slight cellulose-to-glucose yield reduction in FY 2012 compared to FY 2011 is offset by the lower enzyme loading (19 mg/g versus 40 mg/g).

Table 10. Cellulose-to-Glucose Yields From Enzymatic Hydrolysis

	% TS	FY 2009 SOT	FY 2010 SOT	FY 2011 SOT	FY 2012 SOT
Whole-slurry	15%	–	90%	–	–
	17.5%	–	86%	83%	–
	20%	83%	84%	79%	78%*

* Yield is based on demonstration Run 4 data for FY 2012 SOT base case modeling. However, cellulose-to-glucose yields ranged from 78%–92% in the pilot-scale demonstrations, shown in Table 7.

3.2.5 Fermentation Process Design

The fermentation proceeds at 33°C and a pH of 5.8 using an engineered fermentative bacterium. The fermentation residence time is 36 h (1.5 d). Table 11 summarizes the fermentation performance at 20% total solids, using corn steep liquor (CSL) as the only fermentation nutrient source. The combined saccharification and fermentation time remain the same as in the FY 2011 case (5 days), as confirmed in the pilot-scale demonstration runs. Finally, the assumed CSL loading is 0.25%.

Table 11. Fermentation Performance of Fermentation Organism

Process Description	Ethanol Process Yield[a]	Ethanol Yield From Glucose[b]	Ethanol Yield From Xylose[c]	Ethanol Yield From Arabinose	Final Ethanol Titer (g/L)
20% TS, 1.5 days	92%[d]	95%	93%	54%	74

[a] Ethanol produced from initial monomeric glucose, xylose, arabinose, and fructose and glucose derived from sucrose.
[b] Glucose-to-ethanol metabolic yield is assumed to be 95%.
[c] Measured ethanol yield from xylose assuming 95% conversion of glucose to ethanol.
[d] Ethanol yield is based on demonstration run 4 data for FY 2012 SOT base case modeling. However, it ranges from 80%–92% in the pilot-scale demonstrations.

3.2.6 Improved Wastewater Treatment Model

As noted in the 2011 design report, a significant effort was made in FY 2011 to improve the wastewater treatment (WWT) section of the Aspen model, because the concentration of inorganic compounds in the stillage water was potentially too high for standard treatment by anaerobic and aerobic digestion. One of the findings was that nitrification was required in aerobic digestion to remove the high loading of ammonia. When deacetylation was introduced, both acid loading and resultant ammonia loading for conditioning were reduced dramatically (see Table 9 and Table 14). However, the WWT section in the 2011 design report was designed specifically for one level of pretreatment chemical usage and its costs do not accurately scale as acid and ammonia usage are reduced, or as chemical oxygen demand (COD) varies from the base case. Therefore, a subcontract with Brown and Caldwell (a WWT technology) was established early in FY 2012 to quantify the cost implications for the WWT section associated with the revisions made to the pretreatment operations (namely the new black liquor stream from deacetylation and significantly lower amount of ammonia salts associated with the reduced ammonia conditioning demand).

The study with Brown and Caldwell demonstrated WWT capital reduction in a process concept similar to the previous design described in the 2011 design report. Updated capital costs are shown in Table 12. In addition to updated capital quotations for WWT, the scaling method is improved, allowing the new design to more accurately scale as front-end chemical loading changes (rather than merely the total flow rate to WWT, as in the 2011 case). In the previous 2011 WWT design, all equipment costs were scaled on hydraulic flow rate. In the new design, the evaporator system, membrane reactor, and reverse osmosis system are scaled on hydraulic flow rate, while the anaerobic digester, aeration basin, and the other equipment are scaled on total chemical oxygen demand (COD) loading to the WWT system. The newer design is believed to better account for upstream process variations, such as acid and ammonia usage changes. In addition, the operating cost is reduced equivalent to an ethanol cost savings of roughly $0.03/gal, due primarily to lower ammonia going into the WWT system. The power usage matches well with the prediction using the 2011 design model, so does not currently provide additional operating cost savings from the energy demand aspect. The lessons learned from this Brown and Caldwell study have been incorporated into the design report model and this updated model was used for all of our economic calculations in the FY 2012 SOT analysis.

Table 12. Updated Capital Costs for WWT in the FY 2012 SOT Model (2007$)

Equipment	WWT Installed Cost ($MM)	
	2011 Design Model	FY 2012 SOT
Evaporator system	3	4
Membrane bioreactor	5	4
Reverse osmosis system	2	2
Centrifuge	6	1
Anaerobic digester	27	23
Aeration basin	5	6
Others (pumps, conveyer, etc.)	1	1
Total WWT	49	41

3.2.7 Cost Impact of Adopting Deacetylation

Adopting alkaline deacetylation prior to pretreatment adds process complexity and costs for alkaline usage and for new deacetylation tanks. However, the benefits of using this pretreatment concept are numerous:

- By removing acetate as one of the strong inhibitors, the resulting hydrolysate is more fermentable. More than 95% glucose-to-ethanol yield and more than 92% xylose-to-ethanol yield were achieved in the pilot-scale demonstration runs in FY 2012, as well as demonstrated by bench-scale trials in previous studies (Chen et al. 2012; Tao et al. 2012).
- The use of less acid in pretreatment results in lower ammonia usage for neutralization, which brings about a cost savings of almost $0.05/gal ethanol. The purpose of the ammonia conditioning step is to neutralize sulfuric acid and organic acids, such as acetic acid, in the biomass feedstock, such as acetic acid. Based on the pilot-scale demonstration, more than 80% of acetate is removed into the black liquor stream, resulting in much lower ammonia usage.

