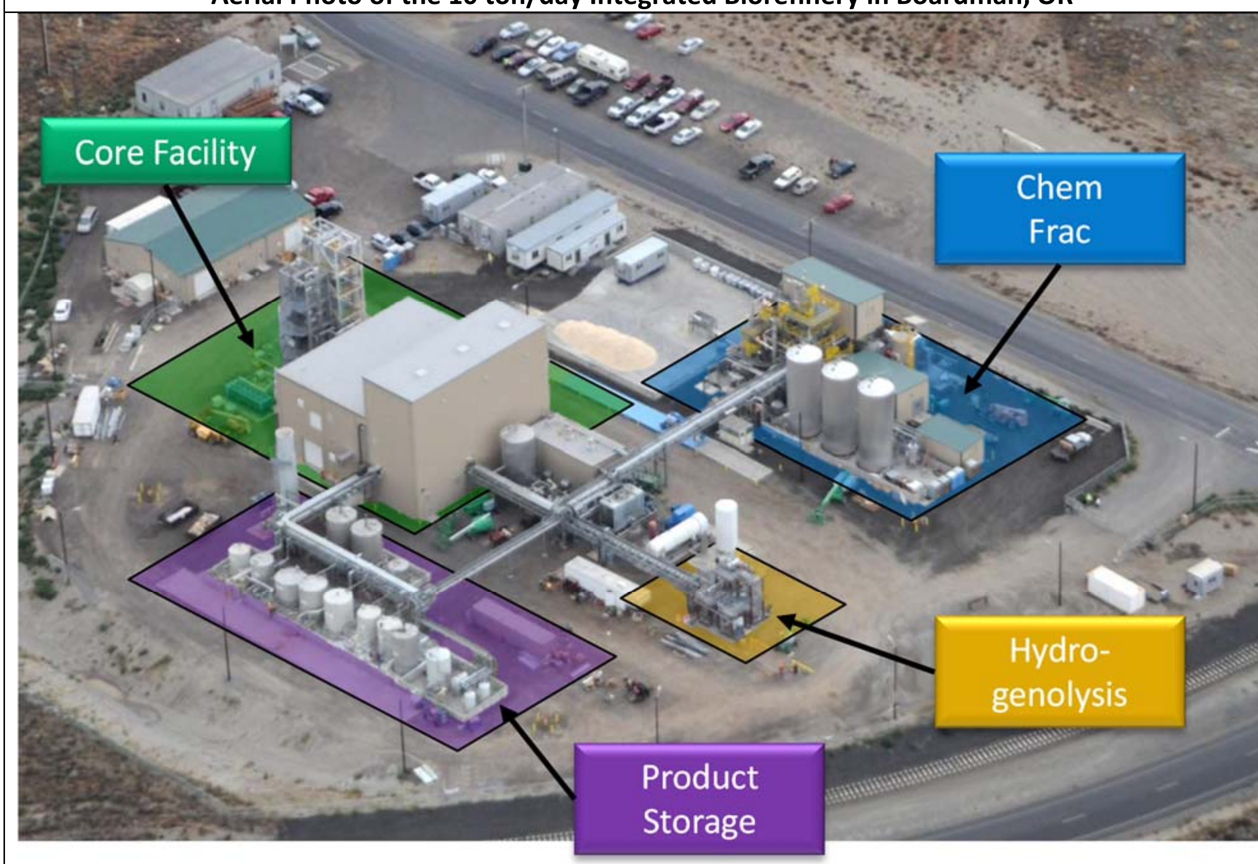
Final Scientific Report

Award Number:	DE-EE0002880
Recipient:	ZeaChem Inc.
Project Title:	High-Yield Hybrid Cellulosic Ethanol Process Using High-Impact Feedstock
Principal Investigator:	Tim Eggeman, Ph.D., P.E.
Project Participants:	Feedstock – GreenWood Resources
	EPC – Burns & McDonnell
	Key Vendors – Andritz, BASF
	Operations – Pacific Ethanol Management Services
Period of Performance:	January 28, 2010 through June 30, 2015
Report Revision:	Rev. 3, August 2016
Report Number:	DOE-ZEACHEM-02880

Aerial Photo of the 10 ton/day Integrated Biorefinery in Boardman, OR



Executive Summary

ZeaChem Inc. and US DOE successfully demonstrated the ZeaChem process for producing sugars and ethanol from high-impact biomass feedstocks. The project was executed over a 5-year period under a $31.25 million cooperative agreement (80:20 Federal:ZeaChem cost share). The project was managed by dividing it into three budget periods. Activities during Budget Period 1 were limited to planning, permitting, and other pre-construction planning. Budget Period 2 activities included engineering, procurement, construction, commissioning, start-up and initial operations through the Independent Engineer Test Runs. The scope of construction was limited to the Chem Frac and Hydrogenolysis units, as the Core Facility was already in place. Construction was complete in December 2012, and the first cellulosic ethanol was produced in February 2013. Additional operational test runs were conducted during Budget Period 3 (completed June 2015) using hybrid poplar, corn stover, and wheat straw feedstocks, resulting in the production of cellulosic ethanol and various other biorefinery intermediates.

The research adds to the understanding of the Chem Frac and Hydrogenolysis technologies in that the technical performance of each unit was measured, and the resulting data and operational experience can be used as the basis for engineering designs, thus mitigating risks for deployment in future commercial facilities. The Chem Frac unit was initially designed to be operated as two-stage dilute acid hydrolysis, with first stage conditions selected to remove the hemicellulose fraction of the feedstock, and the second stage conditions selected to remove the cellulose fraction. While the Chem Frac unit met or exceeded the design capacity of 10 ton(dry)/day, the technical effectiveness of the Chem Frac unit was below expectations in its initial two-stage dilute acid configuration. The sugars yields were low, the sugars were dilute, and the sugars had poor fermentability caused by excessive inhibitors from wood breakdown products, resulting in a non-viable process from an economic point of view. Later runs with the Chem Frac unit switched to a configuration that used dilute acid pretreatment followed by enzymatic hydrolysis. This change improved yield, increased sugar concentrations, and improved fermentability of sugars. The Hydrogenolysis unit met or exceeded all expectations with respect to unit capacity, technical performance, and economic performance.

The US DOE funds for the project were provided through the American Recovery and Reinvestment Act of 2009. In addition to the scientific/technical merit of the project, this project benefited the public through the creation of approximately 75 onsite direct construction-related jobs, 25 direct on-going operations-related jobs, plus numerous indirect jobs, and thus was well aligned with the goals of the American Recovery and Reinvestment Act of 2009.

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Project Overview

Goals and Objectives

The initial project goals were:

- Sufficiently mitigate risks for ZeaChem Inc.'s cellulosic ethanol technology so that a first commercial plant using hybrid poplar chips as feedstock can be financed, constructed and made operational; and

- Demonstrate operations with multiple economically justified high-impact feedstocks so that the technology can be rapidly deployed in follow-on commercial facilities throughout the US.

Both goals were accomplished. In efforts conducted outside the scope of this project, ZeaChem completed the engineering design for a hybrid poplar-to-cellulosic ethanol plant through the Front-End Planning 2 (FEP-2) stage, which means that a complete set of process flow diagrams with material & energy balances were created along with a preliminary set of piping and instrumentation diagrams and a Class 3 cost estimate (accuracy of -20% to +30%). ZeaChem also assembled a financing package that consisted of a combination of debt and equity, with the debt backed by a USDA loan guarantee. Unfortunately, the commercial project lost its feedstock supply when the hybrid poplar tree farm operated by GreenWood Resources was sold in November 2015. This turn of events put the envisioned commercial project on hold. The data collected during the US DOE funded project on operations with wheat straw and corn stover feedstocks (both of which meet the definition of high-impact feedstocks) is being reviewed and these feedstocks considered as possible replacements for hybrid poplar.

The initial objectives of the project were:

- Construct and operate a 10 ton(dry)/day Chem Frac unit to produce sugars from biomass

- Construct and operate a Hydrogenolysis unit to produce ethanol from ethyl acetate and purchased hydrogen

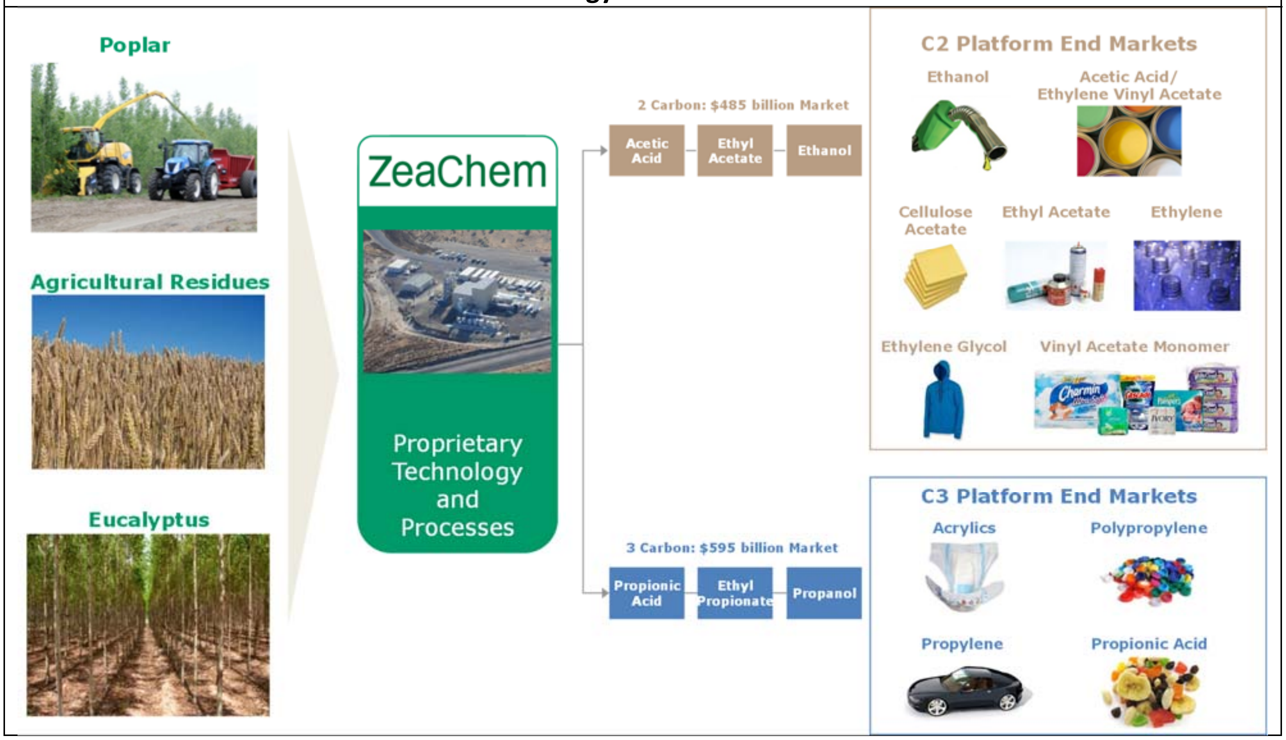
- Operate the facility in an integrated manner, producing ethanol from biomass

All three objectives were accomplished. A Chem Frac unit was constructed and was operated with hybrid poplar chips at a rate as high as 12.5 ton(dry)/day. A Hydrogenolysis unit was constructed and operated to produce ethanol from ethyl acetate and purchased hydrogen at its design capacity of 50 gal/hr. The facility was operated in an integrated manner on several occasions during Budget Period 2 to produce ethanol from wood using the original ZeaChem indirect route through acetic acid. However, the poor technical performance of the Chem Frac unit when in two-stage acid hydrolysis mode forced a change in approach during Budget Period 3 to dilute acid pretreatment followed by enzymatic hydrolysis and direct fermentation of sugars to ethanol so that the necessary technical data for the commercial plant could be collected.

Background

ZeaChem is a developer of biorefinery technology and commercial biorefinery projects. The Company was founded in 2002 and is headquartered in Lakewood, CO. ZeaChem has developed several biorefining product lines that enable it to produce a slate of fuels and biochemicals. Its C2 Platform uses a combination of fermentation and conventional chemical synthesis pathways to produce ethanol, acetic acid, acetate esters, ethylene, and ethylene derivatives. Its C3 Platforms changes the micro-organism used for fermentation, resulting in a slate of analogous three-carbon products such as propanol, propionic acid, propionate esters, propylene, and propylene derivatives.

Flexible Technology and Diversified Markets

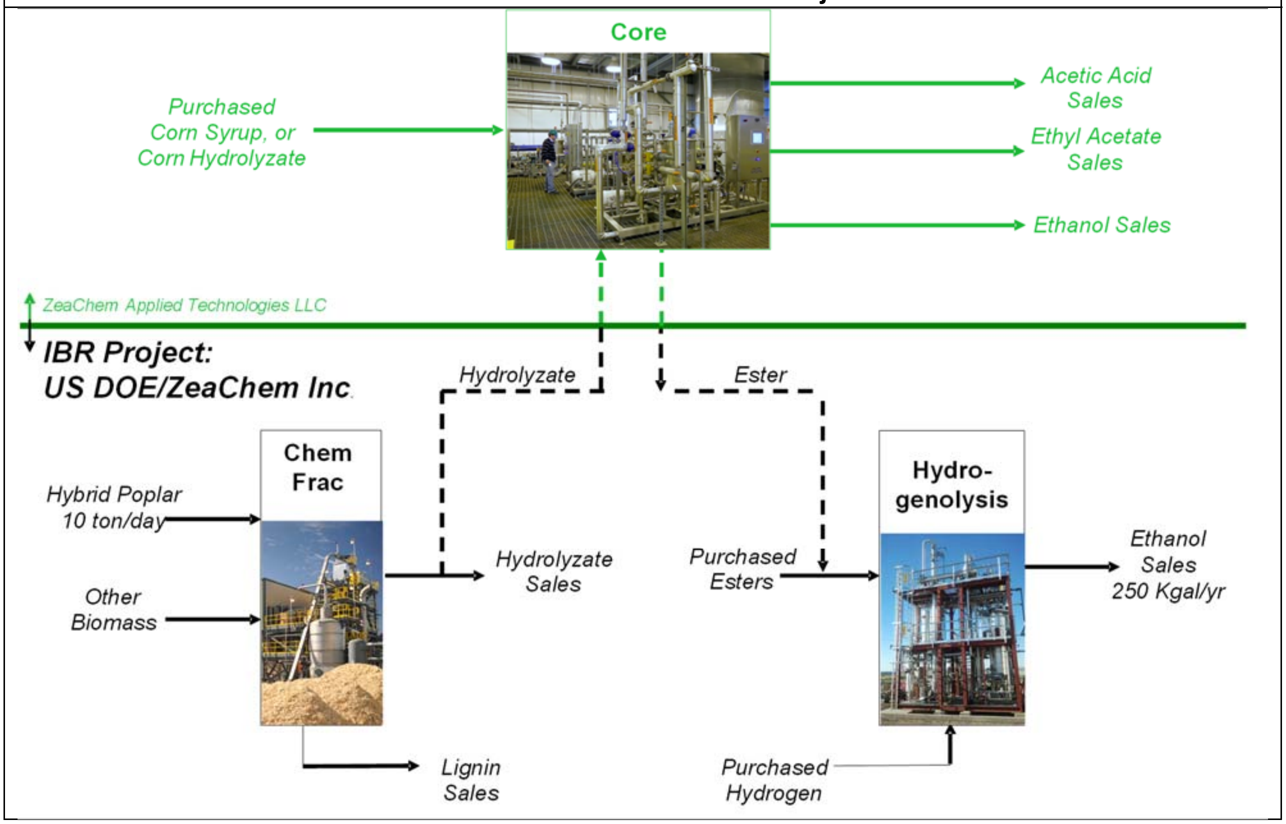


ZeaChem follows two primary tenets with respect to its project development: one, "grow where you go"; and two, "build to serve". This means locating projects adjacent to sustainable economic biomass feedstocks and designing projects and the products they produce to serve defined customers and regional market demands. ZeaChem has successfully built and currently operates a 10 ton per day integrated biorefinery in Boardman, OR. This biorefinery has provided over 4,500 hours of technical performance data that is being used to design ZeaChem's portfolio of commercial plants, thus minimizing technology risk through combined use of conventional technologies and extensive testing.

Project Description

ZeaChem Applied Technology LLC is a subsidiary of ZeaChem Inc. that was established to legally house the core fermentation and recovery assets of the Boardman, OR plant. In 2010, ZeaChem Inc. was selected for award of a $31.25 million cooperative agreement (80:20 Federal:ZeaChem cost share) under the Recovery Act – Demonstration of Integrated Biorefinery Operations (Funding Opportunity DE-FOA-0000096) to expand the capabilities of the existing core "sugars-to-esters" facility in Boardman, OR. As shown in the figure on the next page, the scope of the resulting Cooperative Agreement between US DOE and ZeaChem Inc. (Award Number DE- EE0002880) provided for the addition of two additional units of independent utility: 1) A front end Chemical Fractionation (aka Chem Frac) unit designed to process 10 BDT/d of hybrid poplar and other biomass feedstocks into a sugar-rich hydrolyzate stream and a lignin-rich residue, and 2) A back end Hydrogenolysis Unit that reacts ethyl acetate and hydrogen into 250 KGal/yr of fuel grade ethanol. These two new units can be operated in tandem with the Core facility to convert hybrid poplar into cellulosic ethanol, or the new units can/have been operated independently of the Core to produce hydrolyzate, lignin, and ethanol.

Interface Between the Core and IBR Project Assets



The main biomass feedstock for the project was hybrid poplar. This feedstock qualifies as a high impact feedstock that is domestically available and has the agronomically and ecologically sustainable ultimate availability potential of at least 100 million dry metric tonnes of biomass per year. In addition, wheat straw and corn stover were used as alternative feedstocks so that the necessary data were collected for use in the support of a wider range of future commercial projects.

Project Activities

The project activities were divided into three distinct budget periods with stage gate go-no go decision points between each period. The activities for each budget period are described in more detail below. In addition, the project team participated in annual Comprehensive Project Reviews and biennial Project Peer Reviews.

Original Hypotheses

The original hypotheses presented in the application were: 1) Two-stage acid hydrolysis to release sugars from hybrid poplar and other biomass feedstocks was a feasible alternative to enzymatic hydrolysis, thus avoiding the high cost and other risks associated with the use of cellulase enzymes, and

2) The high yield of the ZeaChem indirect method of ethanol production would prove to be a feasible alternative to ethanol production by direct fermentation of sugars.

