

NARA Goal One

2nd Cumulative Report

April 2013 - March 2014



Sustainable Biojet

Develop a sustainable biojet fuel industry in the Pacific Northwest that uses residual woody biomass as feedstock.

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SUMMARY

All the activities in the NARA project contribute to the goal of providing an industry road-map used to sustainably produce jet fuel (biojet) from wood residuals in the Pacific Northwest, and some activities contribute directly to the technology of this process. The NARA Feedstock and Conversion teams are focused directly on securing the wood residual feedstock and integrating the technologies employed to convert this feedstock into fuel. Specifically, the following efforts provide an integrated approach to creating a viable pathway from forest residuals to biojet:

- 1) Feedstock Logistics team: Integrating feedstock collection, preprocessing, and transportation to deliver cost effective materials suitable for conversion
- 2) Feedstock Development team: Focusing on identifying growing stock varieties amenable to sugar production and delineating traits responsible for increased volume production in Douglas-fir trees
- 3) Pretreatment team: Refining effective pretreatment methods to release sugars from representative forest biomass
- 4) Aviation Biofuels team: Refining the Gevo fermentation/separation and WSU BioChemCat processes to produce aviation biofuels from representative pretreated forest residuals.

FEEDSTOCK LOGISTICS

The NARA feedstock team is divided into two efforts: feedstock logistics and feedstock development. For this reporting period, the feedstock logistics team evaluated methods to improve the costs associated with processing and transporting forest residuals. The processing considerations evaluated were grinding, bulk density and sizing. A collaborative economic analysis indicates that accommodations to process oversized “overs” and undersized “fines” feedstock chips adds little impact to feedstock costs, whereas, methods to improve grinder and transport efficiencies had a significant impact on cost. The feedstock costs associated with grinding can vary as much as \$22

per oven-dried short ton. Lowest grinding costs were achieved by: a) starting with smaller sized pieces, b) grinding to larger final sizes, and c) using (sharp) knife bits instead of (blunt) hammer bits (Task FL-1).

Improved transport efficiencies are correlated with higher bulk density of the load. Blower tests on dry forest residues suggest significant gains in bulk density can be achieved by vertical acceleration of dry grindings. NARA’s goal is to achieve 22 bone dried tons (BDT) per chip truck with a combination of lower moisture content coupled with increased bulk density. This would reduce transport costs up to 33%. In addition, results show that the tops and limbs gave higher bulk densities for all conditions. Methods to improve bulk density could reduce feedstock transport costs by as much as \$10 per oven-dried short ton (Task FL-2.4). A forest residue decision support system (Residue Evaluation and Network Optimization, RENO) was completed and is designed to improve processing and transportation costs (Task FL-2.3).

The range of net feedstock cost variation when considering grinding, bulk density and sizing options together is \$30 per oven-dried short ton. This amount of change to feedstock costs in the NARA base case economic analysis changes total costs by more than \$25 million per year. This variation affects the overall project internal rate of return (IRR) by about 1.4%. (Task FL-1.1).

Additional efforts undertaken to improve the cost of processing forest residuals include developing models to predict biomass availability and moisture content. Fieldwork performed to establish recovery coefficients at various costs for forest residual collection has been completed. A statistical model incorporating these recovery coefficients plus field procedures will be implemented in July 2014 and used to determine recoverable biomass from timber product output (TPO) data (Task FL-2.1). Field data for a forest residue

collection model was completed February 2014 and will be incorporated into the forest residue decision support system RENO (Task FL-2.3). For the past year, temperature, humidity and radiation sensors have recorded data from slash piles, and the moisture content for live branch wood has been recorded. This data collection will conclude July 2014. Preliminary results indicate that live branches have moisture content lower than 50% in most tested sites during fall and winter seasons (Task FL-2.2).

FEEDSTOCK DEVELOPMENT

For this reporting period, the feedstock development effort has initiated screening Douglas-fir seedlings for drought response. To screen Douglas-fir seedlings, a pilot experiment was performed in which 50 Douglas-fir seedlings were screened at the WSU Phenomics facility (Task FD-2). This pilot screening helps establish the protocol used for an experiment planned in the summer of 2014 to screen over 1000 Douglas-fir seedlings.

The data from the drought screening will be combined with data derived from quantifying Douglas-fir chemical composition and recalcitrance among multiple individuals. These data will contribute to developing a Douglas-fir phenotype profile that can be linked to DNA samples assembled on a single nucleotide polymorphism (SNP) chip. The chemical composition and recalcitrance screening has advanced from last year, as 265 new tree samples have been tested. A “recalcitrance factor” parameter has been developed to quantify the degree of recalcitrance among tree samples (Task FD-5). A limited number of Douglas-fir DNA samples will be used to generate a SNP chip, therefore, initial screening steps to narrow the pool of samples has begun. A breeding simulation and testing program has been developed to assist in this process.

Significant Feedstock Team outputs for this reporting period are listed below.

- Slash pile measuring techniques were evaluated. This information is used for biomass availability models and for harvest operations planning (Task FL 2.3). http://www.ingentaconnect.com/content/saf/fs/pre-prints/content-saf_0015749x_forsci13001
- A computerized model, Residue Evaluation and Network Optimization (RENO), was completed. It is used to estimate the cost of processing and transporting forest residuals at the operational level. This model is designed to improve processing and transportation costs and is being introduced to stakeholders (Task FL 2.3). <http://forestprodjournals.org/doi/abs/10.13073/FPJ-D-13-00031>

PRETREATMENT

The Pretreatment team's efforts this year were focused on increasing the scale of operations and selecting a single preferred pretreatment method for continued focus in Year 4 and 5. This effort was informed by technical performance, economics, co-product opportunities, and commercialization potential and was evaluated using a phase and gate analysis. This extensive process culminated with the mild bisulfite (MBS) pretreatment protocol selected as the single pretreatment method used to produce a NARA deliverable of 1000 gallons of jet fuel. Downselection to the MBS process represents a major NARA milestone and allows for a single pretreatment protocol to be considered for the NARA techno-economic analysis (TEA) and life cycle assessment (LCA). The mild bisulfite pretreatment protocol is a modification of the SPORL process and was developed by NARA affiliates Catchlight Energy and the USDA Forest Products Laboratory.

In downselecting to the MBS pretreatment, the MBS and wet oxidation (WOX) protocols were optimized using the FS-10 feedstock sample. Under optimized conditions for each pretreatment protocol, a mass balance was generated, hydrolysate was tested for fermentability, and the solid and liquid residuals were tested for co-product development (Task C-P-3, C-P-

4, C-P-1). The downselection process considered these results along with economic and environmental considerations, plus feedstock and infrastructure compatibility. The NARA Advisory Board and Executive Committee reviewed the comparisons and made recommendations with the final selection made by NARA's Executive Director Ralph Cavalieri.

Improvements to the MBS pretreatment protocol include a reduction of sulfur dioxide applied to wood from 12% to 6% and a cook temperature reduction from 165°C to 145°C. It was also demonstrated that pretreatment efficiencies could occur without significant chip size reduction. These changes improve energy efficiency, reduce chemical cost and generate fewer inhibitors to the fermentation process. An integrated pretreatment/fermentation plant is economically better than a situation where sugar must be shipped to an existing ethanol plant, and producing hydrolyzing enzymes on site is more economical than purchased enzymes (Task C-P-4.1).

Another pretreatment strategy investigated this year involves wood milling. The distinction of wood milling compared to MBS and WOX pretreatment is that no chemicals, water or high temperatures are required. In areas such as the Pacific Northwest where electricity is relatively inexpensive, wood milling may prove to be an attractive pretreatment alternative. The wood milling process has four steps: (1) wood residuals chipping; (2) chip size reduction before drying; (3) chip drying to reduce chip moisture from 50% to 10%; and (4) wood milling.

During this reporting period, FS-10 feedstock samples were reduced by two grinding technologies and characterized for particle size. The reduced samples were then milled and hydrolyzed. The hydrolysate was sent to Gevo for fermentation analysis and to the NARA Co-Products team for co-product development. The carbon dioxide emissions associated with the wood milling drying process were measured and will be used for a later life cycle assessment. Energy usage was calculated for all four steps in the milling process and will be used to generate a techno-economic analysis.

Based on the small-scale milling results generated thus far, the total energy requirement for wood milling could range from 1.45 to 1.73 kWh/oven-dried kg. The energy usage data for this process will be refined when large-scale milling (currently underway) is analyzed.

Significant Conversion Team outputs for this reporting period are listed below.

- MBS (mild bisulfite: based on the SPORL pretreatment protocol) pretreatment temperature at 145°C is superior to a previous 165°C temperature both economically and in reduced inhibitor formation (Task C-P-4). <http://www.sciencedirect.com/science/article/pii/S1359511313007435>
- The SPORL pretreatment protocol was optimized using Douglas-fir forest residuals. <http://www.sciencedirect.com/science/article/pii/S0961953413003607>

AVIATION BIOFUELS

The NARA Aviation Biofuels Team focused on two fermentation technologies: Gevo's fermentation, separation, and upgrading process and Washington State University's (WSU) BioChemCat process managed at the WSU-Bioprocesses, Sciences & Engineering Laboratory (BSEL). During this reporting period, Gevo characterized the pretreated hydrolysate generated by the SPORL, wet-oxidation, mild bisulfite and mill wood pretreatments on FS-10 feedstock. Hydrolysate characterization included sugar and inhibitor analyses and fermentability to isobutanol. These characterizations were considered in the pretreatment downselect process (see Phase Gate model in the Organizational Structure chapter).

The isobutanol producing biocatalysts being developed are stable and continue to improve in growth rate and isobutanol production. Sodium hydroxide was substituted for ammonium hydroxide to balance pH, which reduced impurities significantly. In a 1 liter GIFT® system, biocatalyst growth rates and isobutanol production were measured in 60% hydrolysates derived from varied pretreatment processes. Growth

rates were relatively similar within each hydrolysate; however, isobutanol production varied with the highest isobutanol production and hexose consumption generated from the MBS hydrolysate. A 20L GIFT® pilot scale system was designed and built for future scale-up trials.

Gevo provided NARA mass and energy balance information and capital expenditure (CapEx) costs covering Gevo's process of converting sugars into isobutanol, which is combined to form iso-paraffinic kerosene (IPK). This data will be incorporated into the NARA techno-economic and life cycle assessments. The saccharified biomass sugars are fermented and isobutanol recovered in a process essentially identical to the corn mash process currently being used at Gevo's plant in Luverne, MN. Preliminary analysis of isobutanol quality derived from Douglas-fir biomass and corn starch are very similar.

WSU BSEL fermented carbohydrates, derived from FS-01, FS-03 and FS-10 feedstock samples pretreated by wet oxidation. Calculated yields of volatile fatty acids produced ranged from 410 kg/ton biomass to 510 kg/ton. These results translate to a range of 60.1 gallons to 74.8 gallons of jet fuel per biomass ton, with FS-01 (clean wood chips) producing the highest results.

Three different separation methods: (i) pressurized carbon dioxide extraction; (ii) ion exchange resin-mediated extraction; and (iii) esterification have been investigated for efficiency in extracting organic acids from the fermentation broth.

While the extraction of volatile fatty acids (VFAs) using pressurized carbon dioxide and ion exchange show high recovery rates, the technology at its current stage can be considered expensive. Conversion of VFAs into mixed alcohols by esterification has been initiated. To integrate the fermentation/conversion process into a continuous system, a lab-scale catalytic system has been designed and is currently in the process of being set-up at the BSEL laboratory (Task C-AF-2).

Training

Name	Affiliation	Role	Contribution
Carlos Alvarez-Vasco	WSU, BSEL	PhD Student	Assay development
Ruoshui Ma	WSU, BSEL	PhD Student	Lignin work
Scott Geleynse	WSU, BSEL	PhD student	Pretreatment and enzyme hydrolysis
Roland Gleisner	USFS, FPL	Tech support	Pretreatment and preprocessing
Hongming Lou	USFS, FPL	Post Doc	Enzyme and lignin interaction
JinLan Cheng	USFS, FPL	Visiting Scholar	Pretreatment Studies
Andrea Laguna	University Wisconsin-Madison	Undergraduate Student	Saccharification Studies
Jingzhi Zhang	USFS, FPL	Visiting Student	Saccharification Studies
Rene Zamora	OSU	Post Doc	See Publication List; Task FL-2
Storm Beck	OSU	MS Student	See Publication List; Task FL-2
Francisca Belart	OSU	PhD Student	See Publication List; Task FL-2
Justin Long	OSU	Res. Associate	See Publication List; Task FL-2
Keerthi Srinivas	WSU, BSEL	Post-doc	BioChemCat development
Ben Garrett	WSU, BSEL	Graduate student	BioChemCat development
Ricarda Hoehner	WSU	Post-Doc	Established the screening platform for Douglas-fir including data analysis
Ahmad Zia	WSU	Post-Doc	Helped to Established the screening platform for Douglas-fir including data analysis
Rajib Biswas	WSU, BSEL	Post-doc	Mass balances: Enzymatic hydrolysis

Resource Leveraging

Resource Type	Resource Citation	Amount	Relationship or Importance to NARA
DOE BRDI	To be received March, 2014. Humboldt State University, Lead	\$300,000 sub-award to John Sessions as Co-PI.	Additional Funds for Decision Support
Salary	US Forest Service		Support of J.Y. Zhu & Roland Gleisner
Scholarship	China Scholarship Council		Support Haifeng Zhou, JinLan Cheng, and Jingzhi Zhang
Grant	USDA SBIR Phase II program to Biopulping International (Contract Number: 2010-33610-21589)		
NSF Grant	Award 1067012		Understand biomass recalcitrance: X. Zhang
DOE Port of Benton	Bioreactors	\$100,000	Parallel project terminating end of April, 2014
Equipment Pretreatment	DOE Port of Benton	\$400,000	Provided funding for the equipment

TASK FL-1: FEEDSTOCK SOURCING

Key Personnel

Gevan Marrs

Affiliation

Weyerhaeuser

Task Description

Weyerhaeuser and Oregon State University will work cooperatively to quantify costs and quantities of key Pacific Northwest candidate feedstocks by region; determine feedstock key quality parameters, variation and impact on conversion processes; perform analysis to select optimum feedstock sourcing strategies; and develop and test feedstock supply chain improvements to reduce costs and increase value.

Weyerhaeuser will concentrate on the supply chain value improvement from forest harvest residuals. Weyerhaeuser will work collaboratively with other logistics teams to extend harvesting and delivery options to mill-site processing. Weyerhaeuser will also work with the conversion group(s) to quantify the options for feedstocks specifications (moisture, size, chemical composition, etc.); document the costs and productivity of forest residual feedstock supply chains for the Techno-Economic team; test and document improvements in the supply chain; and coordinate with the sustainable production group on long-term site productivity studies.

Activities and Results

In prior project years this team has sampled, prepared, and characterized ten different potential softwood feedstocks for conversion to biojet by the NARA collaborators. The majority of these have been the most economically viable feedstock – forest harvest residuals. While the sampled materials cover a range of composition levels, three (FS-01, FS-03, and FS-10) have served as reference materials for more exhaustive testing through the pretreatment, hydro-

lysis, and fermentation stages of the NARA process. These results form the basis for economic comparisons between options and lead to significant input for downselecting conversion options as well as assessing the overall economic viability of the process.

The work for April 2013 through March 2014 has focused on quantifying the potential feedstock cost impacts for various comminution and mill-site processing to deliver a “standard sized” product to the conversion mouth. Large samples (several hundred pounds each) of all the 18 treatments for the 2013 grinding trials (Figure FL-1.1) were obtained and shipped to the Weyerhaeuser Technology Center (WTC) for mill-site simulated preparation (Figure FL-1.2). The full results of the grinding portion of the trial are reported elsewhere in the logistics team report – only the additional mill-site preparation, and overall economic impacts quantification are reported in this section.

The grinding portion of the trials had three main impacts on preparation that were translated into economic impacts. These were a) fuel consumption for grinding and relationship to total grinding cost, b) bulk density and impact on hauling costs, and c) particle size distribution and impact on mill-site screening and feedstock costs to the conversion mouth. Each of these will be discussed below in more detail.

FUEL USAGE AND TOTAL GRINDING COSTS

The gallons of fuel used for each grinding test were translated to a specific fuel consumption based upon total wet tons processed and moisture content, yielding gallons per oven-dry short ton (ODST). These results are shown in summary in Figure FL-1.3, where it can be seen that there are very large relative differences in various treatments. Assuming an off-road diesel fuel price of \$3.29, the fuel usage alone was calculated on a per ton basis. All treatments

were indexed to the case most similar to that used for the NARA techno-economic assessment (TEA) since absolute costs were not a concern, for this case, hammer carbide bits with 3 and 4-inch screens on Feed Piece Class 2 pulp logs. Further, since more than just fuel costs vary with specific power usage, it was assumed that the loader and grinder were kept running at 75% of full power each operating hour using a constant 26.5 gallons diesel per hour. Thus, the total hourly costs of \$411 (plus fuel) were allocated to the tons per hour that could be produced, and the variations by treatment shown as differences from the base case (Figure FL-1.4)

BULK DENSITY AND HAULING COST

Bulk density for each treatment was measured in a 2-cubic foot box in a laboratory setting. While these results do not correspond to fully loaded chip vans of normal size, other comparison tests show that they are roughly 15% lower. It is believed that the relative bulk density differences translate to full chip vans, thus by referencing all treatments against the chosen internal base case, the observed differences can be translated into truckload value differences for hauling costs. Figure FL-1.5 shows the actual bulk density differences observed in the grinding tests. As can be seen, there is a significant variation in results, suggesting an important potential impact on a significant cost element for feedstock. The results show that the tops and limbs gave higher bulk densities for all conditions. This is likely due to both higher wood density of this material and the impact of drier material, which could produce more fines. For larger piece sizes (pulp logs and chunks) the chipper bits gave consistently higher bulk density than hammer-carbide bits.

To translate these bulk density differences into a feedstock delivered cost change, it was assumed that (as was the case for these materials) the moisture content is sufficiently low and that the chip vans would fill

volumetrically before hitting the gross vehicle weight (GVW) highway weight limit: for typical bulk densities and chip van configurations, this would occur somewhere around 40% moisture.

The same treatment was considered the base case, and the NARA TEA hauling costs of \$24/ODST for a 75-mile haul were adjusted for the load carried according to bulk density. This factor had considerable economic impact, giving a total range of \$9.50/ODST.

OVERSIZE PRODUCTION AND RE-SIZING COST

The samples from each treatment were screened at the Weyerhaeuser Technology Center on a gyratory screen that would be typical in a pulp mill. The conditions of the grinding treatments, in particular the grinding screen size used, had a significant impact on the amount of oversize rejected above the 1 3/4 inch round-hole screen. Figure FL-1.7 shows how variable the treatments were for oversize rejects. These rejects would not be disposed, but instead re-sized, typically in a hammer hog. Using literature values for total re-sizing costs (amortized capital, power costs, maintenance, etc.), the total cost per ton of re-sized material (\$3.47/ODST oversize) was translated into a differential cost based upon total feedstock, shown in Figure FL-1.8. Since only about 1% to 10% of the feed needs to be resized, when expressed on the basis of feed to the conversion mouth, the effect is nearly trivial – only \$0.38/ODST. This impact is dwarfed by the grinding power and hauling costs effects.

The WTC gyratory screening used a 1/8 inch woven-wire screen mesh to remove fines, as prior work has shown that the concentration of bark and grit will warrant some level of removal to avoid conversion problems and low yields. Figure FL-1.9 shows that the fines reject levels can vary quite dramatically, in particular being high with hammer-carbide bits on (relatively dry) tops and limbs when using a small grinder screen. Logically, larger grinder screens produced more oversize and fewer fines, and vice-versa.

The rejected fines would not be completely devalued, as they can be an energy source—either internally if the facility has a hog fuel boiler, or alternatively sold on the open market. Either way, the valuation can be set by market prices for hog fuel, which in the NARA base case is assumed to be \$45/ODST. The impact of fines rejects is then only the cost differential between the through-the-gate feedstock price (\$68/ODST) and the hog fuel value, but only for the fraction rejected to hog fuel. This cost is then spread back over the tons going to the conversion mouth, expressing the cost change on a basis of feed tons. Figure FL-1.10 shows that this economic impact is not large—mostly due to the relatively small fraction downgraded, but also to the relatively small per-ton value downgrade. The total range of impact on a feed basis is \$2.77/ODST feed.

TOTAL ECONOMIC IMPACT OF ALL FACTORS COMBINED

Since the three factors described above are not independent of each other, and often are in contradictory directions, the net effect for any treatment is non-obvious. For example, smaller grinder screens require more power, but can increase bulk density, lowering hauling costs, and reduce oversize and resizing costs, but increasing fines downgrade cost to hog fuel. Since the same reference case was used for all relative comparisons, the net can be obtained by simply summing all impacts for each treatment. The total impact is shown in Figure FL-1.11, where it can be seen that there is a significant range of impact—the total being about \$30/ODST to the conversion mouth. This amount of change to feedstock alone in the NARA base case changes total costs by more than \$25 million dollars per year and would change the overall project internal rate of return (IRR) by about 1.4% from one extreme to the other. The data show that chipper bits are generally favorable; this arises from the combined effects of lower specific power leading to lower grinding cost and higher bulk density giving lower hauling costs. In particular, it would seem that hammer-type carbide bits used on logs and chunks is not a favorable economic condition. Grinder

screen sizes, somewhat surprisingly, seem to have little overall impact due to counteracting effects.

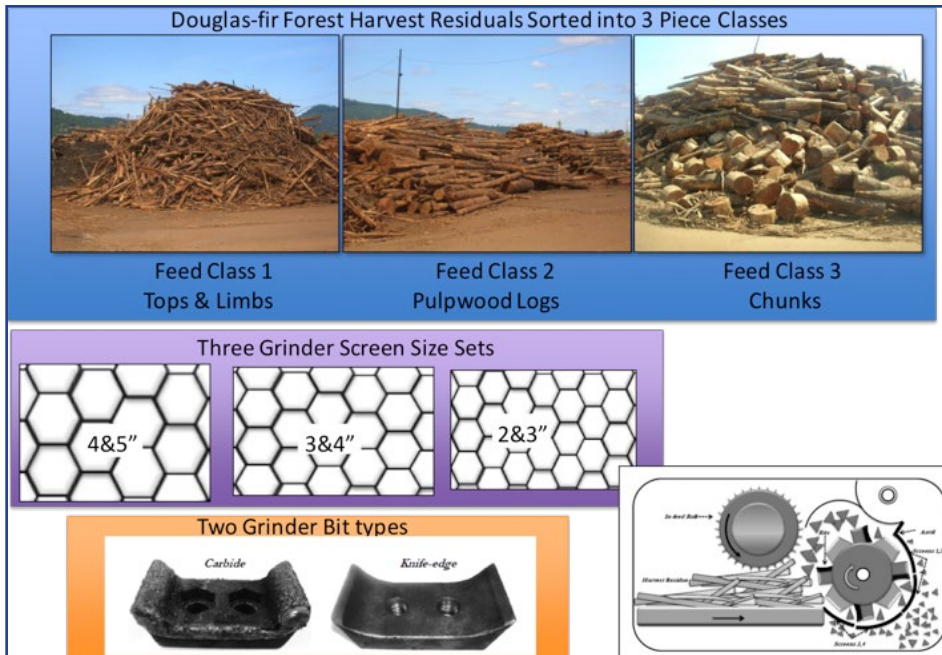


Figure FL-1.1. Overall structure of the woods-simulated Grinding Trials main factors

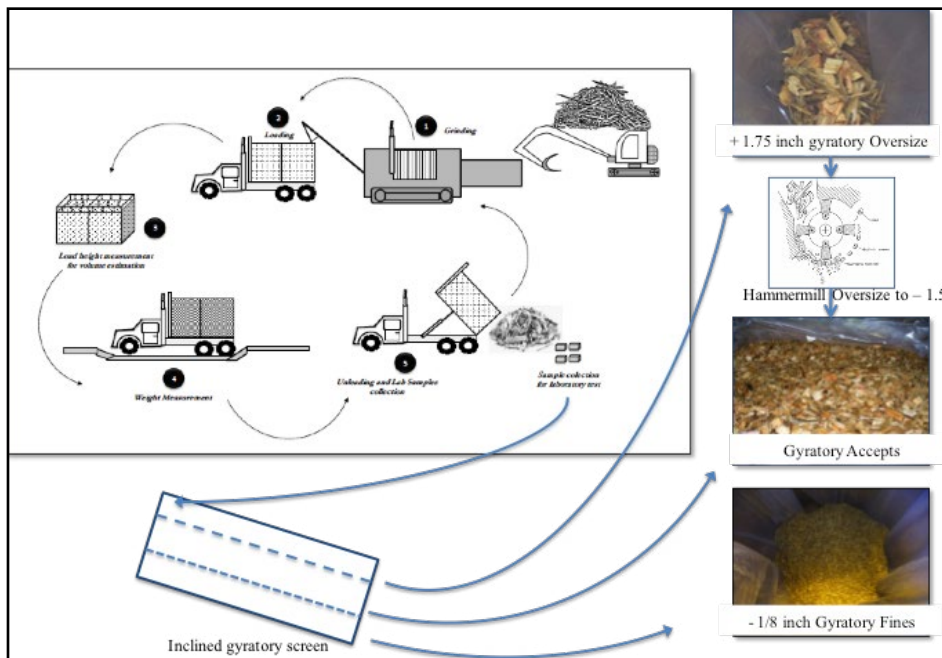


Figure FL-1.2. Overview of the Logistics portion of the Grinding Trials and the subsequent WTC mill-site simulated processing. There were 18 treatments in the trial set.