Roughly 20%–25% of dry biomass material is solubilized into the black liquor upon deacetylation, based on the pilot-scale demonstrations. Removing this material upfront provides an additional benefit of at least 20%–25% dry solids flow rate reduction going into all downstream processing operations, from pretreatment to ethanol recovery, which translates to considerable capital cost savings. For instance, 12 fermentors are required in the 2011 design report, while only nine are needed in the FY 2012 SOT case because of lower process throughput. Additionally, the design specification for pretreatment is 30% TS, so the amount of dilution water is also reduced. Table 13 shows a comparison of the installed capital costs for the FY 2012 SOT case with the 2011 design report.

Table 13. Installed Capital Costs in the FY 2012 SOT Model Compared to the 2011 Design Report (2007$)

Capital Costs ($MM)	2011 Design Report	FY 2012 SOT
Pretreatment	30	25
Neutralization/conditioning	3	4
Saccharification and fermentation	31	25
On-site enzyme production	18	17
Distillation and solids recovery	22	20
WWT	49	41
Storage	5	4
Boiler/turbogenerator	66	68
Utilities	7	7
Total installed equipment cost	**232**	**210**

3.2.8 MESP of FY 2012 SOT Using Pilot-Scale Demonstration Data

Table 14 shows the FY 2012 target case and SOT case performance and cost results, based on Run 4 process conditions and results. As in the 2011 design report, the SOT model includes on-site enzyme production to compute enzyme costs. **The SOT case meets the cost target for FY 2012 with a modeled MESP of $2.15/gal ethanol using $58.50/dry ton feedstock cost.** Cost contribution details from each major process area for the FY 2012 SOT case are presented in Figure 8, while the historical trend in MESP reduction demonstrated since 2007 is shown in Figure 9. Further process and cost details are summarized in Appendix A. Ethanol yield (gal/ton) is roughly 10% lower than the target case; however, this is offset by a similar 10% cost reduction in combined capital and operating costs (Table 13), because of modifications made to the model directly tied to process improvements demonstrated in the pilot plant (e.g., deacetylation, lower severity pretreatment, and associated WWT updates). The roughly equivalent reduction of overall yields and associated capital and operating costs from the original targets lead to a demonstrated per-gallon ethanol cost that achieves the FY 2012 target.

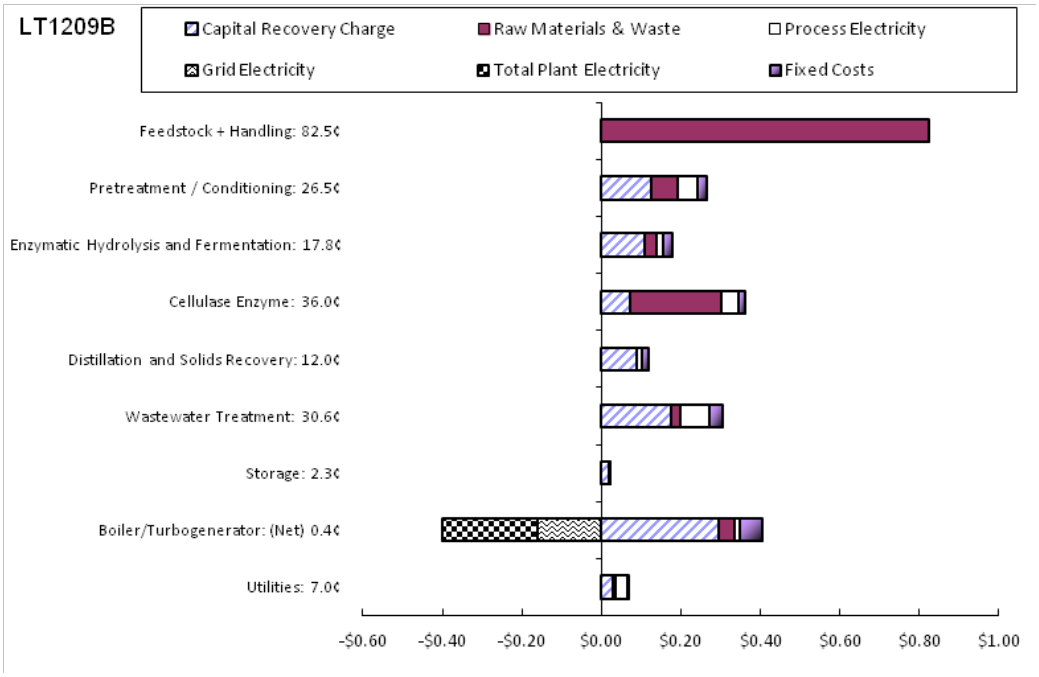

Figure 8. Cost contribution details from each process area (per gallon ethanol)