Approach - Budget Period 1 (BP-1)

The main purpose of BP-1 was to complete all of the front-end planning activities needed prior to construction. The period of performance for BP-1 occurred between January 28, 2010 and September 30, 2011. Highlights of activities during BP-1 include:

- Creation of project management documents including the Budget, Project Management Plan, and the Risk Management Plan with associated Risk Register.
- Completion of vendor trials for the chemical fractionation and hydrolysis units.
- Completion of the Schedule A Design package and project cost estimate, using Burns & McDonnell as the engineering firm.
- Competitive selection of vendors
    - Andritz Pulp and Paper was selected as the vendor for the chemical fractionation unit.
    - Continental Technologies was selected as the equipment vendor for the hydrogenolysis equipment, and BASF was selected as the catalyst supplier.
- Completion of the National Environmental Policy Act (NEPA) review, resulting in a Categorical Exclusion determination for the project.
- Complete Phase 1 of detailed design for the Chem Frac unit, Hydrogenolysis unit, and associated supporting infrastructure systems upgrades needed to support the project.
- Completion of an independent evaluation of the project using the services of Independent Project Analysis, Inc., a well-respected consulting firm that provides benchmarking and other advisory services to improve outcomes of capital projects in the process industries.
- Completed application for Budget Period 2

Approach - Budget Period 2 (BP-2)

The main purpose of BP-2 was to complete construction of the new Chem Frac and Hydrogenolysis units and successfully commission, start-up, and operate the new units through the completion of the Independent Engineer Test Run. The period of performance for BP-2 occurred between October 1, 2011 and September 30, 2014. Burns & McDonnell was selected as the EPCM contractor. Highlights of activities during BP-2 include:

- Completed detailed engineering.
- Completed procurement. Burns & McDonnell issued two large procurement awards, one to Andritz Pulp & Paper for the Chem Frac equipment, the other to Continental Technologies for the Hydrogenolysis skid.

- Completed construction in December 2012. Burns & McDonnell acted as the construction manager, issuing subcontracts to the various construction trades for concrete, structural steel, piping, insulation, etc. The project was managed for compliance with prevailing wage and other requirements of the Davis-Bacon Act.

- Completed operations pre-startup tasks including: writing of operating procedures, completing operator training, completing training for laboratory personnel, and completing environmental health and safety training for the entire staff.

- Completed commissioning and start-up tasks including: field verification of piping and instrumentation diagrams and other specifications, instrument and control system checks, leak tests, motor checks, water test runs for the Chem Unit, and catalyst loading and activation for Hydrogenolysis.

- Completed initial operations, with the first cellulosic ethanol produced in February 2013. Below is a staff photo taken during collection of the first cellulosic ethanol sample. This material was made from hybrid poplar wood chips using the entire set of operations for the high yield ZeaChem process (*i.e.*, wood was converted to sugars and lignin, sugars were fermented to acetic acid and recovered as glacial acetic acid, glacial acetic acid was converted into ethyl acetate, and ethyl acetate was reacted with hydrogen to produce ethanol).

First Cellulosic Ethanol Production – February 2013

- Completed Independent Engineer Test in June 2014. Harris Group was the subcontracted by US DOE to provide independent engineering services throughout the project. The purpose of the Independent Engineer Test was to validate, through a well-defined and witnessed test run, that construction of the Chem Frac and Hydrogenolysis units was complete and the units were fully operational assets.

- Completed application for Budget Period 3

The next page contains a set of aerial photos of the IBR Facility and the adjacent site for the proposed 1st Commercial facility, along with a regional map. Additional photos for each of the major units on the site (Chem Frac, Fermentation, Acetic Acid Recovery and Esterification, and Hydrogenolysis) are also presented.

Approach - Budget Period 3 (BP-3)

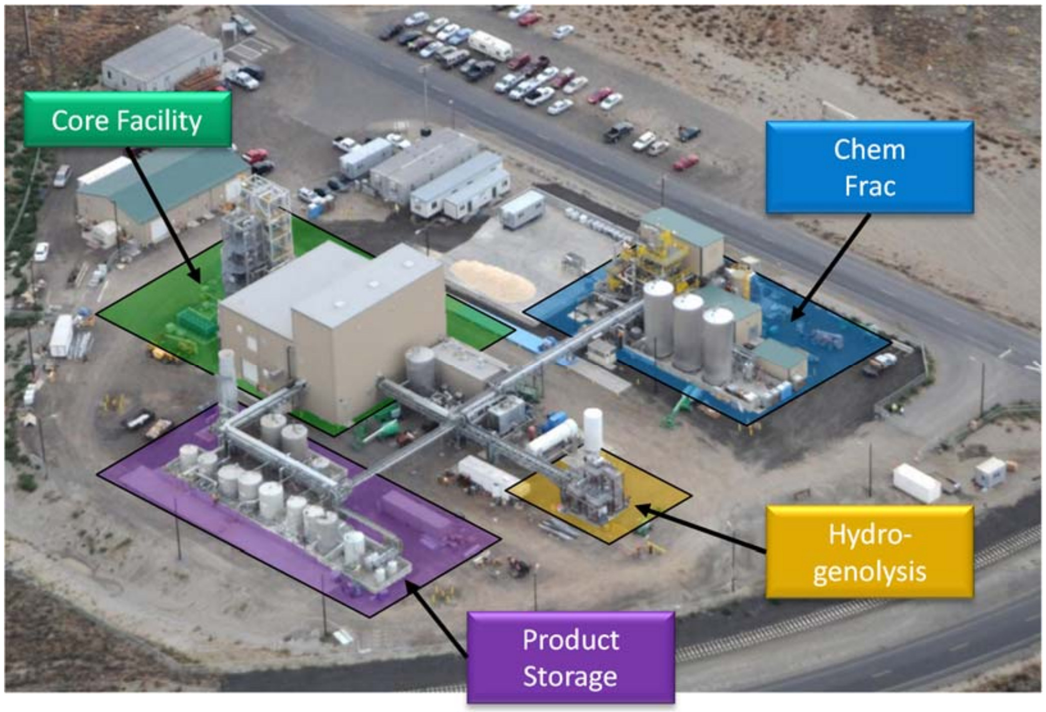
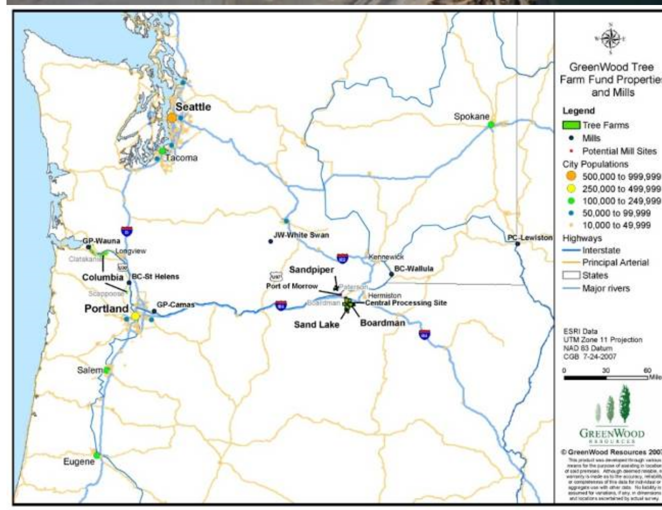
The main purpose of BP-3 was to operate the units and collect the data needed to support commercial scale-up activities. The period of performance for BP-3 occurred between October 1, 2014 and June 30, 2015, however the contract for BP-3 was not actually executed until early May 2015. This created a constraint on the scope of activities during BP-3, given US DOE's hard deadline of June 30, 2015 for completion of projects funded under the American Recovery and Reinvestment Act of 2009. Furthermore, ZeaChem's business plans had changed over the course of time and they no longer included a need for near-term deployment of the ester hydrogenolysis technology. For these reasons, the scope of the BP-3 activities was limited to test run trials of the Chem Frac unit with three feedstocks (*i.e.,* hybrid poplar, corn stove, and wheat straw) plus enzymatic hydrolysis and direct fermentation of the resulting sugars to ethanol.

The entire report for the BP-3 test runs is included in Appendix A. Appendix B contains the report ZeaChem submitted for the Independent Engineer Test at the end of BP-2. These two reports provide a sound technical basis for scale-up of the technologies to commercial operations.

Problems Encountered

The Chem Frac unit was initially operated in two-stage dilute acid hydrolysis as originally planned. The sugar yields, sugar concentration, and sugar fermentability did not meet expectations. For later runs during BP-3, the Chem Frac unit was operated as dilute acid pretreatment followed by enzymatic hydrolysis.

The Hydrogenolysis unit was successfully operated as originally intended. There were no problems encountered with the unit.

Aerial Overview of the IBR Facility and Adjacent 1st Commercial Site (proposed) and Regional Map	
	 

Chemical Fractionation Unit

Fermentation (Core)	
	

Acetic Acid Purification and Esterification (Core)	
	

Hydrogenolysis


Departure from Planned Methodology

The poor technical performance of the Chem Frac unit when operated in two-stage dilute acid mode forced the decision to revert to dilute acid pretreatment followed by enzymatic hydrolysis during BP-3.

Assessment of Impact of Problems on Project Results

Even with the change of operation mode for the Chem Frac unit, the C5-rich liquid hydrolysate from the pretreatment stage was still quite dilute and had poor fermentability, as documented in Appendix A. The C6-rich sugars resulting from enzymatic hydrolysis were of a reasonable concentration and exhibited good fermentability, again as documented in Appendix A.

Products Developed Under the Award

This report is the main technical publication of the results from this project to date. Some additional interim results were provided to the public through the Peer Reviews conferences held by the US DOE Bioenergy Technologies Office in 2011[1], 2013[2], and 2015[3]. ZeaChem plans to use the equipment for its intended purpose beyond the project period of performance. The Company web site (www.zeachem.com) contains general information on the Company. The Company is making the equipment available for use by outside parties, and a flyer describing the capabilities of the equipment is available at http://www.zeachem.com/technology-institute/ . This award fostered a collaboration with the University of Washington and several other universities in the US Pacific Northwest that resulted in a USDA funded Regional CAP grant. It also fostered a second collaboration with the University of Hawaii, Hawaiian Commercial & Sugar Company, and several other universities that resulted in a USDA funded grant under the Biomass Research and Development Initiative (BRDi). No other products (Technologies/techniques, Inventions/patent applications, Databases or collections) were generated.

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[1] See: http://www.energy.gov/sites/prod/files/2014/03/f14/2011_ibr_review.pdf

[2] See: http://www.energy.gov/sites/prod/files/2016/05/f31/ibr_eggeman_55111.pdf

[3] See: http://www.energy.gov/sites/prod/files/2015/04/f22/demonstration_market_transformation_eggeman_34115.pdf

Appendix A - Operations Test Report for Budget Period 3

EE0002880 ZeaChem Integrated Bio-refinery (IBR)

Operations Test Report for Budget Period 3

Objective

Demonstrate operation of the IBR and obtain performance data. This requirement is stated in the Funding Opportunity Announcement DE-FOA-0000096.

Scope of the Cooperative Agreement

The scope of Cooperative Agreement EE0002880 includes construction and operations of two process units in addition to ZeaChem's existing Bio-refinery (Core facility), so that ethanol can be produced from cellulosic feedstocks. The two process units constructed are the Chemical Fractionation unit and the Hydrogenolysis unit.

ZeaChem Staff Organization

Tim Eggeman – Principal Investigator, project oversight.
Brian O'Neill – Project Manager, BP3 operations planning.
Pete Wilhelm – Plant Manager, oversight of Boardman facility and operations.

Operation of the Facility

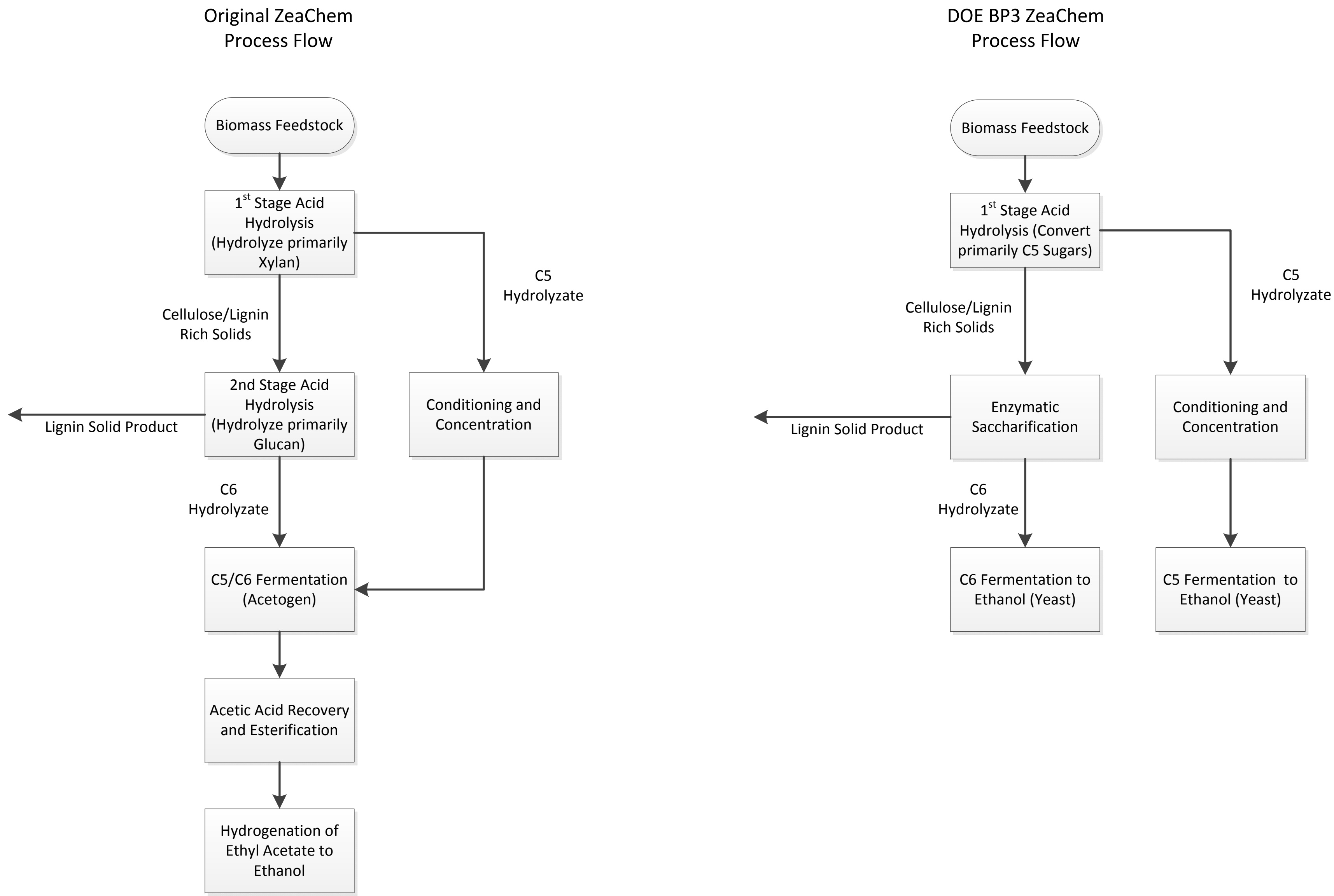
For the purposes of this test, the IBR is comprised of two basic modules:

1. **Chemical Fractionation (Production of C5 Hydrolyzate and C6 Pretreated Solids)**
    - Single-pass processing with a batch-fed continuous reaction.
    - Batch liquid/solid separation of the C5 hydrolyzate/C6 solids products.
    - Storage of feedstock, hydrolyzate and solid products.

2. **Core (Saccharification and Fermentation)**
    - Single-pass C5 hydrolyzate conditioning, run continuously.
    - Batch fermentation of conditioned C5 hydrolyzate.
    - Batch enzymatic saccharification of pretreated C6 solids.
    - Batch fermentation of saccharified C6 slurry.
    - Batch filtration of fermented C6 slurry.

Note: The hydrogenolysis unit was operated and tested during BP2 with results meeting and exceeding the design. Since this BP3 test included yeast fermentation with direct conversion of sugar to ethanol, the hydrogenolysis unit was not included in BP3 operations.

ZeaChem Wood-to-Ethanol Processes



Scope of BP3 Operations

Operations were conducted from the period beginning May 18, 2015 and concluded June 30, 2013. Three biomass feedstocks were used during BP3 operations: hybrid poplar, corn stover, and wheat straw. Each feedstock and its resultant products were processed separately.

The Chemical Fractionation unit which performs biomass pretreatment and C5 hydrolysis was run as a single-stage operation with a batch-fed continuous reaction and a batch filtration process. 14 allocated days of operation were comprised of 5 days feeding hybrid poplar, 4 days feeding corn stover, and 5 days feeding wheat straw. The system was fed at maximum rate that the bulk-density and feed characteristics of each feedstock would allow. The products of the Chemical Fractionation unit were C5 rich hydrolyzate containing high levels of wood extractives and hemicellulose degradation products including acetic acid, and C6 rich pretreated solids with high lignin content. Downstream (Core) processing for the C5 and C6 rich streams was accomplished separately. The C6 rich solids were saccharified using a cellulase enzyme mixture. The produced C6 hydrolyzate slurry from each feedstock was fermented with yeast to produce ethanol.

The mixed HB34-35-36 Poplar C5 rich hydrolyzate was conditioned using solvent extraction to remove acetic acid and other byproducts. The conditioned hydrolyzate was then buffered as needed and fermented with yeast to produce ethanol. Quantities of HB37 Corn Stover C5 rich hydrolyzate and HB38 Wheat Straw C5 rich hydrolyzate were insufficient to operate the extraction unit, so no conditioning was done on those hydrolyzates.

HB36 Poplar C5 rich conditioned hydrolyzate was fermented in a 4000 gallon fermentor and produced ethanol. Due to low hydrolyzate quantities and difficulty with the Poplar C5 fermentation, HB37 Corn Stover C5 rich hydrolyzate was fermented in a 40 gallon fermentor and produced ethanol. Due to time constraints and poor yeast performance with both the HB36 and HB37 C5 fermentations, HB38 Wheat Straw C5 rich hydrolyzate fermentation was not performed.

Run Procedure

Facility Preparation – Several steps were taken in weeks and hours prior to running the tests:

1. Walkdown of sampling points and preparation of sampling plan.
2. Equipment and controls inspected and serviced as required for operation.
3. Safety systems were inspected and serviced as required for operation.
4. Startup walkthrough was performed to verify equipment was ready for operation.
5. Required feedstock, chemicals, and utilities were verified prior to operation.
6. Lab equipment was inspected and calibrated as needed prior to operation.
7. Process tanks were cleaned of material from previous runs.