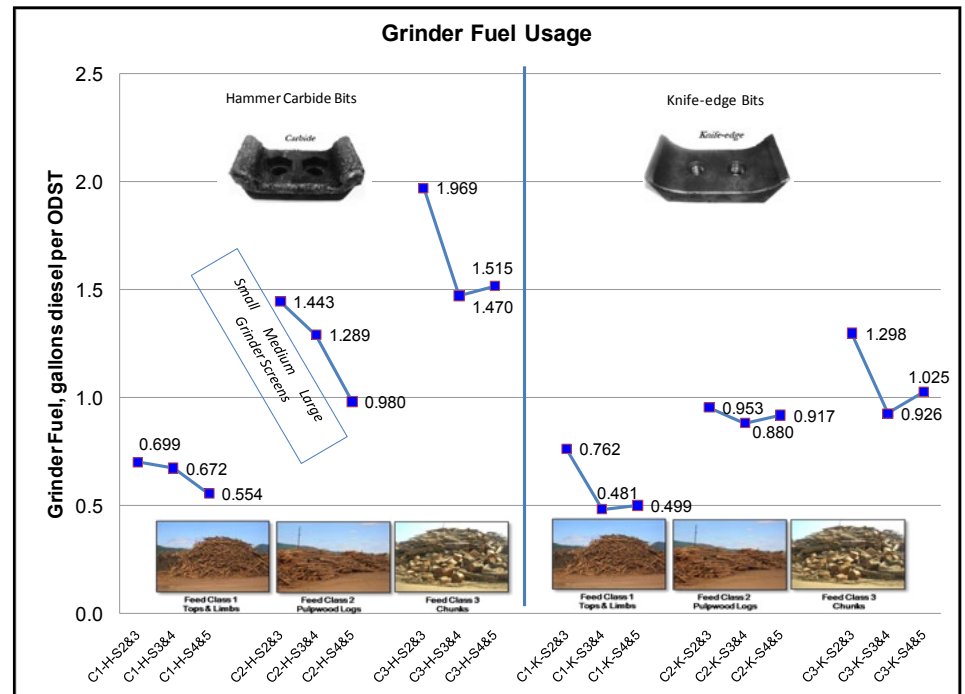


Figure FL-1.3. Horizontal grinder fuel usage for different treatment conditions

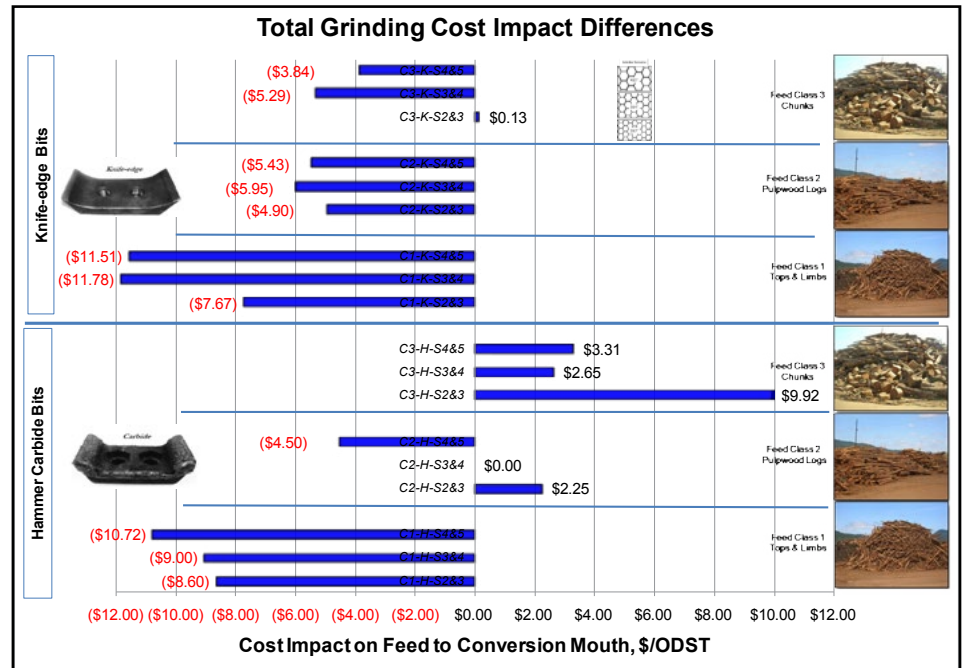


Figure FL-1.4. Total grinding costs by treatment vary significantly – a total impact range of \$22/ODST

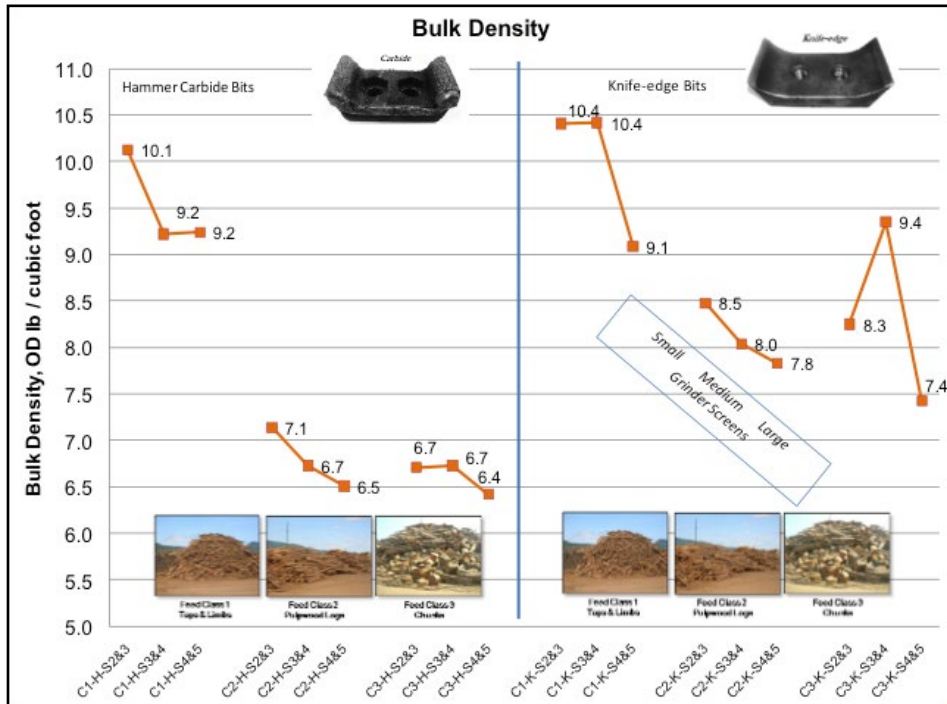


Figure FL-1.5. Laboratory bulk density variations by treatment

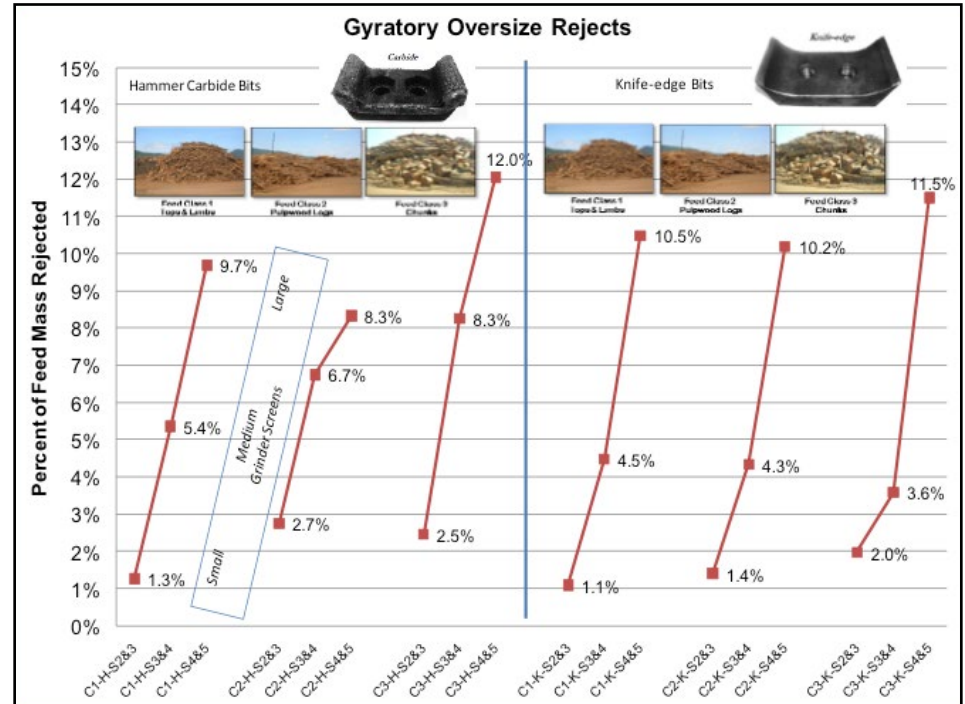


Figure FL-1.7. Grinder screen size had the only, and large, impact on gyratory screen oversize rejects levels

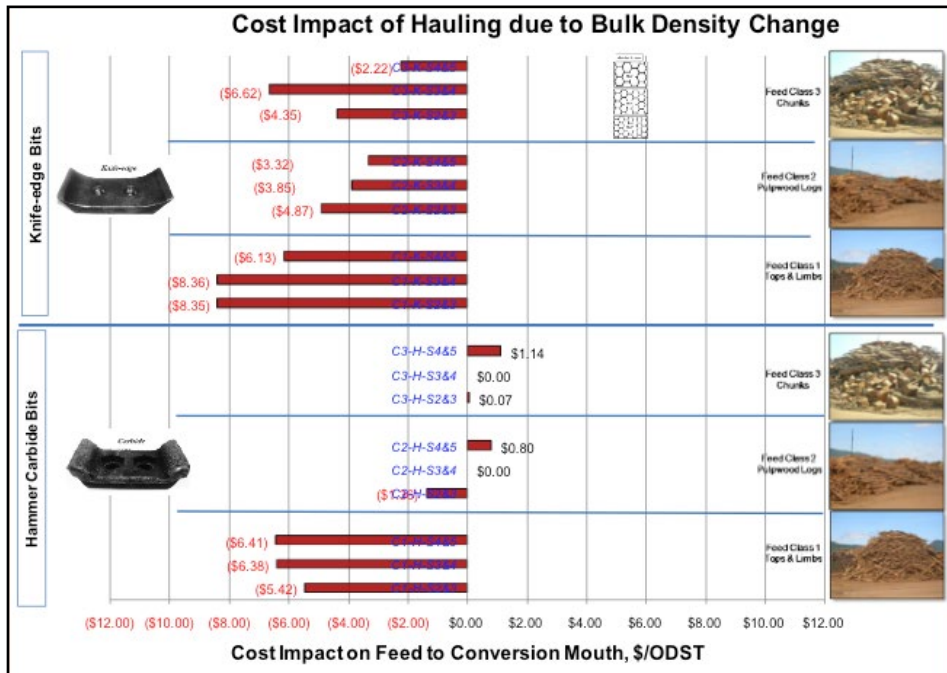


Figure FL-1.6. Bulk density differences impact hauling costs to a significant degree - \$9.50/ODST

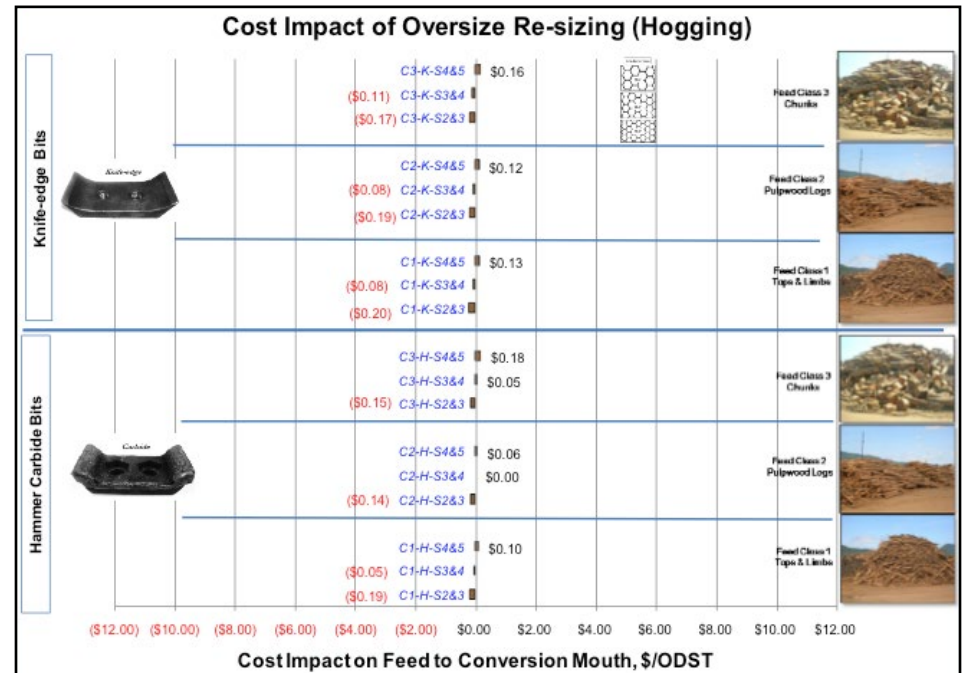


Figure FL-1.8. Surprisingly, when expressed on a feed basis, the oversize re-sizing total costs are almost trivial, ranging only a total of \$0.38/ODST feedstock

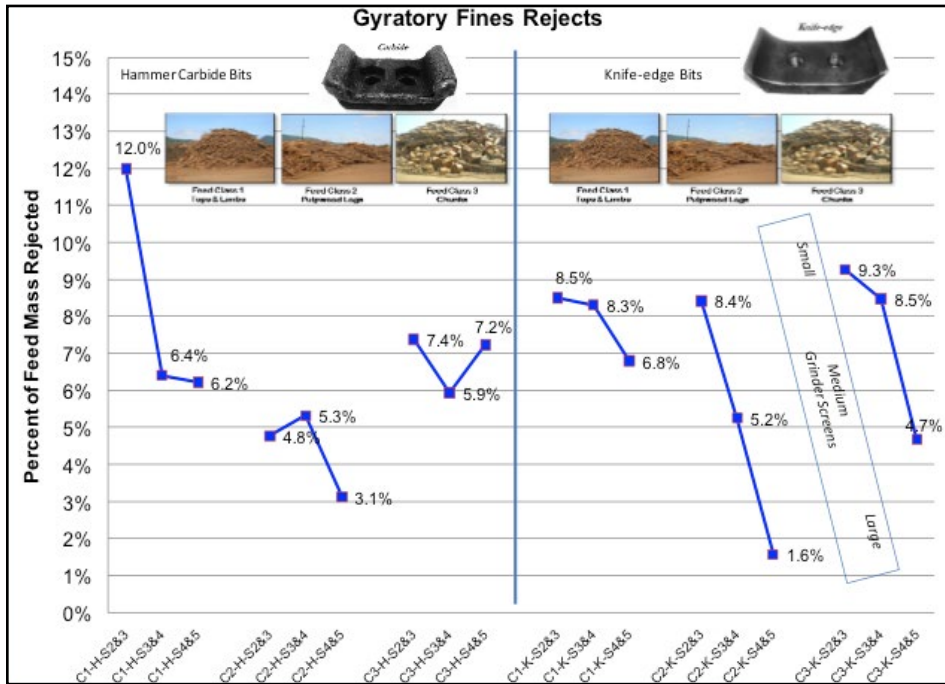


Figure FL-1.9. Rejects to fines can vary quite dramatically, relatively

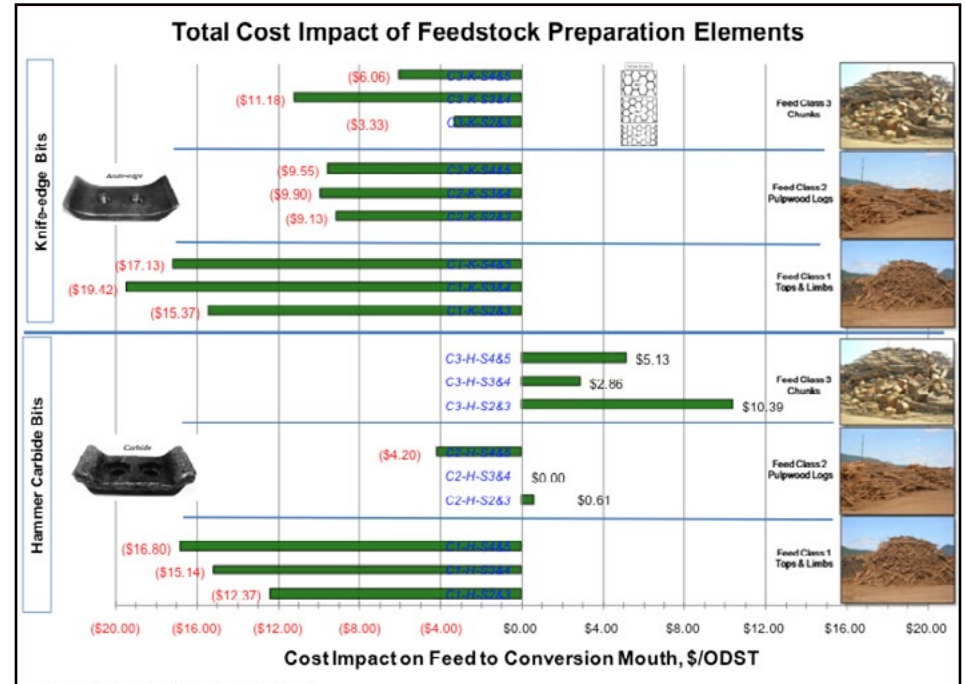


Figure FL-1.11. The net total impact range is about \$30/ODST feedstock to conversion mouth

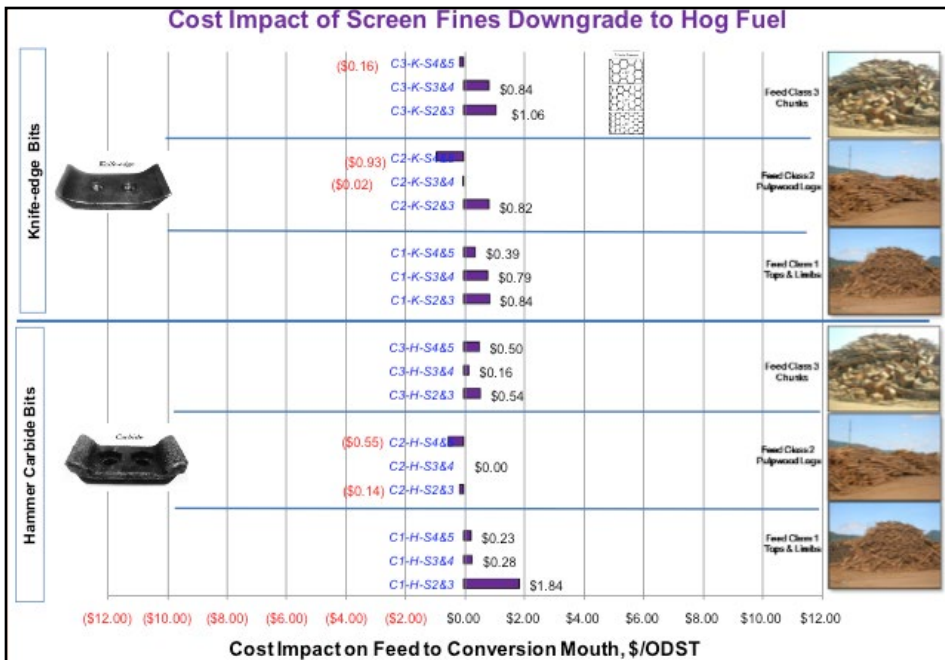


Figure FL-1.10. The cost impact of fines downgrade to hog fuel value has an impact of about \$2.77/ODST feed material

Recommendations | Conclusions

- 1) Lowest grinding power was achieved by: a) starting with smaller piece sizes, b) grinding to larger final sizes, and c) using (sharp) knife bits instead of (blunt) hammer bits.
- 2) Under the assumptions used here, total grinding costs are the largest impact factor, in the directions noted in results, to the tune of \$22 / ODST impact range.
- 3) Highest bulk density was obtained with: a) smaller feed piece size class – tops & limbs, otherwise, with b) knife bits compared to hammer.
 - The reason for higher bulk density with tops and limbs is probably due to some combination of higher wood density, greater fines content due to drier wood and higher bark content.
- 4) Higher bulk density (as long as moisture is low enough) reduces hauling cost and is the second most powerful economic effect, having an impact range of \$10 / ODST.
- 5) Oversize material production is, logically, almost totally controlled by grinder screen size.
 - The economic impact of resizing oversize is very small – impact range of <\$0.40 / ODST.
- 6) Fines downgraded to hog fuel is mostly related to grinder screen size, particularly for tops and limbs with hammer bits.
 - The economic impact of fines downgrade is relatively small – impact range of <\$3 / ODST.
- 7) Overall, the total net impact of variables assessed here can be quite large – the impact range is \$30 / ODST.
 - Because both lower total grinding costs and higher bulk density was achieved consistently with tops and limbs, this Feed Piece Size class was consistently economically favored for both bit types.
 - For other Feed Class Piece Sizes (pulp logs and chunks), knife bits were economically favorable to hammer bits, mostly due to lower grinding costs and higher bulk density for knife bits.

CAVEATS

- 1) Knife bits are somewhat more expensive and likely have higher maintenance costs and these characteristics were not tested in this trial.
- 2) It is probably not realistic to sort material size classes in practice. That is, avoiding grinding material after one is already set up at the harvesting site is not logical, even if slightly more expensive on a per unit cost. What materials remain in residue piles is likely driven by pulp material market demand.
- 3) Some of the bulk density benefit of tops and limbs is probably due to higher bark content creating more fines, which have lower conversion yield and are not accounted for in this analysis.
- 4) While the tops and limbs have cost advantages to conversion, they also will have a lower conversion yield due to higher bark content – that impact has not been quantified here.

RECOMMENDATIONS

- 1) Samples of each material type should be tested via the NARA analytical protocol (that is, added to the feedstock database) so that approximations of economic impacts of higher bark (really, lower total polysaccharides) can be quantified. This impact can be compared to the impacts cited in this report.
- 2) Although a “pulp chip” type size criteria was used for the work here, there is no disciplined analysis of optimum particle size distribution to conversion that trades added cost for preparing smaller particles against the presumed decreased conversion costs. This optimization should be explored for the downselected NARA pretreatment).

PHYSICAL AND INTELLECTUAL OUTPUTS

- Three additional forest harvest residue samples were collected, processed, and characterized for the NARA Feedstocks database.

TASK FL-2: LOGISTICS DECISION SUPPORT AND IMPROVEMENT

Key Personnel

John Sessions
Kevin Boston
Loren Kellogg

Affiliation

Oregon State University
Oregon State University
Oregon State University

Task Description

Synthesize existing feedstock supply chains for collection, preprocessing, storage, and transport to support model development. Develop biomass efficiency recovery factors linked to forest type and harvest methods; quantify grinding and chipping production costs and their ability to meet alternative feedstock specifications; develop and test operational strategies and decision support systems to reduce moisture content for long distance wood transport; work with trailer manufacturer partners to demonstrate advanced trailer configurations to increase load efficiency and performance of chip vans on highway and on forest roads to improve access, reduce weight, and increase capacity; compare mobile versus stationary chipping/grinding strategies under a range of field conditions and operating strategies; evaluate any new processes for worker health and safety.

TASK 1 REVISION FOR YEAR 3 - EXTENDED SCOPE OF WORK

PI: Kevin Boston

This project will develop a double sampling strategy that will combine both the OSU methods to determine the available recoverable volume of biomass following harvesting operations and the Montana Bureau of Industrial Statistics Method to determine the total volume of biomass that is possible. Harvesting units that were measured by the BERR groups to determine the total volume of biomass will have their piles measured by the OSU group to determine the recoverable biomass. The result will create a ratio that can

be used to refine biomass estimates. These ratios will be developed for the two concentration areas, central Oregon and southwestern Washington to further refine the biomass estimates in the woodsheds for the potential sites. Additionally, these differences between these two estimates are useful for other NARA functions, as it will be an estimate of the amount of biomass that remains on site for nutrient cycling and potential wildlife habitat.

- Deliverable: Field procedures and statistical model to develop recoverable biomass from timber product output (TPO) data. Draft field procedures to be developed by June 30, 2014. Final report due September 30, 2014.

Activities and Results

Task FL-2.1 is to develop biomass recovery coefficients for the NARA region. Fieldwork has been completed and the data is currently being summarized to prepare reports to determine the recovery coefficients at various costs for collection. A second study to focus on the southwest Washington and central Oregon areas is being developed. One manuscript has been published. Field procedures and a statistical model to develop recoverable biomass from TPO data are being developed for implementation in July 2014. Analysis to support an additional manuscript to determine the economically available biomass is in preparation.

Task FL-2.2 is to develop moisture management strategies and models. The first logging residue moisture monitoring trial was installed on an Oregon Coast Range Douglas-fir stand. Two slash piles of different sizes were instrumented with temperature, relative humidity and solar radiation sensors recording hourly. Temperature and relative humidity sensors were located at different heights and depth of the pile.

Additionally, a weather station monitoring relative humidity, air temperature, wind speed and precipitation was installed on the same harvest unit. This unit is being visited monthly to check equipment, download data and collect scattered residue samples for wood moisture content determination. This experiment will be finished in July 2014, completing one year of monitoring. Partial results for scattered residue moisture are resented in Figure FL-2.1. As expected, scattered residue has been gathering moisture through the winter and it is starting to dry as spring approaches.

Based on the results from this first installation, monitoring protocols have been modified and field plans are being made for replicated installations in hemlock, wet Douglas-fir, dry Douglas-fir, and ponderosa pine stands in Spring/early Summer of 2014. These will be monitored for one year. Monitoring of live branch wood moisture content on four sites (hemlock, wet Douglas-fir, dry Douglas-fir, and ponderosa pine) will be completed July, 2014. Preliminary results shown in Table FL-2.1 indicate that live branches have moisture content lower than 50% in most tested sites during fall and winter seasons.

Task FL-2.3 is to refine collection and transport models for regional modeling. The forest residue decision support system, RENO, estimates grinding and transport costs and was completed in July, 2013. Three manuscripts were prepared, two of which are in press. Field data for a forest residue collection model were completed February, 2014. Data analysis and model development will be completed Spring 2014. Field data from Boston/Long will be used to estimate collection costs that will be applied to regional modeling. NARA researchers Adams/Latta are currently using this preliminary data for regional modeling.

Task FL-2.4 is to evaluate chipping and grinding production to meet alternative feedstock specifications. Structured grinding tests to quantify grinding

and chipping production costs and its ability to meet alternative feedstock specifications at low moisture contents were completed in August. Two manuscripts are in review and one additional manuscript is in preparation. In general, knife-edge bits produced denser grindings compared to carbide bits. Knife-edge bits produced a higher proportion of fine particles (pieces < 0.95 cm) compared to carbide bits. Oversized particles (> 7.62 cm) were also reduced with the use of knife-edge bits. Significant decreases in fuel consumption can be achieved when using knife-edge bits compared to carbide bits. Although several benefits can be obtained by using knife-edge bits, they are more susceptible to dirt, and they cost approximately \$10 more per hour compared to carbide bits. Although productivity was not measured directly, power requirement is related to fuel consumption. The lower the fuel consumption per unit volume, the higher the potential productivity of the grinder (Table FL-2.2).

In terms of screen size, a smaller screen size combination produced denser material compared to larger screen combinations, however the effect was not as evident as with the bit type. In branches and tops a slight effect of screen size was found. As piece size increased, stronger differences, especially between the small screen compared to the medium and large screen combination, was observed. Although the effect of screen in bulk density did not show the same trend for all the treatments, fuel was highly impacted by the screen size especially when using carbide bits. Since carbide bits lack the ability to cut the material, they depend on the hammering of the residue, and thus they are more affected by a reduction in the screen size than knife-edge bits. The screen size also affected the proportion of oversized particles. In general, a higher proportion of oversized particles were found when processing the material using large screen compared to the small screen combination. Limbs produced denser material compared to the other classes. This was caused in part by the higher specific gravity of this grindings compared to the other size classes. The known presence of reaction wood in branches can be related to this increase in

specific gravity. In addition, the proportion of fines in this class was higher, probably due to the presence of dust, sand and dirt in general. Non-wood content is higher in branches and tops as compared to larger size classes due to larger pieces being partially debarked by the log processor when the log manufacturing was performed at the landing during the harvesting, particularly in the spring when bark is looser on the stem.

Preliminary attempts at increasing bulk density through vertical acceleration of grindings by blowing into a dump truck trailer resulted in significant increases in bulk density (Figure FL-2.2). Blower tests included grindings from standard carbide bits, knife-edge bits, and a combination of standard carbide/knife-edge bits. Grinding of fresh forest residues at high moisture content (50+ percent) and testing of baling green residues was completed in early March. Lab tests are ongoing.