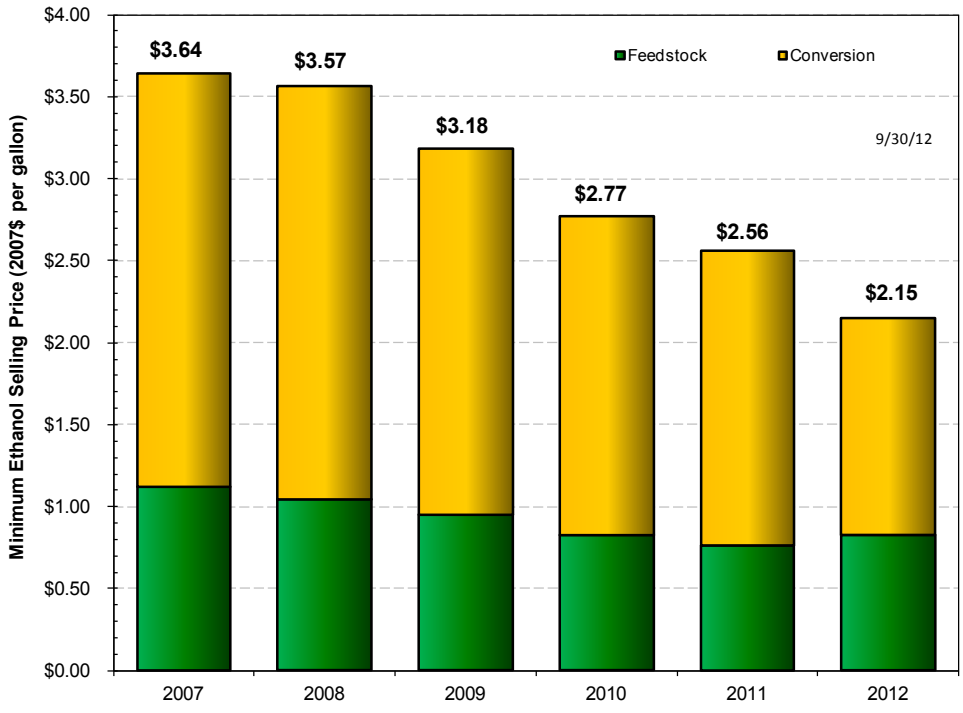

Figure 9. MESP techno-economic modeling results for 2007–2012 SOT cases

Table 14. FY 2012 SOT Case Based on 2011 Design Report Model (with modifications as noted above); Compared to 2007-2011 SOT Cases Back-Cast from 2011 Design Report Model Basis

	2007 SOT	2008 SOT	2009 SOT	2010 SOT	2011 SOT	2012 SOT
Minimum Ethanol Selling Price	**$3.64**	**$3.57**	**$3.18**	**$2.77**	**$2.56**	**$2.15**
Feedstock contribution ($/gal)	$1.12	$1.04	$0.95	$0.82	$0.76	$0.83
Conversion contribution ($/gal)	$2.52	$2.52	$2.24	$1.95	$1.80	$1.32
Yield (gallon/dry ton)	69	70	73	75	78	71
Technical Targets						
Feedstock						
Feedstock cost ($/dry ton)	$77.20	$72.90	$69.65	$61.30	$59.60	$58.50
Pretreatment						
Xylan to xylose (including enzymatic)	75%	75%	84%	85%	88%	81%
Xylan to degradation products	13%	11%	6%	8%	5%	5%
Hydrolysate solid-liquid separation	Yes	Yes	Yes	Yes	Yes	No
Xylose sugar loss	2%	2%	2%	2%	1%	0%
Glucose sugar loss	1%	1%	1%	1%	1%	0%
Enzymatic Hydrolysis and Fermentation						
Enzyme contribution ($/gal ethanol)	$0.39	$0.38	$0.36	$0.36	$0.34	$0.36
Combined saccharification and fermentation time (d)	7	7	7	5	5	5
CSL loading (wt%)	1%	1%	1%	1%	0.25%	0.25%
Overall cellulose to ethanol	86%	86%	84%	86%	89%	74%
Xylose to ethanol	76%	80%	82%	79%	85%	93%
Arabinose to ethanol	0%	0%	51%	68%	47%	54%
Operating Parameters						
Pretreatment solids loading (wt%)	30%	30%	30%	30%	30%	30%
Pretreatment temperature (°C)	190	190	158	158	152	160
Acid loading (mg/g dry biomass)	38.0	30.0	24.5	22.1	15.0	9.0
Secondary oligomer hold step	No	Yes	Yes	Yes	Yes	No
Ammonia loading (g/L of hydrolysate)	12.9	12.9	9.8	4.8	3.8	1.6
Conditioning mode	Liquor	Liquor	Liquor	Liquor	Liquor	Whole-slurry
Saccharification mode	Washed-solids	Washed-solids	Washed-solids	Washed-solids	Washed-solids	Whole-slurry
Enzymatic hydrolysis solids loading (wt%)	20%	20%	20%	17.5%	17.5%	20%
Reference Aspen Model	DW1102F	DW1102E	DW1102D	DW1102C	DW1109B	LT1209B

3.2.9 Cost Analysis of All Pilot-Scale Demonstration Runs

Techno-economic modeling was performed for all five pilot-scale demonstration runs (see Table 15). The FY 2012 SOT case used Run 4 as the basis for techno-economic modeling. The achieved overall ethanol yields for Run 5 (conducted at the same general processing conditions and enzyme loadings) were slightly higher than for Run 4. This leads to a slight reduction in MESP for Run 5 ($2.13/gal ethanol). Thus, the two pilot-scale demonstration runs that utilized an enzyme loading of 19 mg protein/g cellulose either met or *slightly improved upon* the overall cost target.

Table 15. Performance and MESP Summary for All FY 2012 Pilot-Scale Demonstration Runs

Demo Run #	Xylan-to-Xylose	Arabinan-to-Arabinose	Enzyme Loading (mg protein/g cellulose)	Cellulose-to-Glucose	Xylose-to-EtOH[a]	Arabinose-to-EtOH	EtOH Yield (gal/ton)	MESP ($/gal)
Target	90%	90%	20	90%	85%	85%	79.0	$2.15
1[b]	93%	96%	33	92%	–	–	–	–
2	81%	86%	33	85%	86%	62%	73.0	$2.36
3[c]	88%	92%	26	89%	58%	56%	70.4	$2.32
4	81%	95%	19	78%	93%	54%	70.9	$2.15
5	82%	91%	19	82%	91%	51%	72.5	$2.13

[a] Glucose-to-ethanol yields are fixed at 95%
[b] Fermentation terminated due to contamination
[c] Minor contamination was detected

3.2.10 Cost Sensitivity Analysis

Optimization of deacetylation with downstream processing. A range of 2%–6% xylan loss has been observed in the black liquor after the deacetylation step as soluble xylan. Although black liquor is sent to WWT where most of the heating value of the extracted xylan is reclaimed with the integrated energy system in the design, the overall ethanol production is reduced with the amount of xylan lost. The percentage of xylan loss to black liquor is believed to depend on feedstock varieties, as well as the strength of alkaline treatment (combination of alkaline loading, temperature, and duration). Optimization of deacetylation process conditions will be required to maximize overall sugar and ethanol yields. Additionally, process scenarios to recover solubilized xylan, acetic acid, and/or lignin in the black liquor represent an opportunity to reclaim additional value from this stream and to further reduce WWT costs. To quantify the cost implications of xylan loss in the deacetylation step, a sensitivity analysis is performed with 0%–6% loss of xylan during deacetylation to black liquor (see Figure 10), keeping in mind that we assumed 2% loss in our base case model.