Pretreatment/C5 Hydrolysis Runs Summary (Chem Frac Unit Operation):

Hybrid Poplar (run# HB34, HB35, and HB36, May 18-22, 2015) – The total time processing biomass through the reactor was 108 hours, and there was additional time for startup and to filter the resultant slurry. Since this is a continuous reaction and the equipment is pre-heated, "steady state" in the reactor was achieved once it was full of material, about ½ hour after loading biomass feedstock. Sugar concentration increased in the blowtank and hydrolyzate storage throughout the run as filtered hydrolyzate was recirculated back to the blowtank and filter press feed. Before the runs, the blowtank and the hydrolyzate day tank were filled to the required start level with dilute hydrolyzate from a previous run. The reaction acid concentration was adjusted several times and was broken down by the HB34, HB35, and HB36 run numbers. HB36 ran with the optimal parameters and represents the bulk of the run time. The goal of changing conditions at the beginning of a run is to optimize the sugar yields while minimizing over-conversion to xylose degradation products, primarily furfural and formic acid. Changes in the wood composition from lot to lot require adjustment of the reaction severity.

Issues and Resolutions:

- Chip furnish had large pieces – The chips contained a number of large pieces that caused plugging in the lock hopper feed equipment. Adjustments were made to resolve plugging including setting the feed rate to 9.5 BDST/day. Note that while resolving plugging there was additional water being added to the process resulting in lower sugar concentration. Resolution of this issue will involve supplier management and ensuring the supply agreement chip specification which prohibits the large pieces seen during this run is followed. Small quantity deliveries of poplar chips makes it difficult for the supplier to provide on-spec material in a timely manner, an issue which is not anticipated once large-quantity supply streams are established.

Corn Stover (run# HB37, May 26-29, 2015) - The total time processing biomass through the reactor was 20 hours, and there was additional time for startup and to filter the resultant slurry. This was the first time that corn stover was run at the facility. Due to frequent interruptions due to equipment wear, processing of corn stover through the reactor was limited.

Issues and Resolutions:

- High insoluble ash content in the biomass resulted in frequent interruption of the run due to blowline rupturing. It is notable that the corn stover caused considerably higher wear rates than had been previously experienced processing other agricultural residues. The run was stopped as needed while a ruptured pretreatment reactor discharge blowline section was either repaired or replaced. Future runs with agricultural residues will require biomass washing and/or wear-resistant blowlines.

Wheat Straw (run# HB38, June 2, 2015) - The total time processing biomass through the reactor was 52 hours, and there was additional time for startup and to filter the resultant slurry. This was the first time that wheat straw was run at the facility. Due to frequent interruptions due to equipment wear, feeding of corn stover to the reactor was limited.

Issues and Resolutions:

- High insoluble ash content in the biomass resulted in frequent interruption of the run due to blowline rupturing. The run was stopped as needed while the ruptured blowline section was either repaired or replaced. Wheat straw blowline wear rates were high though lower than when processing corn stover. Future runs with agricultural residues will require biomass washing and/or wear-resistant blowlines.

Saccharification of 1st stage acid-pretreated cake summary (Core Unit Operation)

Hybrid Poplar (run# HB36-ST2-E1, June 9-13, 2015) – Saccharification of HB36 1st stage acid-pretreated cake was carried out for 79 hours. Since HB36 represents the optimized parameters, no cake from HB34 or HB35 was saccharified.

Issues and Resolutions: No issues were observed.

Corn Stover (run# HB37-ST2-E1, June 16-17, 2015) – Saccharification of HB37 1st stage acid-pretreated cake was carried out for 32 hours.

Issues and Resolutions: No issues were observed.

Wheat Straw (run# HB38-ST2-E1, June 22-24, 2015) – Saccharification of HB38 1st stage acid-pretreated cake was carried out for 47 hours.

Issues and Resolutions: Agitator went out at hour 37. A recirculating line was assembled and installed on tank to maintain mixing during the remainder of saccharification.

Fermentation of C6 sugars from 1st stage acid-pretreated cake Saccharification (Core Unit Operation) – **June 13-26, 2015**

Hybrid Poplar (run# HB36-ST2-F1, June 13-14, 2015) – Fermentation of C6 sugars produced during saccharification of HB36 1st stage acid-pretreated cake was carried out for 30 hours. Solids remaining following saccharification were not removed prior to fermentation. Fermentation results verified that the sugar slurry produced during saccharification of 1st stage acid-pretreated hybrid poplar was adequate for obtaining high ethanol yields. Solids were separated post fermentation and sent for compositional analysis. Clarified fermentation beer was stored in a designated tote.

Issues and Resolutions: No issues were observed.

Corn Stover (run# HB37-ST2-F1, June 17-18, 2015) – Fermentation of C6 sugars produced during saccharification of HB37 1st stage acid-pretreated cake was carried out for 22 hours. Solids remaining following saccharification were not removed prior to fermentation. Fermentation results verified that the sugar slurry produced during saccharification of 1st stage acid-pretreated corn stover was adequate for obtaining high ethanol yields. Post fermentation beer was stored in a designated tote. Post fermentation solids were not recovered during this run.

Issues and Resolutions: Foaming occurred during fermentation and material was lost due to overflowing. Antifoam was used to try and prevent foaming but this was unhelpful. Agitation was eventually reduced to prevent foaming and further loss of material. Due to the viscosity and low pump ability of this material post saccharification and fermentation, low moisture cake could not be obtained. No solids material could be sent for analysis during this separation step.

Wheat Straw (run# HB38-ST2-F1, June 24-25, 2015) – Fermentation of C6 sugars produced during saccharification of HB38 1st stage acid-pretreated cake was carried out for 22 hours. Solids remaining following saccharification were not removed prior to fermentation. Fermentation results verified that the sugar slurry produced during saccharification of 1st stage acid-pretreated wheat straw was adequate for obtaining high ethanol yields. Solids were separated post fermentation and sent for compositional analysis. Clarified fermentation beer was stored in a designated tote.

Issues and Resolutions: Foaming occurred during fermentation and material was lost due to overflowing. Agitator went out at hour 37 of saccharification. A recirculating line was assembled and installed on tank to maintain mixing during fermentation.

Conditioning of C5-rich Hydrolyzates from Pretreatment (Core Unit Operation)

Hybrid Poplar (run# HB34/35/36, May 27-June 4, 2015) —The hybrid poplar C5-rich hydrolysate was conditioned. It ran intermittently for 5 days, with limited success lowering the acetic acid concentration. The extraction did effectively remove furfural and levulinic acid. No conditioning was done on HB37 or HB38 hydrolyzate due to its relatively low levels of acetic acid and furfural, and based on the results of HB36 conditioning.

Concentration of C5-rich Hydrolyzates (Core Unit Operation)

Hybrid Poplar (run# HB34/35/36, June 9-10, 2015) —The conditioned hybrid poplar C5-rich hydrolysate was concentrated through a crossflow RO membrane. Using an RO membrane minimizes glucose and xylose loss as the RO membrane is excessively tight for sugar. No issues were observed.

Corn Stover (run# HB37, June 15-16, 2015) — Conditioned hybrid poplar C5-rich hydrolysate was concentrated through a crossflow RO membrane. No issues were observed.

Wheat Straw (run# HB38) —Due to poor performance of C5 hydrolyzate fermentation, it was decided not to proceed with HB38 concentration or fermentation.

Fermentation of C5-rich Hydrolyzates (Core Unit Operation)

Hybrid Poplar (run# HB34/35/36-ST3-HP, June 12-17, 2015)—The hybrid poplar C5-rich hydrolysate fermentation ran for 134 hours. After an extended lag period, fermentation began at approximately 90 hours after inoculation. However, fermentation activity ended at ~110 hours. Around the same time ethanol production stopped, glucose content had decreased to about 0 g/L and concentrated ammonia was used for pH control. It is not known if the lack of glucose or the addition of ammonia is responsible for the cease in productivity. Previous work with this organism shows that productivity slows following dilute ammonia additions during pH control. The poor performance exhibited in this run can be attributed to a number of missteps due to protracted timetables. Only 22% of total sugars had been consumed by time fermentation terminated.

Issues and Resolutions: There were no equipment or processing issues, but fermentation efficacy was limited and run length increased to attempt to achieve higher yields.

Corn Stover (run# HB37-ST1-CS, June 18-21, 2015) — The corn stover C5-rich hydrolysate fermentation ran for ~70 hours. Correlating with preliminary results of small scale C5 fermentation during this trial exhibited low performance and produced only ~1 g/L ethanol. This can be attributed to the high inhibitor content of the HB37 corn stover hydrolysate (higher than HB36 hydrolysate), indicated by compositional analysis.

Issues and Resolutions: Though the fermentation of HB37 C5 corn stover hydrolysate was carried out under optimal conditions, poor performance was still observed. This was likely due to the high level of inhibitors present in the material, hindering fermentation as previously described.

Wheat Straw C5-rich Hydrolyzate Fermentation (HB38) — Due to the poor performance exhibited by the selected yeast to ferment C5 sugars in the presence of high inhibitors during previous runs on hybrid poplar and corn stover hydrolyzates, and due to high levels of inhibitors observed in the produced wheat straw hydrolysate, it was decided to forego the fermentation of HB38 C5 hydrolysate material.

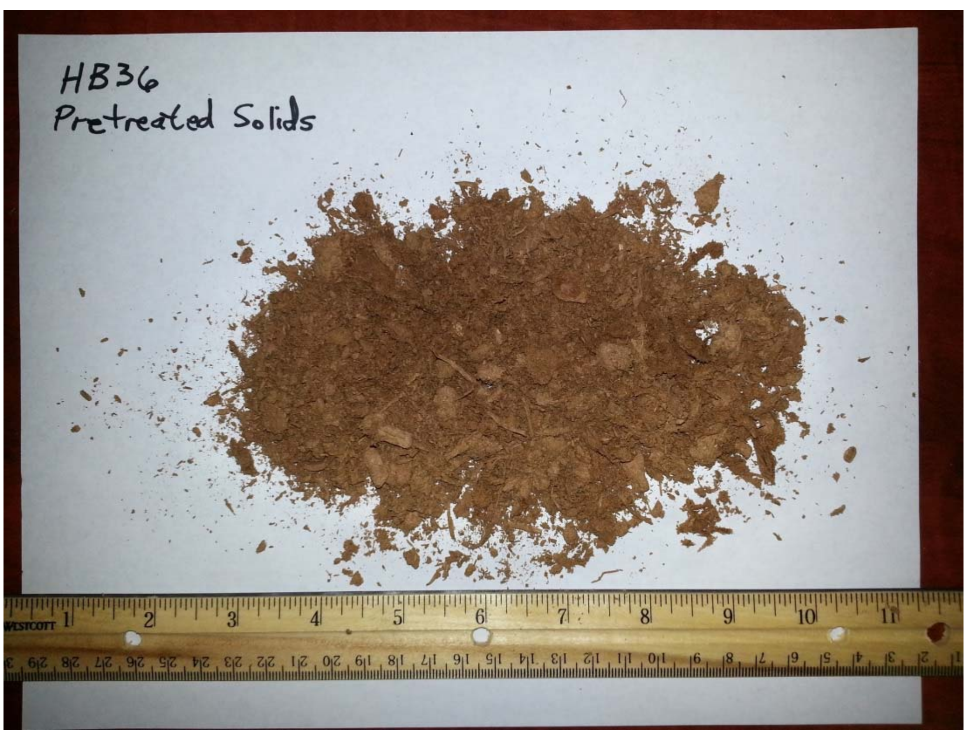
Operations Test Results

Chemical Fractionation (Pretreatment) Summary

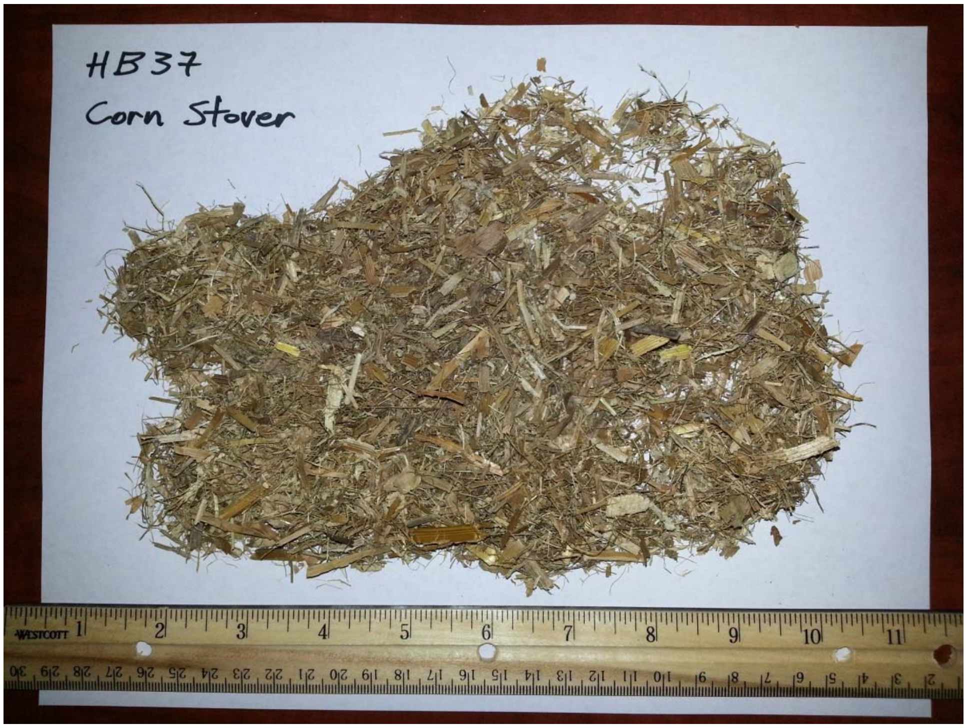
Result	HB34-35-36	HB37	HB38
Pretreatment Reactor Feed			
Delievered Biomass, lb	216220	5060	25680
Biomass Moisture (LOD avg.)	58.5%	9.8%	10.0%
Biomass Processed, BDlb	89644	4564	23112
Biomass Processed, BDST	44.82	2.28	11.56
Biomass Feed Rate (Incremental), BDST/day	12.5	3.6	6.0
Product Hydrolyzate			
Glucose Conc., New Hydrolyzate, g/L	11.5	0.9	5
Glucose Yield, %w/w	6.5%	1.5%	3.6%
Xylose Conc., New Hydrolyzate, g/L	50.7	3.4	9.7
Xylose Yield, %w/w	83.6%	12.2%	13.1%
Formic Acid Conc., New Hydrolyzate, g/L	7.6	0.9	3.5
Acetic Acid Conc., New Hydrolyzate, g/L	19.3	0	4.8
Levulinic Acid Conc., New Hydrolyzate, g/L	3.3	0	0.9
HMF Conc., New Hydrolyzate, g/L	1.5	0.2	1.7
Furfural Conc., New Hydrolyzate, g/L	1.7	0	2.2
Product Pretreated Solids			
Filter Cake Produced, lb	140820	6800	43620
Filter Cake Moisture (avg.)	55.2%	65.1%	59.0%
Filter Cake Produced, BDlb	63087	2373	17884
Pretreatment Biomass Conversion, %w/w	29.6%	48.0%	22.6%