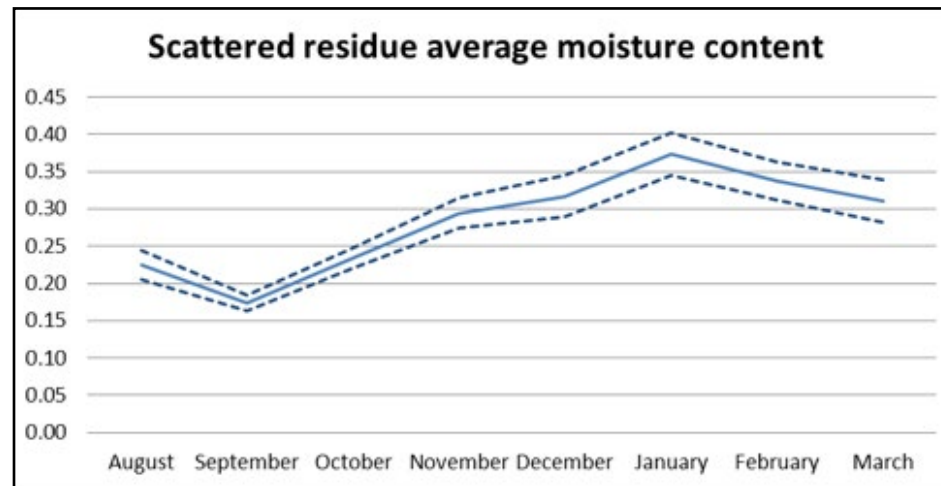


Figure FL-2.1. Average and 95% confidence intervals for scattered residue moisture content (wet basis)

Table FL-2.1. Live branch moisture content (wet basis) for different forest types

Stand	Season	Sample mean	Std Dev	SE	U95CL	L95CL	Max	Min
Valley - D-Fir	Summer	0.45	0.07	0.013	0.47	0.42	0.58	0.23
	Fall	0.48	0.06	0.019	0.51	0.44	0.58	0.28
	Winter	0.47	0.06	0.020	0.51	0.43	0.67	0.28
Sisters-PondPine	Summer	0.44	0.07	0.008	0.45	0.42	0.59	0.13
	Fall	0.48	0.06	0.027	0.53	0.42	0.59	0.31
	Winter	0.51	0.06	0.020	0.55	0.47	0.63	0.25
Newport- WHemlock	Summer	0.48	0.06	0.018	0.52	0.45	0.67	0.29
	Fall	0.49	0.06	0.029	0.54	0.43	0.63	0.33
	Winter	0.51	0.09	0.026	0.56	0.46	0.75	0.33
Dry -D-Fir	Fall	0.48	0.07	0.015	0.51	0.45	0.75	0.26
	Winter	0.50	0.07	0.026	0.55	0.45	0.73	0.31

Table FL-2.2. Estimated productivity (BDT/hr) of a grinder averaging 550 hp output as a function of feedstock, bit type and screen size

Bit Type/Screen Size	2&3"	3&4"	4&5"
Limbs and Tops			
Knife-Edge	36.28	57.45	54.98
Carbide	39.21	41.20	49.96
Pulpwood Logs			
Knife-Edge	28.87	31.23	30.14
Carbide	19.08	21.39	27.98
Large Chunks, Butt Cuts			
Knife-Edge	21.20	29.65	26.83
Carbide	13.97	18.75	18.20

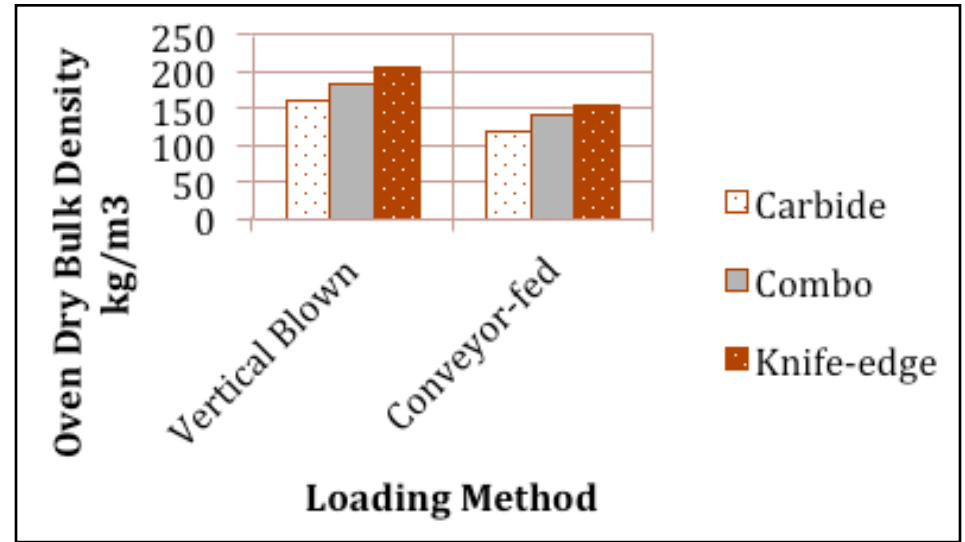


Figure FL-2.2. In-place bulk density as a function of loading method

Recommendations | Conclusions

Grinder efficiency of forest residues at low moisture content showed that grinder parameters and feedstock size have important impacts on grinder fuel consumption per dry ton, grinder production, bulk density, and particle size distribution. A collaborative economic analysis with NARA researcher Gevan Marrs suggests that downstream handling at the mill to accommodate overs and fines adds little cost, and emphasis needs to be placed on potential increases in grinder productivity and transport efficiency. Grinding tests of different feedstock components also suggests that the presence or absence of pulpwood markets will influence grinder productivity, fuel consumption, bulk density, and non-wood components. Non-wood products negatively impact sugar recovery.

Blower tests on dry forest residues suggest significant gains in bulk density can be achieved by vertical acceleration of dry grindings. This is particularly relevant as chip vans loaded with ground residues at less than 40% moisture content are often volume limited. Chip vans carrying ground residues in western Oregon/Washington generally carry about 15-16 BDT. It is

quite possible to achieve 22 BDT with a combination of lower moisture content coupled with increased bulk density. This would reduce transport costs up to 33%. At longer hauls, transport efficiency becomes increasingly important.

The grinding tests indicate that using knife-edge bits will reduce grinding costs by reducing fuel consumption per dry ton processed. Theoretically, grinder productivity should also increase proportionally, but other factors affect grinder utilization including grinder/truck interactions, road accessibility, truck scheduling, and more frequent changes of the bits.

Work in Year 4 will:

- Complete the work extension of Task FL-2.1 to collect biomass data using TPO data.
- Complete the work extension of Task FL-2.1 to collect biomass data using TPO data in collaboration with the BEER group.
- Complete live-branch analysis of moisture content to understand impact of harvest season on beginning moisture content of harvest residues.

- Complete installation of field plots for monitoring field moisture content of piled and unpiled residues and continue data collection.
- Complete the analysis of high moisture residues. It is possible that high moisture residues are less cost effective due to high non-wood content and low net dry wood per truckload. Samples have been shipped to Weyerhaeuser to test Impacts on sugar yield.
- Complete a preliminary supply chain analysis of baled residues.
- Work with industrial cooperators to test methods for increasing bulk density of ground residues
- Install a set of structured tests designed to improve large trailer access to forest sites through controlling road cross slope on horizontal curves.

Physical and Intellectual Outputs

- 59 harvest units were surveyed to measure forest residue piles
- 4 plots of a total of 36 trees were established in four geographical regions to monitor live branch moisture quarterly
- 2 plots of forest residue piles were instrumented for measuring pile temperature, relative humidity, and climatic variables
- 890 tons of forest harvest residues were transported to the Weyerhaeuser TOPS facility in Springfield, Oregon
- 96 samples of forest harvest residues were ground at the Weyerhaeuser TOPS yard and characterized at the Oregon State University, College of Forestry laboratories
- 36 bales of forest harvest residues were made by Forest Concepts at the TOPS yard
- 30 samples of ground forest harvest residues were shipped to Weyerhaeuser for physical and chemical testing.

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Zamora, R., J. Sessions and D. Smith. Effect of Grinder Parameters on Particle Size and Fuel Consumption. In review. Biomass and Bioenergy.

Zamora, R., J. Sessions and K. Boston. Economic Optimization of Forest Biomass Processing and Transport. In review. Forest Science.

Clark, J., Sessions and R. Zamora. Optimizing Knife Change Times for Forest Biomass Chipping Operations. In review. Forest Products J.

Zamora R, Boston K, Sessions J, and Murphy G. Stochastic simulation and optimization of mobile

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Zamora, R., J. Sessions, G. Murphy and K. Boston. 2013. Economic impact of truck-machine interference in forest biomass recovery operations on steep terrain. In press. *Forest Products Journal*. Published online October 2013. (doi: 10.13073/FPJ-D-13-00031)

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Smith, D., J. Sessions, K. Tuers, D. Way and J. Travers. 2013. Characteristics of Forest Derived Woody Biomass Collected and Processed in Oregon. *Forest Products J.* 62(7/8):520-527.

Kim, D.W. and G. Murphy. 2013. Forecasting air drying rates of small Douglas-fir and hybrid poplar

stacked logs in Oregon, USA. *International Journal of Forest Engineering* 24(2): 137-147.

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RESEARCH PRESENTATIONS

Posters:

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Zamora, R. and J. Sessions. 2014. Forest Harvest Residue and Bioenergy Research Program. Prepared for 2014 Annual Meeting of Western Region Council on Forest Engineering. January 16, Valley River Inn, Eugene, OR.

Zamora, R., J. Sessions, and D. Smith. 2013. Structured grinding tests: Increasing bulk density to decrease transportation costs. Prepared for 2013 Annual Meeting for the Northwest Advanced Renewables Alliance (NARA), Sept. 10-12, Corvallis, OR.

Zamora, R., and J. Sessions. 2013. RENO: Residue evaluation and network optimization software. Prepared for 2013 Annual Meeting for the Northwest

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Belart, F., J. Sessions, G. Murphy, M. Jolly and K. Tuers. 2013. Moisture management model for optimal forest biomass delivery in the Pacific Northwest. Prepared for 2013 Annual Meeting for the Northwest Advanced Renewables Alliance (NARA), Sept. 10-12, Corvallis, OR.

Presentations:

Marrs, G., J. Sessions, and R. Zamora. 2014. Economic Impacts of Forest Residue Feedstocks Preparation for Biofuels. In preparation for Bioenergy + Co-products Conference, April 28-30, Red Lion Hotel, Seattle, WA.

Zamora, R. 2014. Improving efficiencies in biomass collection and transportation. Presented at Washington Contract Loggers Association (WCLA) Annual Meeting. March 14, Red Lion Hotel, Spokane, WA.

Long, J, and K. Boston, 2013, Measuring Biomass Using LiDAR, Lasers, and Lucky guesses. Field Technology Conference for Data Collection and Mapping in Forestry and Natural Resources. October 23-24, Portland Oregon.

Zhu, J.Y., C. Zhang, R. Gleisner and J. Sessions. 2013. Upgrading forest residues of Douglas-fir through physical fractionation for fermentable sugar production. Presented at 35th Symposium on Biotechnology for Fuels and Chemicals. April 29-May 2, 2013. Hilton Hotel, Portland, OR.

Sessions, J. 2013. Jet Fuel from Woody Biomass. Presentation at 68th Olympic Logging Conference, May 1-3, Fairmont Empress Hotel, Victoria, BC, Canada.

Sessions, J. 2013. Jet Fuel from Woody Biomass. Presentation at 76th Oregon Logging Conference, Feb 21-23, Lane County Convention Center, Eugene, OR.

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Zamora, R. and J. Sessions. 2013 RENO: Economic optimization of forest biomass and transport. 2013 Western Forestry Graduate Research Symposium. OSU College of Forestry, Corvallis, April 23.

THESIS AND DISSERTATIONS

Zamora, Rene. 2013. Economic Optimization of Forest Biomass Processing and Transport. PhD thesis. Completed May 23, 2013. John Sessions, Advisor.

TASK FD-2: PHENOMICS ANALYSIS

Key Personnel
Helmut Kirchhoff

Affiliation
Washington State University

Task Description

Selected plant lines will be subjected to phenomics analyses. This phenomics system currently relies upon chlorophyll fluorescence analysis, a well-established and versatile tool for studying stress response in plants in situ. In addition to numerous studies on annual plants, this technique has also been applied to study salt-stress responses in poplar trees. Therefore, chlorophyll fluorescence based phenomics will be used as a second screening filter to identify softwood tree individuals, Douglas-fir and western hemlock, that are best adapted to their designated growth habitat. The WSU phenomics facility will speed up the selection process for three reasons: (i) Chlorophyll fluorescence screening can identify stress before it becomes visible; (ii) It is non-invasive, thus screened plants can be further used; (iii) It is fully automated and therefore allows the screening of a large number of plants. Furthermore, due to a fast detection system, screening parameters can be measured multiple times ensuring good statistics and therefore high fidelity data.

The following parameters will be examined because they are expected to be most relevant for growing trees in different designated areas such as: (i) soil composition (N-deprived soil), (ii) fertilizers (iii) salinity, (iv) drought. Softwood trees will be used which passed the first selection criteria for maximal biomass production and quality. Trees to be screened are Douglas fir, at stress conditions. For each of the four parameters, a set of the 500 selected individuals will be grown and screened in parallel (about 2000 plants total). Beside morphometric parameters (leaf size and number, chlorophyll content), the maximal photochemical quantum efficiency, photosynthetic electron

flux, capacity of photoprotective non-photochemical quenching, as well as the degree of photoinhibition of photosystem II will be deduced from chlorophyll fluorescence measurements. This detailed analysis will indicate the stress status of the trees under the different environmental parameters and will allow selection of the most robust plants.

Activities and Results

A large screening on about 1000 Douglas-fir plants in the WSU Phenomics facility is planned for development of the SNP chip. In follow-up discussions with the Feedstock Development group (Dr. Howe, Dr. Jayawickrama, Dr. Kirchhoff), after the 2013 NARA meeting, it was decided to postpone these measurements to early summer 2014. The reason for postponing is that the Douglas-fir trees go into dormancy and therefore the meaning of data collected in the winter on these trees for summer drought resistance is limited. Also, the existing pool of trees at OSU is too valuable to start a large-screen drought experiment with the risk that the plants will not survive this treatment. To be as well prepared as possible for the summer measurements, a pilot experiment with Douglas-fir trees provided by Dr. Jayawickrama (OSU) was conducted.

For the pilot experiment about 50 trees were screened in the WSU Phenomics facility during the reporting period. The trees were acclimated in a greenhouse and transported into the Phenomics facility after two weeks of acclimation. The purpose of this pilot experiment is to gain further experience in collecting data with Douglas-fir and to induce drought stress that will be used for the summer experiment.

Figure FD-2.1 shows an example how the plants were measured in the facility. The arrangement of the plants was organized in a way that avoids overlapping growth. This is essential for assignment of individual plants. Compared to previous measurements, the plants were grown in smaller pots (Fig. FD-2.1).

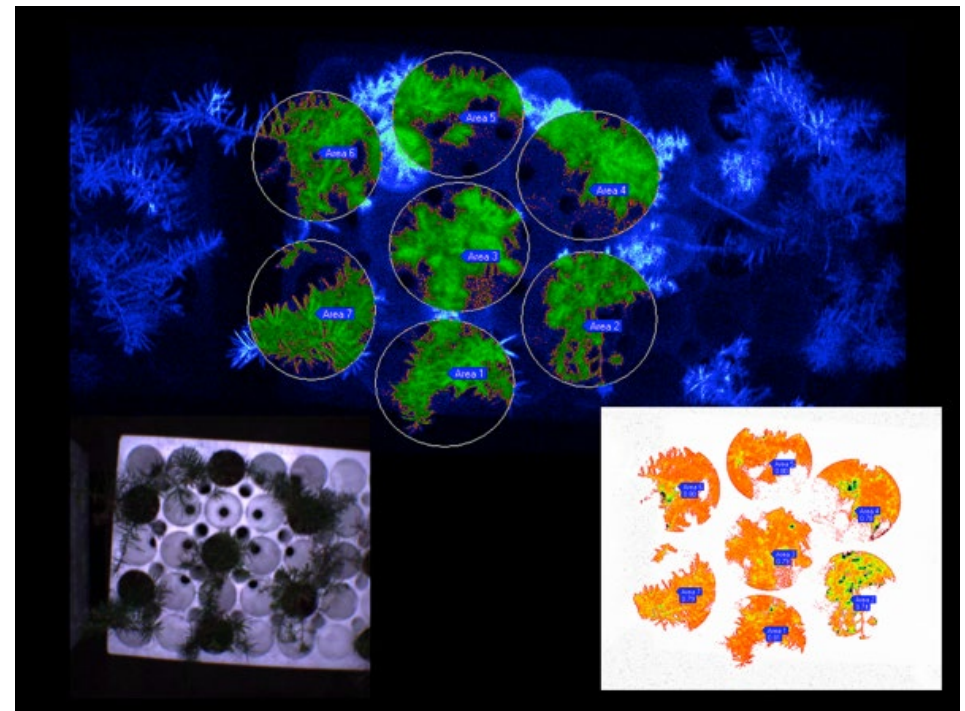


Figure FD-2.1. Example for Screening Douglas-fir in the WSU phenomics facility. Bottom left, top view on Douglas-fir seedlings. The pattern of the plants were optimized to minimize overlap of the individual plants. Top, example of selected plants. Bottom right, False color chlorophyll fluorescence image.

Figure FD-2.2 shows analysis of chlorophyll fluorescence parameters. After watering was stopped (day 0), the maximal photochemical fluorescence parameter (F_v/F_m) starts to decline significantly starting on the fourth day. This decline is well correlated to stress and is accompanied by a decrease in photosynthetic electron flux (Phi II parameter). Furthermore, the decrease in the q_L parameter indicates redox pressure on the photosynthetic electron transport chain caused by less efficient CO_2 fixation by the Calvin-Benson cycle. This in turn is caused by drought-stress induced stomatal closure. The decline in photoprotective high energy quenching is indicated by the qE parameter. That is most likely caused by loss of energization of the photosynthetic membranes. Overall, the photosynthetic parameters measured in the pilot experiment consistently show drought-induced stress symptoms that are caused by closure of stomata. Compared to previous measurements of Douglas-fir in the phenomics facility, the drought response was faster. This is due to the smaller pot size in this round of experiments. The smaller pot size also allows screening more plants. The successful pilot experiment indicates that all is set for the large screen scheduled for summer 2014. Due to the fast plant response, it is expected that the drought period can be shortened to three to four weeks.

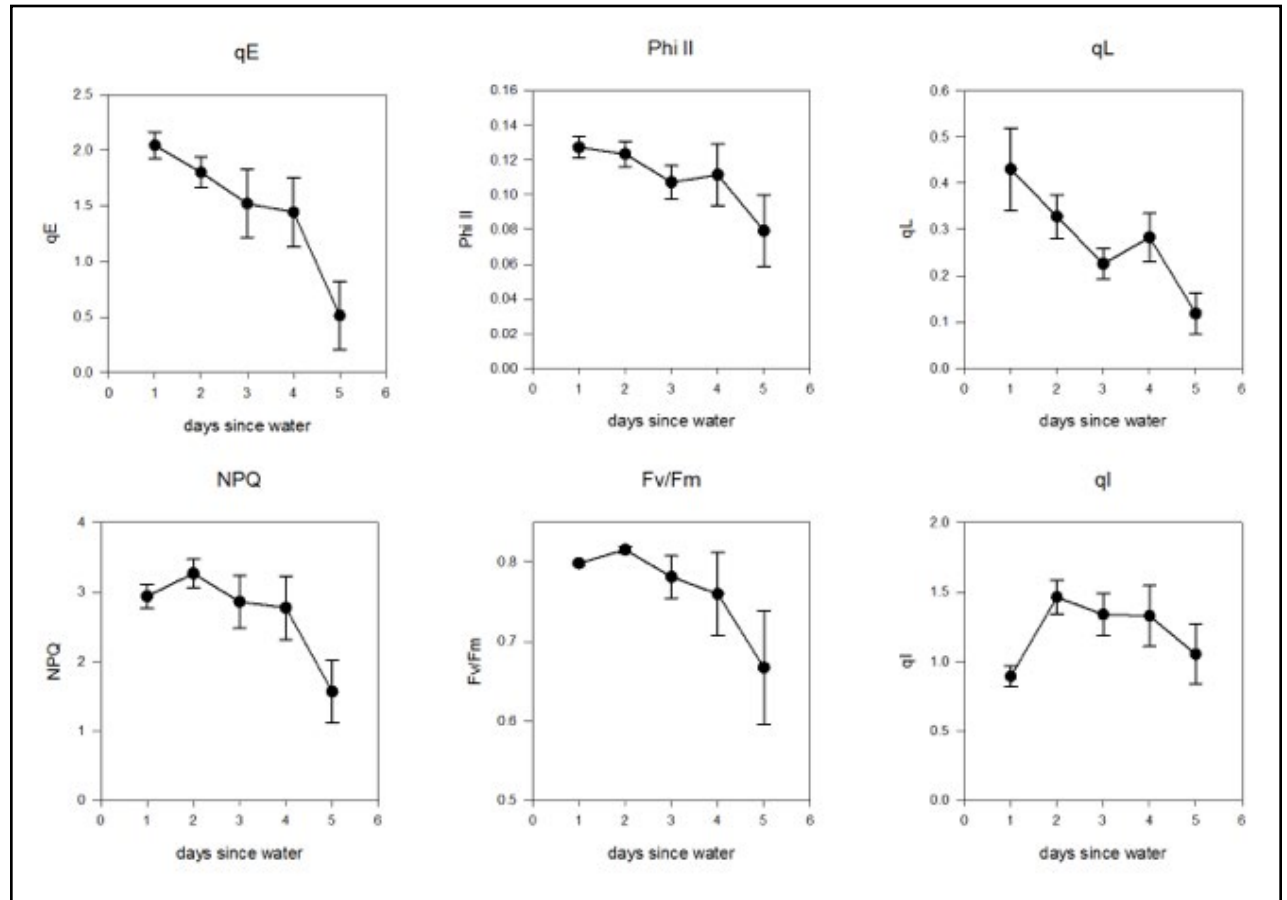


Figure FD-2.2. Analysis of chlorophyll fluorescence parameters. Moderate watered Douglas-fir trees were exposed to drought stress at day '0'

Physical and Intellectual Outputs

REFEREED PUBLICATIONS (ACCEPTED OR COMPLETED)

Kirchhoff, H. 2014. Diffusion of molecules and macromolecules in thylakoid membranes. *Biochim. Biophys. Acta*, 1837(4), pp.495-502. DOI: [10.1016/j.bbabi.2013.11.003](https://doi.org/10.1016/j.bbabi.2013.11.003)

RESEARCH PRESENTATIONS

Hoehner, R. Poster at the 16th International Photosynthesis Congress, Phenomics symposium. August 11-13, 2013, Saint Louis, MO, USA

TASK FD-3: COMBINING GENOMIC AND FIELD-BASED BREEDING AND TESTING METHODS TO IMPROVE WOODY FEEDSTOCK PRODUCTION

Key Personnel

Keith Jayawickrama

Affiliation

Oregon State University

Task Description

Genetic selection and testing has been applied on timber species in the west for over 50 years. One result of that work is data and genetic gain predictions for several traits from replicated, randomized progeny tests for over 30,000 families of Douglas-fir and western hemlock. A range of phenotypic variation, and some level of genetic control, has been demonstrated between families for every trait studied, so variation and genetic control in traits pertaining to biofuel production can be expected. Another result is that over 150,000 timberland acres are reforested annually with seedlings from open-pollinated seed orchards, thus delivering real genetic gains (in whatever traits are selected for) to operational plantations in the west.

Over the last decade, the cost of using genomic and marker-based tools to complement field-based breeding and testing has dropped rapidly in forest tree species. These tools have the potential to improve the efficiency, speed of delivering genetic gain, especially given the long times needed for field-based breeding, and reduce cost. Recent advances by the Conifer Translational Genomics Network (a multi-institution project for major US conifers) can be put to use in this project. It is proposed, as an expanded/strengthened Task 2 (Identify single nucleotide polymorphisms [SNP] genotypes), to use the power of both of these approaches in tandem. A state-of-the-art genotyping array will be developed based on SNP technology for marker-based selection of phenotypes conducive to the production of biofuels from woody residuals. The intent is to add these traits to trees already selected for the production of lumber and other products of saw logs.

The specific objectives of this project are: (1) Quantify the phenotypic variation in biofuel production potential in a subset of Douglas-fir and western hemlock families, pre-selected for commercially important traits such as rapid growth, adaptability, wood specific gravity and wood stiffness; (2) (expanded/strengthened Task 2) Identify SNP genetic markers in Douglas-fir associated with useful variations in biofuel production potential; and (3) Make selections for increased biofuel production in woody residuals of trees developed for use as saw logs using a combination of phenotypic and SNP genetic marker data.

Activities and Results

Task FD-3.1. Collect samples and combine with phenotypic data

Sample collection from the Moon Creek site of the CL98 series, for a total of 360 samples, was completed. All samples were processed and shipped to the Zhang lab at WSU.

Genetic gains for pretreatment yield, pretreated holocellulose, enzymatic hydrolysis yield, and recalcitrance factor have been predicted for 145 trees and 43 parents from the T96 population, and 45 trees from the Moon Creek population. Pretreatment yield, pretreated holocellulose, enzymatic hydrolysis yield and recalcitrance factor have been assessed on 220 more trees from the Moon Creek population. Pretreatment heritability estimates from the CL98 population appeared to be much higher than in the T96 population, so efforts are focused on CL98. The preliminary estimates of predicted genetic gain show that it would be possible to genetically select for decreased recalcitrance factor.

Task FD-3.2. Collect tree tissue samples and obtain marker data

Very good progress was made on identifying markers to be used in the SNP chip, with collaboration from NCGR. Another focus was on narrowing down the optimal population to genotype. Selecting the most appropriate population of trees is very important, since the SNP chip is expensive and only a limited number of trees can be genotyped. First, a simulation program called QMSIM was evaluated but was later modified in-house. This in-house program (Tree Genome Simulator) can simulate the types of mixed-matings that occur in natural populations of Douglas-fir.

A 3-generation pilot genomic selection population with elite genetic material from a cooperative Douglas-fir breeding program was developed. Consent was obtained from the breeding program to use the required seed from 3rd-cycle crosses; for greenhouse space to sow the study in 2014; and for agreement by a large industrial landowner to outplant the study in 2015. The trial was sown at the end of March, 2014.

The pool of trees considered for processing through the array are as follows:

Z (≈ 49) grandparents of 3rd cycle crosses
X (≈ 38) parents of 3rd cycle crosses
 $\sum Y_i$ (≈ 340) 2nd cycle progeny
Total training population = 427

$\approx 1,000$ 3rd cycle progeny (selection population)
 ≈ 400 2nd-cycle T96 and CL98 trees used in wood chemistry analysis
 ≈ 50 Parents of T96 and CL98 trees
Total = 1,920

Foliage samples from the parents and grandparents in the training population have been collected. Arrangements have been made for extracting DNA from all the samples.

OSU intends to award the contract for building the genotyping array to GeneSeek (based in Lincoln Nebraska). The array is to be in the order of 15,000-50,000 SNPs, capable of running about 1,900 samples, and costing in the range of \$144,000 – 150,000. The contract will likely be finalized and signed by April 21. Due to the cost of the contract and OSU contracting rules, a Request for Proposals is in preparation.

