NATIONAL LABORATORY OF THE ROCKIES

# Feedstock/Pretreatment Screening for Bioconversion of Sugars and Lignin Residues

## Cooperative Research and Development Final Report

## CRADA Number: CRD-18-00765 (Project 6)

NLR Technical Contact: Darren Peterson

**The National Laboratory of the Rockies is a national laboratory of the U.S. Department of Energy, Office of Critical Minerals and Energy Innovation, operated under Contract No. DE-AC36-08GO28308.**

This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

**Technical Report**
NLR/TP-2800-98016
January 2026

NATIONAL LABORATORY OF THE ROCKIES

# Feedstock/Pretreatment Screening for Bioconversion of Sugars and Lignin Residues

## Cooperative Research and Development Final Report

## CRADA Number: CRD-18-00765 (Project 6)

NLR Technical Contact: Darren Peterson

**Suggested Citation**
Peterson, Darren. 2026. *Feedstock/Pretreatment Screening for Bioconversion of Sugars and Lignin Residues: Cooperative Research and Development Final Report, CRADA Number CRD-18-00765 (Project 6).* Golden, CO: National Laboratory of the Rockies. NLR/TP-2800-98016. https://www.nlr.gov/docs/fy26osti/98016.pdf.

**The National Laboratory of the Rockies is a national laboratory of the U.S. Department of Energy, Office of Critical Minerals and Energy Innovation, operated under Contract No. DE-AC36-08GO28308.**

This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

**Technical Report**
NLR/TP-2800-98016
January 2026

National Laboratory of the Rockies
15013 Denver West Parkway
Golden, CO 80401
303-275-3000 • www.nlr.gov

**NOTICE**

This work was authored by the National Laboratory of the Rockies for the U.S. Department of Energy (DOE), operated under Contract No. DE-AC36-08GO28308. Funding provided by the U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office. The views expressed herein do not necessarily represent the views of the DOE or the U.S. Government.

This work was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, nor any of their contractors, subcontractors or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or any third party's use or the results of such use of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof or its contractors or subcontractors. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof, its contractors or subcontractors.

This report was produced when the laboratory operated as the National Renewable Energy Laboratory (NREL). The laboratory is now the National Laboratory of the Rockies (NLR).

This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

U.S. Department of Energy (DOE) reports produced after 1991 and a growing number of pre-1991 documents are available free via www.OSTI.gov.

*Cover photos (clockwise from left): Josh Bauer, National Laboratory of the Rockies 61725; Visualization from National Laboratory of the Rockies Insight Center; Getty-181828180; Agata Bogucka, National Laboratory of the Rockies 91683; Dennis Schroeder, National Laboratory of the Rockies 51331; Werner Slocum, National Laboratory of the Rockies 67842.*

National Laboratory of the Rockies prints on paper that contains recycled content.

# Cooperative Research and Development Final Report

**Report Date:** August 22, 2025

In accordance with requirements set forth in the terms of the CRADA agreement, this document is the CRADA final report, including a list of subject inventions, to be forwarded to the DOE Office of Scientific and Technical Information as part of the commitment to the public to demonstrate results of federally funded research.

**Parties to the Agreement:** ExxonMobil Technology and Engineering Company (EMTEC) f/k/a ExxonMobil Research and Engineering Company (EMRE)

**CRADA Number:** CRD-18-00765 (Project 6)

**CRADA Title:** Feedstock/Pretreatment Screening for Bioconversion of Sugars and Lignin Residues

**Period of Performance:** 4/9/2019 – 12/31/2023

**Responsible Technical Contact at NLR:**

Darren Peterson | Darren.Peterson@nlr.gov (for Richard Elander)

**Name and Email Address of POC at Company:**

Changyub Paek | Changyub.Paek@exxonmobil.com (for Giovanni Pilloni)

**Sponsoring DOE Program Office(s):**

Office of Energy Efficiency and Renewable Energy (EERE), Bioenergy Technologies Office

**Joint Work Statement Funding Table showing DOE Commitment:**

No NLR Shared Resources

**Executive Summary of CRADA Work:**

This project will conduct biomass deconstruction (pretreatment and enzymatic saccharification) on two representative biomass feedstocks and four different pretreatment processes, including three high temperature steam/chemical pretreatments and one low temperature chemical/mechanical pretreatment. A third biomass feedstock will undergo biomass deconstruction with three different pretreatment processes, including two high temperature chemical pretreatments and one low temperature chemical/mechanical pretreatment. Several pretreatment conditions will be performed in a screening study using NLR pilot-scale pretreatment equipment to generate a range of pretreated biomass substrates. A selected number of these substrates will be chosen for enzymatic saccharification evaluation, based on standard compositional analysis of the pretreated substrates as a primary indicator of pretreatment efficacy. Resulting enzymatic hydrolysis slurries will be analyzed to determine overall biomass sugar yields. Additional compositional analysis will be performed to determine oligomeric sugar



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

composition and structure, to analyze structural characteristics of solids fractions on native, pretreated, and enzymatically saccharified biomass residues for one of the biomass feedstocks, corn stover. The enzymatically saccharified materials will undergo 2,3-butanediol fermentation in a shaker flask as bench scale. Using relevant process performance data collected in these various conversion steps, technoeconomic analysis activities will be performed to compare the economic potential of the various biomass feedstock and pretreatment processes and to identify key economic drivers and sustainability metrics.

**CRADA benefit to DOE, Participant, and US Taxpayer:**

- Adds new capability to the laboratory's core competencies and/or
- Enhances U.S. competitiveness by utilizing DOE developed intellectual property and/or capabilities.

**Summary of Research Results:**

Notes:

1. National Energy Technology Laboratory, NETL, (Ref. AGMT-0922) was not a part of this project work.
2. Idaho National Laboratory, INL, only supplied the feedstock but was not a part of the analysis portion of the project work.
3. Project 6 task work was modified out of Modification No. 4 resulting in the Project 6 scope developed in Mod No. 37. No work for Project 6 was ever completed under Mod No. 4.

**TASK DESCRIPTIONS:**

**Purpose:** This project will generate a comprehensive set of process data and underlying knowledge for conversion of several wide-ranging biomass types across a variety of deconstruction processes and conditions to generate biomass sugar hydrolysates. These hydrolysates are expected to have widely different properties regarding monomeric sugar concentrations, oligomeric sugar concentrations and structural properties, and other compounds derived from the feedstock and pretreatment conditions that could significantly impact the ability of conversion microorganisms to grow and produced targeted intermediates from these hydrolysates. One crucial aspect of this project is to generate knowledge about the structure-properties relationships across the various biomasses, pretreatment options and sugar compositions. In the long term, such knowledge might be extremely useful for combining the right bioconversion to the appropriate feedstock composition or blend different feeds to accommodate for some of the bioconversion limitations, similarly to what is currently done in the oil and gas industry in the application of catalysts for different crude compositions. The resulting process performance data will be utilized in technoeconomic and life-cycle assessment models to identify overall commercialization potential, with accompanying sensitivity analysis to identify the most important cost drivers for improving overall process performance and economics and reduce emissions. The outcomes of this project will be of significant interest to



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

the broader biomass conversion community, so the dissemination of key findings will occur via presentations at leading scientific conferences and publications in high-impact scientific journals.

**Statement of Work - Task Descriptions and Estimated Completion Dates:**

Below is a list of tasks NLR will perform for the proposed work plan:

Task 1: Hydrolysate Generation (steam, dilute acid, deacetylation-dilute acid pretreatments) (Mod 4)

Task 2: Hydrolysate Generation (deacetylation-mechanical refining (DMR) pretreatment) (Mod 4)

Task 3: Detailed Compositional and Imaging Analysis (Mod 4)

Task 4: Fermentation of Enzymatic Hydrolysate (changed in Mod 37 from Task 4: Lignin Characterization)

Task 5: Process Engineering/Technoeconomic Analysis (Mod 4)

Task 6: Fermentation of hydrolysates (Mod 37)

Task 7: CRADA Final Report (Mod 37)

**Task 1: Hydrolysate Generation (steam, dilute acid, deacetylation-dilute acid pretreatments)**

**Objective:** Task 1 will generate multiple sets of hydrolysate streams from three different feedstocks: corn stover, a representative woody biomass such as poplar, and a representative energy crop feedstock such as switchgrass. Feedstocks will be sourced through Idaho National Laboratory (INL) and will be appropriately pre-processed at INL to achieve a pre-determined specification for each feedstock. Pre-processed feedstock will be shipped to NLR in supersacks. For corn stover and switchgrass, three different high temperature pretreatments will be performed:

- Steam pretreatment
- Dilute acid pretreatment
- Deacetylation-dilute acid pretreatment

For poplar, two different high temperature pretreatments will be performed:

- Dilute acid pretreatment
- Deacetylation-dilute acid pretreatment



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

The high temperature pretreatments will be conducted using NLR's pilot scale continuous pretreatment reactor(s). For each feedstock and high temperature pretreatment method, up to 12 specific pretreatment conditions (combinations of temperature, residence time, and pretreatment chemical loading (as appropriate)) will be conducted by allowing each pretreatment condition to achieve a steady-state operating condition followed by collecting a representative pretreated slurry sample over a 30 minute period. The following steps for each feedstock and high temperature pretreatment method will be conducted:

1. Dilute alkali deacetylation (only for deacetylation-dilute acid pretreatment)
2. Water/chemical pre-impregnation
3. Pilot scale pretreatment (up to 12 conditions)
4. Standard compositional analysis and yield calculations (liquid and solids fractions)

Up to six pretreated slurries from each feedstock and high temperature pretreatment method will be selected for enzymatic saccharification, which will be conducted using a single commercial enzyme (cellulase/hemicellulase) preparation at a single enzyme loading. The selection of the pretreated slurries to be used in enzymatic saccharification will be based upon an assessment of pretreatment performance, including the yield of oligomeric and monomeric sugars in the liquid fraction of the pretreated slurry, the amount of sugar degradation products (primarily furfural and 5-HMF) in the liquid fraction of the pretreated slurry, and the overall carbohydrate component mass closure in the pretreated slurry solid and liquid fractions. The enzymatic saccharification will be conducted in laboratory-scale roller bottle-type reactors at a solids loading of 20% total solids. At the endpoint of enzymatic saccharification, the saccharified slurries will be harvested and samples will undergo standard compositional analysis and sugar yield calculations.