Enzyme loading. Enzyme loading is a well-documented key cost driver in overall MESP results, relative to a baseline of 20 mg/g (Humbird et al. 2011). Given continuously evolving and improving enzyme preparations available from commercial companies, the cost sensitivity to enzyme loading is evaluated relative to the base case utilized here at 19 mg/g. The loading is varied between 10 mg/g as a reasonable improvement in the near-term for developing commercial enzyme cocktails, up to 30 mg/g as an approximation for the upper end of enzyme

loading utilized experimentally in the present effort (assuming all other parameters, including cost of the enzyme production process itself, are held constant). Consistent with prior results, the analysis demonstrates substantial economic sensitivity to enzyme loading over this range, with MESP decreasing by $0.27/gal at the low end and increasing by $0.21/gal at the high end of the enzyme loading evaluated.

While measuring the protein content of cellulase preparations is more repeatable than the traditional filter paper assay, there is some uncertainty in this measurement. To understand the cost impact of this uncertainty, a sensitivity case was run at a lower enzyme protein content of 165 mg protein/g solution (see Figure 10).

Pretreatment reactor cost. The pretreatment technology used in the FY 2012 demonstration runs employs alkaline deacetylation followed by low-acid, low-severity pretreatment. Given measured acid concentrations around 0.3 wt% and pretreatment temperature at 160°C, it is possible that the primary pretreatment reactor metallurgy could be replaced with lower cost alloys or that lower required pressure ratings result in lower reactor costs. However, a conservative approach was taken in the current FY 2012 SOT model to assume the same Incoloy 825 cladded carbon steel as the material of construction used in the 2011 design report. To quantify this potential savings of lower cost alloys and/or additional possible pretreatment reactor cost savings resulting from lower severity dilute acid pretreatment conditions, or escalation in pretreatment reactor cost caused by future inflation of alloy prices, the sensitivity analysis assumes a variation in pretreatment capital on the order of ±25% of the base capital cost for this unit operation, relative to the FY 2012 SOT model; this results in a ~$0.03/gal impact (see Figure 10).

Fermentation contamination loss. For large-scale operations, contamination is an issue that must be addressed. A contaminating bacterium was present during Runs 1 and 3 of the pilot-scale demonstration campaign that produced lactic acid. The 3% loss of fermentable sugars to contamination is still assumed in the FY 2012 SOT model, even though no significant contamination was seen in Runs 2, 4, and 5. The impact of a 10% contamination loss is plotted in Figure 10 simply as an indicator of the importance of this issue, which would increase MESP by $0.14/gal. This demonstrates that engineering considerations are needed to ensure sanitary practices in enzymatic hydrolysis and fermentation operations, as well as to ensure the overall robustness of fermentative processes.

Enzymatic hydrolysis and fermentation residence time. A total of 5 days for enzymatic hydrolysis and fermentation has been demonstrated at the pilot scale. Based on the single point sensitivity analysis (see Figure 10), further shortening the time by 1 day would provide $0.02/gal ethanol reduction in the MESP.

Optimizing sugar and ethanol yields. Major sugar and ethanol yield sensitivities are also considered and summarized in Figure 10. Because the biomass has a high cellulose content (~35% dry basis), cellulose-to-glucose yield has the largest cost impact of all yield cases. If assuming 12% higher than the baseline of 78% (as achieved in Run 4), the cost would reduce by $0.15/gal, and if 8% lower than this baseline, the MESP would increase by $0.12/gal. The pilot-scale demonstration Run 4 achieved 95% glucose-to-ethanol yield. A lower yield of 85% would result in a $0.12/gal cost increase. Similarly, if xylose-to-ethanol yield decreases from 93% (as

demonstrated here) to 80%, the MESP would increase by $0.09/gal. Baseline xylan-to-xylose yield is 81% in the FY 2012 model. Assuming 90% xylose yield in pretreatment would reduce cost by $0.07/gal, while assuming 70% would result in an increase of more than $0.09/gal. If arabinose-to-ethanol yield can be improved from the baseline of 54% to 85%, the cost could be reduced by $0.03/gal.