Task FD-3.4. Provide Douglas-fir seedlings for phenomics study

The preliminary trial at the Kirchoff lab indicated that the drought period applied to the first batch of seedlings was too short for Douglas-fir, leading them to revise the experimental plan for the main group of seedlings. Over 1,000 seedlings from 107 elite families were transplanted to 615A cavities in May 2013.

The main phenomics study was postponed to May 2014 since any drought-hardiness/ stress response results obtained over the winter of 2013-14 (dormant season) would be less valuable and informative than those obtained when the seedlings are actively growing. The seedlings will be kept at their current location (BLM Sprague Seed Orchard in Merlin, OR) till May 2014. At that point, seedlings will be sent to Pullman using a refrigerated truck.

In late November 2013, 50 seedlings were shipped to the Kichhoff lab for preliminary drought application and imaging work.

Recommendations | Conclusions Physical and Intellectual Outputs

The preliminary estimates of heritability and predicted genetic gains show that it would be possible to genetically select for decreased recalcitrance factor. It looks as though a SNP chip can be built with considerably greater capacity than originally estimated.

REFEREED PUBLICATIONS (ACCEPTED OR COMPLETED)

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CONFERENCE PROCEEDING AND ABSTRACTS FROM PROFESSIONAL MEETINGS

Geleynse, S., Alvarez-Vasco, C., Jayawickrama, K., Trappe, M, Garcia, K. and Zhang, X. 2013. Phenotypic variations of biomass recalcitrance in Douglas-fir families. Poster presented at 35th Symposium on Biotechnology for Fuels and Chemicals, April 29- May 2, 2013. Hilton Portland, Portland, Oregon.

RESEARCH PRESENTATIONS

Oral, Posters or Display Presentations

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Jayawickrama, KJS. 2013. Overview of Feedstock Development: NARA Years 1-3. Presentation at 2nd NARA Annual Meeting, September 12, 2013, Oregon State University, Corvallis, OR

Geleynse, S., K. Jayawickrama, K. Garcia, M. Trappe and X. Zhang. Improving Douglas Fir Feedstocks by Screening Families for Biomass Recalcitrance. Poster presentation at the NARA Annual Meeting, Corvallis, OR, September 10, 2013.

TASK FD-4: GENETIC VARIATION UNDERLYING AMENABILITY TO PRETREATMENT/BIOCONVERSION

Key Personnel

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Affiliation

National Center for
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bioinformatic methods, already applied in Douglas-fir genetics, to arrive at a candidate set of new SNPs ready for validation. The goal is to supplement the existing SNP set with new polymorphic variants, with the particular goal of having SNPs in every Douglas-fir gene.

Task Description

NCGR (National Center for Genomic Resources) will build an updated reference transcriptome for Douglas-fir. NCGR will apply its Batched Parallel Assembly (BPA) software to these Illumina reads. The workhorse of the assembly is the ABySS assembler, which applies a De Bruijn graph approach. The ABySS kmer pool is fed into an overlap consensus using Mira to further contiguity and collapse redundancy. If the pool is very large (>several million sequences), a cd-hit-est can be performed to reduce the set to the best two cluster representatives. The final synthetic-EST set is then deredundified further using cd-hit-est if desired, and is fed into alignment using BWA. The existing reference transcriptome contigs can be fed into the BPA pipeline at the Mira stage, which will yield a new transcriptome reference that unifies the available resources.

SNP (Single Nucleotide Polymorphism) discovery and prioritization (Task FD-4.4) will be done by alignment of the newly available Illumina sequence reads to the updated reference transcriptome, and identification of mismatches. NCGR will do this by applying Alpheus, its high-throughput alignment and variant detection pipeline. Alpheus is a parallelized workflow that aligns short reads to the reference using the GSNAP algorithm, and parses the output to tabulate all variants that are discovered along with supporting statistics such as the number of reads having a variant, variant nucleotide quality, and sequence coverage at the variant position. Threshold heuristics are then applied to arrive at a draft set of putative variants that have strong support. Alpheus will be supplemented by

Activities and Results

This task has been modified and incorporated into Task FD-3: Combining Genomic and Field-based Breeding and Testing Methods to Improve Woody Feedstock Production

TASK FD-5: SCREEN AND IDENTIFY SUITABLE PLANT FEEDSTOCKS FOR LARGE SCALE PRETREATMENTS TO PRODUCE HIGH YIELD SUGAR AND HIGH QUALITY LIGNIN

Key Personnel

Xiao Zhang

Affiliation

Washington State University

Task Description

Biomass recalcitrance, a collective term describing the resistances of biomass material toward mechanical and/or biochemical deconstructions, is the key barrier hindering the development of an economically viable biomass conversion process. Despite the larger abundance, softwood and its forest residues are still not an economically viable feedstock for biofuel production. The feedstock collection, transportation and processing all contribute significantly to the overall cost. One effective means of reducing feedstock cost and subsequent conversion cost is to select biomass with high amounts of sugars and low recalcitrance toward releasing sugar.

Our work carried out in the last two years has clearly demonstrated that there is a significant variation in biomass recalcitrance among different Douglas-fir families. A parameter “recalcitrance factor” is introduced to quantify the level of biomass recalcitrance toward sugar production from different Douglas-fir families. The goal of this research is to develop and implement a selective feedstock breeding methodology used to identify and produce “ideal” softwood biomass that provides maximum sugar yields and reduced conversion (pretreatment and hydrolysis) cost.

Activities and Results

Significant progress has been made toward demonstrating a new methodology to determine biomass recalcitrance. From the work done in the previous year, a new methodology for measuring the recalcitrance of woody biomass has been completed on three levels (biomass composition, response to pretreatment, and enzymatic digestibility). This method was applied as a high-throughput screening tool to analyze Douglas-fir from different families. In addition to 150 tree samples previously shown, over 265 new tree samples collected from another plantation site have been fully analyzed in this year. It was found that a significant variation in the tree characteristics across all three biomass recalcitrance levels was observed (Figure FD-5.1). It was further observed that, in general, trees did not follow any major trend between any particular factor and the overall degree of recalcitrance (i.e. while a high lignin content is often considered an indicator of high recalcitrance, it is not necessarily the case here), and each tree can demonstrate differing susceptibility to different pretreatment methods. A parameter, recalcitrance factor, combining both physical and chemical properties contributing to biomass recalcitrance was applied to quantify the difference among different tree samples. The previous results suggest that the chemical compositions such as cellulose and lignin seem to associate with heritability. The additional analysis of the new sample pool from the different plantation site also indicated a relationship between recalcitrance factor and heritability may also exist. Detailed further analysis is currently being carried out at WSU and OSU. The outcome from this project has clearly demonstrated the complexity of the nature of biomass recalcitrance. The multi-level data generated from this new screening methodology can be used to identify and selectively breed low recalcitrance softwood feedstock for biochemical conversion, which will help reduce both feedstock and conversion costs.

Physical and Intellectual Outputs

REFEREED PUBLICATIONS (ACCEPTED OR COMPLETED)

Scott Geleynse, Carlos Alvarez-Vasco, Karissa Garcia, Keith Jayawickrama, Matt Trappe, Xiao Zhang. “A Multi-Level Analysis Approach to Measuring Variations in Biomass Recalcitrance of Douglas fir,” submitted.

RESEARCH PRESENTATIONS

Geleynse, S., K. Jayawickrama, K. Garcia, M. Trappe and X. Zhang. Improving Douglas Fir Feedstocks by Screening Families for Biomass Recalcitrance. Poster presentation at the NARA Annual Meeting, Corvallis, OR, September 10, 2013.

Garcia, K. Not all Douglas fir Trees are Created Equally for Conversion to Biofuels. Poster presentation at the NARA Summer Undergraduate Research Symposium, Pullman, WA, August 2, 2013.

Geleynse, S., “Not All Trees Are Created Equally: Exploring Variations in Douglas Fir Trees for Biofuel Production” WSU showcase, March 28, 2014

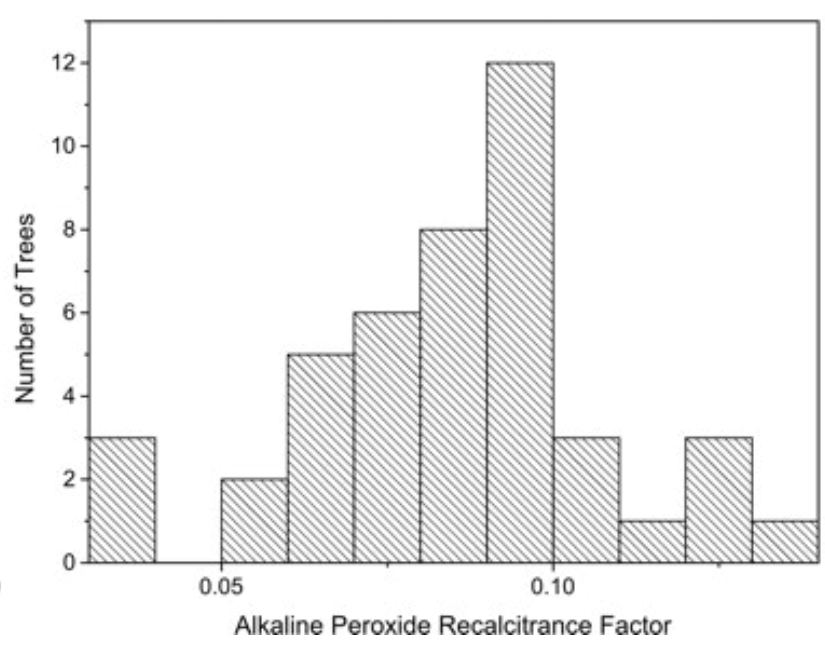
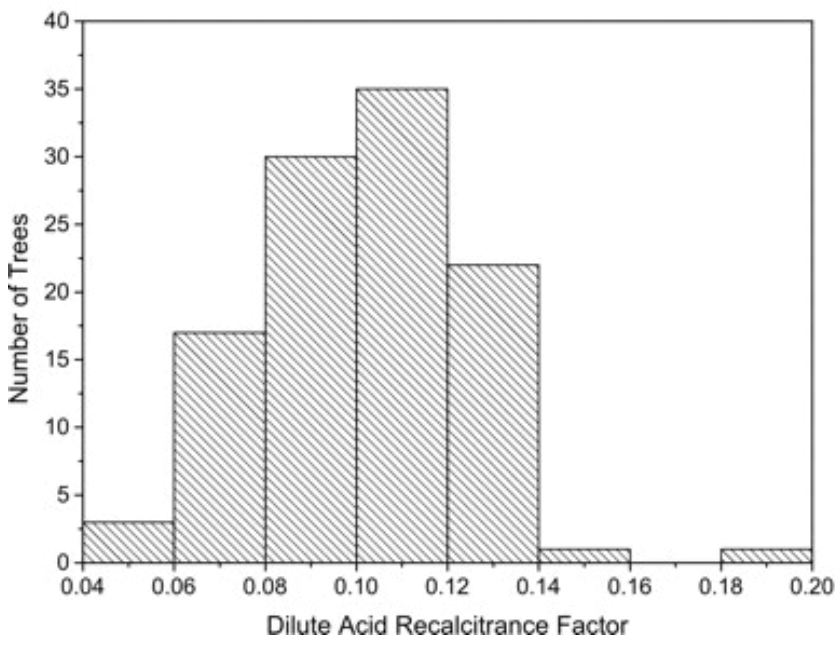
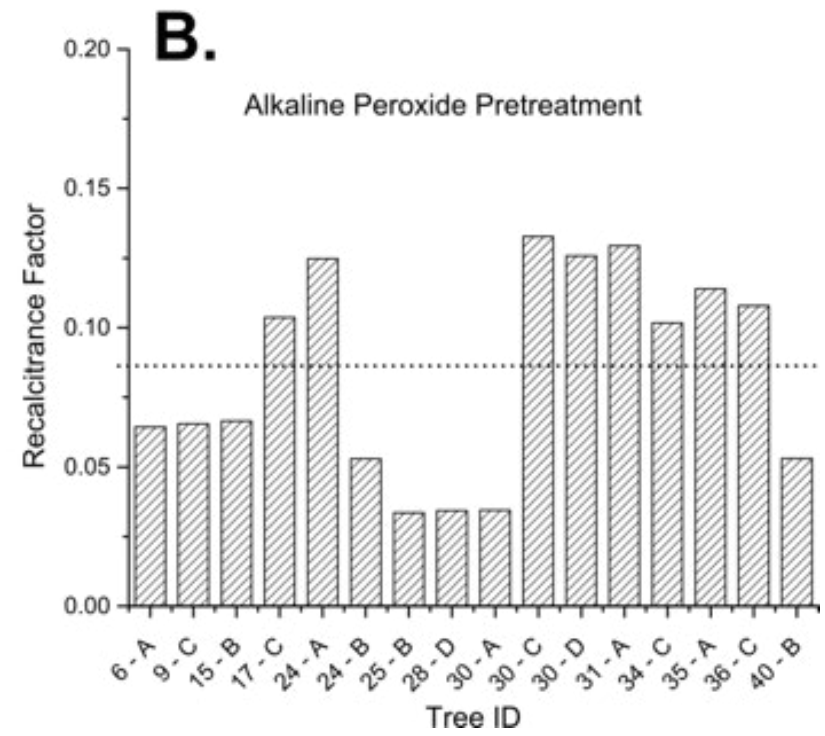
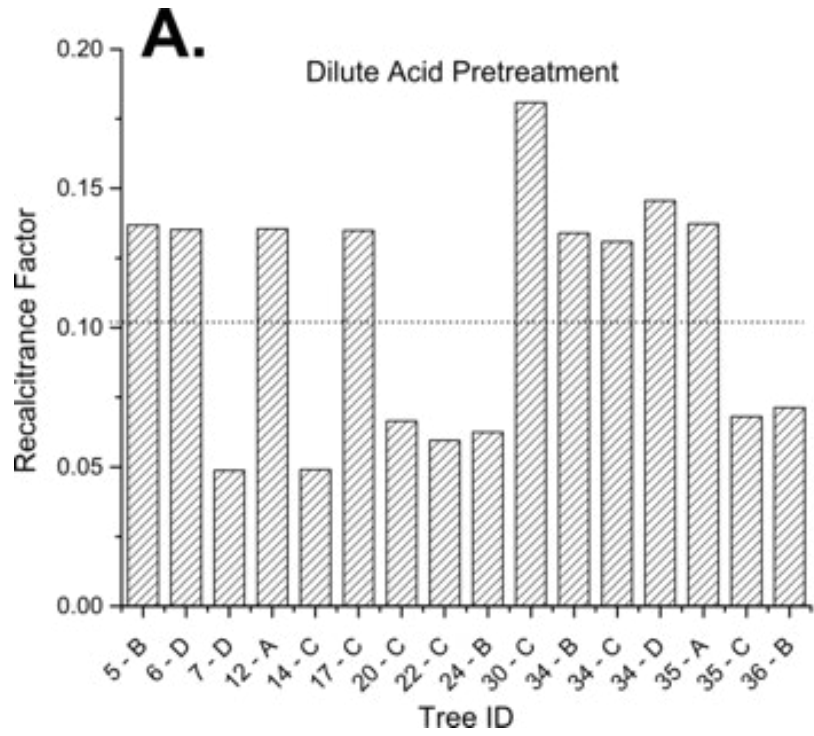


Figure FD-5.1. Recalcitrance Factors Using Dilute Acid (Graph A) and Alkaline Peroxide (Graph B) Pretreatments. The recalcitrance factors represent the overall conversion for each sample including pretreatment and hydrolysis yield combined. This value is the most relevant factor for consideration in choosing and optimizing feedstocks, including through genetic selection

TASK C-P-1: PRETREATMENT TO OVERCOME RECALCITRANCE OF LIGNOCELLULOSE

Key Personnel
Junyong (J.Y.) Zhu

Affiliation
USFS Forest Products Lab

Task Description

Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) has demonstrated robust performance to remove recalcitrance of woody biomass, including softwood species. SPORL outperforms competing technologies in terms of sugar/ethanol yield and energy efficiency/net energy output (Zhu et al., Applied Microbiology and Biotechnology, 86:1355-1365, 2010; Tian et al. Bioresource Technology 101:8678-8685; Lan et al., Bioresource Technology, 127:291-297, 2013). The major work for the proposed study is to demonstrate the performance of the SPORL using Douglas-fir forest residues with relatively high lignin contents, and its scalability at two pilot plant facilities for 1000 gallon biojet fuel production. The focus of the study is on low cost and low value Douglas-fir forest residues to improve economics and sustainability. The specific objectives are: (1) to optimize SPORL pretreatment conditions for Douglas-fir forest residues under laboratory bench scale conditions based on sugar yield after subsequent enzymatic saccharification; (2) to conduct SPORL pretreatments of Douglas-fir forest residues using the Forest Products Lab (FPL) pilot scale pulping facility to realize first step scale-up, to determine optimal conditions based on total sugar yield after subsequent enzymatic saccharification under high solids loadings of approximately 20%; (3) to conduct large scale, approximately ten tons of wood, production of SPORL substrate at an industrial scale facility with a potential one-step production process under optimal conditions through preliminary large scale production study at FPL; (4) to work with Washington State University, Weyerhaeuser and Gevo for large scale biojet fuel production and lignin co-product development using SPORL hydrolysate and lignin fractions.

Activities and Results

The major activities in this reporting period were: (1) optimizing pretreatment of Douglas-fir forest residues, FS-03 and FS-10; (2) developing kinetic based parameters for pretreatment scale-up; (3) conduct pilot scale pretreatment; (4) conducting a laboratory fermentation study to evaluate inhibitor formation; (5) integrating FPL's original SPORL pretreatment with CatchLight Energy's sulfite pretreatment at the FPL pilot scale facility.

In the optimization study, FS-03 was pretreated at 165°C in a range of chemical loadings and durations. Bisulfite loading of 12% and acid loading of 2.2% and pretreatment duration of 75 min were identified as optimal. Enzymatic glucose yield of 345 kg/ton of FS-03 was achieved (Fig. C-P-1.1).

A combined hydrolysis factor (CHF), a pretreatment severity measurer developed previously, was used to scale pretreatment temperature (T) and time (t). A low T and extended pretreatment duration can reduce inhibitor formation with a significant effect on saccharification because the activation energy of sugar degradation is higher than hemicellulose dissolution (Fig. C-P-1.2). Using first order kinetics, sugar degradation (D) can be predicted as a function of pretreatment severity, CHF, and time as shown in Eq.(1).

Equation (1)

$$D = k_d \cdot t \left[1 - \frac{1-\theta}{CHF} (1 - e^{-CHF}) - \frac{\theta}{f \cdot CHF} (1 - e^{-f \cdot CHF}) \right]$$

Using the data derived from Eq. (1), the ratio of sugar degradation at a low and high temperature can be determined in Eq. (2). The symbols E and Ed represent activation energy of hemicellulose dissolution

and sugar degradation respectively.

Equation (2)

$$\frac{D_{T1}}{D_{T2}} = \frac{k_d^{T1}}{k_d^{T2}} \left[\frac{t^{T1}}{t^{T2}} \right] = \exp \left[\frac{E - E_d}{R} \left(\frac{1}{T1} - \frac{1}{T2} \right) \right]$$

While maintaining constant pretreatment severity, CHF, pretreatment temperature and time can be predicted using the below relationship (Eq. 3):

Equation (3)

$$\exp \left(-\frac{E}{RT1} \right) \cdot t^{T1} = \exp \left(-\frac{E}{RT2} \right) \cdot t^{T2}$$

These relationships allow a low temperature pretreatment while balancing sugar yield and inhibitor formation.

The optimal pretreatment conditions were applied to a pilot scale study using the Douglas-fir residual FS-10 sample. The pilot scale reactor used is a 390 liter (L) rotating pulping digester (Fig. C-P-1.3). The pilot scale conditions produced a high sugar yield with low inhibitor furan formation. *S. cerevisiae* was used to ferment the pretreated whole slurry. Fermentation conditions using 21% total solids produced high titer and yield with no apparent inhibition.

Lastly, our laboratory collaborated with NARA affiliate member Catchlight Energy to evaluate pretreatment using sulfur dioxide (SO₂) and calcium hydroxide (Ca(OH)₂). It was found that bisulfite loading can be reduced to 6.5% to achieve equivalent results when pretreatment was conducted at 150°C for 240 min. Data analyses are still in progress.

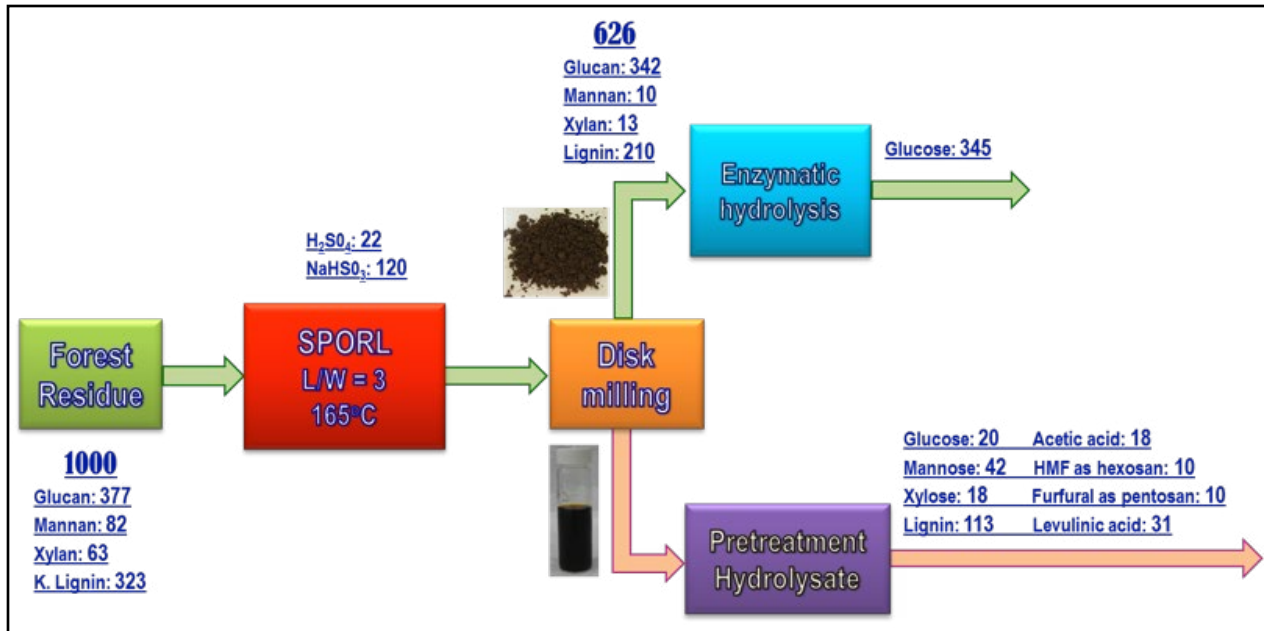


Figure C-P-1.1. Overall mass balance of the optimal pretreatment of FS-03 at lab scale of 150 g at T=165°C, t=75 min, Bisulfite=12%, and Acid = 2.2% with liquid to solid ratio of 3:1. All numbers are expressed in kg

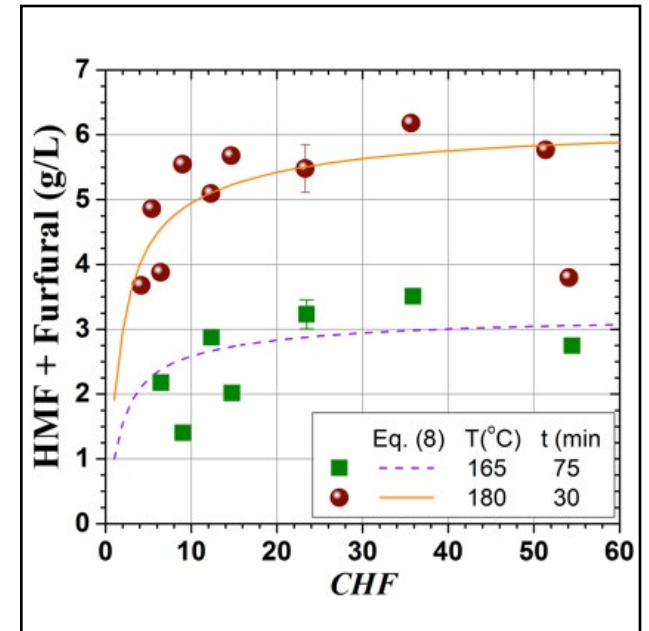


Figure C-P-1.2. Correlation between the HMF and furfural concentration in the pretreatment hydrolysate with the combined hydrolysis factor (CHF) in comparisons with model predictions



Figure C-P-1.3. The FPL pilot scale 390 L rotating reactor for pretreating 50 kg (oven dry) forest residue.

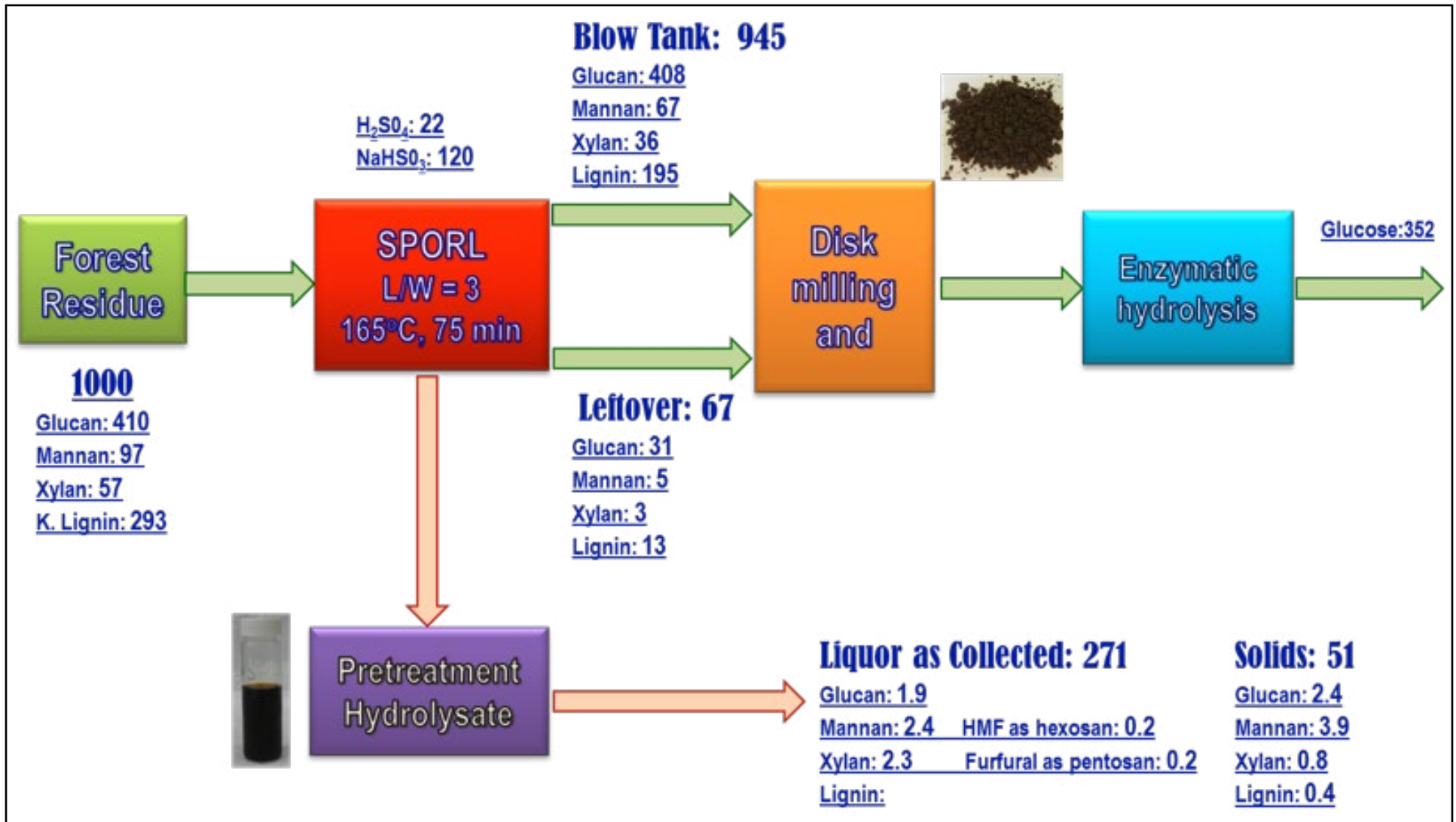


Figure C-P-1.4. Pilot scale 50 kg SPORL pretreatment of FS-10. Overall mass balance of pretreatment at T=165°C, t=75 min, Bisulfite=12%, and Acid = 2.2% with liquid to solid ratio of 3:1. All numbers are expressed in kg

Recommendations | Conclusions

SPORL pretreatment is a very efficient process for pretreating softwood forest residues. Inhibitor formation can be managed by using a low temperature pretreatment. Pretreatment conditions described in this study generate excellent sugar yield with very low inhibitors. Using SO₂ with hydroxide, as the pulp and paper industry does, rather than acid and bisulfite used in the SPORL pretreatment, can reduce chemical loading by 50%. This has been demonstrated in pilot scale experiments. Pilot scale pretreatment of Douglas-fir forest residues achieved excellent sugar yield.