HB36 – Raw Poplar Chips

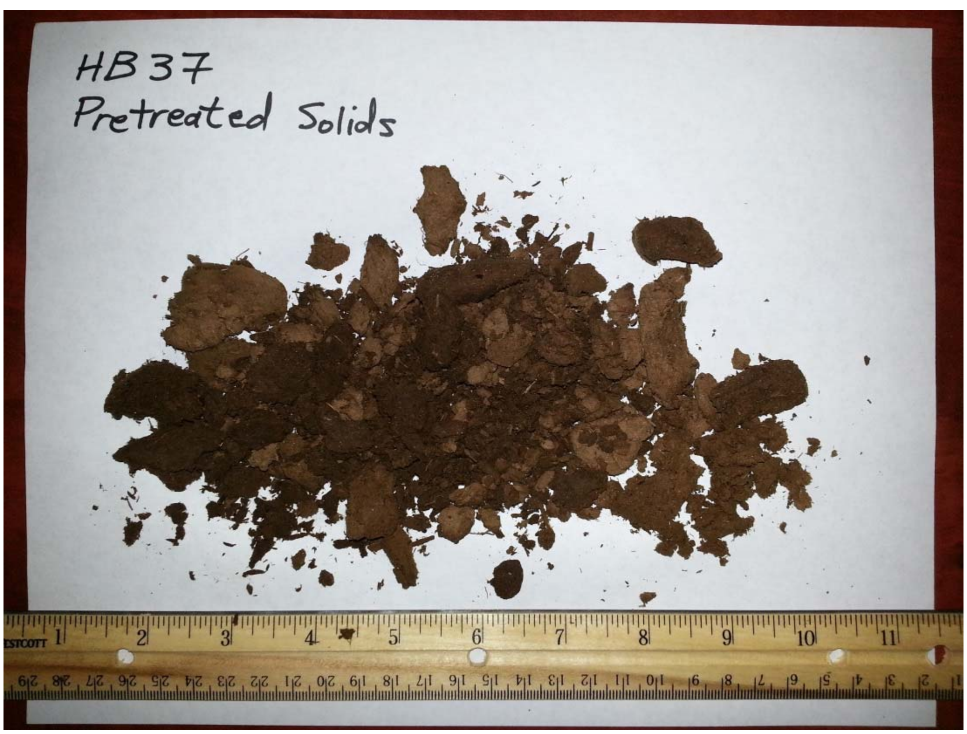
HB36 – Pretreated Poplar Solids



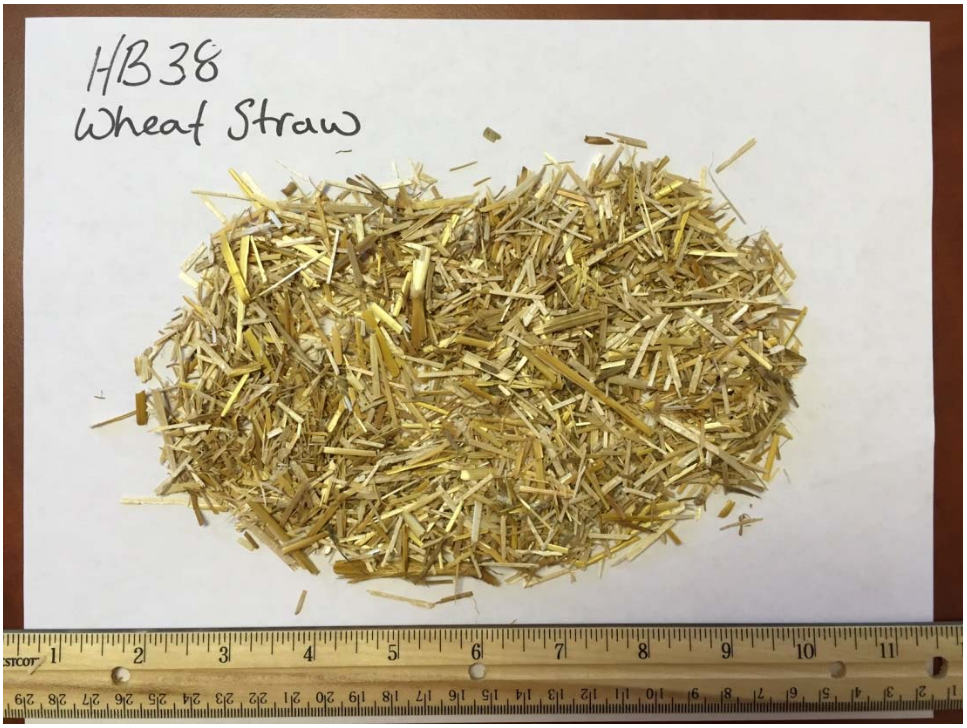
HB37 – Raw Corn Stover



HB37 –Pretreated Corn Stover Solids



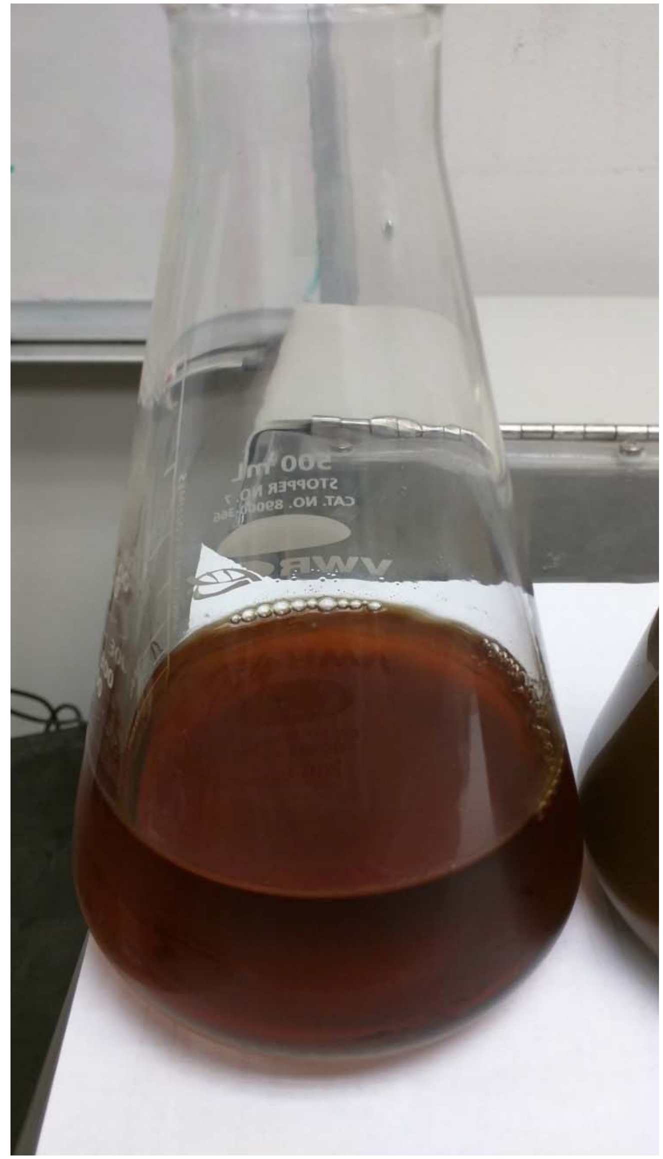
HB38 – Raw Wheat Straw



HB38 –Pretreated Wheat Straw Solids

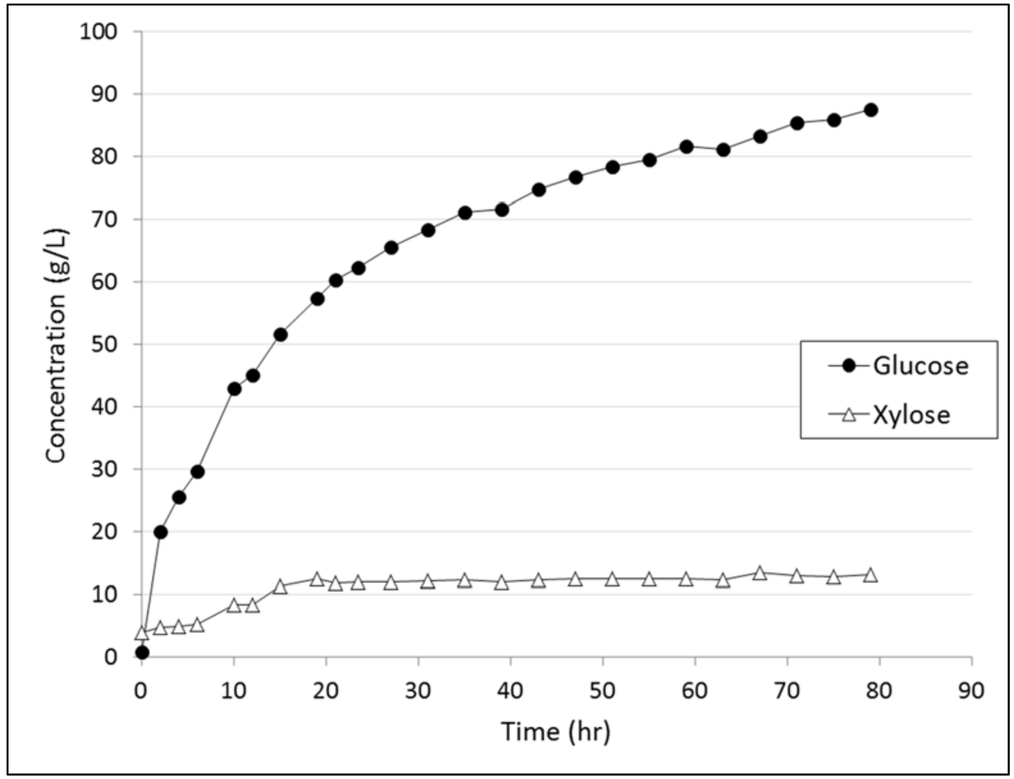
Pretreated Slurry from the Blowtank

Pretreatment Filtered Hydrolyzate



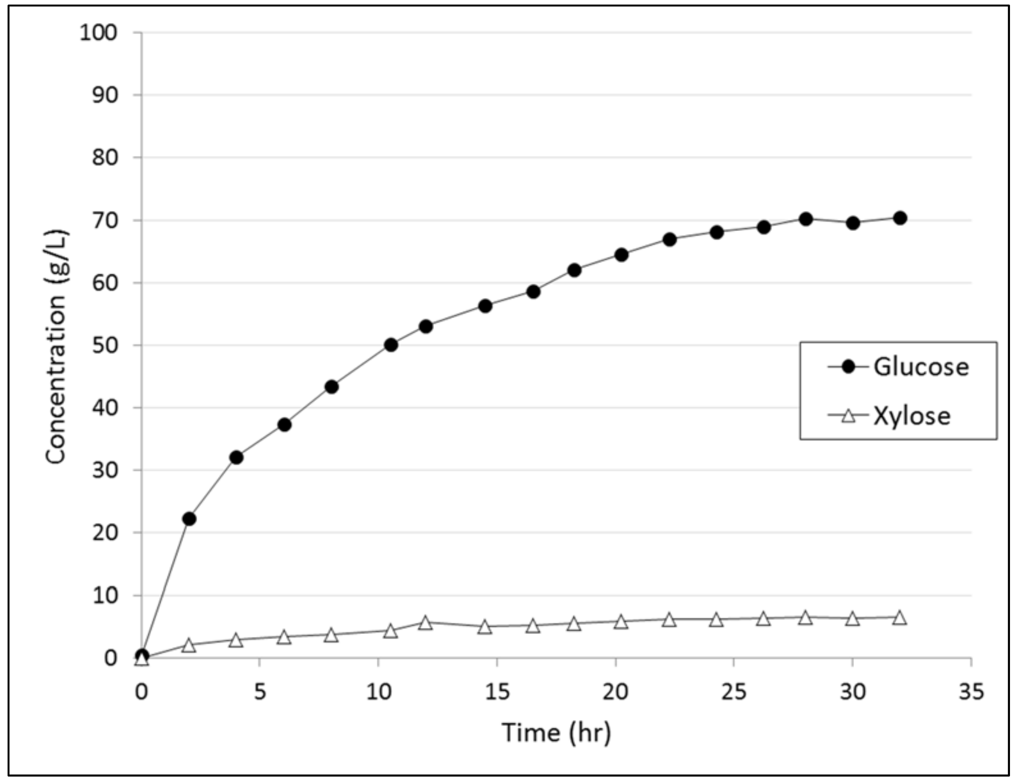
C6 Solids Saccharification Results - HB36-ST2-E1

Time (hr)	Sugars (g/L) Glucose	Sugars (g/L) Xylose
0.00	0.83	3.90
2.00	20.03	4.77
4.00	25.59	4.90
6.00	29.66	5.20
10.00	43.02	8.38
12.00	45.07	8.44
15.00	51.68	11.28
19.00	57.34	12.50
21.00	60.30	11.85
23.50	62.34	12.00
27.00	65.54	12.01
31.00	68.40	12.22
35.00	71.20	12.28
39.00	71.68	12.04
43.00	74.71	12.32
47.00	76.72	12.55
51.00	78.47	12.49
55.00	79.58	12.48
59.00	81.59	12.55
63.00	81.11	12.37
67.00	83.25	13.55
71.00	85.45	13.00
75.00	86.00	12.81
79.00	87.65	13.12



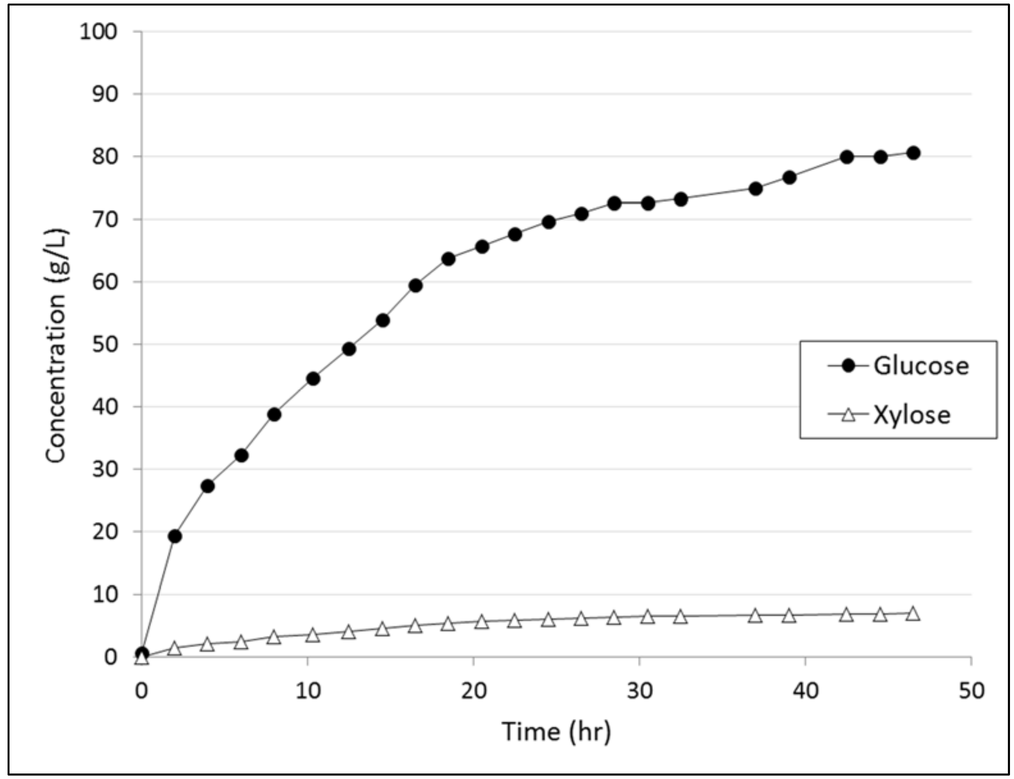
C6 Solids Saccharification Results -HB37-ST2-E1

Time (hr)	Sugars (g/L)	
	Glucose	Xylose
0.00	0.53	0.00
2.00	22.40	2.15
4.00	32.20	2.94
6.00	37.33	3.44
8.00	43.41	3.88
10.50	50.15	4.51
12.00	53.20	5.79
14.50	56.41	5.13
16.50	58.69	5.26
18.25	62.10	5.57
20.25	64.62	5.89
22.25	67.16	6.21
24.25	68.19	6.26
26.25	68.99	6.40
28.00	70.40	6.53
30.00	69.70	6.40
32.00	70.47	6.56



C6 Solids Saccharification Results - HB38-ST2-E1

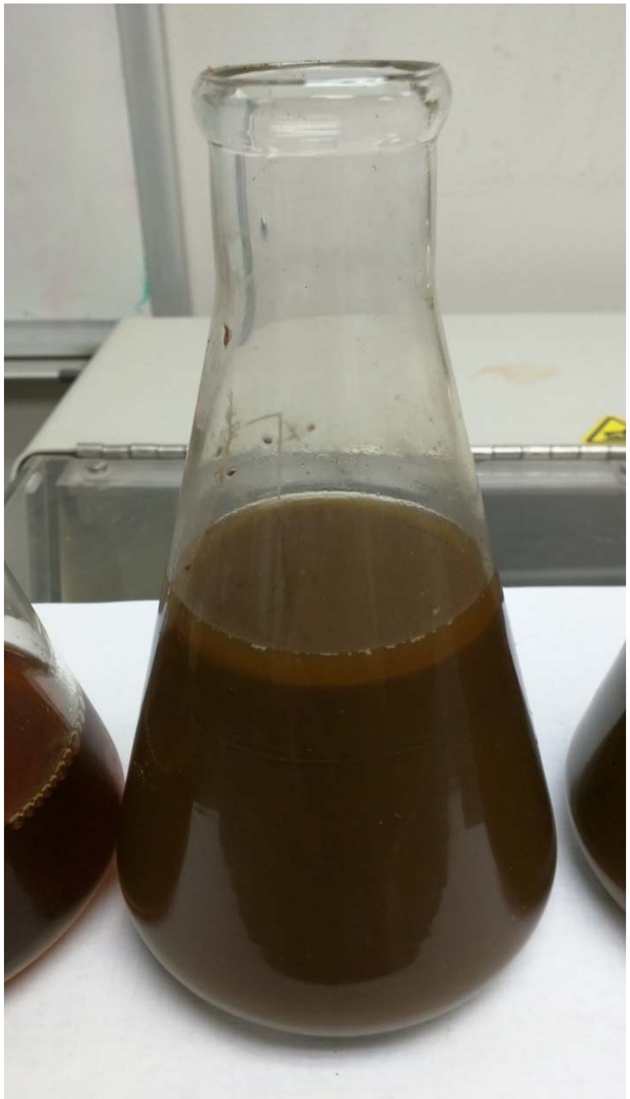
Time (hr)	Sugars (g/L) Glucose	Xylose
0.00	0.66	0.00
2.00	19.35	1.51
4.00	27.41	2.17
6.00	32.30	2.56
8.00	38.96	3.28
10.33	44.69	3.72
12.50	49.36	4.13
14.50	54.01	4.68
16.50	59.59	5.17
18.50	63.85	5.50
20.50	65.83	5.76
22.50	67.71	5.91
24.50	69.70	6.12
26.50	71.04	6.24
28.50	72.73	6.45
30.50	72.61	6.57
32.50	73.38	6.60
37.00	74.97	6.70
39.00	76.82	6.79
42.50	79.98	6.94
44.50	80.02	7.00
46.50	80.65	7.05



C6 Solids Saccharification Summary

Result	HB36 using as-reported solids compositions	HB36 using historical average solids compositions	HB37	HB38	Units
Initial culture volume	1,514.16	1,514.16	1,514.16	1,514.16	L
Base addition Volume	5,430.00	5,430.00	4,970.00	3,435.00	mL
Sample Volume	1,200.00	1,200.00	850.00	1,100.00	mL
Final Culture Volume	1,518.39	1,518.39	1,518.28	1,516.50	L
Saccharification time	79.00	79.00	32.00	46.50	hr
Pretreated Solids Loaded	303.20	303.20	303.20	303.20	kg
Pretreated Solids Glucan	47.59	57.02	60.37	59.51	%
Pretreated Solids Xylan	2.12	5.50	5.29	4.87	%
Pretreated Solids Retained Glucose	0.41	0.41	0.26	0.33	%
Pretreated Glucan loaded	144.29	172.88	183.04	180.43	kg
Pretreated Xylan loaded	6.43	16.68	16.04	14.77	kg
Culture Initial [glucose]	0.83	0.83	0.53	0.66	g/L
Culture Initial [xylose]	3.90	3.90	0.00	0.00	g/L
Starting glucose	1.26	1.26	0.80	1.00	kg
Starting xylose	5.91	5.91	0.00	0.00	kg
Final [Glucose]	87.65	87.65	70.47	80.65	g/L
Final [Xylose]	13.12	13.12	6.56	7.05	g/L
Total Glucose Produced	131.83	131.83	106.19	121.31	kg
Max theo. Glucose	160.33	192.09	203.38	200.48	kg
Glucose Yield	**83.00**	**69.30**	**52.60**	**61.00**	**%**
Total Xylose Produced	14.02	14.02	9.96	10.69	kg
Max theo. Xylose	7.30	18.95	18.23	16.78	kg
Xylose Yield	**191.89**	**73.96**	**54.65**	**63.72**	**%**