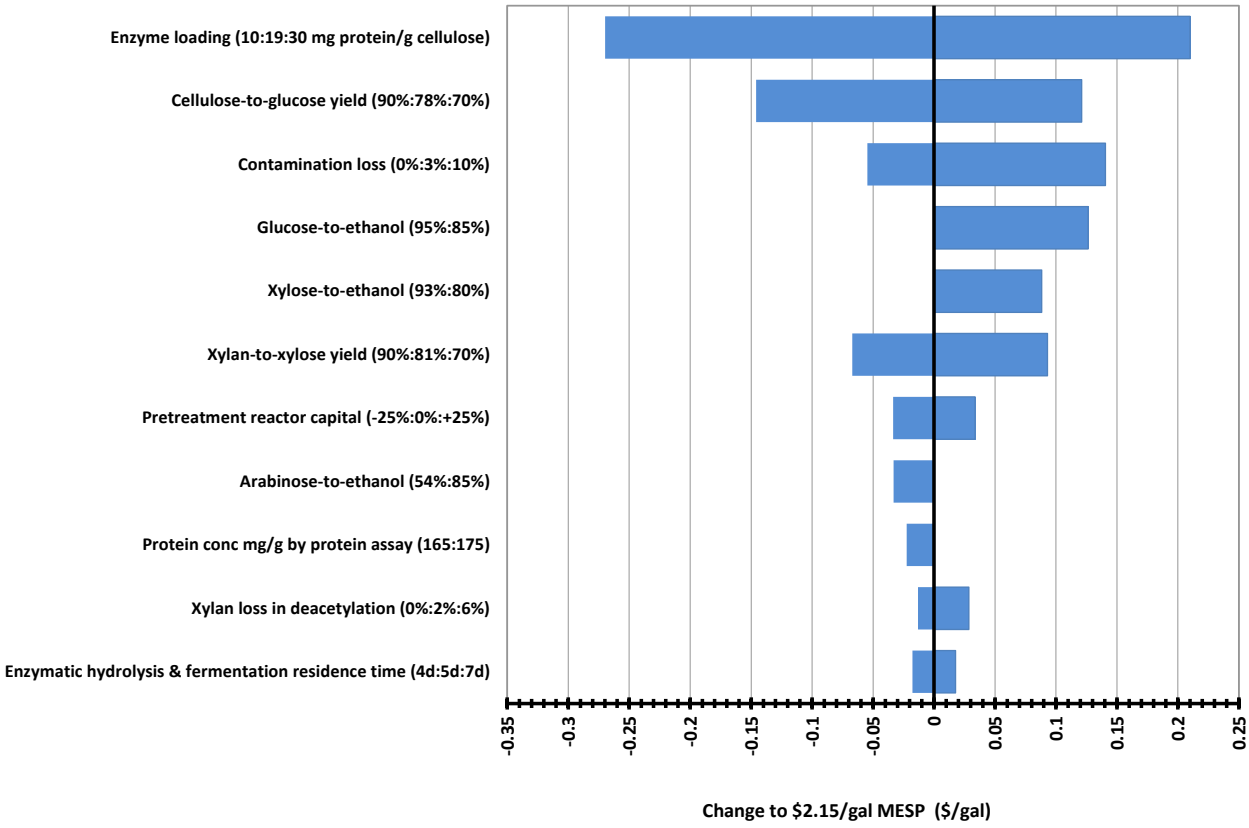

Figure 10. Tornado chart to quantify cost sensitivity relative to FY 2012 SOT baseline

3.3 Sustainability Metrics for Demonstration Case

Life-cycle inventory (LCI) estimates of the biorefinery for the 2011 design case and the current 2012 SOT case models (specifically, pilot-scale demonstration Run 4 associated with the TEA results discussed above) are compiled and summarized in Appendix B. The LCI data consider input and output flows to and from the modeled biorefinery; these data quantify the consumption of natural resources (including water, energy, and raw materials) as well as releases to air, land, and water associated with cellulosic ethanol production. As such, LCI data are important inputs for performing sustainability metrics evaluation and life-cycle assessment (LCA). The LCIs in Appendix B are established by the corresponding Aspen Plus process models discussed above, fixed at a 2,000 dry metric ton/day corn stover feed rate.

The biorefinery also produces excess electricity as a coproduct. Electricity produced exceeds the on-site power demand for the biorefinery; this excess power is assumed to be sold back to the

grid for a coproduct credit. For sustainability analysis purposes, the exported electricity is treated as an avoided product using the product displacement method (Wang et al. 2011), which is based on the concept of displacing the existing product with the new product. The excess electricity coproduct displaces an equivalent amount of grid electricity, thus avoiding significant greenhouse gas (GHG) emissions as well as fossil energy consumption. This assumes an average U.S. electricity grid mixture that is defined by Ecoinvent to carry a GHG emissions burden of 0.78 kg CO_{2e}/kWh and a fossil energy burden of 9.1 MJ/kWh (e.g., per-kilowatt-hour of grid electricity displaced) (Ecoinvent 2010).

To estimate GHG emissions and fossil energy demand of the modeled biorefinery, SimaPro v.7.3 software (Pré Consultants 2011) was used to develop and link units quantifying life cycle impacts as previously documented (Hsu et al. 2010). Based on the LCI information input to SimaPro (Appendix B), the resulting GHG and fossil energy profiles are presented in Table 16, Figure 11, and Figure 12, broken down by process category. Direct refinery emissions make essentially no direct contribution to GHGs, because nearly all the carbon dioxide emissions from the biorefinery are biogenic. Hence, the contributions to GHG emissions for the conversion step are solely attributed to the corresponding underlying processes; e.g., GHG emissions attributed to the production and transport or disposal of chemicals and other materials to and from the biorefinery. The embedded processes with the highest resulting GHG contributions are enzyme production, followed by pretreatment and conditioning chemicals (i.e., sodium hydroxide, sulfuric acid, and ammonia). For the design case model, the embedded processes that emit the most GHGs are WWT chemicals (sodium hydroxide), followed by enzyme production, ammonia conditioning, and flue gas desulfurization chemicals (lime). Cellulase enzyme production contributes 0.43 and 0.41 kg CO_{2e}/gal ethanol for the 2011 design case and the 2012 SOT case, respectively. About 84% of this is attributed to the underlying carbon source (glucose) required for on-site enzyme production, where glucose is assumed to be produced from standard corn wet milling processes. Using a portion of the cellulosic sugars produced within the biorefinery process instead of purchased glucose offers an opportunity to decrease GHG emissions associated with enzyme production; however, this would ultimately translate to a penalty in ethanol yield as it would sacrifice a portion of fermentable sugars; thus, such an approach would have to be balanced against the GHG (and cost) tradeoffs for the overall system. Alternatively, further improving ethanol yield, decreasing pretreatment and conditioning chemical demands, and reducing enzyme loading would also ultimately lower the overall life cycle GHG emissions for the conversion stage.