Physical and Intellectual Outputs

- 15 kg wet pretreated FS-10 at pilot scale using FPL 165°C pretreatment were sent to GEVO for fermentation analysis.
- 1.5 kg of enzymatically hydrolyzed lignin residue from the same above sample was sent to Weyerhaeuser for co-product analysis.
- 15 kg wet pretreated FS-10 at pilot scale using FPL/Catchlight Energy 150°C pretreatment were sent to GEVO for fermentation analysis.
- 1.5 kg of enzymatically hydrolyzed lignin residue from the same above sample were sent to Weyerhaeuser for co-product analysis.
- Lignosulfonate samples were also sent to Weyerhaeuser

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- 1) Zhang, C., Houtman, C.J., Zhu, J.Y. (2014), "Using Low Temperature to Balance Enzymatic Saccharification and Furan Formation during SPORLPretreatment of Douglas-fir", *Process Biochemistry* 49:466-473 DOI: 10.1016/j.procbio.2013.12.017
- 2) Zhang, C., Lei, X., Scott, C.T., Zhu, J.Y., K. Li, (2014), "Comparison of Dilute Acid and Sulfite Pretreatment for Enzymatic Saccharification of Earlywood and Latewood of Douglas-fir", *BioEnergy Research*

- 7:362-370 DOI:10.1007/s12155-013-9376-6
- 3) Leu, S.-Y., Gleisner, R., Zhu, J.Y., Sessions, J., Marrs, G., (2013) "Robust Enzymatic Saccharification of a Douglas-fir Forest Harvest Residue by SPORL", *Biomass and Bioenergy* 59:393-401 DOI:10.1016/j.biombioe.2013.08.014
- 4) Zhou, H.F., Lou, H., Yang, D., Zhu, J.Y., Qiu, X., (2013) "Lignosulfonate to Enhance Enzymatic Saccharification of Lignocelluloses: The Role of Molecular Weight and Substrate Lignin", *Ind. Eng. Chem Res.* 52(25):8464-8470 DOI: 10.1021/ie401085k
- 5) Zhou, H.F., Zhu, J.Y., Luo, X., Leu, S.-Y., Wu, X., Gleisner, R., Dien, B.S., Hector, R.E., Yang, D., Qiu, X., Horn, E., Negron, J. (2013), "Bioconversion of Beetle-Killed Lodgepole Pine Using SPORL: Process Scale-up Design, Lignin Co-product, and High Solids Fermentation without Detoxification", *Ind Eng Chem Res* 52:16057-16065 DOI: 10.1021/ie402873y

CONFERENCE PROCEEDINGS AND ABSTRACTS FROM PROFESSIONAL MEETINGS

Cheng, J. L., Zhou, H.F., Gleisner, R., X.J. Pan, Zhu, J.Y. (2013), "Biorefining of forest residue Using SPORL: Process Scale-up Design, Lignin Co-product, and High Solids Fermentation without Detoxification" 5th Nordic Wood Biorefinery Conference, Stockholm, March, 2014. P 141-146.

RESEARCH PRESENTATIONS

- 1) Zhu, J.Y. (2014), "Biorefining of forest residue Using SPORL: Process Scale-up Design, Lignin Co-product, and High Solids Fermentation without Detoxification" 5th Nordic Wood Biorefinery Conference, Stockholm, Sweden, March 24-27
- 2) Zhu, J.Y. (2014) "Why enzymatic hydrolysis of lignocelluloses should be conducted at elevated pH 5.2-6.2", presented at the 247th ACS National Spring Meeting, Dallas, TX, March 15-21
- 3) Zhu, J.Y. (2014) "Role of lignin on enzymatic saccharification of lignocelluloses", presented at the 247th ACS National Spring Meeting, Dallas, TX, March 15-21
- 4) Zhu J.Y. (2014), "Using low temperature to balance enzymatic saccharification and furan formation

- during SPORL pretreatment of Douglas-fir", presented at the 247th ACS National Spring Meeting, Dallas, TX, March 15-21
- 5) Zhu, J.Y., (2013), "Bioconversion of Forest Residue using SPORL: Process Scale-up Design, Lignin-Coproducts, High Solids Fermentation without Detoxification" Plenary session presentation at the 4th International Conference on Biorefinery to Bioenergy, Xiamen, China, December 2-4
- 6) Zhu, J.Y., H. Lou, T.Q. Lan, (2013), "Why Enzymatic Hydrolysis of Lignocelluloses Should be Conducted At Elevated pH 5.2-6.2" 2013 AIChE Annual Meeting, San Francisco, November 3-8
- 7) Zhu, J.Y., S.-Y. Leu, R. Gleisner, C. Zhang, D. Mann J. Sessions G. Marrs, (2013) "Upgrading Forest Residues of Douglas-fir for Fermentable Sugar Production" 2013 AIChE Annual Meeting, San Francisco, November 3-8
- 8) Zhu, J.Y., Houtman, C.J., Zhang, C., (2013) "Maximize Enzymatic Saccharification and Minimize Sugar Degradation in SPORL Pretreatment of Douglas-Fir At a Low Temperature: A Kinetic Approach" 2013 AIChE Annual Meeting, San Francisco, November 3-8
- 9) Zhu, J.Y., S.-Y. Leu, J. Cheng, R. Gleisner, (2013), "Converting Forest Residue to Biofuel by the SPORL Process", TAPPI 2013 International Conference on Bioenergy and Bioproducts, Green Bay, WI, September 18-20
- 10) Zhu, J.Y., S.-Y. Leu, R. Gleisner, C. Zhang, D. Mann J. Sessions G. Marrs, (2013) "Upgrading Forest Residues of Douglas-fir for Fermentable Sugar Production" TAPPI 2013 International Conference on Bioenergy and Bioproducts, Green Bay, WI, September 18-20
- 11) Leu, S.-Y., J.Y. Zhu, R. Gleisner, J. Sessions, G. Marrs, (2013), "Robust Enzymatic Saccharification of a Douglas-fir Forest Harvest Residue by SPORL", Poster presentation at the 35th Symposium on Biotechnology for Fuels and Chemicals, Portland, OR, April 29 - May 2
- 12) Zhu, J.Y., S.-Y. Leu, R. Gleisner, C. Zhang, D. Mann J. Sessions G. Marrs, (2013) "Upgrading Forest Residues of Douglas-fir for Fermentable Sugar Production" 35th Symposium on Biotechnology for Fuels and Chemicals, Portland, OR, April 29 - May 2

TASK C-P-3: PREPARATION OF PRETREATED BIOMASS

Key Personnel

Brigitte Ahring

Affiliation

Washington State University

Task Description

Using an innovative wet explosion pretreatment process, pretreated samples (up to 100 kg) have been prepared from feedstocks supplied by Weyerhaeuser in accordance with their specific needs (Task 1). The specific operational conditions for the different biomass materials will initially include temperature, pressure and oxygen level. The pretreatment process is fully instrumented, and initial testing under a range of operating conditions will take less than a month per biomass feedstock. During the year of 2013, the pretreatment work has been conducted by a post doctoral fellow, along with a full time technician under supervision of the pilot plant manager. This has allowed WSU BSEL to make full mass balances over feed stock materials and further evaluate the conditions for optimal enzyme hydrolysis of pretreated materials from an economic perspective. The WSU BSEL group will continually consult with the different partners to ensure that the material delivered meets their needs. The group will further evaluate the samples produced for release of C5 and C6 sugars using commercial enzyme products as well as the level of inhibitory compounds such as hydroxymethylfurfural (HMF), furfural and acetic acids. Conversion results from the fermentation and co-product experiments will be reported back to WSU BSEL and used to adjust the pretreatment process to optimize it (Task 2). In year 4, the results will be evaluated against other pretreatment methods used in the project. If our pretreatment method proves most successful, further activities will be planned at that time and a new budget will be determined for further work in the NARA project.

Activities and Results

Extensive optimization of pretreatment and enzymatic hydrolysis along with composition analysis on FS-10 has been conducted throughout the period; the results of this work are summarized in Figure C-P-3.1.

Optimizing WetOx pretreatment for maximum sugar yield was performed using the following conditions (170-190)°C; (10-30)min; (0.5-7.5) % O₂, from the results in Table C-P-3.1.

Maximum total sugar obtained at a minimum resulting concentration of HMF and furfural is obtained at relatively mild conditions (23.9min, 170.5°C, 7.5%O₂). Increasing the temperature slightly (23.1min, 177.5°C, 7.5%O₂) will give a slightly higher total sugar concentration, but still within the standard deviation of the results.

Maximum glucose concentration is obtained at the high end of the severity scale (30min, 189.5°C, 7.5%O₂) and clearly shows that high severity is needed in order to increase the digestibility of the cellulose.

As high severity is needed to access the C6, and low severity is needed to recover the C5, it is clear that a two-step pretreatment is preferred for obtaining the highest possible sugar yield from FS-10. A single test on FS-10, in a two phase pretreatment, was conducted in the fall of 2013 and showed an overall sugar yield of 88%. However, the samples produced were not further tested by Gevo and, therefore, the final test made by Gevo was done on a process which is not optimal for either C5 or C6 sugars.

Recommendations | Conclusions

From the studies performed in 2013, it is evident that a maximum total sugar yield from FS-10 can be obtained when using a two-step WOX pretreatment and that the one-step process which has been evaluated by Gevo is suboptimal. A low severity WOX followed by liquid/solid separation will recover the C5 sugar. The fibers can then be pretreated a second time at high severity to maximize the cellulose accessibility and C6 yield.

It is essential that feedback is provided from our partners, Gevo and Weyerhaeuser to make the optimal pretreatment. Unfortunately, very limited input has been received from the partners during the last 8 months.

Physical and Intellectual Outputs

- (04-16-2013) Lignin sample sent to Weyerhaeuser (FS-03)
- (11-18-2013) 17.1kg of liquid sample of whole hydrolysate was shipped to, and received by, Gevo. (FS-10)
- (11-25-2013) 57kg @ 28.76% dry matter (DM)– Fermented high lignin residual fiber sample at was send to Weyerhaeuser. (FS-10)
- (01-25-2014) 57 lb. of pretreated (not hydrolyzed) FS-10 was shipped to Gevo.
- (01-29-2014) 12 kg of pretreated (not hydrolyzed) FS-10 was shipped to Gevo.

RESEARCH PRESENTATIONS

35 SBFC, Portland, Oregon. April 29 – May 2, 2013.
Pretreatment of Forest Slash using Wet Oxidation.

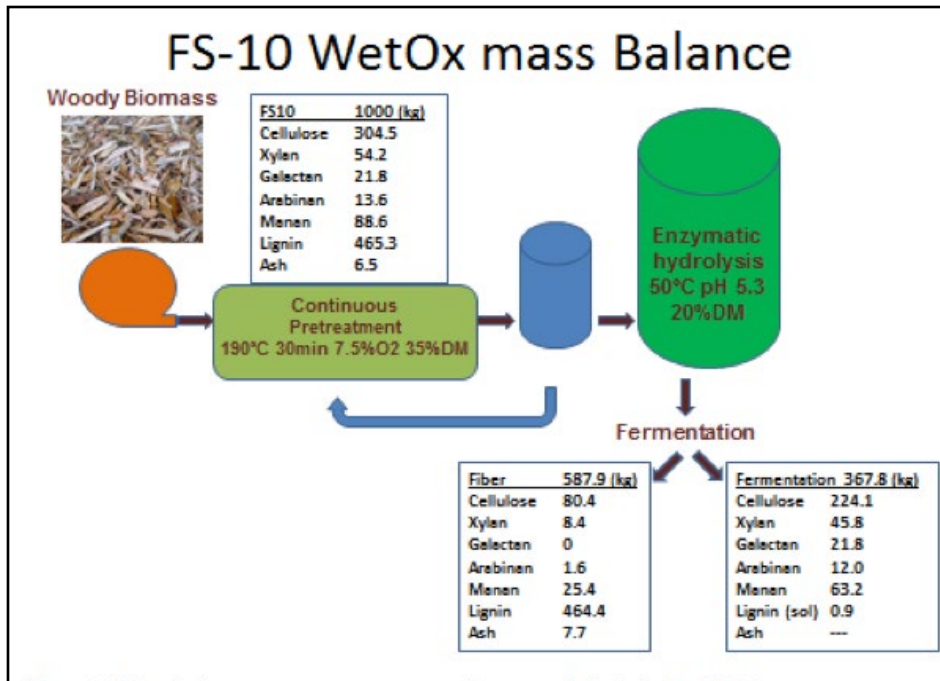


Figure C-P-3.1. Optimized pretreatment and enzymatic hydrolysis has been performed and is shown in figure 2

Table C-P-3.1. Enzymatic hydrolysis performed using 40mg EP/g cellulose Ctec + 10% Htec v/v in 100ml@10%DM in 250ml flasks

Enzymatic hydrolysis performed using 40mg EP/g cellulose Ctec + 10% Htec v/v in 100ml@10%DM in 250ml flasks	
Criteria: Maximum total sugar	23.1min 177.5°C 7.5%O2 (26.6 std1.7 g/l)
Criteria: Maximum total sugar, Minimum HMF+Furfural	23.9min 170.5°C 7.5%O2 (25.8 std1.9 g/l)
Criteria: Maximum glucose	30.0min 189.5°C 7.5%O2 (10.4 std0.8 g/l)

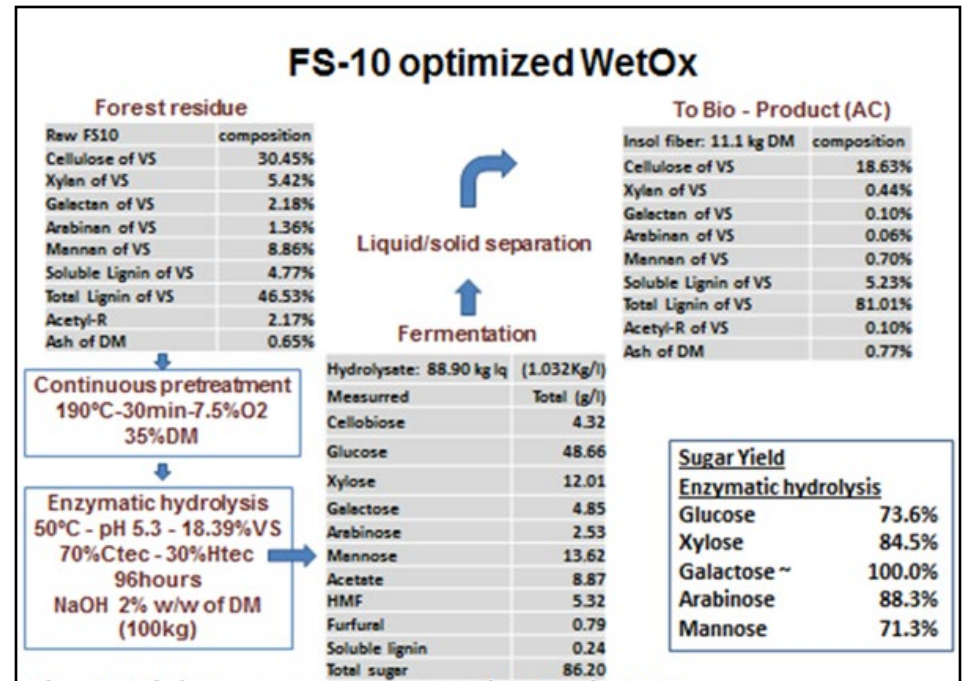


Figure C-P-3.2. Mass balance over pretreatment and enzymatic hydrolysis of FS10

TASK C-P-4: MILD BISULFITE PRETREATMENT OF FOREST RESIDUALS

Key Personnel

Dwight Anderson
Johnway Gao

Affiliation

Catchlight Energy
Weyerhaeuser

Task Description

Catchlight Energy will identify the pretreatment parameters that give the greatest sugar yield for the Mild Bisulfite Pretreatment on a NARA feedstock such as FS-03. Lignin co-product value has the potential for significant contribution to overall economics, so Catchlight Energy will also produce samples to enable the identification of economic opportunities with co-products.

In Year 1, Catchlight Energy participated as a non-funded Member by providing pretreatment data for a NARA feedstock (FS-01) to enable an economic comparison of pretreatments. Catchlight Energy had also provided initial lignin samples to the Co-Products team. Catchlight Energy contributed additional lignin samples from FS-03 to the Co-Products team at Catchlight Energy's own expense.

Year 2 research focused on optimizing Mild Bisulfite pretreatment of softwood forest residues. Pretreatment runs were conducted in a one cubic foot batch pilot digester at Weyerhaeuser Technology Center. The feedstock of choice was softwood residues such as FS-10. The subtasks developed were: a) Identify mild bisulfite pretreatment conditions that optimize the yield of fermentable sugars in the selected feedstock. b) Produce additional samples of lignosulfonate and residual lignin beyond what would be previously provided by Catchlight Energy funding for analysis by the Co-Products team.

Activities and Results

Task CP-4.1. Identify effect of pretreatment process variables on total sugar yield

Catchlight Energy had demonstrated mild bisulfite pretreatment in the pilot scale prior to joining the NARA project, but significant optimization was possible for the following variables:

- Chip Size Distribution – Early development runs were conducted with wood chips that had been re-chipped and screened through a ¾" round hole, with 3 mm fines discarded. This was done as a precaution against the effect of aberrations in chip sizes, but the added handling and loss of material comes at a cost. The first variable explored is the absence of re-chipping and screening. Chips provided by NARA (FS-10) were used as-is, without either screening or further drying. Pilot runs demonstrated that these chips did not act significantly different from the unscreened chips.
- Equivalent sulfur dioxide (SO₂) on wood was reduced from 12% to 6% while maintaining over 80% of the theoretical total sugar yield. This reduces chemical costs and the carbon intensity of the process.
- Temperature and time were varied to achieve lower levels of fermentation inhibitors and demonstrate successful pilot operation at temperatures closer to conventional sulfite pulping. Temperatures investigated include 165°C, 145°C, and 140°C. These results were completed in the last quarter and are described below. The optimal time/temperature appears to be three hours of pretreatment time at 145°C. Using ideal conditions reduces furan formation by 80% compared to the initial condition used as the optimization starting point and achieves 88% of the theoretical hydrolysis possible.
- These results mentioned above can be obtained with 4.0% CTec3 and 0.4% HTec3 enzyme product

loading on pretreated material. There is little advantage to increasing enzyme dosage beyond this, and subsequent optimization may end up showing an optimum at a lower level.

Detailed Description of Optimization Results

Initial Work Demonstrating Ability to Use Chips As-Is

Five pilot pretreatment tests on the FS-10 Douglas-fir softwood residues were completed from April to June, 2013. These pretreatments were performed in a one cubic foot batch pilot digester at Weyerhaeuser Technology Center. Three kilograms of FS-10 chips were used with a pretreatment liquor to OD (oven dry) chip ratio of 4:1. The changes of pretreatment parameters included calcium bisulfite dosage (indicated as equivalent SO₂ on wood), pretreatment temperature and pretreatment time at temperature. The enzyme dosage (CTec3 and HTec3 products) varied from 0.5% to 15% weight of enzyme mixture on the pretreated FS-10 biomass. Table C-P-4.1 summarizes the pretreatment conditions of these five cooks. Liquor samples were collected during each pretreatment to monitor the changes in cooking pH, liquor sugar, and furan formation to guide further optimization of the pretreatment conditions.

Runs 253A and 254A show the extent to which chip size reduction and fines removal affects the results. In development work done by Catchlight Energy before NARA began, chips were re-chipped and screened through a ¾" round hole to ensure that oversized chips weren't biasing results between runs. The re-chipping process produces additional fines, so the 3 mm fines were discarded from the re-chipped wood prior to charging in the pilot digester. Since this has both operational and raw material cost implications, it is worth understanding whether this treatment is necessary. A comparison of runs 253A and 254A suggest that this treatment has no detectable adverse effect:

- Figure C-P-4.1 shows that the pH profile over time is the same between the two cooks. Similarly in Figure C-P-4.2, the usage of equivalent SO_2 profile is the same for these two cooks. In fact, SO_2 residual is needed in the cook to enable sulfonation. Even at the lower SO_2 charge of 6-7% on wood, there remained a residual of 1.1% to 1.4% on the wood. Reducing chip size would decrease the amount of SO_2 residual, but there seems to be little opportunity available for further residual reduction.
- Figure C-P-4.3 shows that sugar forms in the liquor at the same rate; however, there may be a slight hint that the re-chipped wood begins to lose sugar to degradation sooner.
- Figure C-P-4.4 shows that the formation of furans is similar for both samples, with perhaps a hint that the re-chipped wood has more furans, consistent with the hint in Figure C-P-4.2.

In summary, there is no apparent disadvantage to using chips as-is. They behave remarkably similar to the re-chipped wood.

Chemical/Time/Temperature Optimization: The Cumulative Perspective

Building on the success of lower temperature runs from work earlier in this project year, such as Cook 262A and 271A, the pretreatment conditions for the NARA FS-10 feedstock was further optimized with a combination of lower temperature, lower chemical dosage and shorter pretreatment time. The following optimized results at lower temperatures of 145°C and 140°C were compared with the previous results at the higher temperature of 165°C. The results are summarized in Table C-P-4.3. It should be noted that lower temperatures are desirable for reducing inhibitor formation. In conclusion, the most optimal pretreatment conditions are represented by pretreatment run 351A:

- While the cooking temperature was maintained at 145°C and the chemical dosage was kept at 6% SO_2 equivalent on wood (7.3% as calcium bisulfite and 1.4% as free SO_2 on wood), the pretreatment time was reduced from 240 minutes to 180 minutes at

the same cook temperature. This produced a yield of 80.38% on wood. The results are summarized and compared in Table C-P-4.1. Figure C-P-4.1 also shows the total sugar formation in the prehydrolysate liquor, which contains 19.62% of the original wood material.

- Compared to the higher temperature cook at 165°C for 75 minutes, the total furan formation results are shown in Figure C-P-4.3 for the pretreatment times of 240 and 180 minutes at lower temperatures. While a 50% reduction was previously associated with the drop from 165°C to 145°C, an 80% drop for the lower temperature and reduced time is now evident.
- After enzymatic hydrolysis for 96 hours, fermentation was conducted on the optimized pretreated FS-10 feedstock hydrolysate with a standard ethanol yeast NABC Bio-Ferm® XR. The ethanol titer and lignin content in the fermented residuals are shown in Tables C-P-4.2 and C-P-4.3. This data shows that the higher the sugar yields, the higher the ethanol titers are in the fermentation.

The optimization results in Table C-P-4.2 show that while the cooking temperature was maintained at 145°C and the chemical dosage was kept at 6% SO_2 equivalent on wood, the total sugar yield increased from 82% to 88% by reducing cooking time from 240 minutes to 180 minutes at cook temperature. At the same temperature of 145°C and same cooking time of 240 minutes, the total sugar yield increased from 82% to 87% when the SO_2 dosage increased from 6% to 7% on wood. When the cooking time decreases from 180 minutes to 120 minutes at 145°C, the total sugar yield decreased from 88% to 81%. When the cooking temperature further decreased from 145°C to 140°C while the cooking time was the same at 240 minutes, the total sugar yield decreased from 82-87% to 76%.

Figure C-P-4.2 shows the total sugar formation in the prehydrolysate liquor. The pretreatments with higher total sugar yields showed level or increasing total liquor sugar while the formation of furan (hydroxymethylfurfural and furfural) was very low in the

liquor as shown in Figure C-P-4.6. Compared to the higher temperature cook at 165°C for 75 minutes, the furan formation was reduced by 55% in 333A cook and 77% in 351A cook, respectively for 240 and 180 minute cooking time at 145°C.

The hydrolysate samples produced from the pretreated biomass samples, 333A, 336A, 351A and 355A were also used to test the ethanol fermentability with a standard ethanol fermenting yeast, NABC Bio-Ferm® XR *Saccharomyces cerevisiae* (North American Bioproducts Corporation, Duluth, GA). The fermentation was conducted in 100 ml hydrolysate in 250-mL Erlenmeyer shake flask, supplemented with urea (1 g/L) and corn steep liquor (0.5%). The yeast seed was 2 g/L in the hydrolysate. The fermentation temperature was controlled at 33°C. The fermentation went extremely well and was completed within 48 hours. With about 15% pretreated biomass loading after hydrolysis and fermentation, the ethanol titer in the fermentation broth achieved 3.9% (wt/wt) in the hydrolysate samples of 333A and 351A, while the sample from 336A only achieved 3.3% ethanol. The carbohydrate in the fermented residuals was as low as 9.6% and the lignin (Klason lignin and acid soluble) was as high as 73% in the 333A sample. The overall sugar balance is shown in Figure C-P-4.7.

An interesting observation about the effect of temperature on pretreatment is that higher pretreatment temperatures result in denser pulp. The apparent densities of pretreated FS-10 feedstocks are compared at cook temperatures of 165°C, 145°C and 140°C. The results are summarized in Figure C-P-4.8 and Table C-P-4.4.

Various cooked FS-10 biomass samples were tested for apparent densities by filling a graduated cylinder with 20 grams of wet pulp. The apparent pretreated and refined biomass density was calculated by the oven-dried biomass divided by the apparent volume of the biomass in the graduate cylinder (Figure C-P-4.8). The results in table C-P-4.4 show that when the cooking temperature is lower, the pretreated material's apparent density is lighter. This is a useful parameter

for the process design when it comes to equipment selection and specification.