The harvested saccharified slurry samples will be measuring filtration flux rates and washing efficiencies. The liquid fractions will be retained and stored in a frozen state for biological characterization and fermentation process studies (Task 4).

**Task 1 Results:**

1.1.1 Corn Stover Pretreatment

Pretreatment of corn stover was completed using steam, dilute acid (DA), and deacetylated dilute acid (DDA). There were significant delays during pretreatment of corn stover. The first delay was NLR closing for COVID-19 and even when it reopened, labor within the IBRF Pilot Plant was limited for a few months. The second delay was feeding the deacetylated acid impregnated corn stover into the Large Horizontal Reactor (LHR). The deacetylated acid impregnated corn stover did not form a strong enough plug on LHR's screw plug feeder which resulted in multiple blow backs from the steam inside the horizontal reactor. The deacetylated acid impregnated corn stover was switched to the South Vertical Reactor (SVR) where the horizontal section was utilized as a horizontal reactor, see Figure 1. Due to these delays, the corn stover steam/DA/DDA pretreatments were not completed until 2.5 years into the project.



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

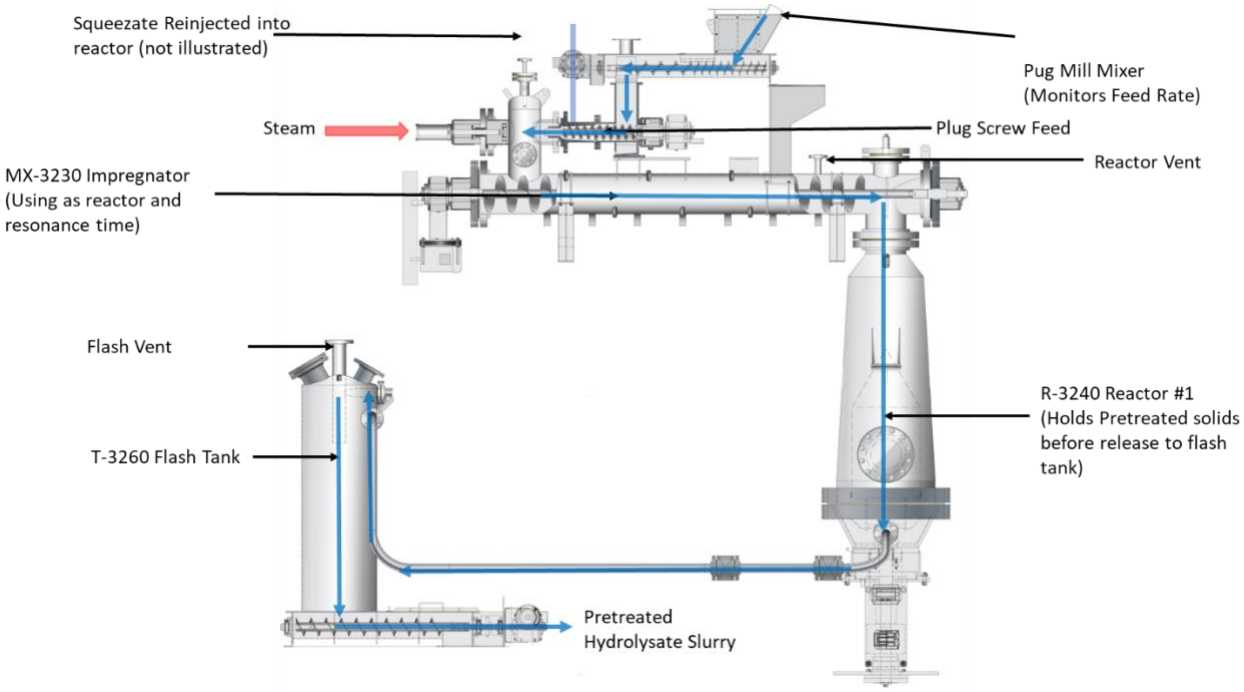


**Fig. 1. Diagram of South Vertical Reactor (SVR)**

Figure 2 displays the xylan yields of pretreated corn stover. Figure 2 has steam pretreatment on the left, DA pretreatment in the middle, and DDA pretreatment on the right. For DA and DDA, the low acid pretreatment (0.5% sulfuric acid versus dry biomass) is on top and the bottom is high acid pretreatment (0.8% sulfuric acid vs dry biomass). All charts start with lowest severity (temperature and residence time) on the left and increase in severity to the right. The nomenclature of the samples are as follows: CS is Corn Stover, Steam/DA/DDA is the pretreatment, 150/160/170/185/200 is the temperature in deg C, 12/20 is the residence time in the reactor in min, and 0.5/0.8 is the sulfuric acid concentration in percent acid per dry biomass by mass. Figure 2 illustrates that with steam pretreatment, most xylan is not solubilized at lower temperatures, but as the temperature increases, xylan is degraded into furfural. DA and DDA pretreatment of corn stover solubilized more of the xylan than steam and converted the oligomeric xylan into monomeric xylose. The DDA pretreatment of corn stover with 0.8% sulfuric acid at 170 deg C was the best performing pretreatment, with over 60% of the xylan converted to monomeric xylose. Yields were calculated using the NLR's Laboratory Analytical Procedures (LAPs), https://www.nrel.gov/bioenergy/biomass-compositional-analysis.html.



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

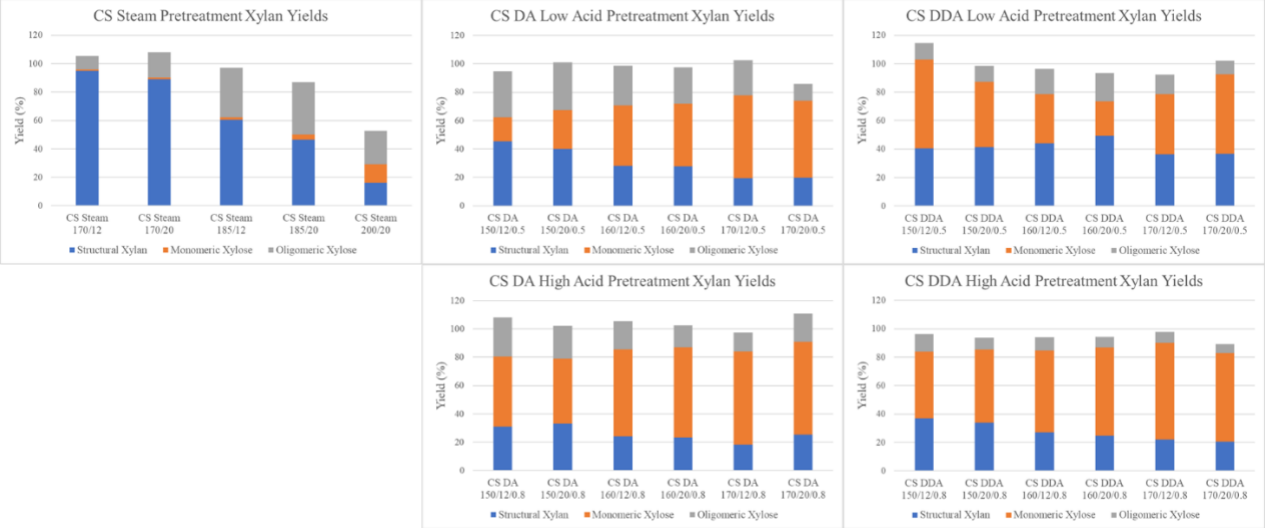


**Fig. 2. Xylan yields of steam/DA/DDA pretreated corn stover**

### 1.1.2 Corn Stover Enzymatic Hydrolysis

Figure 3 displays the enzymatic hydrolysis yields of steam/DA/DDA pretreated corn stover. The enzymatic hydrolysis was performed on the pretreated corn stover slurries. Three pretreatments of each campaign were selected varying in temperature. The highest performing enzymatic hydrolyses were for DA pretreatment at higher severity, specifically CS DA 170/12/0.5, CS DA 160/12/0.8, and CS DA 170/12/0.8 all had glucose yields above 80%. However, of those three, only CS DA 170/12/0.5 had a xylose yield above 80%. The glucose yields for DDA pretreated corn stover peaked at 60% glucose yields. The deacetylation may have left too much salt residue for a sufficient enzymatic digestion.