Table 16. Conversion Process GHG Emissions and Fossil Energy Consumption per Unit of Fuel Product

Category	GHG Emissions		Fossil Energy Consumption	
	2011 Design	2012 SOT	2011 Design	2012 SOT
	kg CO_{2e}/gal	kg CO_{2e}/gal	MJ/gal	MJ/gal
Enzyme production	0.43	0.41	4.58	4.42
Pretreatment chemicals	0.02	0.19	0.31	2.14
Ammonia conditioning	0.31	0.09	6.00	1.67
Flue gas desulfurization chemicals	0.10	0.06	0.53	0.32
Fermentation (nutrients)	0.07	0.06	1.18	0.98
WWT chemicals	0.44	0.02	4.68	0.52
Infrastructure	0.01	0.01	0.14	0.14
Direct refinery emission	0.01	0.01	0.00	0.00
Waste disposal	6.E-03	6.E-03	0.15	0.15
Cooling tower chemicals	7.E-04	8.E-04	0.01	0.01
Boiler water chemicals	5.E-05	5.E-05	7.E-04	6.E-04
Electricity credit	–1.43	–2.05	–16.74	–24.02
Total (excluding electricity credit)	1.40	0.85	17.59	10.36
Net total (including electricity credit)	–0.03	–1.20	0.85	–13.66

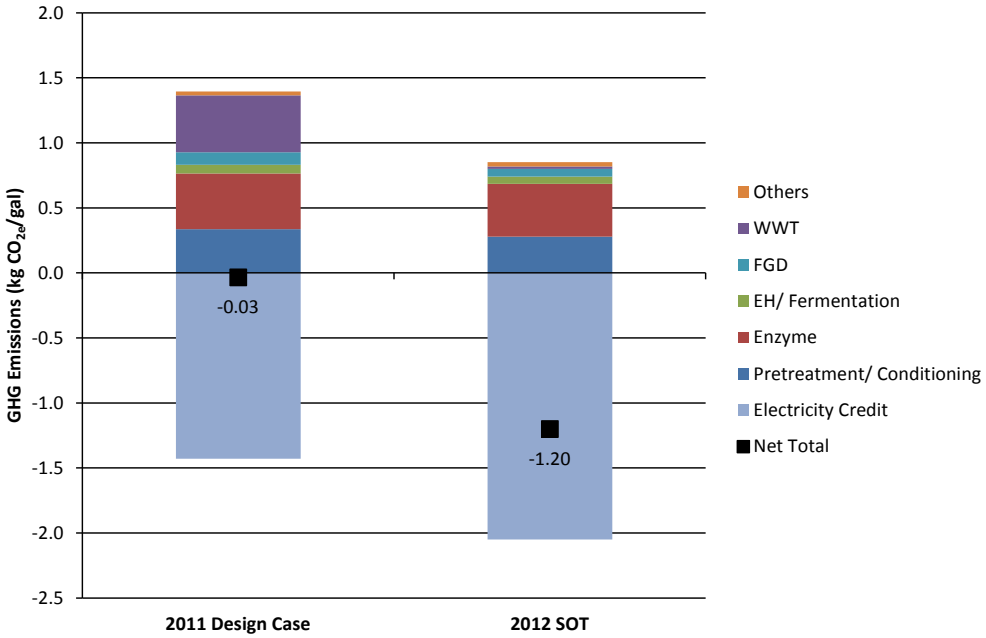

Figure 11. GHG emissions (kg CO_{2e}/gal) for modeled biorefinery (2011 design case vs 2012 SOT case)

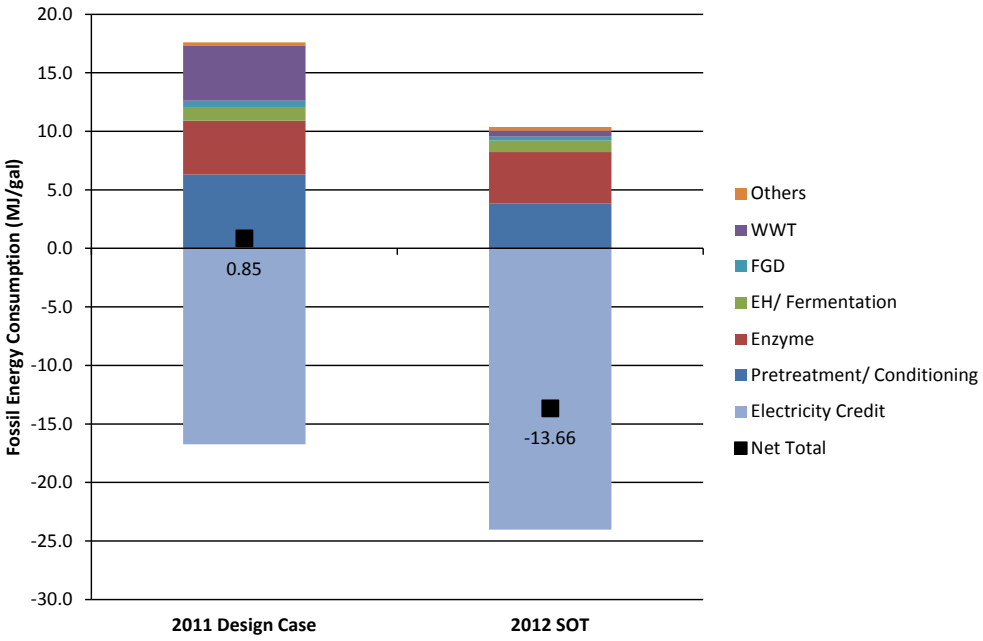

Figure 12. Fossil energy consumption (MJ/gal) for modeled biorefinery (2011 design case vs 2012 SOT case)