Economic Optimization

While primarily focusing on hydrolysis efficiency and the production of inhibitors because of their importance, economic optimization was considered for a number of scenarios related to process conditions. Additionally, the strategy of reducing capital investment by shipping an intermediate sugar product was evaluated and the sensitivity of the process to capital in general was also considered. Since the relative economics among the pretreatment conditions can be reflected in the cost through fermentation, the IPK-to-Jet capital calculation was dropped and everything was compared on the basis of ethanol fermentation because such data are more readily available. Some costs specific to jet fuel may be embedded in other parts of the estimates, so the actual cost of producing ethanol may be less than the number identified in this exercise. The objective is to look for relative differences to guide further optimization. Capital for a four-effect evaporator, based on a confidential Catchlight Energy reference, was included in cases that involved shipping concentrated sugar. Other than these changes, a NARA TEA was used for these calculations: version 3.6.1 for the “boiler only” case with no co-products, and version 6.2 was used when co-products were considered.

Findings:

- The economics of an integrated pretreatment/fermentation plant are better than a situation where sugar must be shipped to an existing ethanol plant. Even though the capital is higher for an integrated plant, the operating cost is lower and more than makes up for the capital due mostly to the cost of transporting syrup a few hundred miles, but also due to the lower energy requirements that result from ethanol distillation compared to evaporating water from syrup. This can be seen in Table C-P-4.5 by comparing cases 1 to 2 and 3, 4 to 5 and 6.
- The economics of using the equivalent 4.4%

CTec2/HTec2 enzyme product with an enzyme produced on site are better than purchasing 1.1% enzymes at the costs disclosed to NARA. Comparing cases 1-3 with 4-6 and cases 7-9 with 10-12 in Table C-P-4.5 support this conclusion. Using the estimated cost of an on-site enzyme plant and the actual conversion efficiency achieved, the on-site enzyme plant at a higher dose is more cost effective. In spite of the greater power generation capability of the low enzyme case due to the higher amount of lignin residuals to burn, the economics of the potential sugar yield override this co-product benefit.

- The economics of the 145°C pretreatments at a longer time are more cost-effective than the 165°C pretreatments for a shorter time. Comparing cases 1-6 with cases 7-12 in Table C-P-4.5 supports this conclusion. The difference is actually more dramatic than shown in this report because the hydrolysis solids loading in cases 7-12 results in 2-3% higher sugar concentration in the combined hydrolysate. The result is less energy required to concentrate syrup when it is sold to an ethanol plant or a higher titer ethanol in an integrated plant. The favorable economics may be counter-intuitive because the lower temperature requires more time. The increase in capital estimated for the digesters, however, was estimated based on a confidential Catchlight Energy reference. It is reflected in the “Pretreatment” capital line which shows a \$30 million difference in capital for these two alterna-

tives. In the 4.4% enzyme cases, the added sugar production makes this trade-off worthwhile. This is not true for the lower enzyme case, however, this case is not optimal.

Task C-P-4.2. Produce lignosulfonate and lignin sample for study by Co-Products team

A fermented residual lignin sample from the cooks 262A and 271A (see Table C-P-4.3 for pretreatment conditions) was sent to the NARA Co-Products team on Sept. 27, 2013. This sample was based on NARA FS-10 feedstock. It was a hydrolyzed and fermented lignin residual sample (~0.9 kg wet; ~30% solid) prepared by 4.4% enzyme dosage and fermented by a standard ethanol yeast strain (NABC Bio-Ferm® XR). The ethanol was driven off at 121°C in the autoclave for 60 minutes. The residuals were prepared from the CLE pretreated biomass by the current best cook condition with low calcium bisulfite (SO₂ equivalent 6.1-7.6% on wood) and at low temperature (145°C).

The prehydrolysate liquor was fermented by the XR ethanol yeast at small scale and sent to the NARA Co-Products team in late November. Upon successful prehydrolysate liquor fermentation, a larger scale liquor fermentation was conducted to supply the Co-Product team with more fermented lignosulfonate sample. At the same time, 5-10 liters of prehydrolysate liquor were sent to Gevo for their isobutanol yeast fermentation test.

Table C-P-4.1. Pretreatment test conditions

No.	Feedstock FS-10 Chip Size	Equivalent SO ₂ (%) on OD Wood	Cooking Temperature (°C)	Cooking Time Hold (min)
253A	BearCat re-chipping with ¾” hole screening & 3mm fine removing	12.4	165	75
254A	No re-chipping	11.8	165	75
257A	No re-chipping	7.9	165	75
262A	No re-chipping	7.6	145	240
271A	No re-chipping	6.1	145	240
Final optimal pretreatment condition		To be completed in 2014		

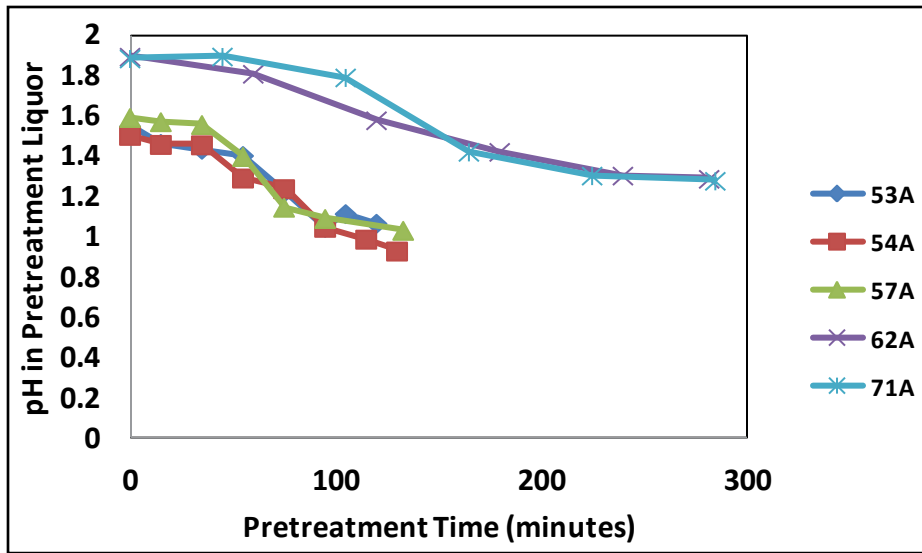


Figure C-P-4.1. pH profiles during pretreatment. Conditions: 253A (SO₂ 12.4%, 165°C, 75 min); 254A (SO₂ 11.8%, 165°C, 75 min); 257A (SO₂ 7.9%, 165°C, 75 min); 262A (SO₂ 7.6%, 145°C, 240 min); 271A (SO₂ 6.1%, 145°C, 240 min).

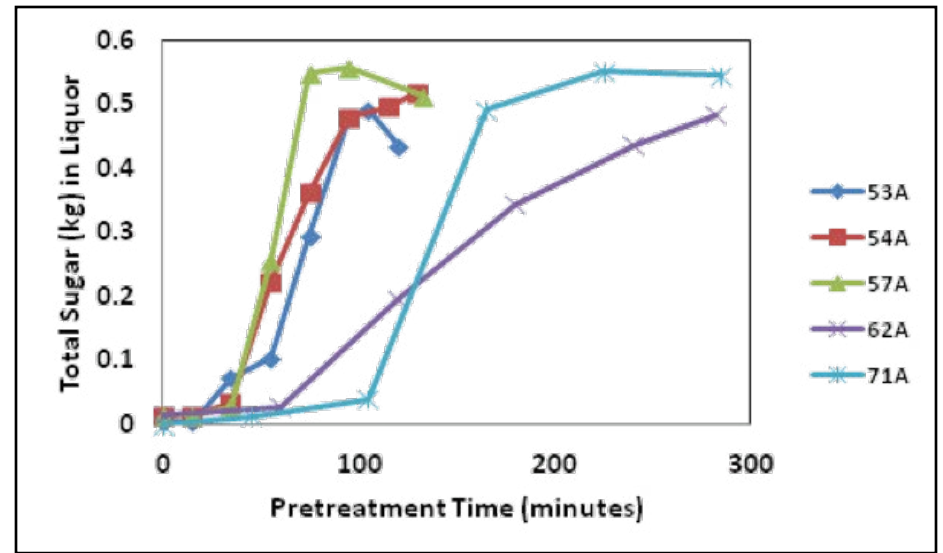


Figure C-P-4.3. Monomeric sugar formation in liquor during pretreatment. Conditions: 253A (SO₂ 12.4%, 165°C, 75 min); 254A (SO₂ 11.8%, 165°C, 75 min); 257A (SO₂ 7.9%, 165°C, 75 min); 262A (SO₂ 7.6%, 145°C, 240 min); 271A (SO₂ 6.1%, 145°C, 240 min).

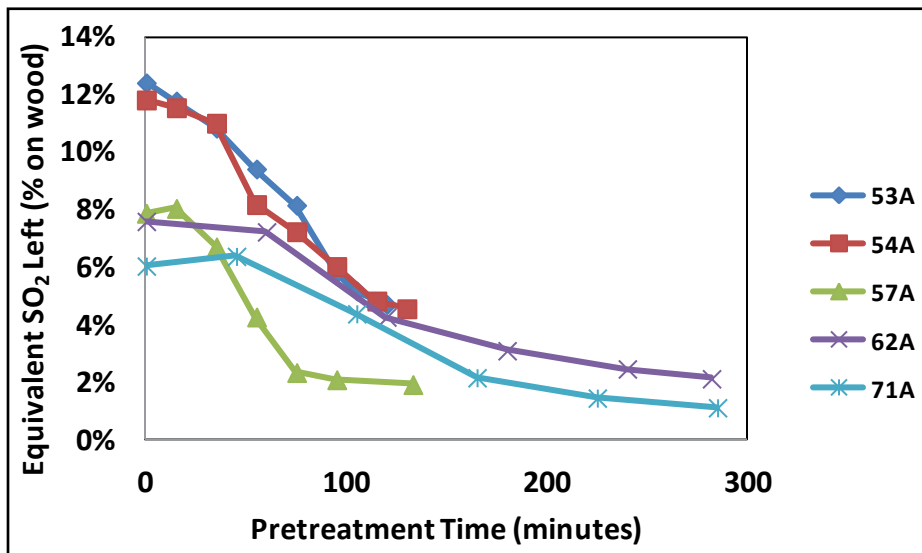


Figure C-P-4.2. SO₂ equivalent amount on wood during pretreatment as residual in the liquor. Conditions: 253A (SO₂ 12.4%, 165°C, 75 min); 254A (SO₂ 11.8%, 165°C, 75 min); 257A (SO₂ 7.9%, 165°C, 75 min); 262A (SO₂ 7.6%, 145°C, 240 min); 271A (SO₂ 6.1%, 145°C, 240 min).

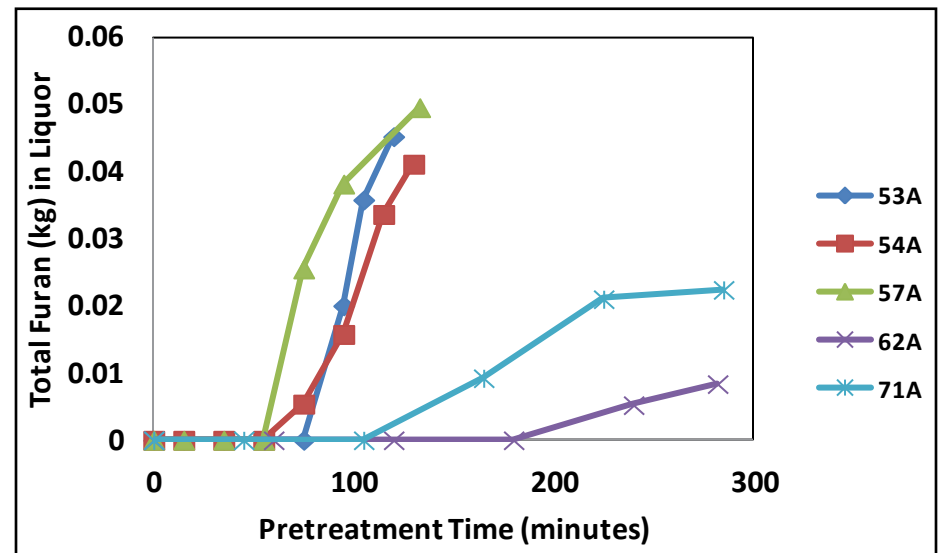


Figure C-P-4.4. Furan (total HMF and furfural) formation in liquor during pretreatment. Conditions: 253A (SO₂ 12.4%, 165°C, 75 min); 254A (SO₂ 11.8%, 165°C, 75 min); 257A (SO₂ 7.9%, 165°C, 75 min); 262A (SO₂ 7.6%, 145°C, 240 min); 271A (SO₂ 6.1%, 145°C, 240 min).

Table C-P-4.2. Pretreatment Summary: 54A pretreated feedstock was air-dried, so this data is inconsistent with other samples

Feed-stocks, analysis based on 100 kg OD wood	Cook No.	Temp., °C	Time at T, min	Equivalent SO ₂ on wood (%)	Pulp Yield, %	Furan formation, % on wood	Acetic acid formation, % on wood	Pulp sugar composition, %	Sugar in liquor, kg	Sugar in hydrolysis, kg	Total Sugar Yield (%)
Douglas-fir, FS-10	253A	165	75	12.4	68.1	1.51	2.13	53.9	9.4	39.6	73
Douglas-fir, FS-10	254A	165	75	11.8	65.7	1.37	2.05	47.9	11.8	24.8	-**
Douglas-fir, FS-10	257A	165	75	7.9	74.4	1.65	1.89	56.7	12.0	42.4	82
Douglas-fir, FS-10	262A	145	240	7.6	78.9	0.28	1.71	55.2	10.9	42.3	80
Douglas-fir, FS-10	271A	145	240	6.1	76.6	0.75	1.93	55.3	12.6	42.4	82
Douglas-fir, FS-10	333A	145	240	7.2	78.2	0.68	1.91	54.4	14.3	43.1	87
Douglas-fir, FS-10	336A	140	240	4.9	79.5	0.24	1.70	52.4	10.6	40.9	76
Douglas-fir, FS-10	351A	145	180	6.0	80.4	0.35	1.88	62.4	10.7	46.4	88
Douglas-fir, FS-10	355A	145	120	6.5	82.3	0.17	1.72	56.8	8.6	45.7	81

Table C-P-4.3. Hydrolysate Fermentation Summary of Pretreated Douglas-fir FS-10 Feedstocks

Cook No.	Pretreated biomass loading at hydrolysis (%)	Ethanol titer (%wt/wt) after fermentation	Carbohydrate in fermented residuals (%wt/wt)	Total lignin in fermented residuals (%wt/wt)
333A	14.7	3.9	9.6	72.0
336A	15.0	3.3	34.0	61.1
351A	14.9	3.9	21.4	70.0
355A	14.9	3.7	25.4	64.9

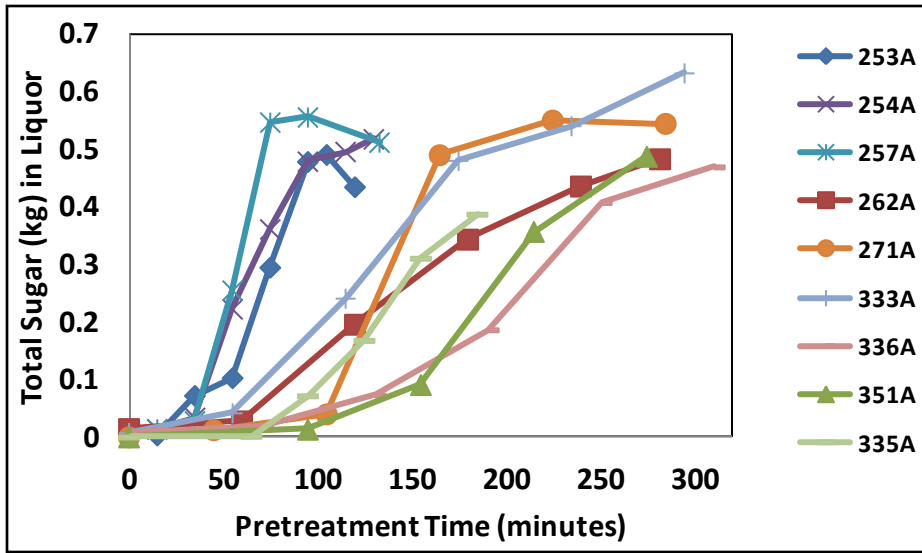


Figure C-P-4.5. Total liquor sugar during pretreatment. Conditions: 253A (SO₂ 12.4%, 165°C, 75 min); 254A (SO₂ 11.8%, 165°C, 75 min); 257A (SO₂ 7.9%, 165°C, 75 min); 262A (SO₂ 7.6%, 145°C, 240 min); 271A (SO₂ 6.1%, 145°C, 240 min); 333A (SO₂ 7.2%, 145°C, 240 min); 336A (SO₂ 4.9%, 140°C, 240 min); 351A (SO₂ 6.0%, 145°C, 180 min); 335A (SO₂ 6.5%, 145°C, 120 min).

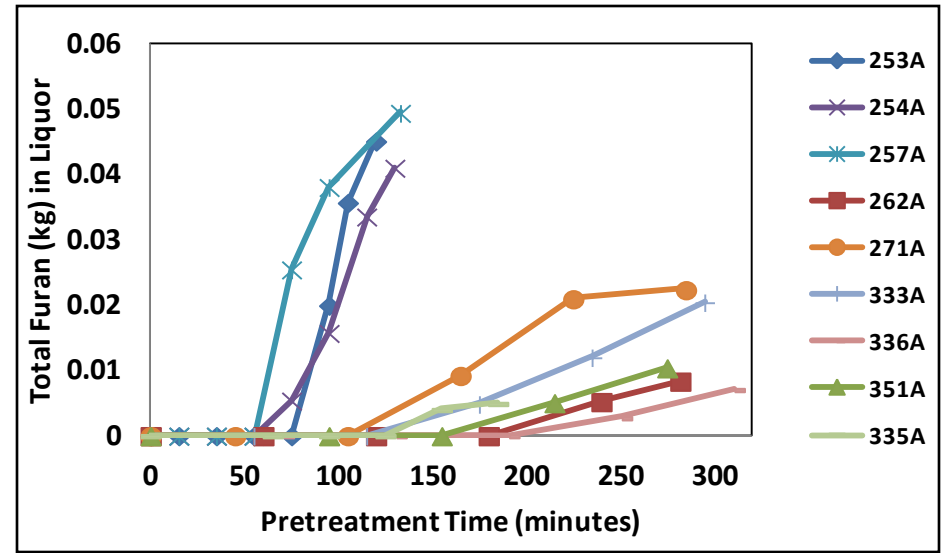


Figure C-P-4.6. Total liquor furan during pretreatment. Conditions: 253A (SO₂ 12.4%, 165°C, 75 min); 254A (SO₂ 11.8%, 165°C, 75 min); 257A (SO₂ 7.9%, 165°C, 75 min); 262A (SO₂ 7.6%, 145°C, 240 min); 271A (SO₂ 6.1%, 145°C, 240 min); 333A (SO₂ 7.2%, 145°C, 240 min); 336A (SO₂ 4.9%, 140°C, 240 min); 351A (SO₂ 6.0%, 145°C, 180 min); 335A (SO₂ 6.5%, 145°C, 120 min).



CLE Bisulfite Pretreatment Mass Balance for Aspen



By Johnway Gao, Dwight Anderson
March 11, 2014

Items	Values	Units	Polymer Sugar Composition (%)	
Pulp Yield	80.3	%		
Solid Out	80.3	kg OD		
Polymer Sugar Output				
Glucan	41.32	kg	51.40	
Xylan	2.99	kg	3.71	
Mannan	4.39	kg	5.46	
Arabinan	0.34	kg	0.42	
Galactan	1.12	kg	1.39	
Total	50.14	kg	62.38	
Other components in pretreated biomass				
			Klason lignin (%)	23.45
			Acid soluble lignin (%)	0.48
			Extractives (%)	14.2
			Ash (%)	2.93

Mass Balance Unit		
Biomass	100	kg
Polymer Sugar Input		
Glucan	39.67	kg
Xylan	5.19	kg
Mannan	11.23	kg
Arabinan	1.16	kg
Galactan	2.84	kg
Total	60.09	kg

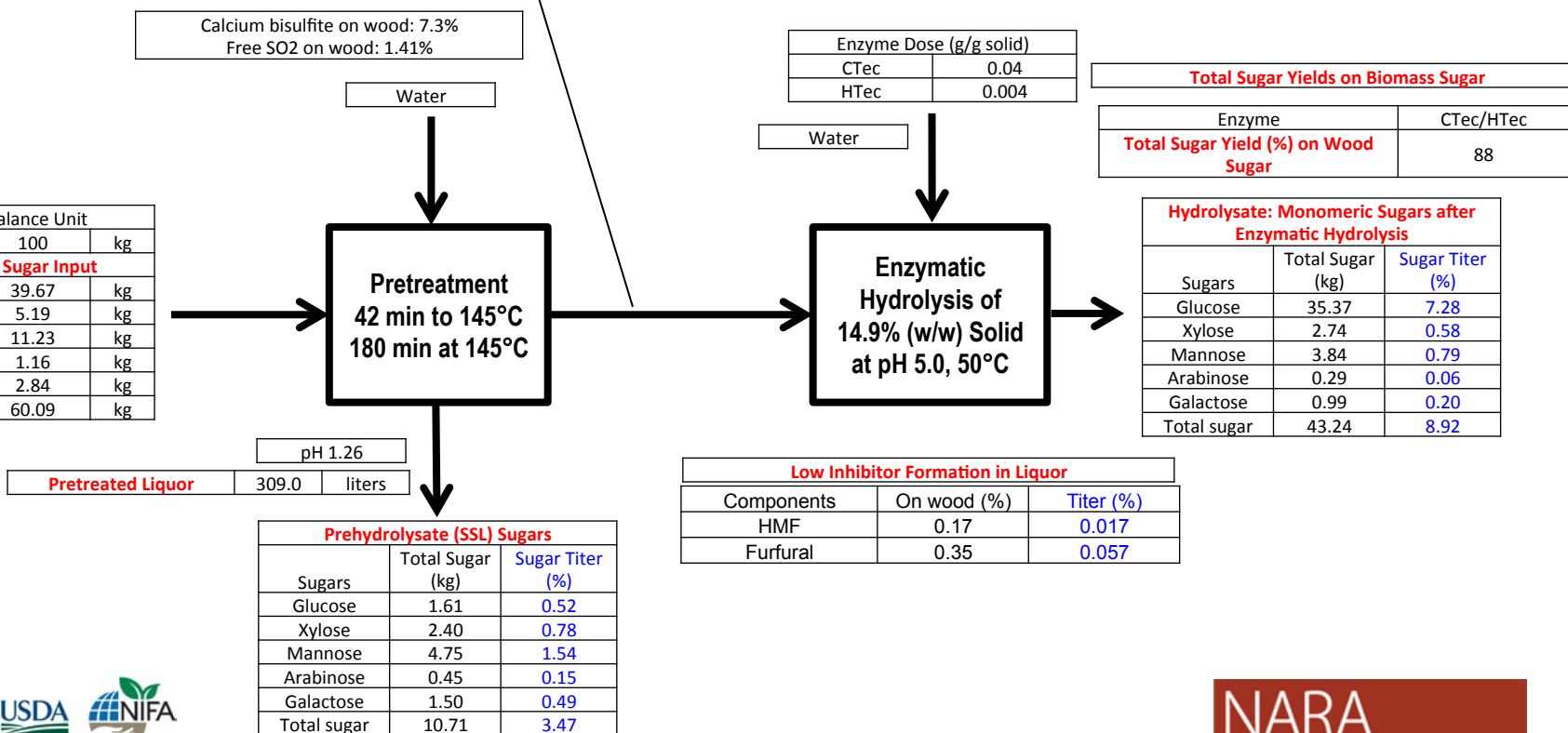


Figure C-P-4.7. Overall Sugar Balance for Optimized Pilot Run on FS-10.

Table C-P-4.4. The apparent densities of pretreated FS-10 feedstocks

Cook No.	Temp., °C	Time at T, min	Equivalent SO ₂ on wood (%)	Apparent pretreated and refined biomass density (dry g /ml)
257A	165	75	7.9	0.216
333A	145	240	7.2	0.130
336A	140	240	4.9	0.093

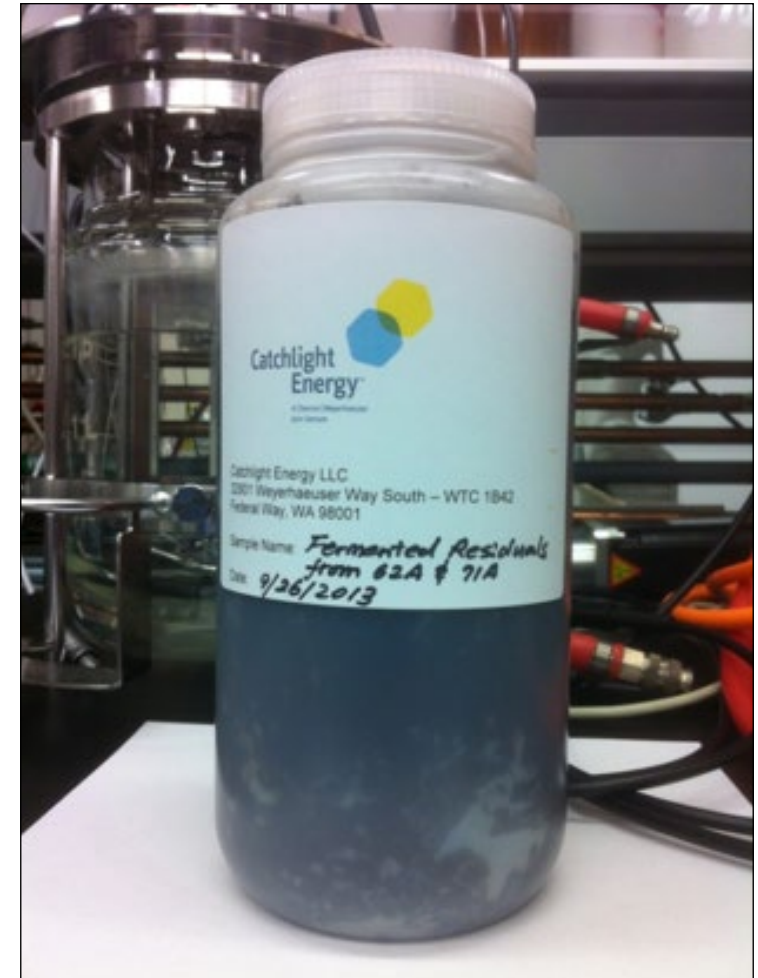
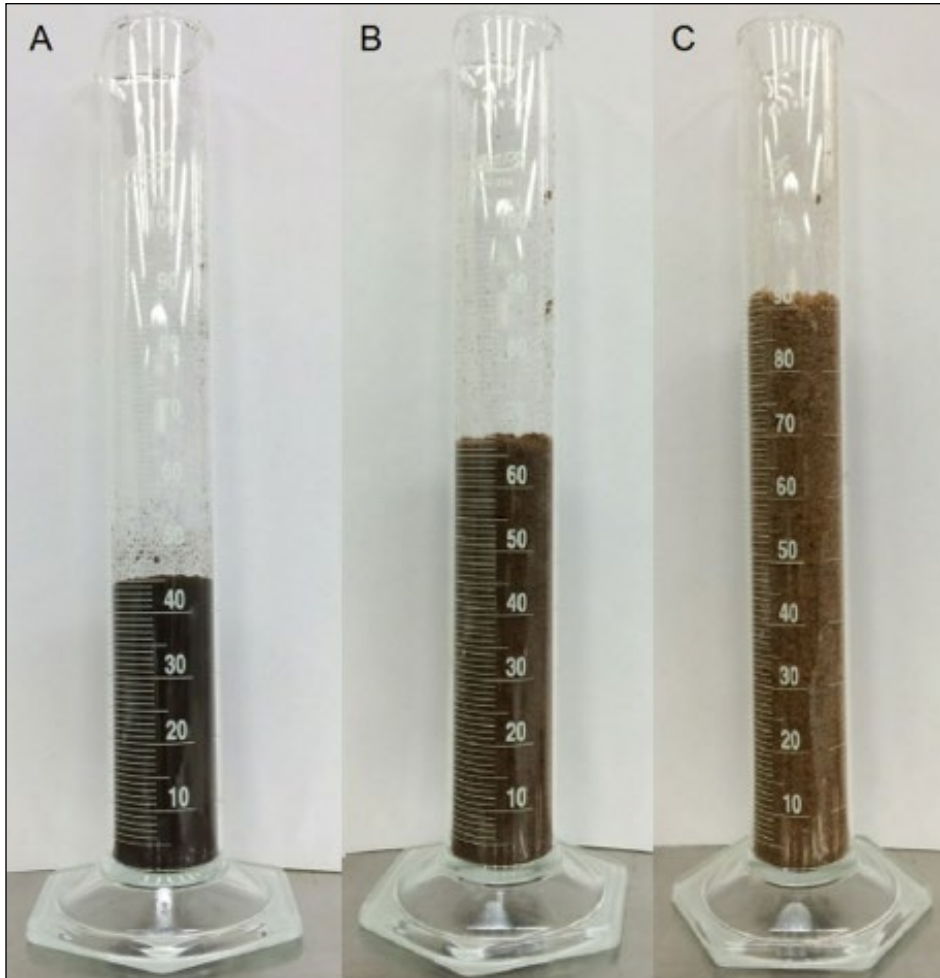


Figure C-P-4.8. Higher Temperature Densifies Pulp - The apparent densities of pretreated FS-10 feedstocks. A: 257A (SO₂ 7.9%, 165°C, 75 min), 20 gram wet pulp; B: 333A (SO₂ 7.2%, 145°C, 240 min), 20 gram wet pulp; C: 336A (SO₂ 4.9%, 140°C, 240 min), 20 gram wet pulp.