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

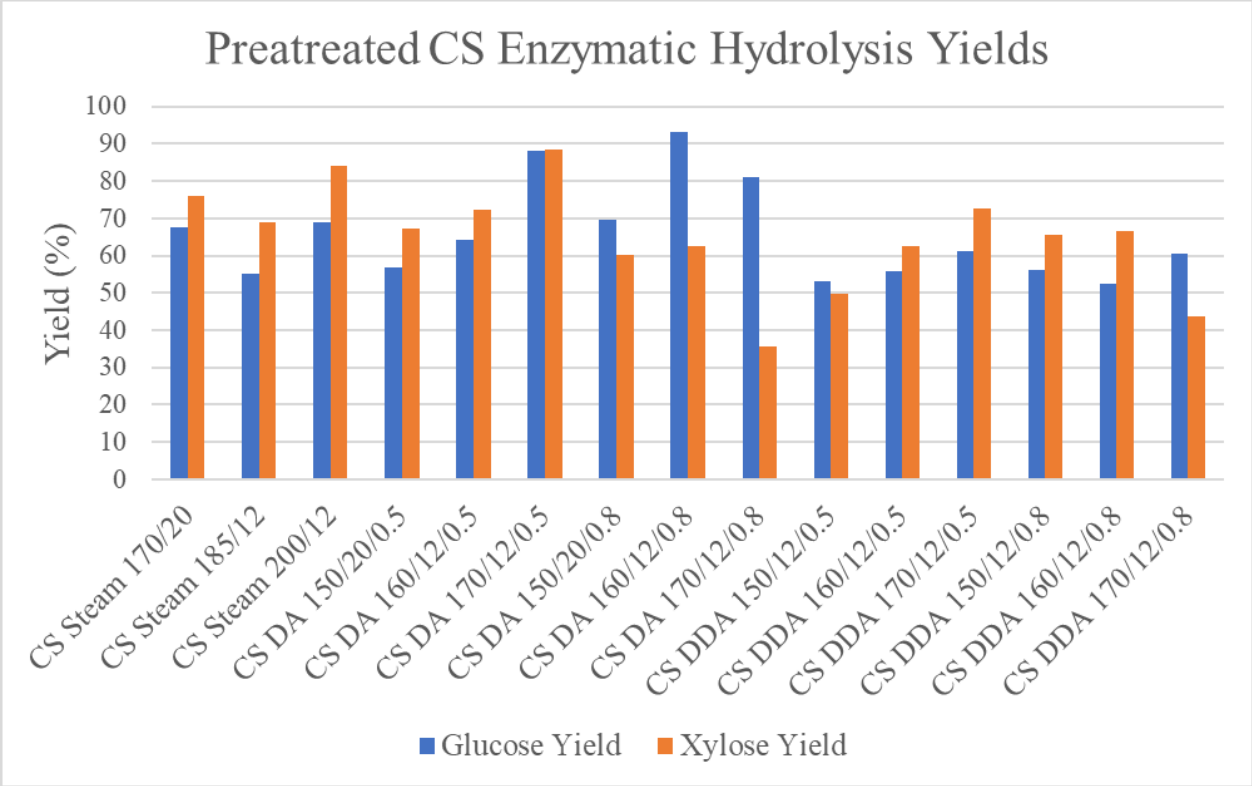


**Fig. 3. Glucose and xylose yields of pretreated corn stover enzymatic hydrolysis**

1.2.1 Poplar Pretreatment

The only poplar pretreatment performed was DA Low Acid. The poplar pretreatment was scheduled to run immediately following the corn stover pretreatment campaigns. Unfortunately, the corn stover was not completed until 2.5 years into the project. On the second campaign for poplar, DA High Acid, the poplar plug failed and blow back occurred that was severe enough to shut down the SVR for six months. Due to the poor performance of the initial poplar pretreatment compared to corn stover and switchgrass, limited time and funding, the remaining DA/DDA pretreatment poplar campaigns were abandoned.

Figure 4 displays the xylan yields of pretreated poplar. The pretreated poplar had very small yields of monomeric xylose and the majority of the xylan remained with the solids. The yields did not follow much of a trend during pretreatment. The poplar appears to be more recalcitrant than the herbaceous feedstocks studied, like corn stover and switchgrass. The nomenclature is the same as corn stover, except Pop is poplar.



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

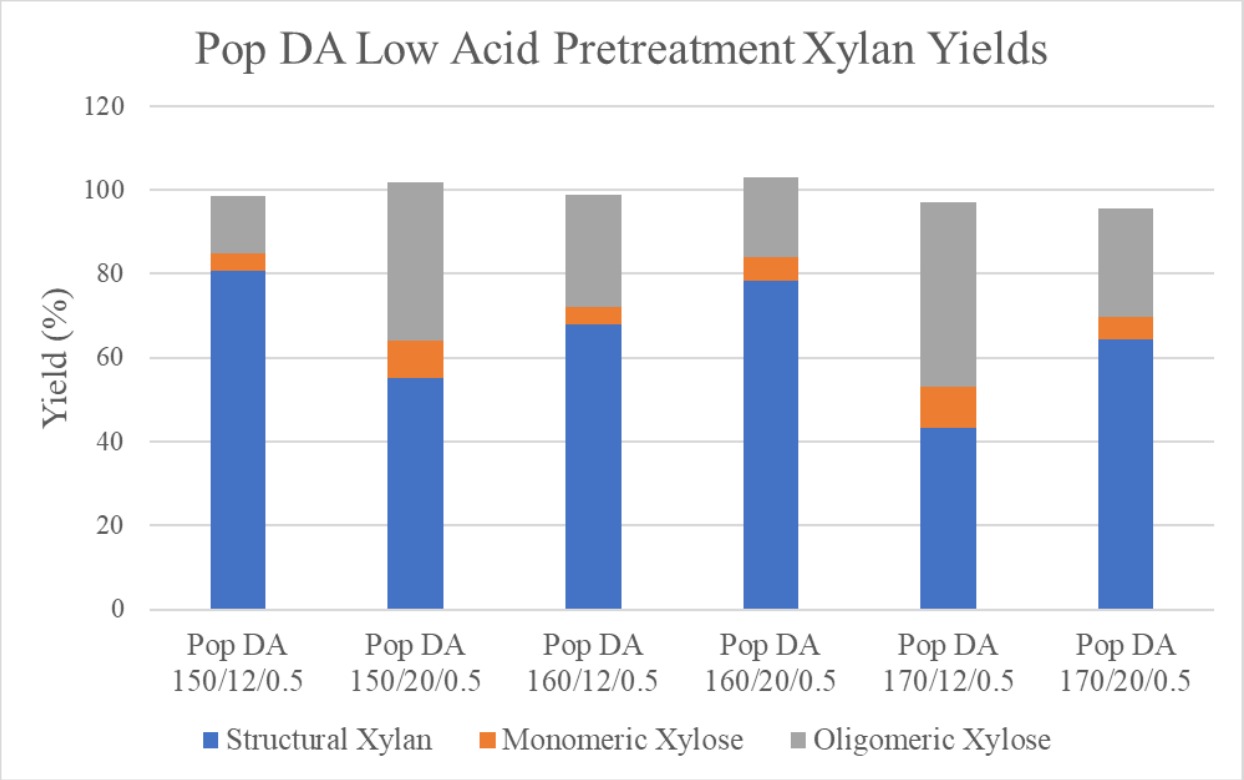


**Fig. 4. Xylan yields of DA pretreated poplar**

1.2.2 Poplar Enzymatic Hydrolysis

Figure 5 displays the enzymatic hydrolysis yields of DA low acid pretreated poplar. None of the enzymatic hydrolyses were able to exceed 20% glucose yields. This supports the hypothesis that the poplar is more recalcitrant than the herbaceous feedstocks studied in this project. Due to these poor performances, it was determined that what time and money remained in this project were better spent on switchgrass and deacetylated mechanical refined (DMR) pretreatments.