Enzyme Saccharified Slurry



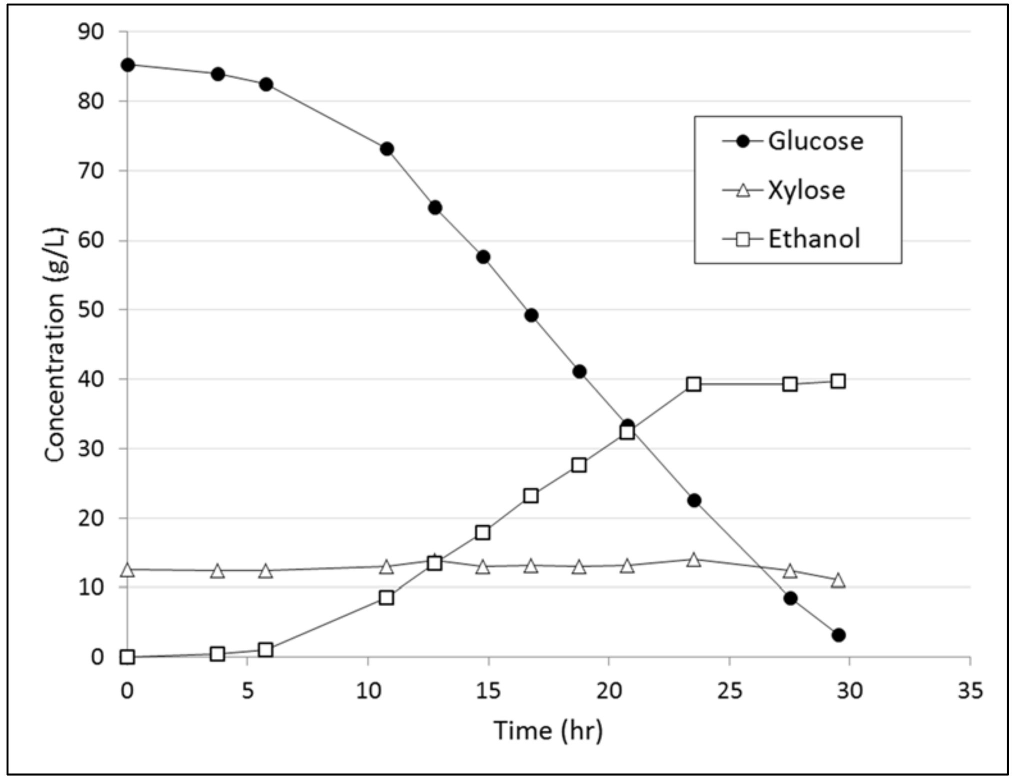
Post-Saccharification Filtered Hydrolyzate

HB36 Poplar Enzyme Saccharified Solids
(Filtered Post-Fermentation)

HB38 Wheat Straw Enzyme Saccharified Solids
(Filtered Post-Fermentation)

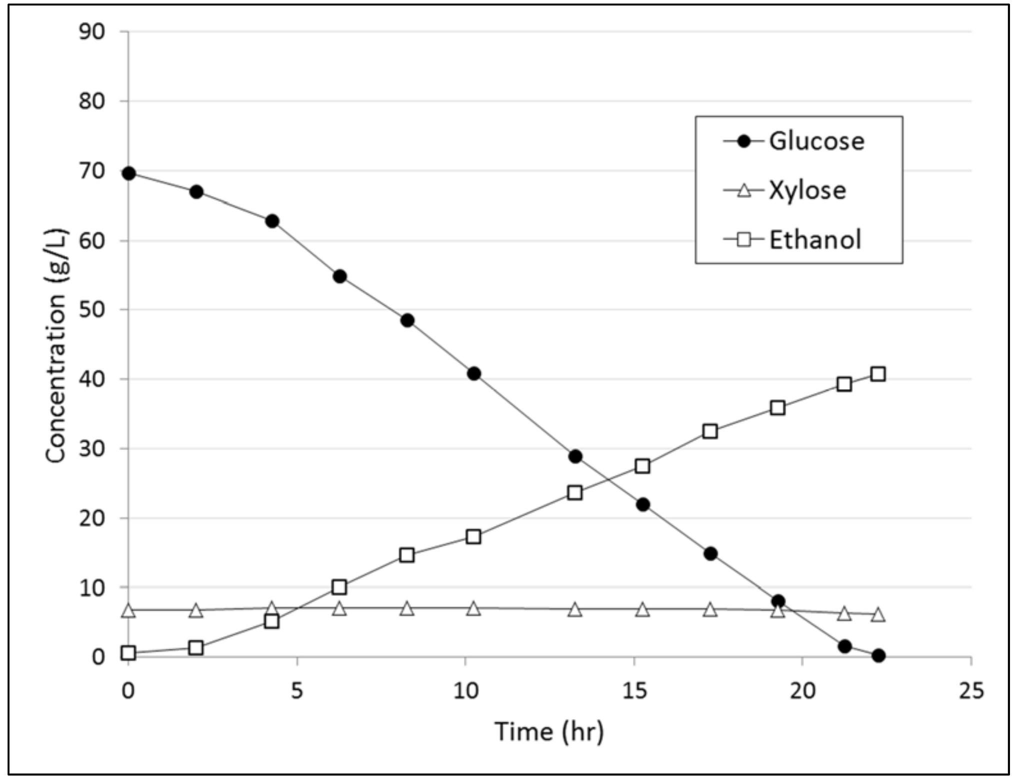
C6 Fermentation Results – HB36-ST2-F1

Time (hr)	Sugars (g/L) Glucose	Sugars (g/L) Xylose	Ethanol (g/L)
0.00	85.28	12.53	0.00
3.75	83.93	12.46	0.41
5.75	82.56	12.49	1.03
10.75	73.26	13.01	8.65
12.75	64.81	13.95	13.40
14.75	57.79	13.07	17.93
16.75	49.38	13.17	23.20
18.75	41.18	13.06	27.64
20.75	33.34	13.13	32.39
23.50	22.65	14.00	39.28
27.50	8.57	12.37	39.30
29.50	3.34	11.07	39.69



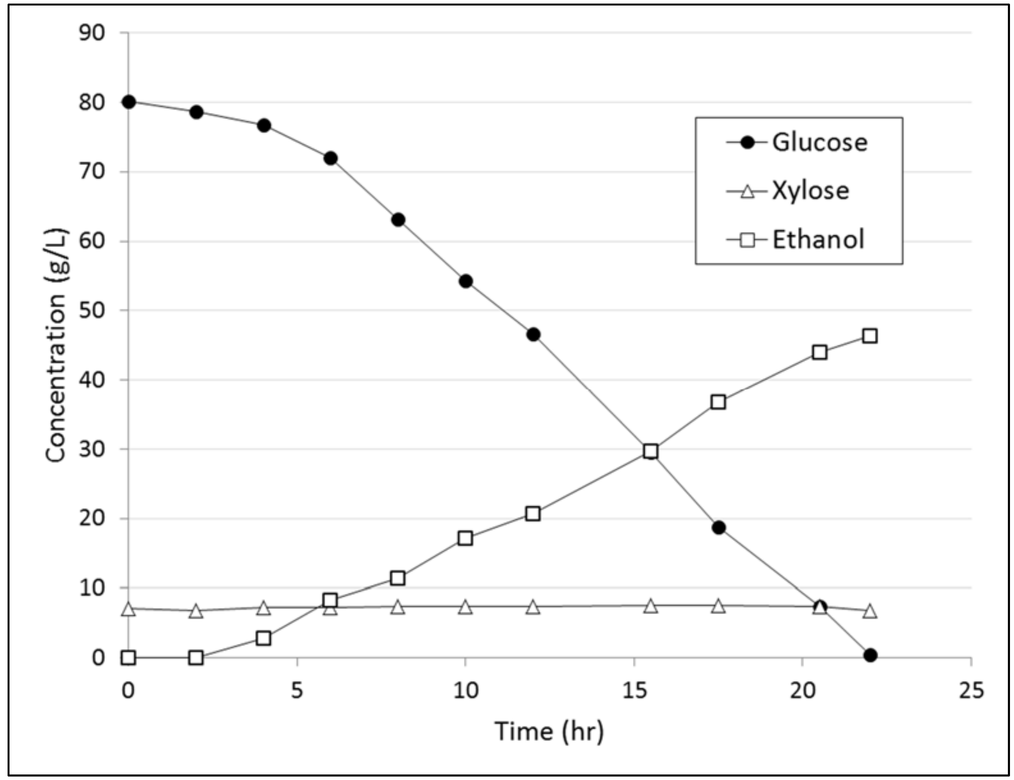
C6 Fermentation Results – HB37-ST2-F1

Time (hr)	Sugars (g/L) Glucose	Sugars (g/L) Xylose	Ethanol (g/L)
0.00	69.63	6.86	0.63
2.00	66.95	6.89	1.37
4.25	62.84	7.08	5.23
6.25	54.92	7.16	10.08
8.25	48.54	7.09	14.65
10.25	40.90	7.11	17.34
13.25	28.94	6.98	23.58
15.25	21.95	6.94	27.50
17.25	14.87	7.02	32.49
19.25	8.16	6.84	35.95
21.25	1.65	6.34	39.25
22.25	0.31	6.16	40.70



C6 Fermentation Results – HB38-ST2-F1

Time (hr)	Sugars (g/L) Glucose	Sugars (g/L) Xylose	Ethanol (g/L)
0.00	80.09	7.05	0.00
2.00	78.68	6.75	0.00
4.00	76.72	7.23	2.90
6.00	72.09	7.20	8.27
8.00	63.15	7.42	11.40
10.00	54.33	7.40	17.20
12.00	46.66	7.43	20.66
15.50	29.50	7.61	29.65
17.50	18.83	7.49	36.76
20.50	7.45	7.39	44.00
22.00	0.53	6.83	46.40



C6 Fermentation Summary

Result	HB36	HB37	HB38	Units
Initial culture volume	1518.4	1518.3	1516.5	L
Culture Initial [glucose]	85.3	69.6	80.1	g/L
Feed Volume	0.0	0.0	0.0	L
Feed [glucose]	0.0	0.0	0.0	g/L
Base addition Volume	1500.0	3200.0	6850.0	mL
Inoculum Volume	360.0	360.0	360.0	mL
Inoculum titer	10e+06	10e+06	10e+06	cells/mL
Sample Volume	600.0	600.0	550.0	mL
Final Culture Volume	1519.7	1521.2	1523.2	L
Fermentation time	29.5	22.5	22.0	hr
Total glucose added	129488.3	105717.8	121456.1	g
Final [Glucose]	3.34	0.31	0.53	g/L
Glucose Consumed	124412.7	105246.3	120648.8	g
P-Final [EtOH]	39.7	40.7	46.4	g/L
P-Final EtOH	60314.9	61914.5	70674.4	g
Alcohol by volume	5.03	5.16	5.88	%
Q(P) - Productivity	1.35	1.81	2.11	g/L/hr
Ethanol Yield	**91.1**	**114.5**	**113.8**	**%**

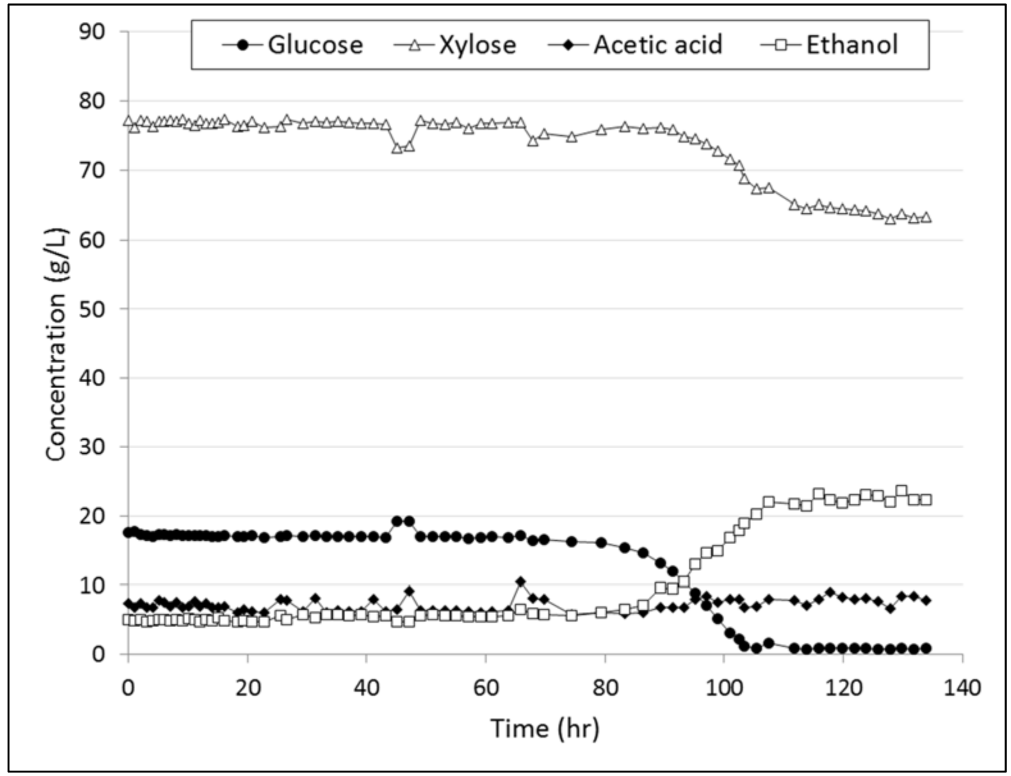
C5 Conditioning and Concentration Summary

Result	Glucose, (g/L)	Xylose, (g/L)	Acetic, (g/L)	Levulinic, (g/L)	Ethanol, (g/L)	HMF, (g/L)	Furfural, (g/L)	Phenols, (g/L)	TOTAL, g
HB36 C5 Hydrolyzate	7.66	33.47	9.42	0.66	0.02	1.05	1.46	9.53	63.27
HB36 C5 Hydrolyzate Post Conditioning	7.16	31.56	8.04	0.00	6.98	0.10	0.00	5.41	59.25
HB36 C5 Hydrolyzate Post RO Conc.	21.57	96.60	7.09	0.00	5.67	0.13	0.00	7.70	138.76
HB37 C5 Hydrolyzate	7.49	29.13	4.57	0.23	0.00	NM	NM	NM	NM
HB37 C5 Hydrolyzate Post RO Conc.	17.60	68.32	7.68	1.19	0.00	NM	NM	NM	NM

C5 Fermentation Results – HB36-ST3-HP

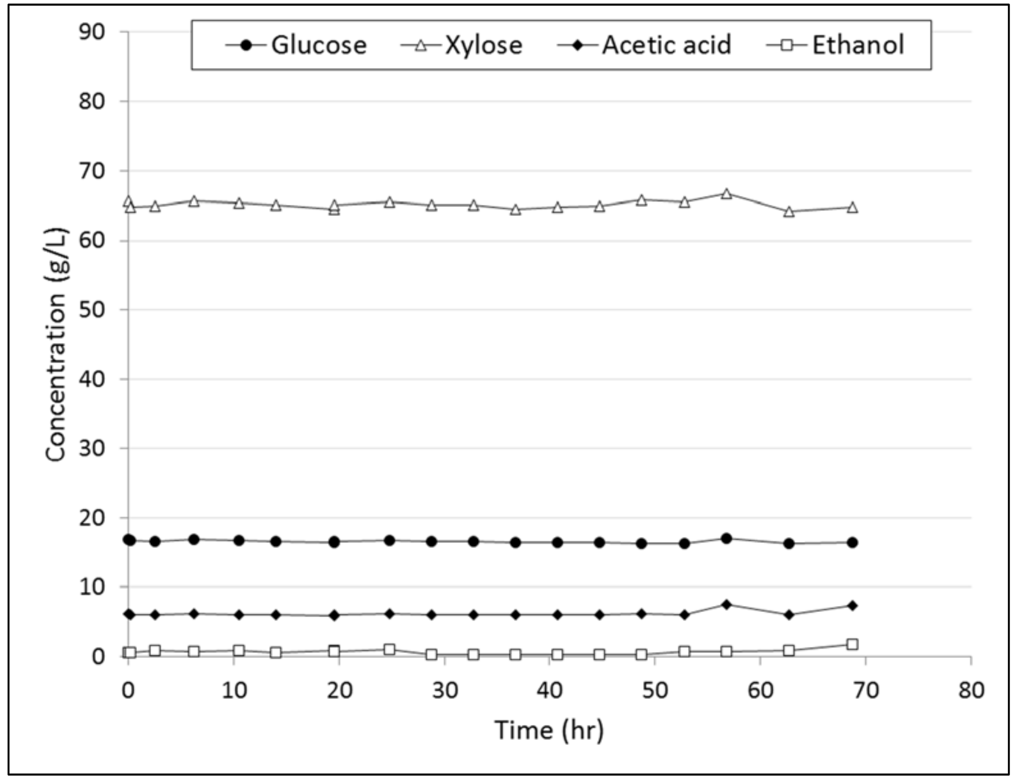
Ferm Time (hr)	Sugars (g/L) Glucose	Xylose	Acetic acid (g/L)	Ethanol (g/L)
0.00	17.66	77.25	7.45	5.00
1.08	17.75	76.20	6.89	4.93
2.08	17.28	77.13	7.40	5.09
3.08	17.21	77.04	6.85	4.78
4.08	17.06	76.33	6.86	4.92
5.08	17.26	77.01	7.82	5.05
6.08	17.25	77.01	7.50	5.03
7.08	17.18	77.14	6.90	4.90
8.08	17.23	76.99	7.55	5.08
9.08	17.19	77.32	6.86	4.91
10.08	17.09	76.79	6.94	5.16
11.08	17.09	76.43	7.69	5.12
12.08	17.17	77.16	6.91	4.73
13.08	17.10	76.79	7.38	4.98
14.08	17.03	76.74	6.82	4.90
15.08	17.05	76.87	6.83	5.28
16.08	17.17	77.34	6.92	4.91
18.33	17.03	76.36	6.15	4.73
19.33	17.00	76.42	6.52	4.96
20.83	17.14	77.02	6.28	4.70
22.83	16.91	76.10	6.09	4.72
25.50	16.98	76.36	8.02	5.57
26.67	17.17	77.32	7.89	5.07
29.33	17.00	76.73	6.23	5.79
31.33	17.10	77.07	8.09	5.27
33.33	16.99	76.86	6.10	5.73
35.08	17.02	76.97	6.32	5.73
37.08	17.03	76.87	6.20	5.61
39.08	16.96	76.73	6.21	5.72
41.08	17.01	76.69	8.01	5.52
43.17	16.90	76.62	6.29	5.70
45.08	19.17	73.23	6.57	4.79
47.08	19.22	73.53	9.21	4.70
49.08	17.05	77.23	6.44	5.69
51.08	16.98	76.68	6.31	5.72
53.08	16.94	76.61	6.34	5.65
55.08	16.97	76.87	6.31	5.60
57.08	16.76	76.00	6.16	5.51
59.08	16.91	76.72	6.12	5.50
61.08	16.93	76.80	6.21	5.54
63.83	16.90	76.85	6.35	5.60
65.83	17.20	76.88	10.51	6.59
67.83	16.38	74.17	8.11	5.86
69.83	16.54	75.24	8.05	5.83
74.33	16.20	74.78	5.83	5.69
79.33	16.06	75.84	6.01	6.03
83.33	15.44	76.27	5.99	6.47
86.33	14.57	76.04	6.05	7.08
89.33	13.18	76.10	6.88	9.56
91.33	11.96	75.87	6.76	9.42
93.33	10.57	74.84	6.81	10.47
95.17	8.86	74.58	8.07	13.04
97.00	7.04	73.82	8.37	14.60
99.00	5.24	72.81	7.58	14.88