Figure C-P-4.9.

Table C-P-4.5. Modeling Relative Pretreatment Condition Economics

Case	Group A: Improved Pretreatment, but low hydrolysis solids						Group B: Higher Titer Hydrolysis					
	1	2	3	4	5	6	7	8	9	10	11	12
Pretreatment ID#	71A	71A	71A	71A	71A	71A	57A	57A	57A	57A	57A	57A
Description	Integrated	Ship 31% Sugar	Ship 50% sugar	Integrated, low	Ship 31% sugar	Ship 50% Sugar	Integrated	Ship 31% Sugar	Ship 50% sugar	Integrated, low	Ship 31% sugar	Ship 50% Sugar
Feedstock ODSTPD (NARA FS-10)	2200	2200	2200	2200	2200	2200	2200	2200	2200	2200	2200	2200
% Glucan	39.67	39.67	39.67	39.67	39.67	39.67	39.67	39.67	39.67	39.67	39.67	39.67
% Galactan	2.84	2.84	2.84	2.84	2.84	2.84	2.84	2.84	2.84	2.84	2.84	2.84
% Mannan	11.23	11.23	11.23	11.23	11.23	11.23	11.23	11.23	11.23	11.23	11.23	11.23
% Arabinan	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16
% Xylan	5.19	5.19	5.19	5.19	5.19	5.19	5.19	5.19	5.19	5.19	5.19	5.19
Base (Ca w/out recycle, Mg w/90% recycle)	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca
Pretreatment Conditions												
Cook Temp (°C)	145	145	145	145	145	145	165	165	165	165	165	165
Heat Up Time (min.)	45	45	45	45	45	45	58	58	58	58	58	58
Pretreatment Time (min.)	240	240	240	240	240	240	75	75	75	75	75	75
Total SO ₂ % on wood	6.06	6.06	6.06	6.06	6.06	6.06	7.89	7.89	7.89	7.89	7.89	7.89
% of total SO ₂ bound to bisulfite cation	66.3	66.3	66.3	66.3	66.3	66.3	59.9	59.9	59.9	59.9	59.9	59.9
Enzymes Purchased? (Y/N)	N	N	N	Y	Y	Y	N	N	N	Y	Y	Y
% CTEC2/HTEC2 on Pretreated	4.4%	4.4%	4.4%	1.0%	1.0%	1.0%	4.4%	4.4%	4.4%	1.1%	1.1%	1.1%
Hydrolytase Sugar Production (ODTPD)	742.2	742.2	742.2	374.1	374.1	374.1	656.0	656.0	656.0	408.6	408.6	408.6
Prehydrolytase Sugar Production (ODTPD)	329.7	329.7	329.7	251.4	251.4	251.4	260.2	260.2	260.2	260.2	260.2	260.2
Combined Sugar Production (ODTPD)	1,071.8	1,071.8	1,071.8	625.5	625.5	625.5	916.2	916.2	916.2	668.8	668.8	668.8
Combined Sugar Concentration (% Sugar)	8.2	8.2	8.2	5.4	5.4	5.4	10.8	10.8	10.8	8.2	8.2	8.2
Final Sugar Concentration (% Sugar)	9.0	31.0	50.0	9.0	31.0	50.0	10.8	10.8	10.8	9.0	31.0	50.0
TPD water removed for concentrating	1,161.9	9,613.6	10,927.5	4,633.2	9,565.3	10,332.1	-	5,491.5	6,614.6	697.4	5,971.0	6,790.8
Ethanol production (equiv. MM gal/year)	49.8	49.8	49.8	28.6	28.6	28.6	42.6	42.6	42.6	31.1	31.1	31.1
Steam Demands (MM Btu/hr)												
Net Pretreatment MM BTU/HR	42.8	42.8	42.8	42.8	42.8	42.8	54.2	54.2	54.2	54.2	54.2	54.2
Concentrate Sugar MM BTU/HR	77.5	640.9	728.5	308.9	637.7	688.8	0.0	366.1	441.0	46.5	398.1	452.7
Lignin Drying MM BTU/HR (+AC mfg)	57.3	57.3	57.3	82.7	82.7	82.7	64.2	64.2	64.2	77.8	77.8	77.8
Ethanol Production MM BTU/HR	162.6	85.5	85.5	93.4	49.1	49.1	73.1	73.1	73.1	53.3	53.3	53.3
Total Demand at Site of Sugar Plant	340.1	741.0	828.5	527.8	763.2	814.3	191.4	557.5	632.4	178.5	583.4	638.1
Steam Supply (MM Btu/hr)												
Sulfur burning	17.5	17.5	17.5	17.5	17.5	17.5	22.8	22.8	22.8	22.8	22.8	22.8
Lignin Residual Burning	663.6	663.6	663.6	958.1	958.1	958.1	743.5	743.5	743.5	901.7	901.7	901.7
Lignosulfonate Burning	178.6	178.6	178.6	178.6	178.6	178.6	203.6	203.6	203.6	203.6	203.6	203.6
Power Demand (kW)												
Sugar Plant (8 AC plant when applicable)	24818	24818	24818	24961	24961	24961	23876	23876	23876	23877	23877	23877
Ethanol Plant	2661	2661	2661	1529	1529	1529	2275	2275	2275	1660	1660	1660
MM BTU/HR steam for on-site power	375	338	338	361	340	340	357	326	326	348	326	326
Power Supply												
kW generated	38098	8704	2281	45928	28669	24921	57084	30240	24750	69626	39937	35929
OR Purchased Steam (MM BTU/HR) if > 0 or kW sold from Sugar Plant if > 0	-145	220	307	-265	-51	1	-422	-87	-12	-601	-219	-164
	10618	-16114	-22537	19438	3709	-39	30934	6364	874	44089	16060	12053
L/ODT	261	261	261	150	150	150	223	223	223	163	163	163
kWh/L sold (if >0)	0.44	(0.67)	(0.94)	1.41	0.27	(0.00)	1.51	0.31	0.04	2.95	1.08	0.81
Operational Costs (\$/gal ethanol)												
Feedstock	1.09	1.09	1.09	1.89	1.89	1.89	1.27	1.27	1.27	1.74	1.74	1.74
Natural Gas	-	0.18	0.24	-	-	0.02	-	0.02	0.02	-	0.02	0.02
Power Cost (not including credit)	-	0.03	0.03	-	0.03	0.03	-	0.03	0.03	-	0.03	0.03
Enzymes	-	-	-	0.20	0.20	0.20	-	-	-	0.20	0.20	0.20
Chemicals	-	-	-	-	-	-	-	-	-	-	-	-
Sulfur Consumption	0.04	0.04	0.04	0.06	0.06	0.06	0.05	0.05	0.05	0.07	0.07	0.07
CaCO ₃ Consumption	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
MgO Make-Up	-	-	-	-	-	-	-	-	-	-	-	-
Maintenance Materials	0.32	0.28	0.28	0.54	0.48	0.48	0.34	0.29	0.30	0.46	0.39	0.39
Labor	0.18	0.18	0.18	0.30	0.30	0.30	0.20	0.20	0.20	0.28	0.28	0.28
Co-product opex (from TEA 6.2)	-	-	-	-	-	-	-	-	-	-	-	-
Transport ethanol and (if needed) sugar	0.06	0.60	0.43	0.06	0.60	0.43	0.06	0.60	0.43	0.06	0.60	0.43
Waste Disposal	0.03	0.03	0.03	0.05	0.05	0.05	0.04	0.04	0.04	0.05	0.05	0.05
Sub-Total \$/gal	1.70	2.42	2.31	3.12	3.64	3.48	1.97	2.52	2.35	2.88	3.41	3.23
By-Product Credits												
50% Calcium Ligno-Sulfonate	-	-	-	-	-	-	-	-	-	-	-	-
Activated Carbon	-	-	-	-	-	-	-	-	-	-	-	-
Power (sell)	0.10	-	-	0.32	0.06	-	0.34	0.07	0.01	0.67	0.24	0.18
Sub-Total \$/gal	0.10	-	-	0.32	0.06	-	0.34	0.07	0.01	0.67	0.24	0.18
Capital SMM (Based on NARA TEA)												
Feedstock handling	43.9	43.9	43.9	43.9	43.9	43.9	43.9	43.9	43.9	43.9	43.9	43.9
Pretreatment	133.0	133.0	133.0	133.0	133.0	133.0	133.0	103.5	103.5	103.5	103.5	103.5
Enzyme Production and Hydrolysis	39.6	39.6	39.6	25.4	25.4	25.4	39.6	39.6	39.6	25.4	25.4	25.4
Fermentation and Separation	61.7	0.0	0.0	44.6	0.0	0.0	61.7	0.0	0.0	61.7	0.0	0.0
Concentrator (4 effect, not in NARA)	11.6	41.4	44.7	27.6	42.6	44.6	0.0	30.5	34.1	8.9	32.1	34.7
IPK to Jet or co-products, as needed	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wastewater Treatment	49.4	49.4	49.4	49.4	49.4	49.4	49.4	49.4	49.4	49.4	49.4	49.4
Product Storage, Blending, Distribution	29.8	14.2	10.7	29.8	14.2	10.7	29.8	12.9	9.7	29.8	12.9	9.7
Power Boiler	48.4	48.4	48.4	57.9	57.9	57.9	51.9	51.9	51.9	56.9	56.9	56.9
Utilities	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9
Other Direct (based on ISBL)	50.7	45.1	45.7	48.0	42.9	43.2	43.5	38.1	38.7	42.6	35.9	36.3
Indirect Cost	285.0	253.1	253.3	279.9	249.7	249.0	258.1	226.0	226.6	257.4	220.2	220.1
Fixed Capital Investment (land, working)	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
Sub-Total SMM	785	700	701	772	691	689	714	628	630	712	613	612
Sub-Total \$/gal, 12.5% Capital Recovery Factor	1.97	1.76	1.76	3.37	3.02	3.01	2.09	1.84	1.85	2.86	2.46	2.46
Total Cost \$/gal	3.57	4.18	4.07	6.17	6.59	6.49	3.72	4.29	4.18	5.07	5.63	5.51

Recommendations | Conclusions

While further optimization is certainly possible, the most optimal condition in the Year 3 scale up work conducted jointly by Forest Products Lab and Catchlight Energy was targeted. The preferred conditions are as shown for the 145°C pretreatment, with 4.0% CTEC2 on pretreated feedstock and 0.4% HTEC2 on pretreated feedstock, with 6% total SO₂ on wood that includes 6-7% calcium bisulfite on wood. A total cook time of 3 hours is adequate, although 4 hours was used in the first run by Forest Products Lab to produce the final, large lignin residual and liginosulfonate samples required by Gevo and the Co-Products team.

Although separate sugar production transported to another location is not as cost-effective as an integrated plant, we will continue to explore a trial alternative that makes use of the Cosmo mill in Cosmopolis, Washington to do a demonstration or off-site production.

Physical and Intellectual Outputs

RESEARCH PRESENTATIONS

Poster Presentation at NARA Annual Meeting in Corvallis, OR in September 2013: Mild Bisulfite Pretreatment, by Dwight Anderson and Johnway Gao Gao, Johnway; Anderson, Dwight; "Optimization of Mild Bisulfite Pretreatment on Softwood," Harvesting Clean Energy Conference, Helena, Montana, February 4-6, 2014.

Gao, Johnway; Anderson, Dwight; "Optimization of Mild Bisulfite Pretreatment on Pacific Northwest Softwood," Northwest Wood-Based Biofuels and Co-Products Conference, Seattle, Washington, April 28-30, 2014.

TASK C-P-5: CLEAN SUGAR AND LIGNIN PRETREATMENT TECHNOLOGY

Key Personnel

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Task Description

A majority of current pretreatment technologies use chemical or thermal treatments to alter the lignocellulosic structure and enhance saccharification. Many of these methods have been proven to be effective with high yield. But the disadvantage of these methods involve the costs of chemicals, chemical recycling, or capital cost to achieve efficient scale; environmental consequence of the chemical use; and contamination of both the pretreated feedstock or lignin co-products. Evidence exists in the literature that milling of lignocellulosic materials to micron- or nano-scale can produce good hydrolysis performance without the use of any chemicals in the pretreatment. To effectively utilize this approach to mechanical pretreatment, energy efficient methods of milling are needed. Recent advances in ball mill designs have yielded commercial equipment that potentially can mill wood in the sub-micron size range with low energy input. This technique has been investigated in preliminary trials and found to yield adequate sugar production in an un-optimized condition. A preliminary techno-economic analysis estimates nearly a 40% savings in total pretreatment costs compared to the current bisulfite processes. This technology also demonstrates a strong potential for cost effectiveness at small scales, thereby facilitating deployment in depot settings.

While moving towards a feasibility validation of this mechanical pretreatment method, the goals for Year 3 were to investigate the potential yield improvements, verify energy consumption, and environmental burdens by manipulating process parameters. The tasks were as follows:

- Comprehensive literature study of wood milling technologies

- Conduct life cycle analysis (LCA) of wood milling
- Verify energy consumption in tandem mill at [redacted]
- Produce small-scale clean sugar and lignin for Gevo fermentation and lignin co-product evaluation
- Optimize hydrolysis
- Produce large-scale clean sugar and lignin production for Gevo fermentation and lignin co-product evaluation

Expected Outputs are:

- Report on the feasibility of the mechanical pretreatment method with specific yield, cost, and environmental performance metrics

Activities and Results

The clean sugar and lignin pretreatment project was initiated in October, 2013 under the NARA Year 3 funding. All the activities are outlined above and focused on the feasibility and validation of a mechanical pretreatment method:

- A literature study was performed on relevant wood or biomass chipping and milling technologies and on commercial machinery. With the techno-economic analysis, the milling process for clean sugar production and lignin fuel production seems plausibly feasible if the overall energy consumption ranges from 1.45-1.73 kWh/oven dry (OD) kg wood from wood chips to sugar and lignin products, where 0.56-0.84 kWh/OD kg wood is the targeted energy for wood milling.
- The LCA was performed on the wood milling process. The wood drying step, either by natural gas or by wood residuals as fuels, contributes to the emission of carbon dioxide equivalent (CO₂e), while the mechanical wood chipping or milling by abundant hydroelectricity in the Northwest does not emit

CO₂ to the environment.

- An energy consumption evaluation was undertaken during an on-site visit to a tandem ring milling operation in [redacted] in October 2013. Milled FS-10 feedstock samples were obtained during the visit. The efficient milling is being optimized in [redacted] after more Douglas-fir chips FS-01 were sent to [redacted] in March, 2014. More milling optimization results are expected towards end of April and more milled wood samples will be obtained for validation from May to June. All the results will be reviewed for the final feasibility study and report.
- Small scale clean sugar samples and un-fermented and fermented lignin samples were produced. The sugars were provided to Gevo for fermentation evaluation and the lignin was given to the NARA co-product team for co-product testing.
- Various milled wood samples were analyzed for its sugar and lignin composition. In addition, higher milled wood solid was hydrolyzed and fermented with ethanol-producing yeast for hydrolysis evaluation. More hydrolyses testing will be conducted for optimization.

The targeted energy consumption by wood milling, which is being optimized, is used for the techno-economic analysis (TEA) for clean sugar and lignin production from wood residual chips. Based on the TEA, if the targeted energy consumption of wood milling is met or achieved within the range of 0.56-0.84 kWh/OD kg wood at a sugar conversion yield of about 58-65%, the economics by milling as a pretreatment technology looks plausible.

WOOD MILLING TECHNOLOGY REVIEW

In current milling literature of lignocellulosic sugar conversion from biomass, quantitative analyses showed that in some instances, wet-disk milling (WDM) can achieve high cellulose saccharification yield of 78.5% from milled rice straw with an energy consumption

of 5.4 MJ/kg biomass (Hideno et al., 2009). In some other instances, WDM can only achieve 44.7% of cellulose saccharification yield when bagasse is milled, and a 59.5% yield when sugarcane straw is milled (da Silva et al., 2010). Resized biomass particles of 2 mm were as used and fed into the WDM milling machine with a 20-40 micron gap between the upper and lower grinders, with a biomass to water ratio of about 1 to 20. The water need is large in the WDM. The WDM process is material sensitive and the energy consumption is very high. The energy consumption of WDM process is not yet economical for lignocellulosic sugar conversion from biomass.

In 2008, a tandem ring milling machine was introduced by Takahashi et al. (2008). The milling is a dry milling process that does not require water. Japanese cedar of less than 20% moisture was tested in the milling machine. A mean wood particle size of about 30 microns was achieved in 60 minutes with 71% cellulose saccharification yield. The initial goal for economical energy milling was estimated at around 0.9 MJ/kg wood by [redacted]. When the large scale trial is reviewed by [redacted], it is estimated that the targeted energy consumption of Douglas-fir milling will be around 2.0 MJ/kg wood.

LCA OF WOOD MILLING

In the Pacific Northwest, hydroelectricity is used for wood chipping and milling energy. However, wood chip drying can use either natural gas or wood residuals as the energy source, which was evaluated for its life cycle assessment (LCA). The LCA reported by ICF International (2012) on natural gas estimated that 78.5 g CO₂e/MJ will be generated by upstream natural gas processing and combustion for heating. Thus, the chip drying energy of 0.8278 kWh/OD kg wood (see tandem wood milling section) translates into an emission of 234 g CO₂e/kg OD wood, summarized in Table C-P-5.1.

If wood residuals are used as fuel for wood drying, the report by Bowyer (2012) shows that the emission

of 24.6 g CO₂e/kWh or 6.8 g CO₂e/MJ will be generated. Thus, the chip drying energy of 0.8278 kWh/OD kg wood translates into an emission of 20.4 g CO₂e/kg OD wood as shown in Table C-P-5.2.

TANDEM WOOD MILLING

The wood milling process has four steps before the milled wood powder can be enzymatically hydrolyzed for clean sugar and lignin production. These four steps include: (1) Wood residuals chipping; (2) Chip size reduction before drying; (3) Chip drying to reduce chip moisture from 50% to 10%; and (4) Wood milling. The first three steps are well known technologies and the equipment is commercially available. The last step of large scale wood milling, with high performance and optimized energy efficiency, is being tested and will be validated for this project in May and June 2014. The current investigation and analysis results are shown in the tandem milling section.

(1) WOOD RESIDUALS CHIPPING:

Commercial chipping machine is available for wood residual chipping operation. Table C-P-5.3 shows the scale and energy requirement for wood residual chipping.

Two pieces of equipment were reviewed for chip size reduction, one is a wood resizing machine, Crumbler™ M24 by Forest concepts LLC, and the other is commercially available sawdust making machine. For the tandem wood milling process, wood chips with a 2 mm mean size is required.

(2.1). CRUMBLER™ M24 MACHINE

On September 24, 2013, Douglas-fir FS-10 feedstock chips with a geometric mean dimension (Xgm) of 13.42 mm and a moisture content of 10% were resized into small chips with a Xgm of 1.29 mm in the Crumbler™ M24 machine at Forest Concepts LLC (Auburn, WA). The energy consumption was recorded by LabView® software Universal and analyzed via Excel® based

program Comminution Energy Rev16.5. Figure C-P-5.1 shows the FS-10 sample before and after resizing. Table C-P-5.4 shows the specific energy consumption to resize FS-10 chips to 1.29 mm Xgm, which were used in small scale milling trials at [redacted].

To calculate the specific energy required to convert raw FS-10 wood chips through a cascading multi-stage process involves measuring the energy consumed during subsequent passes and applying that energy to the diminishing mass of material to be re-processed, as shown in Table C-P-5.4. The 13.2 mm chips were first resized down to about 5 mm before the chips are further reduced to 2.21 mm (Xgm).

The specific energy required for the first pass of the raw wood chips through the Crumbler™ M24 with 4.8 mm cutters was 27 MJ/OD Mg (7.5 kWh/OD tonne) (Table C-P-5.4). Processing all the material through recirculation until it passed the No. 10 screen (Xgm = 1.29 mm) consumed 162 MJ/OD Mg or 0.162 MJ/OD kg (45 kWh/tonne). Note that these values would have been considerably lower had the chips been processed at moisture levels above the fiber saturation point. It is expected that a minor increase in target screen size to 2.4 mm will require fewer passes through the Crumbler™ machine and will still yielded a geometric mean particle size of less than 2 mm. It is estimated that increasing the screen to 2.4 from 1.6 mm would reduce the specific energy to approximately 130 MJ/OD Mg or 0.130 MJ/OD kg (36 kWh/tonne).

(2.2). SAWDUST MACHINE

The sawdust machine is commercially available for the production of 2 mm size chips, without requiring two steps in the size reduction process. Table C-P-5.5 shows the scale and energy requirement for wood residual chipping. This sawdust machine does not require initial wood chipping to 5-13 mm size.

(3). WOOD CHIPS DRYING:

Tandem wood milling or wood milling to fine wood particles requires wood chip moisture of about 10% or less to be efficient. A wood chip drum dryer with a natural gas burner to provide heat energy for wood drying is commercially available. Table C-P-5.6 shows the actual energy needs to dry the wood chips from 50% moisture to 10% moisture. The specific energy requirement is 1375 Btu/lb water (Savovic Z, TSI, Lynwood, WA, March 2014, personal communication).

(4). TANDEM WOOD MILLING

(4.1) Large scale tandem milling machine

In October 2013, NARA members visited the team that developed the tandem ring milling technology. They evaluated the energy consumption of the first tandem ring milling machine with a capacity of one metric ton per hour (MT/hr), which was under optimization studies for design and performance.

The 1 MT/hr tandem machine milling machine (Model HV-70) requires twin 6-meter-long tandem milling chambers equipped with two motors, each capable of 100 kW actual running power. The 6-meter chamber tandem ring milling machine was first built in 2011 and tested for its structural stability. It was found that the 6-meter machine was not quite stable in operation, in part from excessive sympathetic vibration. Subsequently, an improved HV-70 version was built in 2012 with twin 3 meter chambers and a 0.5 MT/hr capacity. The improved HV-70 was found to be stable during operation. Large scale optimizations in power, wood chip loading (productivity) and performance have not been completed yet but are ongoing. The design goal of energy consumption in large scale tandem milling was 0.9 MJ/kg OD wood. At low solid loading of Japanese cedar chips (apparent density = 0.1 kg/L) at 144 kg/hr and 240 kg/hr, the energy consumption would be estimated as 2.5 and 1.4 MJ/kg OD wood, under an assumption that half of the equipped motor power for the 6-meter milling ma-

chine could be needed for the 3-meter improved HV-70 tandem ring milling machine. The best enzymatic hydrolysis yields were 62.5% and 37.6%, respectively. Upon large scale optimization, it is plausible that lower energy consumption will be sufficient to achieve a good enzymatic hydrolysis yield.

The design plan for the tandem ring milling machine will eventually achieve a processing capacity of 1 to 5 metric tons/hr. From October 2013 to December 2014, trials will be conducted with (1) the Improved HV-70 model (3-meter model); (2) a new TR-3000 model (100 kg/hr, under construction now); (3) large scale prototype design/production/test. This program will be reviewed before March 2015 to determine the feasibility of large scale design.

It should be noted that large scale tests or trials require more than 100 kg OD chips of 2 mm Xgm size.

(4.2) Small scale tandem milling trials

Douglas-fir FS-10 feedstocks of 10 kg with 1.29 Xgm were used for milling trials. Small scale milling screening trials were conducted in the small scale tandem ring milling machine (1.6 kg capacity). This small machine has much higher energy consumption as compared to the 0.5 MT/hr machine due to the small machine's heavy frame design vs. its small capacity. However, the small trials could help understand the particle size distribution vs. milling time and lignocellulosic conversion yield to sugar.

(4.3) Douglas-fir tandem milling energy optimization

We reviewed the large scale tandem milling design and Japanese cedar mill trial results. The Japanese cedar fine chips have a density of 0.1 kg/L while the Weyerhaeuser Douglas-fir chips have a density of 0.26 kg/L. Thus, the treatment of powder volume factor is $0.26/0.1 = 2.6$, indicating that less energy is needed for a more dense material. The estimated power consumption target for Douglas-fir chips milling is ~2 MJ/OD kg wood chips.

The large scale milling test is being conducted on 2250 pounds of dry Douglas-fir FS-01 chips in the improved HV-70 tandem milling machine (0.5 MT/hr) to validate the estimated power consumption of ~2 MJ/OD kg wood chips.

In summary, if the fine wood milling energy consumption target will be met at 2 MJ/OD kg wood or even 3 MJ/OD kg wood (0.56-0.84 kWh/OD kg biomass), the total energy requirement for wood milling could range from 1.45 to 1.73 kWh/OD kg, summarized in Table C-P-5.7.

SMALL-SCALE CLEAN SUGAR AND LIGNIN PRODUCTION

To provide Gevo with a hydrolysate of milled FS-10 feedstocks and the Co-Product Team with lignin rich residuals from the hydrolyzed milled FS-10 powder, larger scale and higher solid hydrolyses at 15% consistency with 5.5% CTec3/HTec3 enzyme product were conducted in 1000 ml shake flasks with 700 ml working volume. After hydrolysis, a simple vacuum filtration was used to separate hydrolysate from the lignin rich solid residuals. The hydrolysate has a sugar titer of about 7.5% total sugar, which was further concentrated to 10% total sugar titer with a vacuum evaporator at 61-63°C. The generated 5 liter hydrolysate, as shown in Figure C-P-5.2, was sent to Gevo for a preliminary isobutanol fermentation evaluation. More hydrolysate of 1.8 liters (Figure C-P-5.3) was sent to Gevo on March 25, 2014 for GIFT fermentation system testing.