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

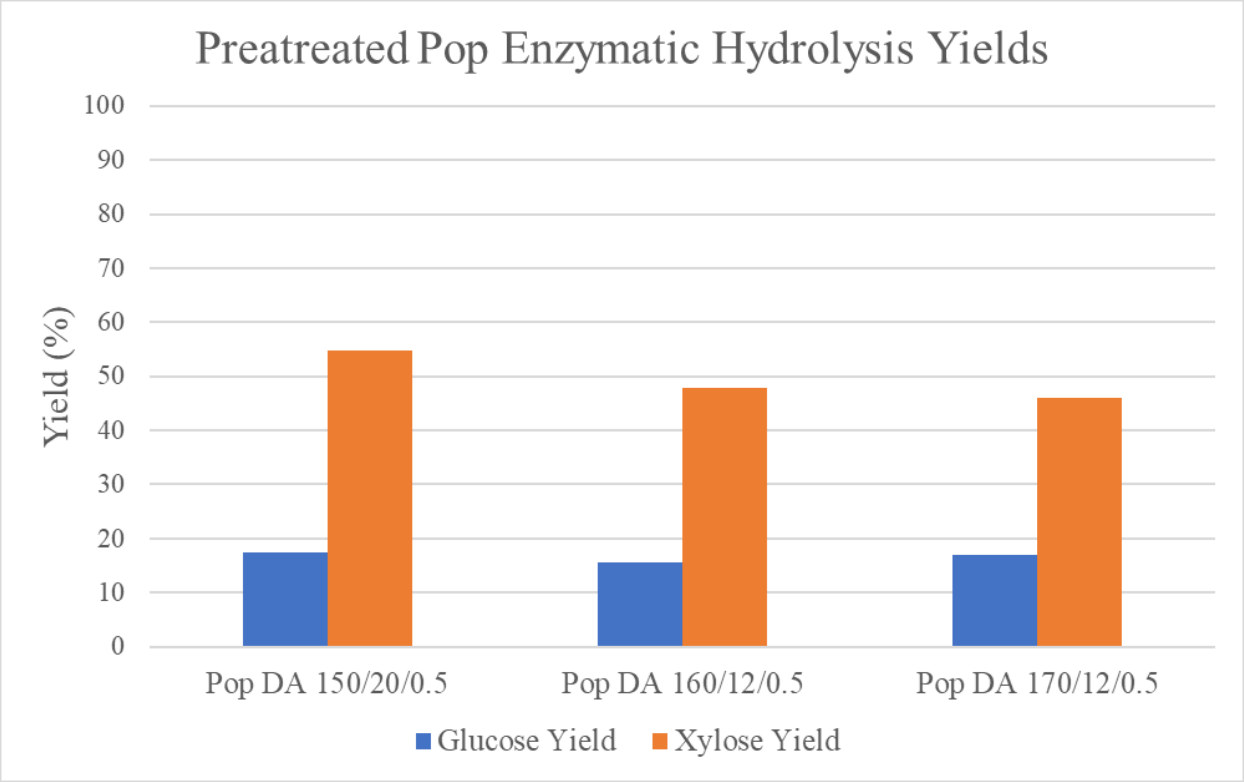


**Fig. 5. Glucose and xylose yields of pretreated poplar enzymatic hydrolysis**

1.3.1 Switchgrass Pretreatment

Due to the delays discussed previously, three switchgrass pretreatments were prioritized based on corn stover results. The steam and DA low acid pretreatment campaigns were dropped.

Figure 6 displays the xylan yields of pretreated switchgrass. Figure 6 has DA pretreatment on the left, and DDA pretreatment on the right. For DDA the low acid pretreatment (0.5% sulfuric acid versus dry biomass) is on top and the bottom is high acid pretreatment (0.8% sulfuric acid vs dry biomass). All charts start with lowest severity (temperature and residence time) on the left and increase in severity to the right. The nomenclature is the same as corn stover except SWG is switchgrass. The analytical methods for these pretreatments were not completed in time to notice that there was a malfunction with the 20 minute residence time during pretreatment. The 20 minute residence time forced the biomass into the vertical section of the SVR where the temperature was not as high and did not have as severe of pretreatment as the 12 minute residence time. The DA pretreatment of switchgrass had higher monomeric xylose yields than the DDA pretreatments. For all pretreatments, the higher severity produced higher monomeric xylose yields.



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

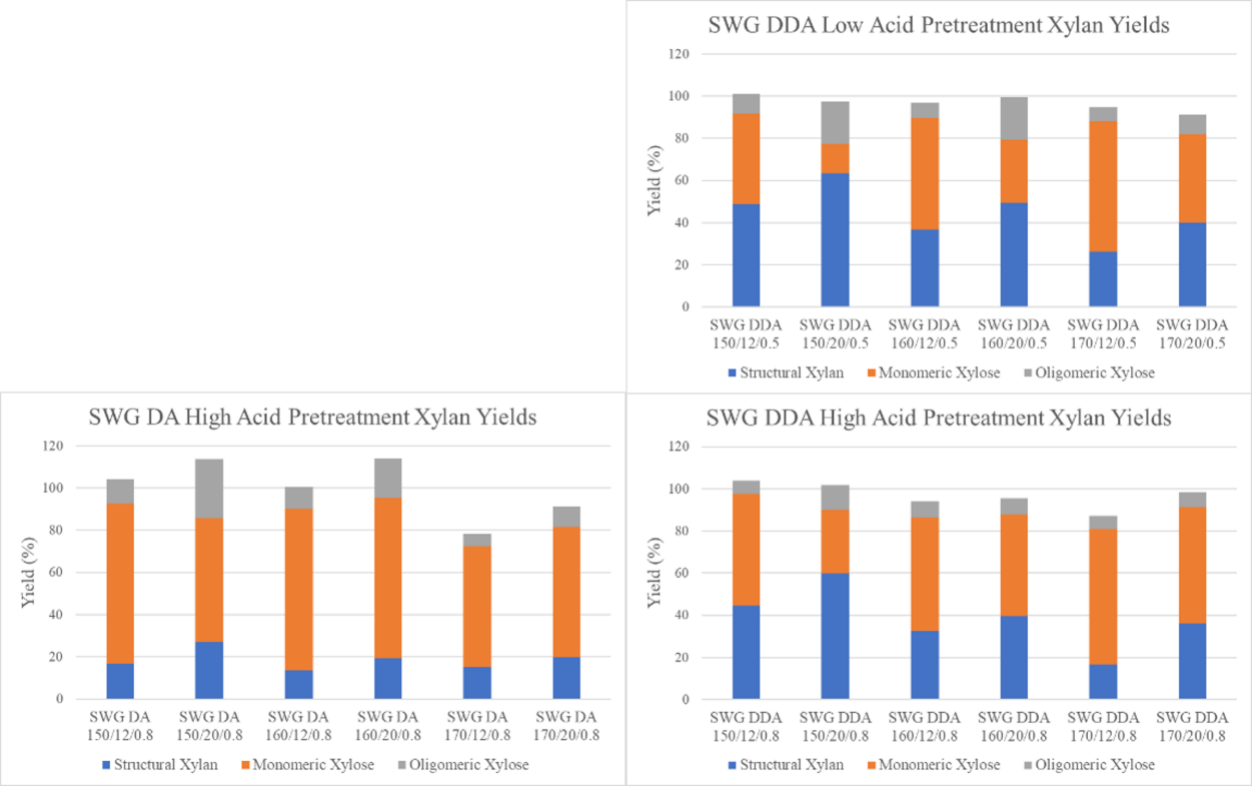


**Fig. 6. Xylan yields of DA pretreated switchgrass**

### 1.3.2 Switchgrass Enzymatic Hydrolysis

Figure 7 displays the enzymatic hydrolysis yields of DA/DDA pretreated switchgrass. SWG DA 160/12/0.8 was the only sample with a glucose yield above 80%. The DA pretreated switchgrass all had glucose yields that reached 70%. The DDA pretreated switchgrass had higher glucose yields as the severity of pretreatment increased. However, the lower acid concentration led to better enzymatic hydrolysis performance.