The total net GHG emissions associated with the conversion stage for the 2011 design case and the current SOT demonstration case are *negative* at –0.03 and –1.20 kg $CO_{2\text{-eq}}$/gal, respectively. The negative (offset) value in this case is primarily driven by the coproduct displacement credit for excess electricity, at –1.43 kg $CO_{2\text{-eq}}$/gal ethanol for the 2011 design case and –2.05 kg $CO_{2\text{-eq}}$/gal for the SOT case. The SOT process has been modified from the 2011 design case, as discussed previously. As a result, the SOT case uses fewer chemicals and generates more electricity coproduct credits. Primary changes to the SOT process relative to the 2012 target case include: (1) the use of deacetylation to remove the majority of acetic acid by adding sodium hydroxide; (2) lower severity pretreatment, which is primarily associated with lower acid loadings present in the pretreatment reactor and lower subsequent caustic (ammonia) neutralization demand; and (3) lower chemical demand for WWT, which is associated with a change in wastewater quality and composition, including lower nitrogen/ammonia loading (a function of lower upstream ammonia neutralization demand). The lower nitrogen loading negates the previously required nitrification step, which required caustic (NaOH), and instead now requires a lesser amount of supplemental nitrogen by way of ammonia or urea. Consequently, most of the chemicals required in the SOT process are used at a lower rate than in the design case; for example, ammonia for conditioning, lime for flue gas desulfurization, and caustic for WWT (see Appendix B); the lower chemical loadings translate to lower embodied GHG emissions contributions to the overall system. Additionally, as ethanol yield for the SOT case (70.9 gal/dry ton) is lower than for the target case (79.0 gal/dry ton), more unconverted carbohydrates are ultimately combusted in the boiler, leading to more steam production and electricity generation and thus a higher electricity coproduct export. The export electricity is roughly 13,400 kW for the 2011 design case and 17,200 kW for the SOT case (Appendix B). It is also important to point out that results in Table 16, Figure 11, and Figure 12 do not include

biomass feedstock contributions; i.e., GHG emissions and fossil energy consumption associated with the production and processing logistics of the biomass (including fertilizers, harvesting, preprocessing, and transportation).

Table 16 and Figure 12 also show the fossil energy consumption for the conversion process. The modeled conversion process does not require direct fossil energy input to the biorefinery, for example by way of natural gas import. Fossil energy inputs are associated with the embedded input/output LCI processes only. The overall fossil energy profile for the biorefinery is significantly offset by the excess electricity coproduct credit (i.e., displacing an equivalent amount of grid electricity produced from fossil-intensive sources). The total net fossil energy consumption for the 2011 design case and the SOT case are 0.85 and –13.66 MJ/gal, respectively. Similar to the GHG emissions, the majority of the fossil energy demand for the biochemical process is attributed to enzyme production and the use of required chemicals.

Table 17 summarizes the key sustainability metrics for the bioconversion step associated with both the 2011 design case and the 2012 SOT case. On an energy basis, the GHG emissions at the conversion stage for the 2011 design case and the 2012 SOT case are –0.42 and –14.88 kg CO_{2e}/GJ, respectively. Similarly, the fossil energy consumption for the two cases is 0.011 MJ/MJ and –0.170 MJ/MJ, respectively. Consumptive water use, ethanol fuel yield, and carbon-to-fuel efficiency are also reported in Table 17. Among these sustainability metrics, GHG emissions and fossil energy consumption for the SOT case are considerably more favorable than those for the 2011 design target case, driven largely by lower inventory demand for key inputs and higher electricity coproduct credit, relative to the 2011 design targets.

Table 17. Summary of Sustainability Metrics for the Modeled Biorefinery

	2011 Design Case	2012 SOT
GHG emissions (kg CO_{2e}/GJ)	–0.42	–14.88
Consumptive water use (m^3/day)	3283	3890
Consumptive water use (gal/gal ethanol)	4.95	6.58
Total fuel yield (gal ethanol/dry ton)	79.0	70.9
Carbon-to-fuel efficiency (C in ethanol/C in biomass)	30.2%	27.1%
Net fossil energy consumption (MJ/MJ)	0.011	–0.170

4 Conclusions

Pilot-scale cellulosic ethanol production data from corn stover were generated in the Integrated Biorefinery Research Facility at performance levels that demonstrated achievement of the FY 2012 State of Technology MESP target of $2.15/gal (2007$, $58.50/ton feedstock cost). Improved processing technologies, including feedstock deacetylation, reduced severity pretreatment conditions, high solids enzymatic hydrolysis at targeted enzyme loadings, improved fermentation yields at high ethanol titers, and reduced wastewater treatment costs brought about by upstream deacetylation were all features of the integrated pilot-scale process. Five integrated runs were completed, including two runs at enzyme loadings of 19 mg protein/g cellulose that resulted in a calculated MESP that was equal to or slightly below the FY 2012 cost target. An additional run was able to achieve higher overall ethanol yields using a higher enzyme loading (26 mg/g) that resulted in an MESP lower than the cost data when using data from a parallel bench-scale fermentation control, as the pilot fermentation vessel in that run became slightly contaminated.

For the FY 2012 SOT base case (using Run 4 data), the overall ethanol yield is roughly 10% lower than the target. The lower yield is offset by a similar 10% cost reduction in combined capital and operating costs, due to modifications made to the model directly tied to process improvements demonstrated in the pilot plant, including use of a feedstock deacetylation operation that enables several downstream process improvements that reduce costs. While the NREL ethanol program did not formally have official sustainability metric "targets" similar to MESP cost targets, the key sustainability metrics for GHG emissions and fossil energy demand associated with the NREL 2011 design case were improved upon for the 2012 SOT process model; namely, GHG emissions for the 2012 SOT case were estimated at –1.2 kg CO_{2e}/gal ethanol produced, while fossil energy consumption was estimated at -13.7 MG/gal (compared to the original design case metrics at –0.03 kg CO_{2e}/gal and 0.85 MJ/gal respectively); both metrics improved primarily due to increased coproduction of excess electricity that was assumed to displace standard grid electricity (driven by more residual unconverted carbon reaching the boiler). These metrics were evaluated for the conversion stage only (biorefinery model), and thus do not represent a full well-to-wheel (WTW) LCA analysis.