101.00	3.09	71.58	7.96	16.91
102.45	2.25	70.63	8.01	17.86
103.45	1.25	68.74	6.83	18.88
105.45	0.86	67.25	7.00	20.18
107.45	1.60	67.43	8.06	22.04
111.87	0.89	65.06	7.79	21.78
113.87	0.82	64.51	7.12	21.47
115.87	0.88	65.08	8.04	23.16
117.87	0.88	64.69	9.08	22.24
119.87	0.85	64.48	8.28	21.86
121.87	0.89	64.39	8.02	22.32
123.87	0.85	64.17	8.20	23.10
125.87	0.84	63.74	7.65	22.93
127.87	0.74	63.03	6.74	21.95
129.87	0.85	63.75	8.44	23.57
131.87	0.83	63.23	8.39	22.35
133.87	0.85	63.36	7.90	22.27



C5 Fermentation Results – HB37-ST1-CS

Ferm Time (hr)	Sugars (g/L)		Acetic acid (g/L)	Ethanol (g/L)
	Glucose	Xylose		
0.00	16.85	65.61	6.24	0.61
0.17	16.70	64.74	6.06	0.69
2.50	16.62	64.95	6.11	0.89
6.25	16.79	65.66	6.16	0.79
10.50	16.69	65.36	6.10	0.95
14.00	16.62	65.13	6.13	0.68
19.50	16.45	64.50	5.99	0.85
19.50	16.51	65.03	6.05	0.78
24.75	16.71	65.56	6.16	1.05
28.75	16.49	65.14	6.05	0.28
32.75	16.54	65.13	6.12	0.31
36.75	16.39	64.57	6.07	0.30
40.75	16.43	64.83	6.08	0.30
44.75	16.44	64.94	6.08	0.32
48.75	16.33	65.77	6.20	0.38
52.75	16.20	65.60	6.14	0.81
56.75	17.00	66.77	7.57	0.78
62.75	16.24	64.14	6.05	0.86
68.75	16.42	64.77	7.46	1.75



C5 Fermentation Summary

Result	HB36	HB37	HB38	Units
Initial culture volume	1514	151	NA	L
Culture Initial [Glucose]	17.7	16.9	NA	g/L
Culture Initial [Xylose]	77.3	65.6	NA	g/L
Inoculum Volume	360.0	360.0	NA	mL
Inoculum titer	10e+06	10e+06	NA	cells/mL
Sample Volume	600.0	600.0	NA	mL
Final Culture Volume	1513.8	150.8	NA	L
Fermentation time	29.5	22.5	NA	hr
Total Glucose added	26737.2	2544.4	NA	g
Total Xylose added	116956.5	9907.1	NA	g
Final [Glucose]	0.85	0.31	NA	g/L
Final [Xylose]	63.63	64.77	NA	g/L
Glucose Consumed	25450.5	2497.6	NA	g
Xylose Consumed	20636.0	0.0	NA	g
P-Final [EtOH]	17.3	0.9	NA	g/L
P-Final EtOH	26248.6	134.2	NA	g
Alcohol by volume	2.20	0.11	NA	%
Q(P) - Productivity	0.59	0.04	NA	g/L/hr
Ethanol Yield	**35.7**	**2.1**	NA	**%**

Solids Composition Summary

Sample Description	Water Extractives, Other (%)	Ethanol Extractives (%)	Lignin (%)	Glucan (%)	Xylan (%)	Galactan (%)	Arabinan (%)	Mannan (%)	Acetyl (%)	Total Ash (Included in "Other" (%)
Raw Biomass										
Hybrid Poplar Clean Chip, HB36	2.16	1.81	22.49	50.56	17.12	ND	ND	2.67	3.19	0.89
Corn Stover, HB37	12.87	3.32	18.21	41.47	19.94	ND	3.20	0.62	0.38	11.12
Wheat Straw, HB38	3.49	1.92	20.26	44.81	23.13	ND	3.17	0.57	2.65	10.98
Pretreated Solids										
Pretreated Hybrid Poplar Chip, HB36	4.88	10.84	15.30	47.59	2.12	0.00	0.74	0.00	0.37	0.53
Pretreated Hybrid Poplar Chip, HISTORICAL AVERAGE	4.47	11.36	20.27	57.02	5.50	0.00	0.59	0.29	0.52	0.58
Pretreated Corn Stover, HB37	0.97	7.85	25.08	60.37	5.29	ND	ND	ND	0.44	21.40
Pretreated Wheat Straw, HB38	3.71	11.05	20.30	59.51	4.87	ND	ND	ND	0.56	14.39
Post-Fermentation Solids										
Post-Fermentation Saccharified Solids, HB36	9.18	10.95	68.90	9.59	0.96	ND	ND	0.21	0.21	0.90
Post-Fermentation Saccharified Solids, HB38	4.59	6.67	63.68	20.87	3.33	ND	ND	0.40	0.45	16.56

Wood to Ethanol Yield Summary

Result	HB36 using as-reported solids compositions	HB36 using historical average solids compositions	HB37	HB38	Units
Pretreated Biomass C6 Solids Processing					
Pretreatment Solids Retained	74.2	74.2	51.7	77.4	%w/w, BD Solids/BD Raw Biomass
Saccharification Glucose Yield	83.0	69.3	52.6	61.0	%w/w, Actual/Theoretical
Saccharification Glucose Yield	43.9	43.9	35.3	40.3	%w/w, Glucose/BD Pretreated Solids
Fermentation EtOH Yield (Based on Glucose only)	91.1	91.1	114.5	113.8	%w/w, Actual/Theoretical
Fermentation EtOH Yield	46.6	46.6	58.6	58.2	%w/w, EtOH/Glucose
Fermentation EtOH Yield	46.0	46.0	32.4	55.1	gal/BDST Raw Biomass
Pretreatment C5 Hydrolyzate Processing					
Pretreatment Biomass Conversion	25.8	25.8	48.3	22.6	%w/w, Filtrate from Wood/BD Raw Biomass
Glucose Yield in Pretreatment Hydrolyzate	6.5	6.5	1.5	3.6	%w/w, Actual/Theoretical
Glucose Yield in Pretreatment Hydrolyzate	3.7	3.7	0.7	1.8	%w/w, Glucose/BD Pretreated Solids
Xylose Yield in Pretreatment Hydrolyzate	83.6	83.6	3.4	9.7	%w/w, Actual/Theoretical
Xylose Yield in Pretreatment Hydrolyzate	16.3	16.3	0.7	2.6	%w/w, Xylose/BD Pretreated Solids
Glucose in Conditioned Hydrolyzate	100.0	100.0	100.0	100.0	%w/w, Glucose retained
Xylose in Conditioned Hydrolyzate	100.0	100.0	100.0	100.0	%w/w, Xylose retained
Fermentation EtOH Yield (Based on Glucose and Xylose only)	35.7	35.7	2.1	NR	%w/w, Actual/Theoretical
Fermentation EtOH Yield	18.5	18.5	1.1	NR	%w/w, EtOH/Glucose+Xylose
Fermentation EtOH Yield	2.9	2.9	0.0	NR	gal/BDST Raw Biomass
Combined C5 & C6 Ethanol Yield					
Total EtOH Yield (Based on Glucose and Xylose only)	**57.1**	**45.4**	**28.7**	**49.7**	**%w/w, Actual/Theoretical**
Total EtOH Yield	**48.9**	**48.9**	**32.4**	**55.1**	**gal/BDST Raw Biomass**

Discussion of Results

HB36 Hybrid Poplar Pretreatment - The pretreatment (Chem Frac Unit) processing of HB36 hybrid poplar feedstock was largely uninterrupted over 5 days of operation, which was expected as poplar is the feedstock ZeaChem has used for the large majority of previous processing trials. The reaction severity was changed two times toward the beginning of the week to optimize the C5 sugar yields for the lot of wood received. The HB36 C5 rich hydrolyzate produced was 50.7 g/L xylose, in line with a 50 g/L target concentration, though the final concentration was lower due to a large amount of dilute hydrolyzate used to charge the system at the beginning of each run. Xylose yield was 83.6% of theoretical, which is a good result.

HB37 Corn Stover Pretreatment - HB37 C5 hydrolyzate produced was low concentration due to frequent run interruptions due to blowline wear and non-optimized processing conditions, which with more processing time could be significantly improved. High rates of blowline wear had been seen previously when processing other agricultural residue feedstocks, but on the order of 10x less than corn stover and wheat straw. In high production processing, cleaned biomass and improved blowline design would be needed. All steam-exploded solids appeared fine (though no particle size analysis was done) and saccharified well. The composition of both the C5 hydrolyzate and pretreated solids could be improved with higher reaction severity.

HB38 Wheat Straw Pretreatment - HB38 C5 hydrolyzate also had low concentration for the same reasons as HB37, though an improved blowline bend in the wear area was used resulting in longer runtime than during HB37. All steam-exploded solids appeared fine and saccharified well. The composition of both the C5 hydrolyzate and pretreated solids could be improved with higher reaction severity.

C6 Pretreated Solids Saccharification - Pretreated solids saccharification yields were good but are under scrutiny due to questionable pretreated solids composition results. The solids glucan was 10% lower and lignin 5% lower than typically seen and the sample total solids was also low. A second sample analysis yielded results with extremely high ethanol and water extractives and were thrown out. With these results in question, historical averages were used to analyze the saccharification effectiveness. Though the solids composition results affect saccharification analysis, they do not affect the wood-to-ethanol yield calculation. Using historical averages, the HB36 xylose yield was 73.96%, which is fairly consistent with 54.65% and 63.72% for HB37 and HB38 respectively. Lower HB37 and HB38 yields could be due to under-severe pretreatment as indicated by C5 hydrolyzate yields for those runs. The 191% xylose yield in the results could be due to amplification of solids composition analysis error since xylan accounted for only 2.1% of the solids. Enzymatic saccharification results did verify that HB36 acid-pretreatment of hybrid poplar was sufficient to allow enzymatic access to cellulose for conversion to glucose.

C6 Saccharified Slurry Fermentation - The results tables show good Ethanol yields from C6 Saccharified Solids. The HB36 C6 Fermentation had no issues and had a 91.1% Ethanol yield, which is in the expected range. HB37 and HB38 calculated EtOH yields were >100%, which is of course not possible. Based on

HB36 results it is thought that HB37 and HB38 EtOH yields were similar to HB36, but both HB37 and HB38 fermentations foamed severely and there was significant mass loss through the tank overflows. The overflowed foam composition is unknown. Based on the high yields it is thought the foam contained little or no ethanol. Antifoam was used without success to reduce foaming, and reduced agitation improved but did not eliminate the foaming. It is thought that optimized agitator blade style, configuration, and speed can significantly reduce or eliminate foaming.

C5 Hydrolyzate Conditioning - HB36 hydrolyzate was conditioned with partial success and a few issues. Since there was limited hydrolyzate and too little acetic acid to accumulate quickly, acetic acid could not be built up, creating a system imbalance. It is also notable, though not an issue, there was ethanol mixed with the EtAc solvent which was introduced to the hydrolyzate. Acetic concentration was only lowered slightly, though furfural and levulinic acid were both completely removed. In the future it may be helpful to find a more effective solvent and to pre-charge the system with acetic acid. Since the HB36 conditioning was minimally effective at removing acetic acid, it was decided to not condition HB37 hydrolyzate.

C5 Hydrolyzate Concentration – HB36 and HB37 hydrolyzates were concentrated successfully using a conventional crossflow RO membrane with no appreciable sugar loss. The concentrations were increased and met the desired concentrations for fermentation with no issues.

C5 Hydryolyzate Fermentation - HB36 Hydrolyzate was fermented with very limited success. The target time for a C5 fermentation is 72 hours or less. The HB36 fermentation made little progress but appeared to still be viable at 72 hours so was continued through 134 hours but with only 35.7% ethanol yield, over half of which came from glucose consumption. The proprietary organism chosen for C5 fermentation had unproven performance at this scale and in the presence of high inhibitor levels. The hydrolysate used contained a high amount of acetic acid which is well known to cause fermentation lag depending on concentration. It is believed that the relatively high concentration of acetic acid along with phenols and other fermentation inhibitors (e.g. HMF and furfural) had an additive effect to the observed lag phase. Removal of these inhibitors prior to fermentation could have improve ethanol yields and possibly reduce lag during fermentation. Additionally, due to logistical issues surrounding receipt of the preferred antibiotic, chloramphenicol, an alternative antibiotic mix, virginiamycin and penicillin, commonly used in the fuel ethanol industry was supplemented. As a result of the sensitivity of the proprietary organisms used here, the alternative antibiotic used here might have further contributing to the increased lag phase and low productivity. The HB37 C5 fermentation followed the poor performance of the HB36 C5 fermentation, and after the first 72 hours of fermentation the run was terminated. The poor performance exhibited by the selected yeast to ferment C5 sugars in the presence of high inhibitors during previous runs on hybrid poplar and corn stover hydrolyzates, and due to high levels of inhibitors observed in the produced wheat straw hydrolysate, it was decided to forego the fermentation of HB38 C5 hydrolysate material.

Discussion of IBR Operations

The BP3 Operations Test was the culmination of the IBR project efforts and the next progression following the BP2 phase of the project during which a number of lessons were learned. During both BP2 and the beginning of BP3 many of those improvements were implemented affecting operations procedures, technical process parameters, and equipment configurations.

Lessons Learned and Processing Improvements Implemented in BP3 (Chem Frac Unit):

- The raw biomass feedstock neutralization capacity changes with different types of biomass and also by lot. The pretreatment severity is adjusted with each run to optimize C5 sugar concentrations and reduce unwanted degradation byproducts.
- Addition of a chip feed bin weigh scale and blowtank vent condensate flow meter improved the ability to close the run mass balance.
- Control system hardware and logic improvements resulted in better control over biomass, water, and acid additions, higher sugar concentrations, and improved ability to close a run mass balance.
- Movement of steam injection lines eliminated localized over-heating and resulted in lower undesired sugar byproduct concentrations in the pretreatment hydrolyzate.
- Insulation of Blowtank and piping reduced condensate, resulting in higher sugar concentrations.
- Improved boiler feed water system resulting in uninterrupted runs which allowed better mass balance closure and higher pretreatment hydrolyzate sugar concentrations.

It is notable that BP3 operations included the first processing at ZeaChem for both corn stover and wheat straw feed stocks. It is also notable that ZeaChem and DOE did not enter into contract for BP3 until May 4, 2015 and the deadline for the end of BP3 operations was June 30, 2015, which allowed limited time to optimize processing, implement previously identified improvements, and complete a satisfactory operational run. It also precluded initial trials to shake out processing of corn stover and wheat straw which could have improved the BP3 operations runs significantly.

Appendix B - IE Performance Test Run Report

EE0002880 ZeaChem 10 Bone Dry Ton/Day Integrated Bio-refinery (IBR)

Report for Performance Test for conclusion of Budget Period 2

Objective

Through an operational run of the IBR and production of cellulosic ethanol, witnessed by the Independent Engineer, demonstrate that the EPC portion of the project is complete. This requirement is stated in the Funding Opportunity Announcement DE-FOA-0000096. Because the Budget Period 2 performance test is intended to only demonstrate that construction is complete, the scope and duration of this test are minimized. In Budget Period 3 an additional longer performance test will be conducted to obtain comprehensive technical, operating, and financial information, as stated in the FOA.

Scope of the Cooperative Agreement

The scope of Cooperative Agreement EE0002880 includes construction of two process units in addition to ZeaChem's existing Bio-refinery (Core facility), so that ethanol can be produced from cellulosic feedstocks. The two process units are the Chemical Fractionation unit and the Hydrogenolysis unit.

ZeaChem Staff Organization

Tim Eggeman – Principal Investigator, project oversight
Brian O'Neill – Project Engineer, BP2 performance test run planning and execution coordination.
Pete Wilhelm – Plant Manager, oversight of Boardman facility and operations.
Angela Boatman – Operations Superintendent, IE test operations leader.

Operation of the Boardman Integrated Bio-Refinery (IBR) Facility

The IBR is comprised of 3 basic modules with the capability to run independently or in sequence:

3. **Chemical Fractionation (Hydrolyzate production)**
    - 2 separate processing stages, each run continuously, with both stages utilizing the same equipment, requiring intermittent processing runs.
    - Due to material storage constraints, the maximum continuous run-time for an individual stage is about 4 days.
    - Batch storage includes: Feedstock Storage, Hydrolyzate Storage, solid product storage.

4. **Core (Ethyl Acetate production).** ***NOTE: The Core operations are not within the boundary of this test or the IBR project. Core operations are not included in this test plan other than as mentioned for reference. The BP2 Performance Test did not include Core operations.***

5. **Hydrogenolysis (Ethanol production)**
    - Continuous processing.

The Chemical Fractionation (Hydrolysis) unit is designed to be operated in a two-stage mode where material is run through the processing equipment twice, with different processing parameters for each stage. There is a continuous reaction process followed by a batch filtering process. During the first stage operation cellulosic feedstock is processed, and primarily C5 sugars (xylose) are produced along with a solid residue product. During the second stage operation the residue product from the first stage operation is processed and primarily C6 sugars (glucose) are produced along with a lignin product. The C5 and C6 sugar streams are combined into a single stream (hydrolyzate). For the purposes of this test, the hydrolyzate was analyzed, but not further processed.

The Hydrogenolysis unit is primarily a continuous reaction and distillation process. The ethyl-acetate (EtAc) feedstock is reacted with purchased hydrogen in order to produce ethanol. The EtAc will be purchased since Core operations are not a part of this test.

Scope of the BP2 Performance Test

The Chemical Fractionation (Hydrolysis) unit was operated and demonstrated 2-stage acid hydrolysis. The first stage biomass feedstock was hybrid poplar wood chips. The first stage processing goal was to achieve steady state with a biomass feed rate of 8 Bone-Dry-Standard-Tons (BDST) per day minimum (10 BDST per day is the targeted design rate). The second-stage processing goal was also achieve steady state operating at a minimum rate of 8 BDST per day. After the completion of the second-stage hydrolysis run, hydrolyzate and lignin products were collected and not processed further.

Core operations were not included in this test, and cellulosic EtAc was not produced or used as the feedstock for Hydrogenolysis operation in this test.