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

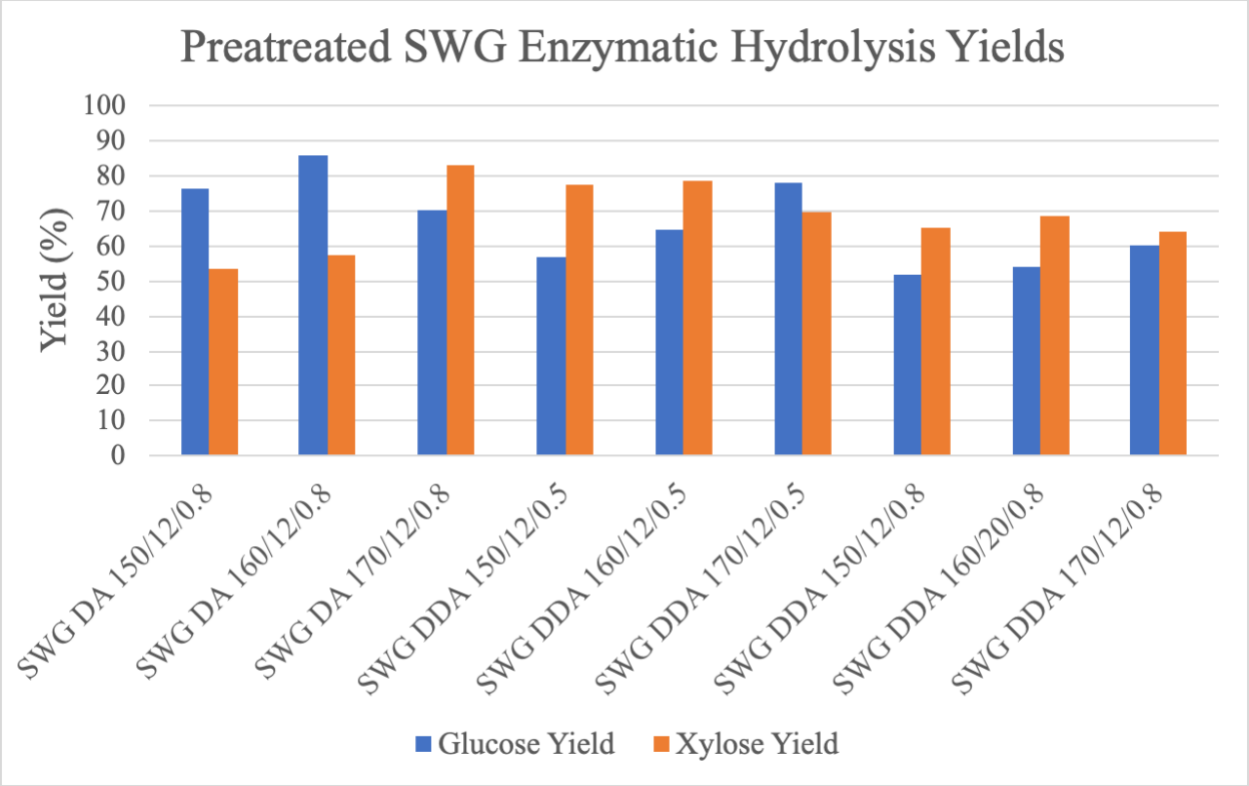


**Fig. 7. Glucose and xylose yields of pretreated switchgrass enzymatic hydrolysis**

**Task 2: Hydrolysate Generation (deacetylation-mechanical refining (DMR) pretreatment)**

**Objective:** Task 2 will generate hydrolysate streams from the same three feedstocks utilized in Task 1. In Task 2, a low temperature pretreatment process involving dilute alkali deacetylation followed by a mechanical refining operation will be used. The mechanical refining step will be performed in a 12-inch pilot-scale disk refiner.

For each feedstock, up to 12 specific pretreatment conditions (combination of deacetylation conditions, such as residence time, alkali concentration, and mechanical refining conditions) will be conducted to collect representative pretreated samples. Each pretreatment sample will be enzymatically saccharified using a single commercial enzyme (cellulase/hemicellulase) preparation at a single enzyme loading. The enzymatic saccharification will be conducted in laboratory-scale roller bottle-type reactors at a solids loading of 20% total solids. At the endpoint of enzymatic saccharification, the saccharified slurries will be harvested and samples will undergo standard compositional analysis and sugar yield calculations. The predictor of pretreatment efficacy used in the Task 1 high temperature pretreatment processes (solubilization profile of carbohydrate components and associated component mass closure of carbohydrate components in pretreated slurry solid and liquid fractions) cannot be used for the deacetylation-mechanical refining pretreatment due to limited carbohydrate solubilization occurring in that pretreatment process. Therefore, the enzymatic saccharification results from the Task 2 pretreated substrates will be utilized along with more detailed compositional activities in Task 3 (including imaging and structural analysis) to develop a predictive function of saccharification performance.



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

The harvested saccharified slurry samples' lignin-rich residual solids will be separated from the sugar-rich liquid fraction using laboratory-scale filtration devices. Filtration flux rates and washing efficiencies will be measured. The liquid fractions will be retained and stored in a frozen state for biological characterization and fermentation process studies (Task 4).

**Task 2 Results:**

2.1 Deacetylation & Disc Refining

Figure 8 displays the soluble lignin and acetic acid yields during deacetylation and disc refining for the DMR process. Deacetylation was performed using 5kg of dry biomass in a 90L paddle reactor. The concentration of NaOH, residence time was varied during deacetylation. The lignin rich black liquor was separated from the deacetylated slurry and the remaining solids were disc refined in a 12 inch disc refiner. The space between discs was varied during disc refining. The nomenclature of the samples are as follows: CS/Pop/SWG are the feedstocks corn stover/poplar/switchgrass, 60/80/100 is the concentration of NaOH in g per kg of dry biomass, 120/240 is the residence time during deacetylation in min, and 0.001/0.005/0.015 is the gap space during disc refining in in. The poplar had to have smaller gap space during disc refining because the 0.015 in gap was too large and the poplar fell through the disc. Deacetylation is meant to remove inhibitory acetic acid and lignin while not producing furfural and Hydroxymethylfurfural (HMF) during pretreatment. Figure 8 illustrates that all of the acetic acid was removed from the biomass solids during deacetylation. The acetic acid yields are larger than 100% because the compositional analysis of the raw biomass underestimates acetyl functional groups. The corn stover lignin yields are over 100%, indicating that all of the lignin was removed. However, at this time, the current methods for measuring lignin in black liquor are inadequate and overestimate the concentrations and thus overestimate the yields. The removal of lignin from corn stover during deacetylation was much more effective than that of poplar and switchgrass. Higher severity deacetylation removed all the lignin from corn stover, according to this data, which it peaked at 55% and 60% respectively for poplar and switchgrass.



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

**Fig. 8. Soluble lignin and acetic acid yields during DMR pretreatment**

## 2.2 Enzymatic Hydrolysis

Because DMR sugar yields can only be determined after enzymatic hydrolysis, all of the DMR slurries underwent enzymatic hydrolysis. The results of the enzymatic hydrolyses are displayed in Figure 9. Corn stover was clearly the best performing feedstock from DMR enzymatic hydrolysis. Once the NaOH concentration reached 80 g/kg the xylose yield was near or above 90% and the glucose yields near or above 70%. The poplar xylose yield peaked at 60% and the switchgrass peaked at 80%. The poor enzymatic hydrolysis performance for poplar in DMR was another reason the DA/DDA pretreatment campaigns for poplar were abandoned.



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

**Fig. 9. Glucose and xylose yields enzymatic hydrolysis of DMR pretreated biomass**

## Task 3: Detailed Compositional and Imaging Analysis

**Objective:** While NLR standard Laboratory Analytical Procedures will be performed on samples generated in Tasks 1 and 2, additional detailed analysis methods will also be performed on selected samples generated in Tasks 1 and 2.

Liquid-phase sample analysis performed under Task 3 will involve detailed oligomeric sugar speciation and structure analysis, including degree of polymerization and branching pattern of high temperature pretreated corn stover. This information will help identify the differences in oligomeric sugar from corn stover in different pretreatment processes and conditions and can identify the types of bond cleavage and associated enzymatic activities necessary to produce monomeric sugars from these oligomer structures.



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

**Task 3 Results:**

Oligomeric sugar speciation was determined using an Liquid Chromatography Mass Spectrometry (LCMS) with a zinc column and an Evaporative Light Scattering Detection (ELSD). Due to branching of the polymers and unknown response factors of those branches, quantification of the polymers was not possible. This was an exercise to determine how large the polymer chains of pentose and hexose were in the liquid fraction of the corn stover pretreated slurries. The results are displayed in Figure 10. Figure 10 has steam pretreatment on the left, DA pretreatment in the middle, and DDA pretreatment on the right. For DA and DDA, the low acid pretreatment (0.5% sulfuric acid versus dry biomass) is on top and the bottom is high acid pretreatment (0.8% sulfuric acid vs dry biomass). All charts start with lowest severity (temperature and residence time) on the left and increase in severity to the right. CS Steam 170/20 was an underperforming pretreatment, which is displayed with pentose chains with 12 degrees of polymerization. There was also only monomeric hexose present in all the steam pretreated corn stover samples. Most of the liquors' longest pentose chains ranged from 8 to 11 degrees of polymerization. The DA pretreated corn stover hexose's longest chains ranged from 5 to 8 degrees of polymerization, while the DDA pretreated corn stover ranged from 3 to 5 degrees of polymerization for hexose.

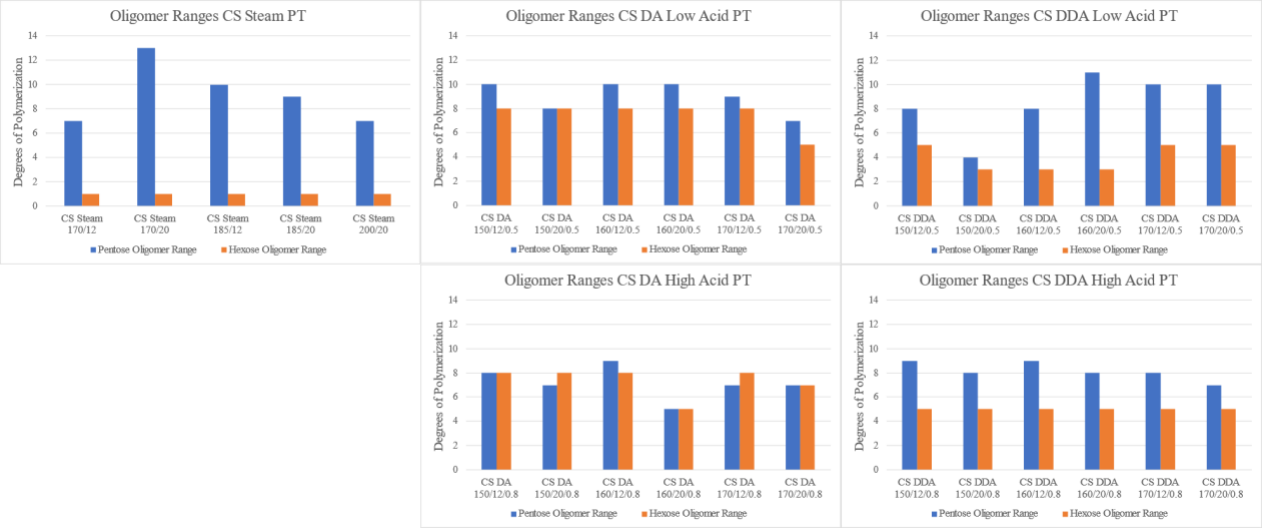


**Fig. 10. Oligomeric ranges of pretreated corn stover**

**Task 4: Fermentation of Enzymatic Hydrolysate (modified from Task 4: Lignin Characterization in Mod 4)**

**Objective:** The various pretreatment approaches used in this project will generate different types of sugar rich enzymatic hydrolysates with different monomeric sugar concentrations as inhibitory and degradation product concentrations such as acetic acid and furfural. To measure the digestibility of the various hydrolysates, each enzymatic hydrolysate will undergo laboratory scale batch fermentation using NLR's 2,3-butanediol strain of Zymomonus mobilis. Each fermentation will be sampled throughout the fermentation process to provide data on production



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

rates, and total fermentation conversion. Work in Task 4 is structured to occur in sequence with the generation of samples from Task 1 and Task 2 activities.