Several cost sensitivity cases were developed to show the relative significance of key conversion parameters and costing assumptions on MESP. A number of potentially achievable scenarios were shown that could result in additional significant MESP reductions. These include lower pretreatment capital costs resulting from less corrosive pretreatment conditions at lower operating pressures and the validation of more effective pre-commercial enzyme preparations that could significantly lower enzyme loading requirements in the future. Such improvements would not only benefit cellulosic ethanol processes, but also infrastructure-compatible biofuel processes that utilize sugar or sugar-derived intermediates pathways. Such improvements will be considered in further detail going forward.

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Appendix A: Process and Cost Details for 2012 State of Technology Model

Ethanol Production Process Engineering Analysis

Corn Stover Design Report Case: 2012 model LT1209B-demo run 4
Dilute Acid Pretreatment with Enzymatic Hydrolysis and Co-Fermentation
All Values in 2007$

Minimum Ethanol Selling Price (MESP):	**$2.15**	**/gal**
Gasoline-Equivalent MESP:	**$3.27**	**/gal gasoline equivalent**
Contributions: Feedstock	**$0.83**	**/gal**
Enzymes	**$0.36**	**/gal**
Non-Enzyme Conversion	**$0.97**	**/gal**
Ethanol Production	54.8	MMgal/yr (Ethanol at 68 °F)
Ethanol Yield	70.9	gal / dry U.S. ton feedstock
Feedstock + Handling Cost	$58.50	/dry U.S. ton
Internal Rate of Return (After-Tax)	10%	
Equity Percent of Total Investment	40%	

Capital Costs	
Pretreatment	$24,600,000
Neutralization/Conditioning	$4,100,000
Saccharification & Fermentation	$25,100,000
On-site Enzyme Production	$16,600,000
Distillation and Solids Recovery	$20,200,000
Wastewater Treatment	$40,600,000
Storage	$4,300,000
Boiler/Turbogenerator	$67,600,000
Utilities	$6,800,000
Total Installed Equipment Cost	$209,900,000
Added Direct + Indirect Costs	$171,100,000
(% of TCI)	45%
Total Capital Investment (TCI)	$381,000,000
Installed Equipment Cost/Annual Gallon	$3.83
Total Capital Investment/Annual Gallon	$6.95
Loan Rate	8.0%
Term (years)	10
Capital Charge Factor (Computed)	0.131
Denatured Fuel Production (MMgal/yr)	55.3
Denatured Fuel Min. Sales Price	$2.18
Denaturant Cost ($/gal denaturant)	$2.10
Maximum Yields (100% of Theoretical)	
Ethanol Production (MMgal/yr)	80.3
Theoretical Yield (gal/U.S. ton)	103.9
Current Yield (Actual/Theoretical)	68.2%

Manufacturing Costs (cents/gal ethanol)	
Feedstock + Handling	82.5
Sulfuric Acid	3.1
Ammonia	1.8
Glucose (enzyme production)	21.0
Other Raw Materials	10.9
Waste Disposal	2.5
Net Electricity	-16.0
Fixed Costs	18.2
Capital Depreciation	21.9
Average Income Tax	12.4
Average Return on Investment	57.0

Manufacturing Costs ($/yr)	
Feedstock + Handling	$45,200,000
Sulfuric Acid	$1,700,000
Ammonia	$1,000,000
Glucose (enzyme production)	$11,500,000
Other Raw Materials	$6,000,000
Waste Disposal	$1,300,000
Net Electricity	-$8,800,000
Fixed Costs	$10,000,000
Capital Depreciation	$12,000,000
Average Income Tax	$6,800,000
Average Return on Investment	$31,200,000

Specific Operating Conditions	
Enzyme Loading (mg/g cellulose)	19
Saccharification Time (days)	3.5
Fermentation Time (days)	1.5
Ethanol titer (wt%)	6.6%
Excess Electricity (kWh/gal)	2.7
Plant Electricity Use (kWh/gal)	4.4
Plant Water Usage (gal/gal)	6.6

Appendix B: Life Cycle Inventory for Design and State of Technology Case Models

	2011 Design Case	2012 SOT	
Products	**Production Rate**	**Production Rate**	
	kg/h	kg/h	
Neat ethanol	21808	19455	
Denatured ethanol	22273	19994	
Grid electricity	13441	17209	kW
Resource Consumption	**Flow Rate**	**Flow Rate**	
	kg/h	kg/h	
Biomass (corn stover) - wet	104167	104167	
Biomass (corn stover) - dry	83333	83333	
Sulfuric acid	1981	2240	
Sodium hydroxide (caustic soda)	2252	750	
Ammonia	1166	372	
Corn steep liquor	1321	1021	
Diammonium phosphate	142	105	
Sorbitol	44	33	
Glucose	2845	2354	
Host nutrients	67	66	
Sulfur dioxide	16	16	
Polymer	0	8	
Boiler water chemicals	2.5.E-01	2.0.E-01	
FGD lime	896	486	
Cooling tower chemicals	2	2	
Makeup water	137528	162962	
Air demand	530420	605597	
Denaturant (gasoline)	465	417	
Antifoam agent	13	13	
Air Emissions	**Flow Rate**	**Flow Rate**	
	kg/h	kg/h	
Water (H_2O)	214357	234644	
Nitrogen (N_2)	416398	471699	
Oxygen (O_2)	61913	76759	
Carbon dioxide (CO_2)	100333	104213	
Methane (CH_4)	3	3	
Nitrogen dioxide (NO_2)	64	71	
Carbon monoxide (CO)	62	69	
Ethanol	4	4	
Sulfur dioxide (SO_2)	54	29	
Waste Streams	**Flow Rate**	**Flow Rate**	
	kg/h	kg/h	
Ash disposal	5726	5035	
Wastewater (Brine)	9904	9865	