The Hydrogenolysis unit was operated to demonstrate hydrogenolysis processing at steady state with an EtAc feed rate of the 50 gal/hr (50 gal/hr is the design rate). Since the Core operations were not part of this test and no cellulosic EtAc was produced, the Hydrogenolysis feedstock was purchased non-cellulosic EtAc. Test data will be provided to compare the purchased EtAc to cellulosic EtAc previously produced by ZeaChem at the Boardman facility.

The Independent Engineer was on site to observe both the first-stage and second-stage operation of the Chemical Fractionation unit, and operation of the Hydrogenolysis Unit.

Procedure

Facility Preparation – Several steps were taken prior to the testing in weeks and hours prior to running the tests:

8. Walkdown of sampling points was done with IE.
9. Equipment and controls were checked and fully functional during operation.
10. Safety systems were checked and fully functional during operation.
11. Startup walkthrough was performed to verify equipment was ready for operation.
12. Required feedstock, chemicals, and utilities were verified prior to operation.
13. Lab equipment was calibrated and fully functional.

Hydrolysis Run Summary – The two hydrolysis stages were run at different times. Stage 1 was run on March 5th and 6th 2014, and Stage 2 was run on June 26th and 27th 2014. The March Stage 1 product was not used as feedstock for the June Stage 2 run, however feedstock for the Stage 2 run was produced in June under processing conditions identical to the March Stage 1 run:

Stage 1 Operation (run# HB08) – The total time processing biomass through the reactor was 26.5 hours, and there was additional time for startup and to filter the resultant slurry. Since this is a continuous reaction and the equipment is pre-heated, "steady state" is achieved once the reactor is full of material, or about ½ hour from the first loading of the biomass feedstock (The same applies to Stage 2). Sugar concentration builds in the system as filtered hydrolyzate is recirculated back to the blowtank and filter press feed, and approaches maximum concentration in about 12 hours.

Stage 1 Issues and Resolutions:

- Boiler feedwater pump failed – The pump casing cracked just over a day into the test. There was no backup pump available and the Independent Engineer determined sufficient runtime had passed to demonstrate steady state operation. A different design replacement pump and backup pump were procured and installed.
- Chip furnish had large pieces – The chips contained a number of large pieces that caused plugging in the lock hopper feed equipment. Adjustments were made to resolve plugging but net result was an average feed rate for the entire run at 69% of the 9 BDST/day steady-state feed rate. While resolving plugging there was additional water being added to the process resulting in lower sugar concentration.

Stage 2 Operation (run# HB09) – The total time processing biomass through the reactor was 24 hours, and there was additional time for startup and to filter the resultant slurry and pelletizing/drying. Part of the resultant solids produced in the HB08 Stage 1 run were used as feedstock for the HB09 Stage 2 run – Additionally, the HB09 run was a Stage 1 run to produce feedstock for Stage 2 - The HB09 Stage 1 processing conditions were identical to the HB08 Stage 1 processing conditions. A portion of the resultant lignin-rich filtered solids were fed to the lignin dryer system to demonstrate the pelletizer and dryer operation, and ~10 gal of pelletized and dried pellets were produced. The dryer was at the setpoint temperature for ~6 hours, with dry pellets successfully being produced for ~ 1/2 hour. The total lignin produced was not weighed as the facility did not have equipment to load and weigh it. In the future, product to be sold will be loaded on a trailer and weighed on the facility truck scale.

Stage 2 Issues and Resolutions:

- Lignin Dryer Pelletizer Feed - The original lignin pelletizer/dryer feed system could not be made to operate. That system used a positive displacement pump to transport 2nd Stage filter cake (primarily lignin) from the filter press discharge to the pelletizer. It was determined prior to this test that the OEM system would not accomplish the task, so a series of belt conveyors were installed which successfully conveyed the lignin material to the inlet of the pelletizer. The incremental feed rate was difficult to control using the belts, and not all of the filtered lignin was processed through the dryer.

- Lignin Dryer Pelletizer Drive - There was also an OEM belt pre-installed on the pelletizer that was inadequate and slipped with only minimal torque applied. This was identified as an issue after a number of attempts to feed the pelletizer and other adjustments such as slowing the feed rate. A new belt was purchased and installed giving better results.

Hydrogenolysis Run Summary – The hydrogenolysis run was performed the same week as the 2nd Stage hydrolysis run. The run length was 15 hours total and was constrained by the consumption of the entire available tube-trailer of hydrogen – This includes only time where we were feeding ethyl-acetate (EtAc) through the reactor and does not include system warmup. The unit was run at several different EtAc feed rates and at the 50 gal/hr feed rate for 4.5 hours. Note "steady state" operation is achieved within 1 hour. A total of 160 gallons of product ethanol and 621 gallons of off-spec ethanol were produced.

Since the Core operations were not part of this test and no cellulosic EtAc was produced, the Hydrogenolysis feedstock was purchased non-cellulosic EtAc. The purchased EtAc and cellulosic EtAc produced on site are sufficiently equivalent to be interchangeable as feedstock for the Hydrogenolysis unit - Below is a table with Gas Chromatograph (GC) analysis results for both:

Operator	Date	Time	Sample	Acidity				HAc	EtAc	EtOH	H2O
				g Sample used	mL of .02 N NaOH	mol NaOH used	OH equivalent (mol/kg)	weight % by Titration (Assumption 100% Acetic Acid)	weight %	weight %	weight % by KF
Matt	02/27/13	16:00	V7010 EtAc Feed (cellulosic EtAc)	1.07	1.10	2.1793E-05	0.020463	0.12%	99.62%	0.047%	0.16%
Matt	06/25/14	9:30	V7010 EtAc Feed (purchased EtAc)	2.03	1.70	3.3681E-05	0.016592	0.10%	99.83%	0.000%	0.07%

We can also compare the purchased EtAc and cellulosic EtAc and use the reaction conversions as an indicator of the interchangeability for unit testing purposes. In the 2/27 hydrogenolysis test run there was a 95.0% conversion, and in this test run there was a 94.6% conversion.

Hydrogenolysis Issues and Resolutions:

- T7110 pressure control – The pressure control response was to slow to react to pressure changes – PID settings were adjusted.
- P7010 charge pump plugged at check valves – Debris plugged the Reactor EtAc Feed pump. Upon inspection the debris appeared to be rubber. There is a strainer upstream of the plug location which was inspected and fully intact, so the rubber piece may be construction debris which finally broke loose.

- FT7002 EtAc flow meter not reading properly – Until troubleshooting on controls was performed flow was monitored by watching T7010 tank level. 185 gallons not recorded by the flowmeter were fed to the system.

Data Collection

- Key processing parameters and sampling for both hydrolysis and hydrogenolysis are detailed in the list of key measurements
- Lab analysis data was manually logged and is contained in the Lab Master Data File for both Hydrolysis and Hydrogenolysis:
- Hydrolysis biomass feedstock composition testing – The wood composition was determined from a hybrid poplar sample taken in a previous run (HB03), assuming only small variation in the composition between lots of clean chip - This is used as the basis for 1st Stage Processing. The composition basis for 2nd Stage Processing is calculated using the wood composition and accounting for component losses based on 1st Stage hydrolyzate composition.

Test Results

Hydrolysis Results – The hydrolysis test runs achieved the goal of this test by running at steady state and at a feed rate of 8 BDST/day or higher producing resultant pelletized lignin cake and hydrolyzate. The feed rate achieved for 1st Stage using Wood Chips was 9 BDST/day, and as detailed in the table below 5.9 BDST were fed to the reactor. The chip furnish had a large number of very large pieces which tend to plug the feed system at a high feed rate, and so the feed rate was intentionally kept low. The feed rate achieved for 2nd Stage using the 1st Stage residue was 20 BDST/day with a total of 7.0 BDST fed to the reactor. The feed rate constraint is based on volume, so accounting for bulk density differences between wood chips and residue, the 20 BDST/day rate equivalent for wood chips is 11.8 BDST/day. Based on the 2nd stage feeding, it is anticipated that in future 1st Stage runs with a wood chip furnish free of large pieces, a feed rate >10BDST/day will be achieved.

Equipment	Parameter	HB08 Stage 1	HB09 Stage 2	Comments
Biomass Staging	Biomass Description	Poplar Chips	1st Stage Residue	
Biomass Staging	Bulk density (5-gal bucket test), kg/m^3	281	486	
Biomass Staging	Biomass moisture, %	55.5	56.5	
Biomass Staging	Bulk density (5-gal bucket test), BDkg/m^3	125.0	211.4	
Feed Bin	Total # Telehandler Scoops	95	66	Stage 1b includes 2 Scoops of 1st Stage Wood Residue product to carry remaining chips through the system.
Feed Bin	Volume per scoop, approx., m^3	0.453	0.453	
Feed Bin	Total biomass (calculated), BDkg	5381	6320	
Feed Bin	Total biomass (calculated), BDST	5.9	7.0	
Lock Hopper	Volume per cycle, m^3	0.186	0.186	
Lock Hopper	Total # cycles	271	141	
Lock Hopper	Total biomass (calculated), BDkg	6303.0	5544.4	1st Stage Yield calculated using Feed Bin loading due to issues with lock hopper plugging. 2nd stage Yield also calculated using Feed Bin loading since easily compacted feed material makes bulk density difficult to determine.
Lock Hopper	Total biomass (calculated), BDST	6.9	6.1	
Lock Hopper	Feed Rate, BDST/day	9	20	
Lock Hopper	Wood Chip equivalent Feed Rate, BDST/day	9	11.8	Accounts for higher Bulk Density of residue vs. wood chips

The yields under these processing conditions had mixed results, with fair yields of xylose and low yields of glucose. Subsequent runs will reach higher final concentrations by charging the system with relatively concentrated hydrolyzate rather than weak hydrolyzate or water as was done in this run, and also by adjusting operating parameters. High levels of furfural in the 1st Stage indicate processing conditions too severe, and subsequent runs will be performed with reduced severity. A better chip furnish free of large pieces will also allow faster feeding and higher concentrations for 1st Stage. The 2nd Stage results showed both low glucose and HMF levels, indicating processing conditions not severe enough, and subsequent runs will be performed under more severe conditions. Combined sugar yield in this run for glucose was 4.9 w/w% BD wood, and yield for xylose was 9.6 w/w% BD wood, for a total usable sugar yield of 14.5 w/w% BD wood. Based on the processing deficiencies listed above, it is expected to reach the performance targets shown in the table "**Hydrolysis Results vs. Performance Targets**" below.

Yields, 1st Stage (HB08)

FEEDSTOCK	Composition of Hybrid Poplar Clean Chip (HB03 Basis)	Composition of HB08 Wood Processed, kg	Theoretical 100% Yield Sugars, kg
Glucan	45.30%	2437.5	2708.3
Galactan	0.60%	32.3	35.9
Mannan	2.50%	134.5	149.5
Xylan	14.00%	753.3	856.0
Arabinan	0.30%	16.1	18.3
Acid Insoluble Material	23.50%	1264.5	
DCM Extractives	0.61%	32.8	
Other	13.19%	709.7	

BLOWTANK SAMPLE YIELD CALCULATION*	
*Based on 3/6/14 10am sample	
1089	Blowtank Fill rate, L/hr
0.1869	L.H. Volume, m3
9.1	# L.H./hr, avg.
125	Bulk Density, BDkg/m3
195.2	Biomass in Blowtank, as fed basis, BDg/L
4.03	10am blowtank sample Glucose, g/L
4.6%	Glucose Yield, w/w% theoretical sugars
2.1%	Glucose Yield, w/w% BD wood
16.37	10 am blowtank sample Xylose, g/L
59.9%	Xylose Yield, w/w% theoretical sugars
8.4%	Xylose Yield, w/w% BD wood

Yields, 2nd Stage (HB09)

FEEDSTOCK	Composition of 1st Stage Residue Feedstock	Composition of 1st Stage Residue Processed, kg	Theoretical 100% Yield Sugars, kg
Glucan	49.75%	3144.4	3493.7
Galactan	0.28%	17.5	19.4
Mannan	1.15%	72.9	81.0
Xylan	6.46%	408.3	464.0
Arabinan	0.14%	8.8	9.9
Acid Insoluble Material	27.04%	1709.1	
DCM Extractives	0.00%	0.0	
Other	15.18%	959.3	

BLOWTANK SAMPLE YIELD CALCULATION*	
*based on 6/27/14 1pm sample	
715	Blowtank Fill rate, L/hr
0.1869	L.H. Volume, m3
4.9	# L.H./hr, avg.
232.2	Bulk Density, BDkg/m3
297.4	Biomass in Blowtank, as fed basis, BDg/L
9.61	1pm blowtank sample Glucose, g/L
6.5%	Glucose Yield, w/w% available sugars
2.8%	Glucose Yield, w/w% BD wood
4.1	1pm blowtank sample Xylose, g/L
21.3%	Xylose Yield, w/w% available sugars
1.2%	Xylose Yield, w/w% BD wood

Hydrolysis Results vs. Performance Targets*

Key Parameter	Performance Target	Test Result
1st stage hybrid poplar feed rate	8-10 BDST/day	9 BDST/day
1st stage hydrolyzate glucose concentration	13.9 g/L	4.03 g/L
1st stage hydrolyzate glucose yield	5 %	4.6 %
1st stage hydrolyzate xylose concentration	32.1 g/L	16.37 g/L
1st stage hydrolyzate xylose yield	83.4 %	59.9 %
2nd stage wood residue feed rate	8-10 BDST/day	20 BDST/day
2nd stage hydrolyzate glucose concentration	78.4 g/L	9.61 g/L
2nd stage hydrolyzate glucose yield	91.2 %	6.5 %
2nd stage hydrolyzate xylose concentration	0.7 g/L	4.1 g/L
2nd stage hydrolyzate xylose yield	7 %	21.3%

*The above table lists performance targets and actuals achieved during this run, however there are no performance requirements in this IE test run other than feed rates.

Hydrogenolysis Results – The hydrogenolysis run achieved the goal of the test by running at steady state with an EtAc feed rate of 50 gal/hr producing a resultant ethanol product as detailed in the tables below. Though unused EtAc and off-spec ethanol can be recycled back and reprocessed, that was not done during this test (single-pass processing). The hydrogen was consumed at a rate of 0.050 lb/lbEtOH produced, or at 66% hydrogen efficiency. Hydrogen losses are due partly to system leakage and partly to unreacted hydrogen loss to the system vent. Overall hydrogenolysis reaction efficiency was 94.1%, which again could be improved by reprocessing EtAc and EtOH.

Balance, Entire Run

Input	Input, lb	Output, lb
EtAc	6060	328
Hydrogen	302	40
Ethanol	0	5989
Total	6362	6357

Weight Balance, 4 Hrs at Design Rate

Input	Input, lb	Output, lb
EtAc	1520	90
Hydrogen	74	4 Note 1
Tower	0	33 Note2
Ethanol	0	1474
Total	1594	1601

Note 1 - Solution and Seal Loss

Note 2 - Tower Holdup

Product Analysis at Design Rate

Time	Acid (%)	EtAc (%)	EtOH (%)	Water (%)
8:00 PM	0.023	0.1	99.667	0.21
9:00 PM	0.028	0.8	99.002	0.17
11:00 PM	0.027	2.553	97.33	0.09

12:00 PM	0.027	0.12	99.773	0.08

Hydrogenolysis Results vs. Performance Targets*

Key Parameter	Performance Target	Test Result
EtAc feed rate	50 gal/hr	50 gal/hr
Ethanol product concentration	99.83%	99.85 %
Ethanol yield, per pass	96%	95.3%

*The above table lists performance targets and actuals achieved during this run, however there are no performance requirements in this IE test run other than feed rates.

The Boardman Hydrogenolysis unit performed as designed and with conversions consistent with and exceeding the BASF pilot trials:

BASF Pilot Plant vs. IE Test

Run	Temp., ºC	Pressure, BarG	H_2:EtAc, %w/w	LHSV, (L/h)/L	EtAc Conversion, Actual/Equilibrium, (%)
BASF 1	200	40	10	1	89.5/96.3
BASF 2	220	40	10	1	95.2/95.0
IE Test	205	39.2	12.5	0.92	95.4/95.9

<u>Theoretical Overall Run Efficiency</u>

To calculate the theoretical gallons of ethanol produced per BDST of wood processed for this test, we can take the 14.5% sugar yield from hydrolysis, 94.1% hydrogenolysis efficiency, and assume a nominal 61% Core efficiency to convert sugar to EtAc. If we use a 168.1 gal/BDST theoretical 100% yield:

168.1 x 14.5% x 61% x 94.1% = calculated equivalent of 14.0 gal/BDST wood

That results could be dramatically improved with better hydrolysis sugar yields (the largest contributor to the low figure), improved Core yields, and by reprocessing unreacted EtAc and off-spec EtOH.

<u>Calculations for Reported Results</u>

1. [Feedstock Consistency, %] = [dry weight] / [wet weight]
2. [Bulk Density, lb/ft^3] = [wt. of 5 gal feedstock, lb] / [0.6684 ft^3/gal]
3. [Feed Rate (lock hopper), BDST/day] = [Bulk Density, lb/ft^3] x [lock hopper fill volume, ft^3] x [lock hopper cycle time, sec] x [84,600 sec/day] / [2000 lb/BDST]
4. [Feed Rate (loader), BDST/day] = [Bulk Density, lb/ft^3] x [16 ft^3/bucket] x [# scoops] / [# days]
5. [Hydrolysis C5 Yield, % of theoretical] = [hydrolyzate C5 conc., mol/L] x [hydrolyzate vol., L] / ([feedstock total wt., g] x [xylan composition, %] x [xylan mol. wt., g/mol])
6. [Hydrolysis C6 Yield, % of theoretical] = [hydrolyzate C6 conc., mol/L] x [hydrolyzate vol., L]

/ ([feedstock total wt., g] x [glucan composition, %] x [glucan mol. wt., g/mol])

7. [Hydrogenolysis Yield, % of theoretical] = [ethanol product conc., mol/L] x [ethanol vol., L] / [EtAc feedstock total, mol]

Appendix B-J

ZeaChem Bookends Processing – Key Measurements

Process Area	What is being measured?	How is it being measured?	Frequency	Calibration method
Chem Frac	Wood Chip feed rate BDST/d	Calculated from volume, density, and moisture content	Daily	Calculated
Chem Frac	Wood Chip feed rate (volume)	Operations logs the buckets as they are loaded into the feed hopper.	Each bucket is recorded on a log sheet, and the total number of buckets is summed for each shift.	Bucket volume is 15 ft^3/bucket
Chem Frac	Wood Chip feedstock bulk density	5-gal bucket is weighed on a scale	Once per wood chip delivery	Scale calibrated with a weight standard prior to each use. Instrument accuracy +/- 5g.
Chem Frac	Wood Chip feedstock moisture content	Moisture Analyzer	Once per wood chip delivery	Scale calibrated with a weight standard prior to each use. Instrument accuracy +/- 5g.
Chem Frac	Residue feed rate BDST/d	Calculated from volume, density, and moisture content	Daily	Calculated
Chem Frac	Residue feed rate (volume)	Operations logs the buckets as they are loaded into the feed hopper.	Each bucket is recorded on a log sheet, and the total number of buckets is summed for each shift.	Bucket volume is 15 ft^3/bucket
Chem Frac	Residue feedstock bulk density	5-gal bucket is weighed on a scale	Once per batch	Scale calibrated with a weight standard prior to each use.
Chem Frac	Residue feedstock moisture content	Moisture Analyzer	Once per batch	Scale calibrated with a weight standard prior to each use.
Chem Frac	Steam flow rate to Steam Mixing Conveyor	FIT-014	Continuous DCS monitoring, running average logged every 5 seconds	Factory calibrated - Will be calibrated on a 6-month schedule by the PEMS instrument technician using a Hart calibrator.
Chem Frac	Steam pressure in Steam Mixing Conveyor	PT-050	Continuous DCS monitoring, running average logged every 5 seconds	Factory calibrated - Will be calibrated on a 6-month schedule by the PEMS instrument technician using a Hart calibrator.