**Task 4 Results:**

4.1 Steam/DA/DDA Pretreated Corn Stover

The scope was modified from lignin analysis to fermentation because it was determined that understanding the fermentation conversion of the material after enzymatic hydrolysis provided more value than lignin analysis.

Fermentations were performed in shake flasks and therefore were not pH controlled. Samples were collected at various points during the procedure. Fermentations were performed after enzymatic hydrolysis. Figure 11 displays the final process yields for fermenting steam/DA/DDA pretreated corn stover. This strain of *Zymomonus mobilis* (*Z. mobilis*) produces 2,3-butanediol (2,3-BDO) and acetoin. Both compounds can be upgraded and are thus both considered products. The maximum process yields for both 2,3-BDO and acetoin from both glucose and xylose is 50%. The low temperature steam pretreated corn stover has the strongest fermentation, reaching 44% yield. This is due to the lack of furfural present during fermentation. Overall, the DDA pretreated corn stover was most consistent and had less affected by pretreatment conditions, all over which had process yields greater than 35%.

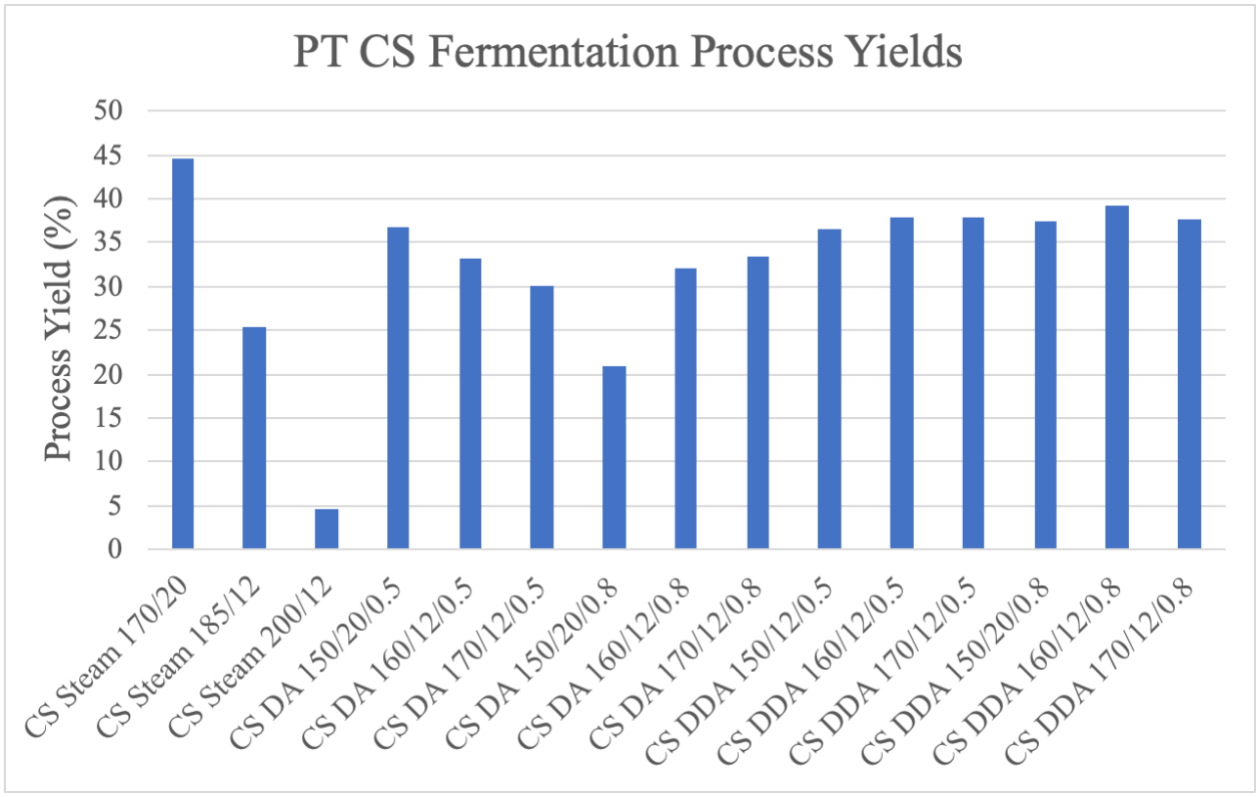


**Fig. 11. Fermentation process yields of steam/DA/DDA pretreated corn stover**



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

4.2 DA/DDA Pretreated Poplar

For the reason previously mentioned, the poplar pretreatment campaigns were dropped to prioritize more promising feedstocks and pretreatments.

4.3 DA/DDA Pretreated Switchgrass

Figure 12 displays the fermentation of DA/DDA pretreated switchgrass. The DA pretreated switchgrass had very poor results, with a maximum process yield of 10%. This was most likely due to an excess amount of acetic acid present in the pretreated liquor. Both DDA pretreatments of switchgrass at 170 deg C also had poor fermentation performance. The pretreatments had degradation products like furfural present which appear to have inhibited the fermentation. The best performing fermentation was the poorest pretreatment of switchgrass, SWG DDA 150/12/0.5 and SWG DDA160/20/0.5. While these pretreatment conditions had poor pretreatment and enzymatic hydrolyses results, the lack of inhibitors allowed for optimal fermentation conditions. Even these two samples did not perform as well as steam pretreated corn stover. In hindsight, it would have been optimal to pretreat switchgrass with steam to see how it would perform compared to corn stover.

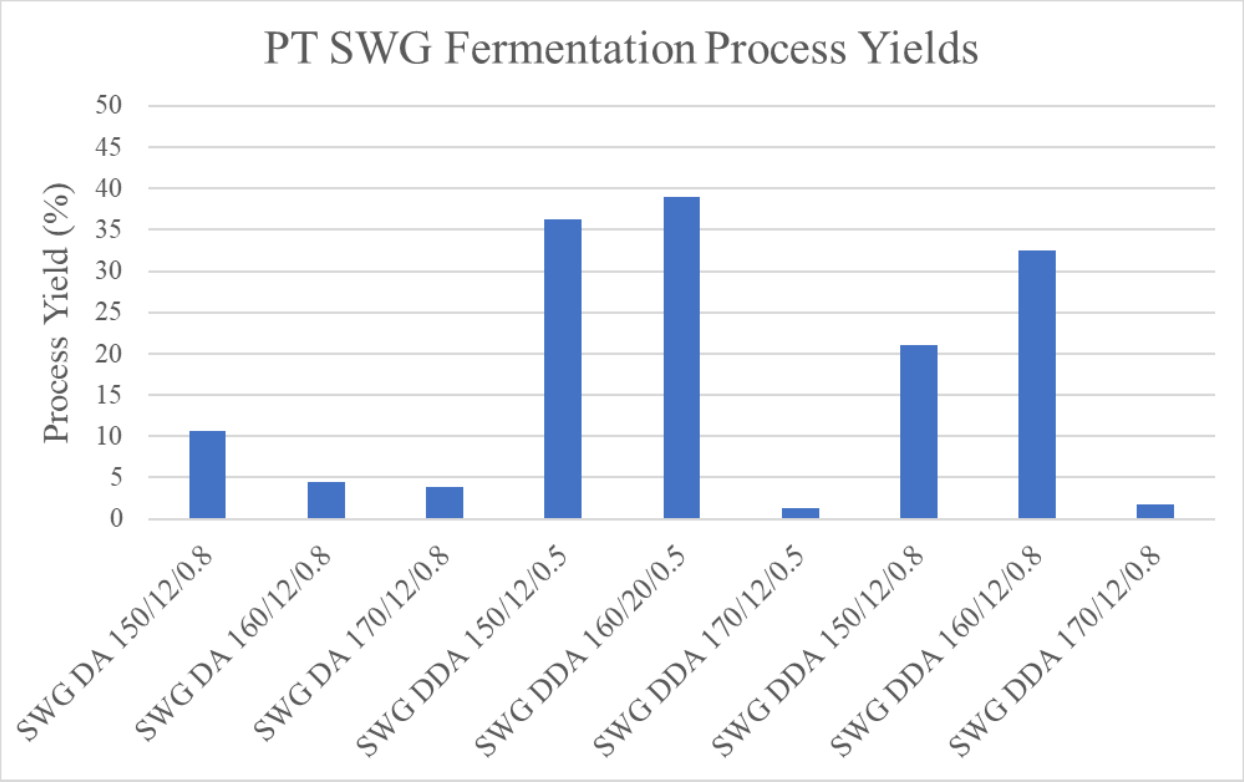


**Fig. 12. Fermentation process yields of DA/DDA pretreated switchgrass**



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

## 4.4 DMR Pretreated Biomass

Figure 13 displays the fermentation results for all DMR pretreated feedstocks. Some of the poplar fermentation could not be run because the enzymatic hydrolysis did not produce enough liquor to run a fermentation. The majority of the DMR pretreated feedstocks had better fermentation performance than the steam/DA/DDA pretreated biomass. However, there are a few unique observations. Both corn stover and poplar consistently had process yields above 45%. However, both had worse fermentations when the deacetylation had NaOH concentrations of 100 g/kg and had retention times of 240 min. Those deacetylations may have been too severe for optimal fermentation and produced too many salts. Switchgrass reached 40% process yield for all but two fermentations, but none of the fermentations reached 45%. Sample DMR SWG 80/120/0.005 appears to be an outlier from the rest of the switchgrass fermentations with a process yield less than 30%.

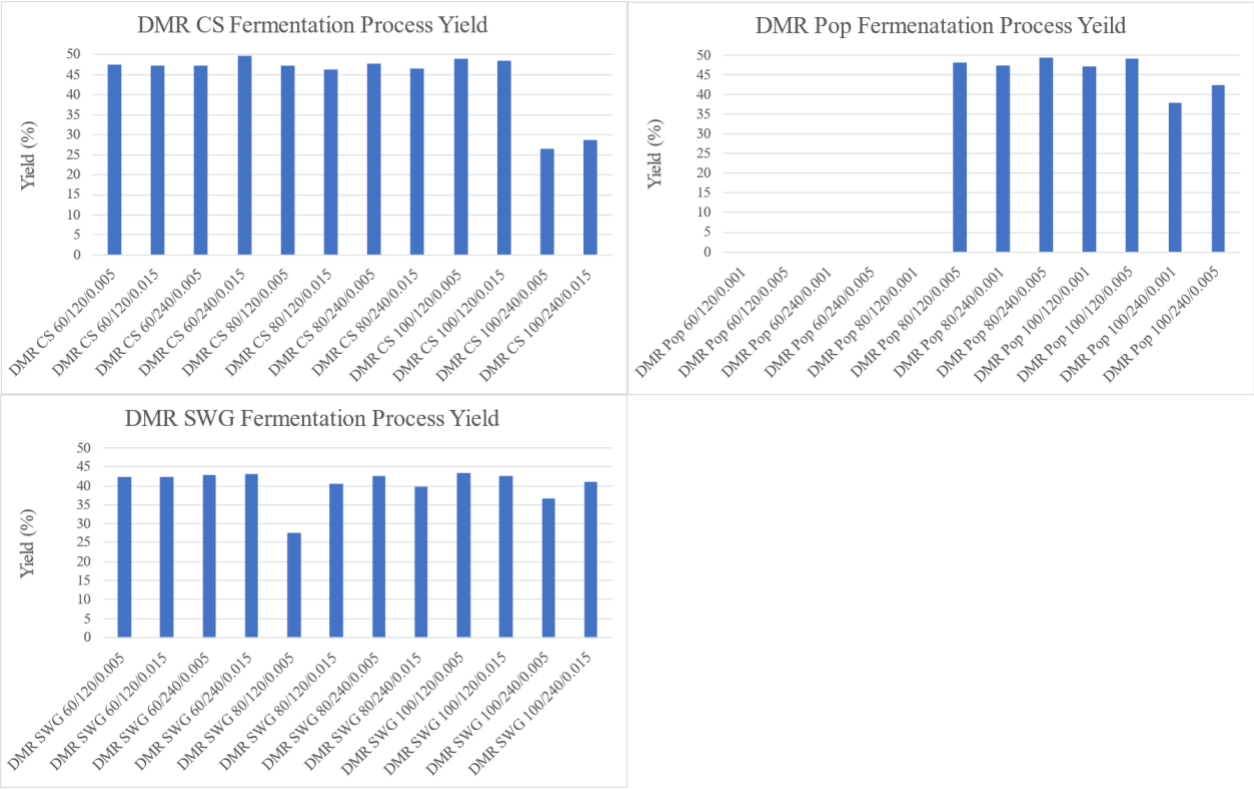


**Fig. 13. Fermentation process yields of DMR pretreated biomass**



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

## Task 5: Process Engineering/Technoeconomic Analysis

## Task 5 Results:

Technoeconomic analysis activities compare the economic potential of the various biomass feedstock and pretreatment processes and to identify key economic drivers and sustainability metrics. Material and energy balances for each feedstock and pretreatment process were constructed that utilize NLR's existing Aspen Plus process simulation models. Appropriate financial parameter assumptions were developed in collaboration between NLR and EMRE to allow for reporting of modeling results in a mutually useful manner.

Sensitivity analysis involving selected individual and collective process variables were conducted to identify the most significant factors that impact overall process operating and capital costs. This information will provide a basis for strategic evaluation of process potential based on technoeconomic and life-cycle considerations. Work in Task 5 was structured to occur in sequence with the generation of process conversion results and characterization from the other project tasks. Technoeconomic analysis (TEA) based on prior task results provided an understanding of the entire process and provide a direct comparison between feedstocks and other sugar sources that are commercially available.

5.1 Steam/DA/DDA Pretreated Corn Stover

NLR's ASPEN model was utilized to provide a TEA report for all pretreated feedstocks that underwent enzymatic hydrolysis. Because the fermentations were performed in a shake flask, the TEA report was used to estimate cost and production cost of the cellulosic sugars in for each feedstock and conditions.

For steam/DA/DDA pretreated corn stover, the TEA results were generally found to be most favorable for the DA pretreatment cases, followed by steam and then DDA pretreatment, largely reflecting similar trends in sugar yields. For a hypothetical commercial-scale biorefinery processing 2,000 dry metric tonne per day of biomass under nth-plant economic projections, the DA cases translated to modeled minimum sugar selling prices (MSSPs) ranging between 21 to 28 cents/lb total sugars with one outlier at 32 cents/lb, while the steam pretreatment cases varied 28 to 32 cents/lb and the DDA cases from 31 to 34 cents/lb. The DDA cases were somewhat further confounded by higher capital and operating expenses than the other pretreatment scenarios, tied to elevated capital costs for wastewater treatment attributed to generation of deacetylation black liquors as well as elevated operating costs for the use of deacetylation caustic. Results are displayed in Figure 14.



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

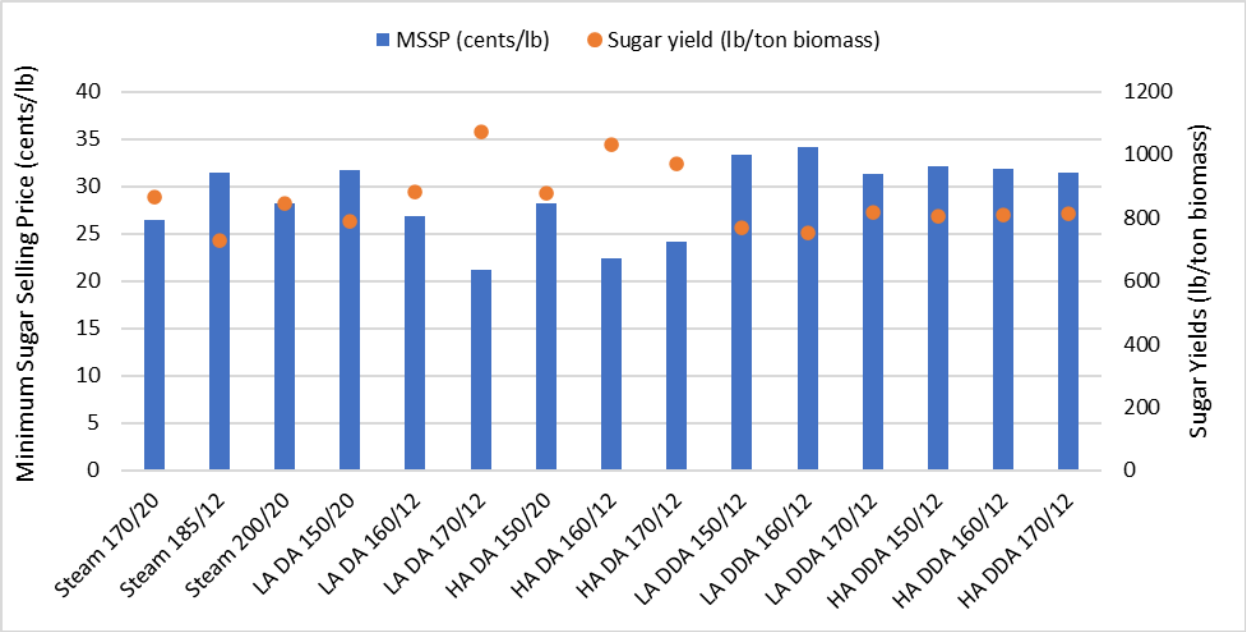


**Fig. 14. Sugar TEA results for steam/DA/DDA pretreated corn stover**

5.2 DA/DDA Pretreated Poplar

For the reason previously mentioned, the poplar pretreatment campaigns were dropped to prioritize more promising feedstocks and pretreatments.