Appendix B-J, cont.

ZeaChem Bookends Processing – Key Measurements

Process Area	What is being measured?	How is it being measured?	Frequency	Calibration method
Chem Frac	Steam Mixing Conveyor Temperature	TT-072	Continuous DCS monitoring, running average logged every 5 seconds	Factory calibrated - Will be calibrated on a 6-month schedule by the PEMS instrument technician using a Hart calibrator.
Chem Frac	Reactor Residence Time	Calculated by DCS from known # of flights and RPM	Continuous DCS monitoring, running average logged every 5 seconds	Visual verification of RPM
Chem Frac	Acid concentration	Titration in lab	Tank is sampled once per tank batch upon filling	Calculated
Chem Frac	Acid flow rate	FIT-082	Continuous DCS monitoring, running average logged every 5 seconds and integrated into totalizer.	Factory calibrated - Will be calibrated on a 6-month schedule by the PEMS instrument technician using a Hart calibrator.
Chem Frac	Hydrolyzate sugar content (xylose and glucose)	HPLC analysis in lab	Samples are collected from the blowtank every 4 hours and from the storage tank at the end of every stage run	The HPLC is calibrated on an irregular schedule about every 2 weeks using a purchased standard.
Chem Frac	Hydrolyzate Production Rate	Calculated using storage tank levels vs. time - LT-018, LT-028, LT-038	Continuous DCS monitoring, running average logged every 5 seconds	Calibrated during commissioning and will be calibrated on a 6-month schedule by the PEMS instrument technician using a Hart calibrator.
Hydrogenolysis	Ethyl Acetate feed rate	FT-7002	Continuous DCS monitoring, running average logged every 5 seconds	Factory calibrated - Will be calibrated on a 6-month schedule by the PEMS instrument technician using a Hart calibrator.
Hydrogenolysis	Ethyl Acetate composition	Gas Chromatagraph analysis	Once at beginning of the run	The GC is calibrated against a range of standards before and after each cylinder changeout.

Appendix B-J, cont.

ZeaChem Bookends Processing – Key Measurements

Process Area	What is being measured?	How is it being measured?	Frequency	Calibration method
Hydrogenolysis	Hydrogen flow rate	FT-7010	Continuous DCS monitoring, running average logged every 5 seconds	Factory calibrated - Will be calibrated on a 6-month schedule by the PEMS instrument technician using a Hart calibrator.
Hydrogenolysis	Reactor Feed Temperature	TT-7026	Continuous DCS monitoring, running average logged every 5 seconds	Factory calibrated - Will be calibrated on a 6-month schedule by the PEMS instrument technician using a Hart calibrator.
Hydrogenolysis	Reactor Temperature	TT-7040	Continuous DCS monitoring, running average logged every 5 seconds	Factory calibrated - Will be calibrated on a 6-month schedule by the PEMS instrument technician using a Hart calibrator.
Hydrogenolysis	Reactor Pressure	PT-7040	Continuous DCS monitoring, running average logged every 5 seconds	Factory calibrated - Will be calibrated on a 6-month schedule by the PEMS instrument technician using a Hart calibrator.
Hydrogenolysis	Hydrogen to Feed ratio	DCS calculation based on the two flow transmitters, FIC-7010 (H_2) and FIC-7002 (EtAc)	Continuous DCS monitoring, running average logged every 5 seconds	Factory calibrated - Will be calibrated on a 6-month schedule by the PEMS instrument technician using a Hart calibrator.
Hydrogenolysis	Ethanol composition	Gas Chromatagraph analysis	Sample is collected every three hours	The GC is calibrated against a range of standards before and after each cylinder changeout.
Hydrogenolysis	Ethanol production rate	FT-7132	Continuous DCS monitoring, running average logged every 5 seconds	Factory calibrated - Will be calibrated on a 6-month schedule by the PEMS instrument technician using a Hart calibrator.
Utilities	Water usage	Plant site meter	Check and beginning and end of each unit operation and stage for each feedstock	Maintained by vendor

Appendix B-J, cont.

ZeaChem Bookends Processing – Key Measurements

Process Area	What is being measured?	How is it being measured?	Frequency	Calibration method
Utilities	Electricity usage	Plant site meter	Check and beginning and end of each unit operation and stage for each feedstock	Maintained by vendor
Utilities	Natural gas usage	Plant site meter	Check and beginning and end of each unit operation and stage for each feedstock	Maintained by vendor

Appendix B-K

Lab Data – Hydrolysis

			Run Info			Bulk Density					HPLC (g/L)											
Lab Tech Initials	Sample Location, Description	Date/Time Sample was Taken	Run#	Process Stage, 1 or 2	% Consistency Average	Warehouse scale, Wet Density (kg/L)	Dry Density, Calculated (BDkg/L)	Dry Density, Calculated (BDlb/ft^3)	pH	% H2S04	Glucose	Xylose	Lactic	Formic	HAc	Levulinic	Butyric	Ethanol	HMF	Furfural	Ave. L.H./hr for previous 24 hours	Ave blowtank fill rate,previous 12 hours gal/hr
MWL	1st Stage Hydrolyzate	3/6/14 11:00	HB07	1							1.85	4.50	0.00	0.40	0.28	0.00	0.00	0.00	0.14	0.18		
	T1011 and Blowtank Starting Concentrations, calculated	3/12/14 1:00	HB08	1							1.75	4.25	0.00	0.38	0.26	0.00	0.00	0.00	0.13	0.17		
MWL	Hybrid Poplar Chip Pile	3/5/14 10:30	HB08	1	44.5%	0.282	0.125	7.83														
DPF	T9820, Sulfuric Acid	3/6/14 2:00	HB08	1						8.55%												
MWL	V1020, Hydrolyzate	3/5/14 14:00	HB08	1	2.8%				3.05		1.45	4.28	0.00	0.25	0.19	0.00	0.00	0.00	0.13	0.24		
DPF	V1020, Blow Tank Hydrolyzate	3/5/14 18:00	HB08	1	3.4%				3		1.44	4.17	0.00	0.30	0.21	0.00	0.00	0.00	0.00	0.00		
DPF	V1020, Blow Tank Hydrolyzate	3/5/14 22:00	HB08	1	5.3%				2.61		2.57	9.72	0.29	0.41	2.00	0.00	0.00	0.00	0.00	0.00		
DPF	V1020, Blow Tank Hydrolyzate	3/6/14 2:00	HB08	1	6.4%				2.54		3.10	13.06	0.52	0.77	3.00	0.00	0.00	0.00	0.40	0.00		
DPF	V1020, Blow Tank Hydrolyzate	3/6/14 6:00	HB08	1	6.5%				2.44		3.53	14.78	0.55	0.81	0.00	0.00	0.00	0.00	0.45	0.78		
MWL	V1020, Blow Tank Hydrolyzate	3/6/14 10:00	HB08	1	6.6%				2.64		4.03	16.37	0.61	0.89	4.23	0.00	0.00	0.00	0.52	0.89	9.1	287.8
MWL	V1020, Blow Tank Hydrolyzate	3/6/14 14:00	HB08	1	8.4%				2.71		4.01	15.57	0.55	0.84	3.99	0.00	0.00	0.00	0.53	0.88		1089.441
DPF	V1020, Blow Tank Hydrolyzate	3/6/14 22:00	HB08	1	4.5%				2.33		3.99	15.82	0.47	0.72	4.07	0.00	0.00	0.00	0.52	0.83		L/hr
MWL	V1080, Condensate	3/5/14 14:00	HB08	1							0.00	0.00	0.00	0.15	0.05	0.00	0.00	0.00	0.00	2.32		
DPF	V1080, Condensate	3/5/14 18:00	HB08	1							0.00	0.00	0.00	0.43	2.08	0.00	0.00	0.00	0.00	5.33		
DPF	V1080, Condensate	3/5/14 22:00	HB08	1							0.00	0.00	0.00	0.48	2.12	0.00	0.00	0.00	0.00	0.00		
DPF	V1080, Condensate	3/6/14 2:00	HB08	1							0.00	0.00	0.00	0.36	1.65	0.00	0.00	0.00	0.00	4.70		
DPF	V1080, Condensate	3/6/14 6:00	HB08	1							0.00	0.00	0.00	0.54	2.85	0.00	0.00	0.00	0.00	6.19		
MWL	V1080, Condensate	3/6/14 10:00	HB08	1							0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80		
MWL	V1080, Condensate	3/6/14 14:00	HB08	1							0.00	0.00	0.00	0.29	1.13	0.00	0.00	0.00	0.00	3.46		
MWL	T2010	3/10/14 10:00	HB08	1							1.75	5.67	0.00	0.18	0.63	0.00	0.00	0.00	0.19	0.33		
MWL	HB08 Final Sample, T2010	3/12/14 0:20	HB08	1							2.40	8.06	0.21	0.24	1.69	0.00	0.00	0.00	0.25	0.46		
MJM	Sulfuric Acid Day Tank	6/24/14 20:00	HB09	1						13.98%												
MWL	1st Stage Solids	6/25/14 0:00	HB09	2	43.5%	0.486	0.211	13.20														
MWL	V1020, Blow Tank Hydrolyzate	6/25/14 15:00	HB09	1	8.3%				2.67		5.10	6.93	0.53	0.72	1.72	0.23	0.00	0.00	0.58	0.62		
MWL	V1020, Blow Tank Hydrolyzate	6/26/14 7:00	HB09	1	8.0%				2.64		6.00	9.18	0.00	0.96	2.69	0.68	0.00	0.00	0.69	0.77	7.5	226.5
MWL	V1020, Blow Tank Hydrolyzate	6/26/14 11:00	HB09	1	9.9%				2.61		5.18	8.42	0.00	0.56	2.34	0.55	0.00	0.00	0.62	0.66		857.3954
MJM	V1020, Blow Tank Hydrolyzate	6/26/14 21:00	HB09	2	5.0%				1.9		8.91	5.03	0.37	1.66	0.88	3.20	0.00	0.00	0.75	0.44		
MJM	V1020, Blow Tank Hydrolyzate	6/27/14 1:00	HB09	2	6.4%				1.7		11.05	4.50	0.37	2.33	0.67	5.19	0.00	0.00	0.81	0.39		
MJM	V1020, Blow Tank Hydrolyzate	6/27/14 5:00	HB09	2	5.3%				1.74		11.76	4.01	0.36	2.40	0.49	5.29	0.00	0.00	0.83	0.42		
MWL	V1020, Blow Tank Hydrolyzate	6/27/14 9:00	HB09	2	5.4%				1.81		9.49	4.00	0.00	1.72	0.38	3.61	0.00	0.00	0.40	0.40	4.9	188.8
MWL	V1020, Blow Tank Hydrolyzate	6/27/14 13:00	HB09	2	4.9%				1.79		9.61	4.10	0.00	1.82	0.48	0.37	0.00	0.00	0.74	0.40		714.6854
MM	V1020, Blow Tank Hydrolyzate	6/27/14 21:00	HB09	2	7.7%				1.93		8.62	3.03	0.27	1.56	0.05	3.30	0.00	0.00	0.00	0.31		L/hr
MJM	V1080, Condensate	6/25/14 3:00	HB09	1																		
MWL	V1080, Condensate	6/25/14 15:00	HB09	1							0.00	0.00	0.00	0.28	1.24	0.00	0.00	0.00	0.00	4.82		
MWL	V1080, Condensate	6/26/14 7:00	HB09	1							0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	2.27		
MWL	V1080, Condensate	6/26/14 11:00	HB09	1							0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.24		
MJM	V1080, Condensate	6/26/14 21:00	HB09	2							0.53	0.00	0.05	1.33	0.00	0.00	0.00	0.00	0.00	2.75		
MJM	V1080, Condensate	6/27/14 1:00	HB09	2							0.53	0.00	0.15	2.17	0.00	0.16	0.00	0.00	0.00	3.08		
MJM	V1080, Condensate	6/27/14 5:00	HB09	2							0.00	0.00	0.00	1.81	0.00	0.00	0.00	0.00	0.00	3.51		
MWL	V1080, Condensate	6/27/14 9:00	HB09	2							0.00	0.00	0.00	0.39	0.00	0.00	0.00	0.00	0.00	1.78		
MWL	V1080, Condensate	6/27/14 13:00	HB09	2							0.00	0.00	0.00	0.27	0.00	0.00	0.00	0.00	0.00	1.40		
MM	V1080, Condensate	6/27/2014 end	HB09	2							0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	1.16		
MWL	T2020 Hydrolyzate	8/26/14 0200	HB09	1&2							7.05	4.79	0.52	1.03	0.61	2.06	0.00	0.00	0.56	0.00		

Appendix B-K, cont.

Lab Data – Hydrogenolysis

Operator	Date	Time	Sample	Acidity				HAc	EtAc	EtOH	H2O
				g Sample used	mL of .02 N NaOH	mol NaOH used	OH equivalent (mol/kg)	weight % by Titration (Assumption 100% Acetic Acid)	weight %	weight %	weight % by KF
Matt	02/27/13	16:00	V7010 EtAc Feed (cellulosic EtAc)	1.07	1.10	2.1793E-05	0.020463	0.12%	99.62%	0.047%	0.16%
Matt	06/25/14	9:30	V7010 EtAc Feed (purchased EtAc)	2.03	1.70	3.3681E-05	0.016592	0.10%	99.83%	0.000%	0.07%
Matt	06/25/14	15:00	T7110 Bottoms, Neat EtOH Product	2.13	0.30	6.0586E-06	0.002844	0.02%		4.954%	0.21%
Matt	06/26/14	13:38	T7110 Bottoms, Neat EtOH Product	3.90	2.50	5.0488E-05	0.012962	0.08%	2.31%	97.48%	0.13%
Matt	06/26/14	16:55	T7110 Bottoms, Neat EtOH Product	3.41	0.25	5.0488E-06	0.001483	0.01%	0.19%	99.68%	0.12%
Michael	06/26/14	19:15	T7110 Bottoms, Neat EtOH Product	4.64	1.00	2.0195E-05	0.004354	0.03%	0.19%	99.28%	0.12%
Michael	06/26/14	20:00	T7110 Bottoms, Neat EtOH Product	4.73	0.90	1.8176E-05	0.003844	0.02%	0.10%	99.67%	0.21%
Michael	06/26/14	21:00	T7110 Bottoms, Neat EtOH Product	4.36	1.00	2.0195E-05	0.004630	0.03%	0.80%	99.00%	0.17%
Michael	06/26/14	23:00	T7110 Bottoms, Neat EtOH Product	4.44	1.00	2.0195E-05	0.004552	0.03%	2.55%	97.33%	0.09%
Michael	06/26/14	0:00	T7110 Bottoms, Neat EtOH Product	4.11	0.90	1.8176E-05	0.004424	0.03%	0.12%	99.77%	0.08%
Michael	06/26/14	1:00	V7030 Bottoms, Reactor Product	4.61	1.00	2.0195E-05	0.004382	0.03%	4.56%	95.324%	0.09%
Michael	06/26/14	2:00	T7110 Bottoms, Neat EtOH Product	4.69	1.00	2.0195E-05	0.004308	0.03%	0.00%	99.85%	0.12%
M att	07/07/14	12:30	T9941, Neat Ethanol Product	15.00	1.80	3.5662E-05	0.002377	0.01%	1.24%	98.47%	0.27%

Appendix B-L

Hybrid Poplar Composition

Work Order

Client	**ZeaChem**
W.O.#	**B13108**
Log #	2066548
Date rec'd	Feb 12/2013
Your P.O. #	**MP 1118, invoice to PEMS**
Results to	Ning Xu
Ph	650-543-8127, 952-374-8201
E-mail	nxu@zeachem.com

econotech

due date: **feb 20**

e-mail:

description of samples: One (1) Sawdust, & Six (6) Wood Pulp Samples

PRELIMINARY RESULTS-Final copy is hard copy

Analysis	unit	Analytical Method	**HB03 - Sawdust** Jan 30 - Feb 7/13	ESM #	Tech/Date
Moisture Content	%, as-received basis	ASTM - D3173	61.4	107B	BM/130215
Carbohydrates	%, dry weight basis	ESM - 310D	attached	310D	LV/130219
Cellulose (by calculation)	%, dry weight basis		attached		
Hemicellulose (by calculation)	%, dry weight basis		attached		
Klason Lignin	%, dry weight basis	Tappi - T222	21.7 (21.4;22.0)	093B	BM/130218-20
DCM Extractives	%, dry weight basis	Tappi - T204	0.61 (0.60;0.62)	077b	bm/190218-19

QUOTATION: **US $** INVOICE: U.S $

REMARKS: Moisture: #17, all other results: #18

Carbohydrates: samples were prepared according to Tappi T249 with analysis by Dionex ion chromatography. Results are calculated relative to extractive free oven dry sample weight. #27.

	Carbohydrates, % (oven dry basis)					Acid			
SAMPLE I.D.	Arabinan	Xylan	Mannan	Galactan	Glucan	Insoluble Materials	Total%	Hemicellulose (by calculation)	Cellulose (by calculation)
HB03 - Sawdust	0.3	14.0	2.5	0.6	45.3	23.5	86.2	18.2	44.4

Hemicellulose (by calculation): Arabinan+Xylan+Mannan+Galactan+Mannan/3

Cellulose (by calculation): Glucan-Mannan/3