5.3 DA/DDA Pretreated Switchgrass

The NLR TEA sugar models were exercised to incorporate experimental data generated across 36 pretreatment cases for deconstruction of switchgrass biomass to hydrolysate sugars. After updating key financial/cost parameters, the assessment found that sugar costs for DA pretreatment are most favorable largely reflecting similar trends in relatively higher sugar yields. The models will also be provided to EMRE collaborators upon request. Sugar yield is the dominant key cost driver. For a hypothetical commercial-scale biorefinery processing 2,000 dry metric tonne per day of biomass under nth-plant economic projections, MSSP ranges from $0.21-$0.25/lb for DA pretreatment and ranges from $0.26-$0.34/lb for DDA pretreatment. Results are displayed in Figure 15.



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

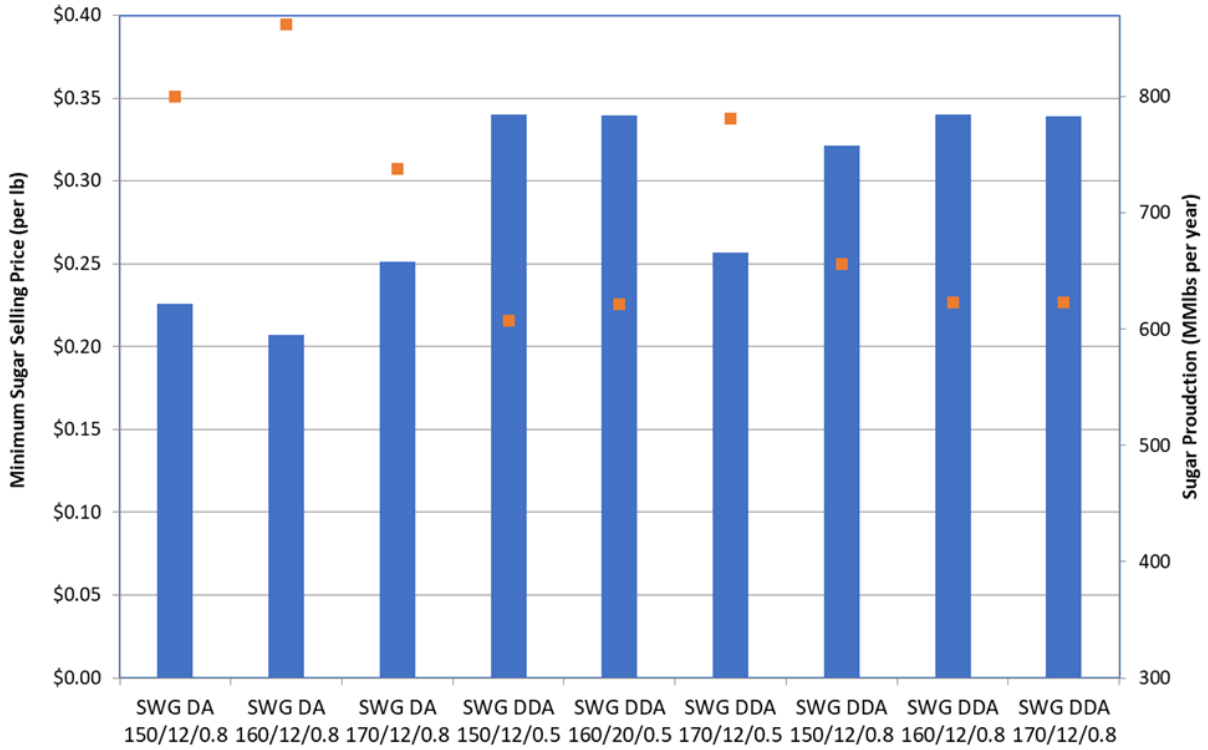


**Fig. 15. Sugar TEA results for DA/DDA pretreated switchgrass**

5.4 DMR Pretreated Biomass

The NLR TEA sugar models were exercised to incorporate experimental data generated across 36 pretreatment cases for deconstruction of corn stover, poplar and switchgrass biomass to hydrolysate sugars. After updating key financial/cost parameters, the assessment found that sugar costs for corn stover and switchgrass using DDR pretreatment are most favorable largely reflecting similar trends in sugar yields. Sugar cost for poplar is less favorable because sugar yields were found generally lower using poplar. For a hypothetical commercial-scale biorefinery processing 2,000 dry metric tonne per day of biomass under nth-plant economic projections, the corn stover cases translated to modeled minimum sugar selling prices (MSSPs) ranging between 29 to 49 cents/lb total sugars, while the switchgrass cases resulted in MSSPs ranges from 33 to 53 cents/lb total sugars. However, the poplar cases translated to modeled minimum sugar selling prices (MSSPs) unfavorably ranging between 66 to 1.52 cents/lb total sugars, mainly due to lower sugar yields. The MSSP trends largely were a function of sugar yields. Results are displayed in Figure 16.



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.

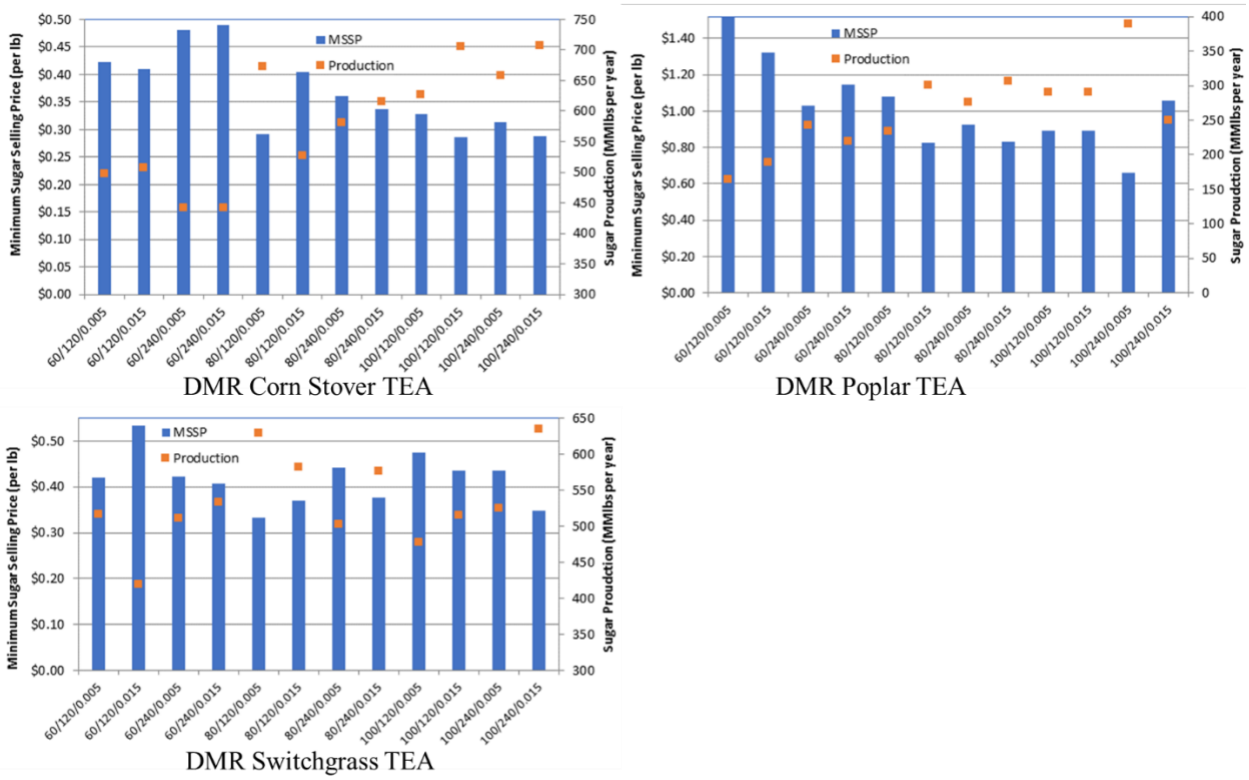


**Fig. 16. Sugar TEA results for DMR pretreated biomass**

**Task 6: Fermentation of hydrolysates (Mod 37)**

**Task 6 Results:**

This task work was realigned and merged with Task 4 scope. See Task 4 Results.

**Task 7: CRADA Final Report (Mod 37)**

**Task 7 Results:**

This report serves to meet the requirement for the CRADA Final Report with preparation and submission in accordance with the agreement's Article X.

**Reference:**

Darren J. Peterson, Changyub Paek, Ling Tao, Ryan Davis, Xiaowen Chen, Roman Brunecky, Matthew Fowler & Richard Elander, "Feedstock/pretreatment screening for bioconversion of sugar and lignin streams via deacetylated disc-refining," BioMed Central Ltd. (*BMC), part of Springer Nature.* April 5, 2024, 52 (2024): Pages 17+, https://doi.org/10.1186/s13068-024-02492-7.

**Subject Inventions Listing:** None.

**ROI #:** None.



This report is available at no cost from the National Laboratory of the Rockies (NLR) at www.nlr.gov/publications.