The hydrolysis-generated 1.1 OD kg equivalent lignin residuals (3x washed by deionized water at equal wet weight each time and shown in Figure C-P-5.4) was sent to the NARA Co-Product team on November 21, 2013 for characterization and application analyses. In addition, fermentation by regular ethanol producing yeast was conducted on milled wood hydrolysate without the separation of hydrolyzed wood residuals from the hydrolysate. The resulting fermented milled residuals of 0.75 kg OD, as shown in Figure C-P-5.5,

was produced and sent to the NARA Co-Product team on January 30, 2014 for evaluation.

OPTIMIZATION OF MILLED WOOD HYDROLYSIS

Particle distribution under various milling times

Small scale tandem milling produced various FS-10 milled wood samples as shown in Figure C-P-5.6. There is some particle size variation in milling between the left chamber and the right chamber, as shown in Figure C-P-5.7. The left chamber was cooled to a lower temperature than the right chamber due to a serial cooling connection (left chamber first, and then to right chamber) rather than a parallel cooling connection. Within 30 minutes of milling, the mean particle diameters of wood powder are 32-38 microns in the left chamber and 34-43 microns in the right chamber. Within 60 minutes of milling, the mean particle diameters of wood powder are 29-34 microns in the left chamber and 41-44 microns in the right chamber. Longer milling of 80-100 minutes seems to cause wood particle agglomerations even with sonication prior to particle size analysis and counting.

The crystallinity of the FS-10 milled Douglas-fir wood was measured by X-ray diffraction. The results are shown in Figure C-P-5.8. After 60 minutes of milling, the milled wood crystallinity shows no further or significant reduction, which means 60 minutes of milling is sufficient.

All the FS-10 milled wood sugar compositions were analyzed and shown Table C-P-5.8. Results show that there is small sugar loss after the milling process. Some samples were analyzed for lignin, and the lignin composition shows some proportional increase due to small sugar loss in the milled samples (Table CP-5.8).

HYDROLYSIS OPTIMIZATION

The enzymatic hydrolysis of milled FS-10 was con-

ducted at a solid of 2% consistency with 5% Meicelase enzyme product (solid enzyme product) on wood powder. The hydrolysis results at 48 hours are shown in Figure C-P-5.9. The low solid loading at 2% consistency achieves saccharification efficiencies or yields of holocellulose (cellulose and hemicellulose) of 67% by 30 minutes of milling and of 71-81% by 60 minutes of milling as shown in Figure C-P-5.9.

The hydrolysis of higher solid loading at 10% consistency was conducted at the Weyerhaeuser Technology Center. The hydrolyses achieved saccharification efficiencies of holocellulose of 53-56% by 30 minutes of milling and of 56-72% by 60 minutes of milling, as shown in Figure CP-5.10. Milling longer, at 80-100 minutes, only shows a slight yield increase and the total yield ranging from 60-75%.

The small scale FS-10 milled Douglas-fir was also hydrolyzed at 20.6% consistency by 6% CTec2 and 0.6% HTec2 enzyme dose on milled wood achieving a hydrolysis yield of 58% on a four day hydrolysis with total sugar titer of 7.7%, as shown in Figure CP-5.11. Hydrolysate fermentation by ethanol yeast can achieve 4.2% ethanol at 24-hr fermentation. More hydrolysis optimization will be conducted in the next trimester.

TECHNO-ECONOMIC ANALYSIS (TEA) FOR CLEAN SUGAR AND LIGNIN PRODUCTION

The targeted wood milling energy consumption of 0.56-0.84 kWh/OD kg wood, which is being tested and optimized, is used for the techno-economic analysis (TEA) for clean sugar and lignin production from wood residual chips.

With the NARA depot concept, a depot size sugar and lignin pellet fuel plant is assumed to process annual softwood wood residuals of 222,000 BDT/year. The techno-economic analysis is conducted for this depot sized plant. Natural gas is used for wood drying. The plant parameters are summarized in Table C-P-5.10 and the commodity cost or product sale

price information is shown in Table C-P-5.11.

A cost stack bar chart is shown Figure C-P-5.12 for the sugar cost with lignin pellet fuel as co-product. The total plant cost is \$23.2 million for the production of 97,794 BDT sugar/year and 133,977 BDT lignin pellet/year, with an annual expense of \$14.8 million. The lignin pellet fuel has a positive credit of \$0.0463 per lb of sugar produced.

The sugar product cost is very competitive at \$0.1340/lb when the feedstock price is at \$65/BDT. At the market sugar price of \$0.169/lb, the net profit would be \$0.035/lb sugar or a total profit of \$6,965,974/yr, indicating a good TEA case. The feedstock cost is very significant in sugar production, and the production cost can be reduced to \$0.1170/lb if the feedstock price can be reduced from \$65/BDT to \$50/BDT. In addition, if at \$50/BDT feedstock price, the sugar production cost is \$0.1285/lb although the milling energy is 0.84 kWh/OD kg wood, which is still very competitive. In certain location, if the feedstock cost is \$45/BDT, the sugar production cost can be as low as \$0.1106-0.1235/lb. At sugar production cost of \$0.1106/lb, the net profit would be \$0.0584/lb sugar or a total profit of \$10 million/yr, indicating a very good TEA case.

Various cases of cost analyses are summarized in Table C-P-5.12. The TEA analysis is being refined, and the full TEA report will be issued in the next trimester.

Table C-P-5.1. Carbon dioxide equivalent emission at natural gas production and its conversion to wood chip drying by natural gas

Emission Amount	kg CO ₂ e /MWh	(g CO ₂ e /MJ)	(g CO ₂ e /OD kg wood drying)
CO ₂ e*	282	78.5	234

*Sum of upstream and end combustion emission for natural gas production

Table C-P-5.2. Carbon dioxide equivalent emission at wood chip drying using residual wood as fuel

Emission Amount	kg CO ₂ e /MWh	(g CO ₂ e /MJ)	(g CO ₂ e /OD kg wood drying)
CO ₂ e	24.6	6.8	20.4

Table C-P-5.3. Specific energy consumption by a wood drum chipper

Items	Output Size (mm)	Specific Energy Consumption (kWh/OD kg wood)	Scale (metric ton/hr)	Commercial Product
Wood chipping	38	0.0098	27.2	Yes†

†Parameters provided by (redacted)



Figure C-P-5.1. Raw Douglas-fir FS-10 chips with 13.42 mm Xgm and finished feedstocks with 2 mm Xgm

Table C-P-5.4. Comminution energy results of raw Douglas-fir FS-10 feedstock chips with 13.42 Xgm to finished feedstocks with 1.29 mm Xgm

Line	Sample ID	Description	GMD (Xgm) mm	Comminution Ratio	% Retained from prior run	% Original Mass	Specific E (MJ/OD Mg)	
							Run	Cumulative, Original mass basis
1	2013.09.23.001	Raw Chips	13.4	-	-	100	-	-
2	2013.09.23.001. A-C	1st Pass 4.8mm	5.66	2.4	-	100	27	27
3	2013.09.23.001. A-C.A	2ndPass 4.8mm	4.12	1.4	-	100	16	43
4	2013.09.23.001. A-C.B	1st Pass 1.6mm	2.21	1.9	-	100	67	110
5	2013.09.23.002	2ndPass 1.6mm	2.21		73	73	37	137
6	2013.09.23.003	3rd Pass 1.6mm	2.21		70	51	26	150
7	2013.09.23.004	4th Pass 1.6mm	2.21		62	32	20	157
8	2013.09.23.005	5th Pass 1.6mm	2.21		58	18	17	160
9	2013.09.23.006	6th Pass 1.6mm	2.21		56	10	14	161
10	2013.09.23.007	Final Product	1.29		53	5	12	162

Table C-P-5.5. Specific energy consumption by a sawdust machine

Items	Output Size (mm)	Specific Energy Consumption (kWh/OD kg wood)	Scale (metric ton/hr)	Commercial Product
Size reduction by sawdust machine	2	0.0505	2	Yes†

†Parameters provided by (redacted)

Table C-P-5.6. Specific energy consumption by a sawdust machine

Items	Output Size (mm)	Specific Energy Consumption (kWh/OD kg wood)	Scale (metric ton/hr)	Commercial Product
Chip drying	NA	0.8278	8	Yes‡

‡Parameters provided by (redacted). The specific energy includes 0.0186 kWh/OD kg as the mechanical energy for the rotary drum dryer.

Table C-P-5.7. Wood milling energy requirement in each size reduction step

Steps	Output Size (mm)	Specific Energy Consumption (kWh/OD kg wood)	Scale (metric ton/hr)	Commercial Product
1. Wood chipping	38	0.0098	27.2	Yes†
2. Size reduction	2	0.0505	2	Yes†
3. Chip drying	NA	0.8278	8	Yes‡
4. Fine milling*	0.030-0.050	0.5587-0.8368 (target)	0.12-0.5	Not yet
Total Energy (kWh/OD kg)	1.45-1.73			

†Parameters provided by [redacted]‡Parameters provided by (redacted).
 The specific energy includes 0.0186 kWh/OD kg as the mechanical energy for the rotary drum dryer.
 *The fine milling is being tested in [redacted]

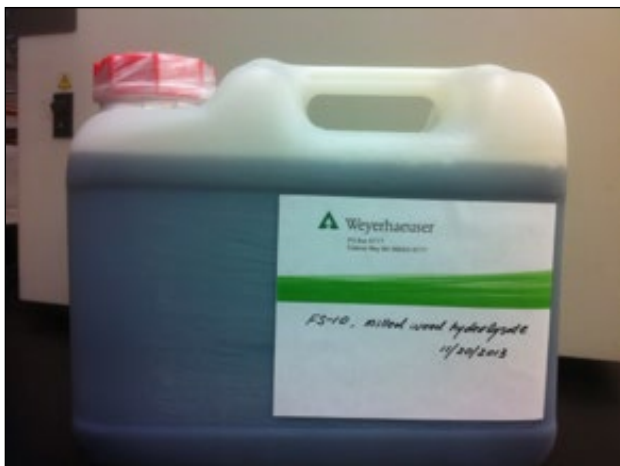


Figure CP-5.2. Hydrolysate of 5 liters with 10% total sugar concentration, sent to Gevo on November 21, 2013



Figure CP-5.3. More hydrolysate of 1.8 liters, sent to Gevo on March 25, 2014 for the GIFT fermentation system testing.

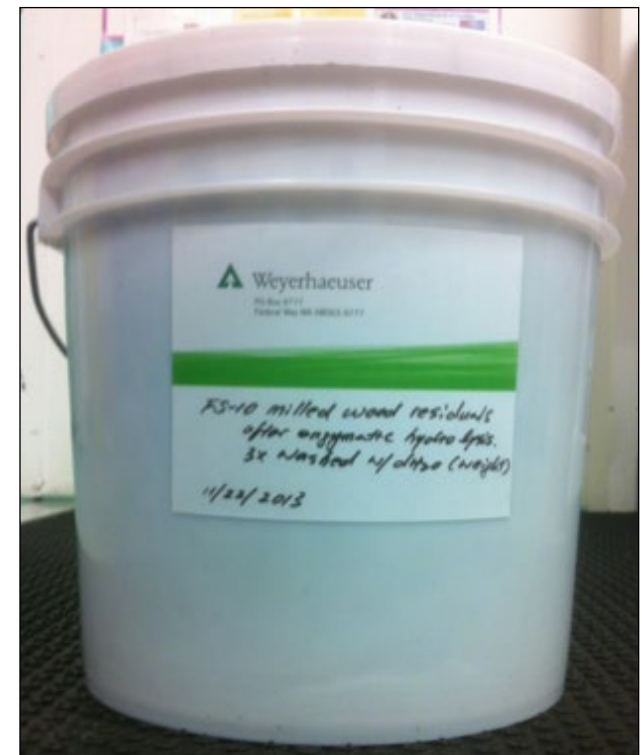


Figure C-P-5.4. Lignin rich residuals of 1.1 OD kg equivalent, sent to the Co-Product Team on November 21, 2013

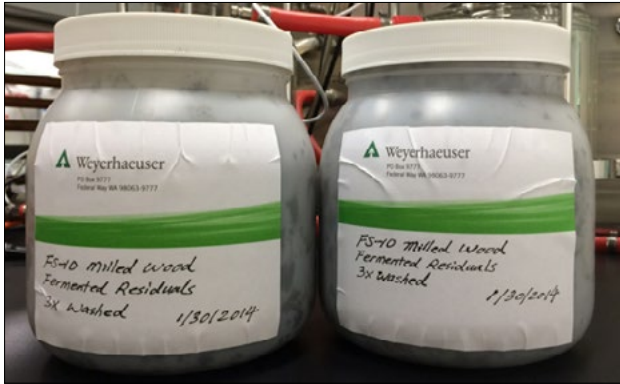


Figure C-P-5.5. Lignin rich residuals of 0.75 OD kg equivalent, sent to the Co-Product Team on January 30, 2014



Figure C-P-5.6. Milled wood powders of FS-10 chips, under various milling time, 30, 60, 80, and 100 minutes. L-from left milling chamber; R- from right milling chamber. Lighter color of milled wood in L-chamber indicates milling temperature was cooler than that in the R-chamber (onsite photo during our visit to [\[redacted\]](#))

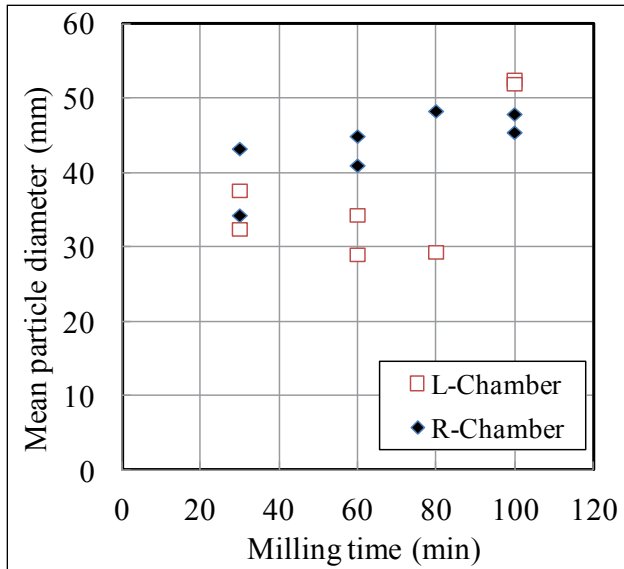


Figure C-P-5.7. Mean particle diameter of milled FS-10 under various milling times

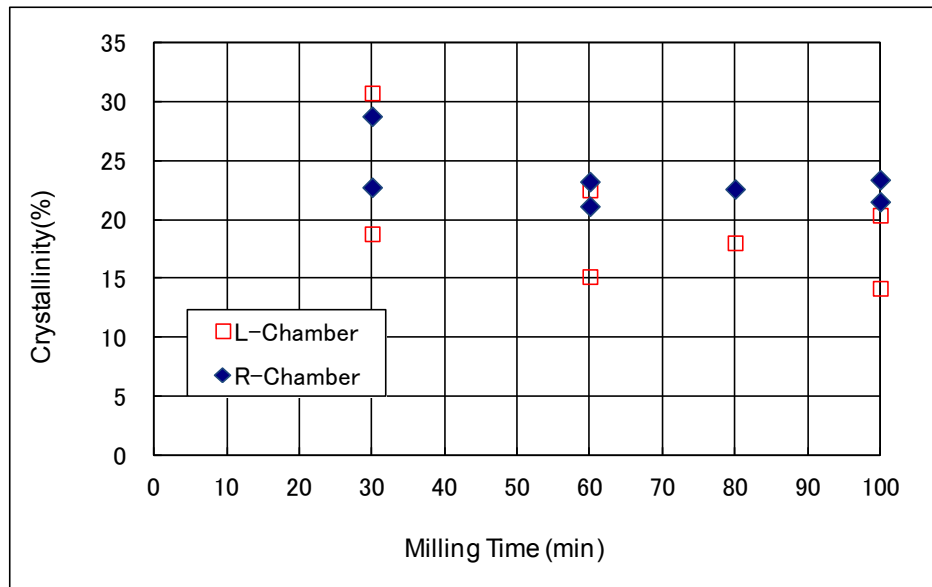


Figure C-P-5.8. Cellulose crystallinity of milled FS-10 under various milling times

Table C-P-5.8. FS-10 milled wood carbohydrate composition under various milling conditions

FS-10 Sample ID	Milling Time (min)	Total Polymer Sugar (%)	Loss (%)
2 mm chips	0	60.40	NA
1.L30	30	59.85	0.91
2.R30	30	60.28	0.20
3.L60	60	59.10	2.15
4.R60	60	58.48	3.18
7.L80	80	58.99	2.33
8.R80	80	58.41	3.29
11.L100	100	58.85	2.57
12.R100	100	58.92	2.45

Table C-P-5.9. FS-10 milled wood lignin composition under various milling conditions

FS-10 Samples	Milling Time (min)	Total Lignin (%)	Increase (%)
2 mm chips	0	27.34	NA
L/R 60	60	27.96	2.2
L/R 100	100	28.23	3.3

Note: L-left milling chamber; R-right milling chamber; L/R-left and right data average.

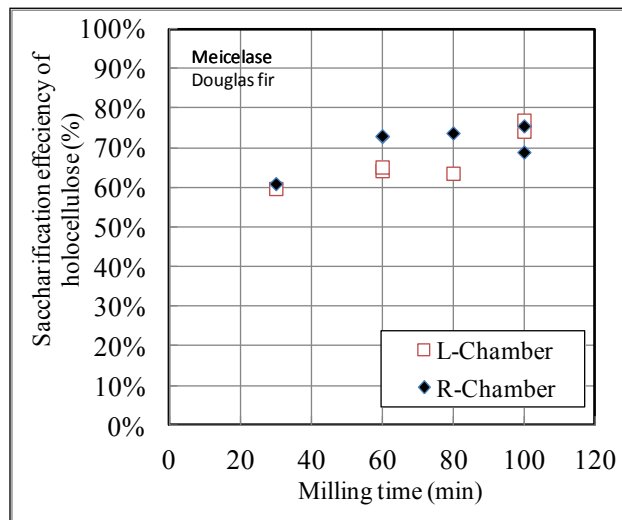


Figure C-P-5.9. Enzymatic hydrolysis efficiency or yield of low solid concentration at 2% consistency with 5% Meicelase enzyme product on wood powder. Hydrolysis time = 2 days

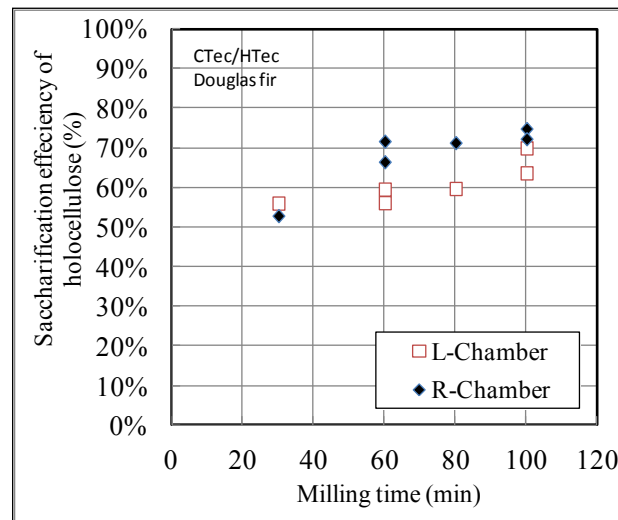


Figure C-P-5.10. Enzymatic hydrolysis efficiency or yield of higher solid concentration at 10% consistency with 4.4% CTec3/HTec3 enzyme product on wood powder. Hydrolysis time = 4 days

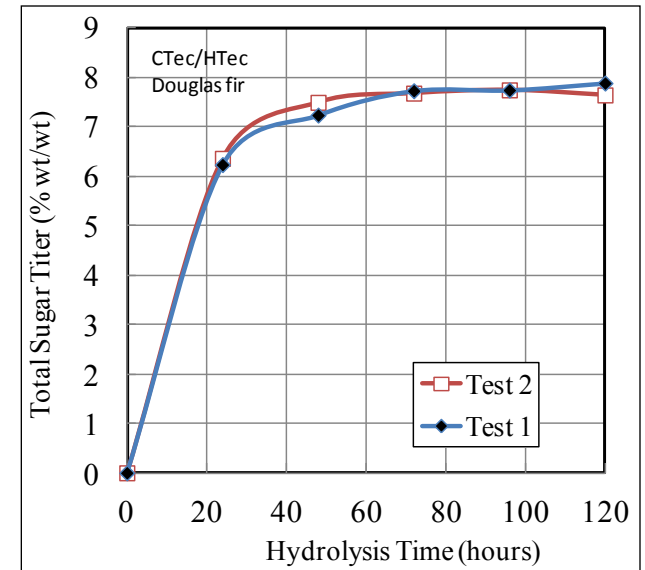


Figure C-P-5.11. The small scale milled Douglas-fir FS-10 hydrolysis sugar titer profile. Conditions: milled wood at 20.6% (wt/wt) from 60-minute milling sample; milled wood sugar composition at 58.48%; enzyme dose at 6% CTec2 and 0.6% HTec2 enzyme product on milled wood; pH at ~5.0-5.2; temperature at 50°C; shaking at 200 rpm

Table C-P-5.10. Sugar production cost under various assumptions

Items	Amount
Biomass Input (bdt/yr)	222,000
Operation days (/yr)	330
Plant staff (FTE)	42
Sugar yield from biomass (%)	65%
Sugar output on dry basis (bdt/yr)	97,794
Lignin pellet fuel on dry basis (bdt/yr)	133,977
Total Capital	\$9,773,180
Installed Capital	\$14,659,770
With 20% Contingency	\$17,591,724
With 20% OSBL	\$21,110,068
With 10% owner's cost	\$23,221,075
Annual Recovery Rate (%)	12%
Annual Capital Recovery (\$/yr)	\$2,786,529
Annual Operation and Labor (\$/yr)	\$12,057,110
Grant Annual Expense (\$/yr)	\$14,843,639

Table C-P-5.11. Commodity cost information for the TEA analysis

Items	Cost/Sale Price (\$)
Biomass cost at depot door (\$/bdt)	65
Electricity cost (\$/kWh)	0.0401
Natural gas cost (\$/MMBtu)	4.5
Lignin pellet fuel bulk price (\$/lb)	0.070
Sugar price (dry basis equivalent) (\$/lb)	0.169

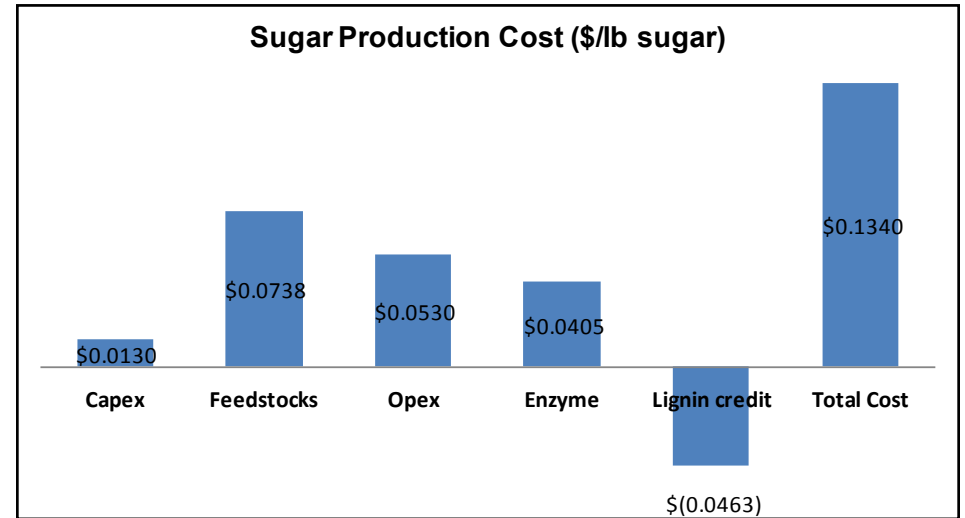


Figure C-P-5.12. Sugar production cost stack bar chart. Analysis conditions: feedstock cost at \$65/ton; plant size 222,000 bdt wood/year; sugar yield from milled wood 65%; lignin pellet selling price at \$0.070/lb; NW electricity cost at \$0.0401/kWh; installed capital with additional 20% contingency cost, 20% OSBL cost and 10% owner's cost; annual capital recovery at 12%; sugar and lignin plant staffed with 42 FTE; wood drying by natural gas price at \$4.50/MMBtu; operated for 330 days/year.

Table C-P-5.12. Sugar production cost under various assumptions

Case No.	Feedstock Cost (\$/bdt)	Milling Energy (kWh/OD kg wood)	Sugar Yield (%)	Sugar Production Cost (\$/lb)
1	65	0.56	65	0.1340
2	50	0.56	65	0.1170
3	50	0.84	65	0.1285
4	50	0.56	58	0.1215
5	50	0.84	58	0.1344
6	45	0.56	58	0.1106
7	45	0.84	58	0.1235

Recommendations | Conclusions Physical and Intellectual Outputs References

The targeted energy consumption by wood milling, which is being optimized, is used for the techno-economic analysis (TEA) for clean sugar and lignin production from wood residual chips. Based on the TEA, if the targeted energy consumption of wood milling is met within the range of 0.56-0.84 kWh/OD kg wood at a sugar conversion yield of about 60%, the economics by wood milling as a pretreatment technology looks very plausible for clean sugar and lignin fuel.

Recommendations

- Wait for the large scale milling energy consumption results to further calibrate the techno-economic analysis results on sugar production cost.
- Need to characterize and understand more on energy-efficient milling to achieve high yield milling (saccharification) by understanding more wood particle characterization.
- Need to optimize enzymatic hydrolysis on milled wood.
- Need to understand market feasibility on wood pellet, since the sugar production cost is largely dependent upon the lignin credit (high Btu lignin-rich wood pellet).

Conclusions

The clean sugar and lignin pretreatment technology by wood milling can produce excellent hydrolysate with almost no impact on isobutanol organism growth and with enhancement on isobutanol production. The potential global warming gas emission is minimal in this pretreatment technology. No chemical is used in the pretreatment. No water is needed in wood milling pretreatment technology. Wood milling could become a unique depot size technology for biomass pretreatment and for sugar and lignin fuel production.

- In September 2013, 100 kg FS-10 chips were reduced to 2 mm mean size and the energy consumption for this size reduction was tested at Forest Concepts LLC, Auburn, WA.
- In October 2013, 10 kg FS-10 2 mm size chips were milled into powder by tandem milling machine.
- In November 2013, 5 liters of milled wood hydrolysate was sent to Gevo for fermentation test.
- In November 2013, 1.1 kg (oven dry based) of un-fermented hydrolyzed FS-10 milled wood residuals was sent to the NARA Co-Product team for lignin evaluation.
- In November 2013, 100 kg FS-10 chips were shipped to [redacted] for some wood milling tests.
- In January 2014, 0.75 kg (oven dry based) of fermented hydrolyzed FS-10 milled wood residuals was sent to the NARA Co-Product team for lignin evaluation.
- In March 2014, an additional 1.8 liters of milled wood hydrolysate was sent to Gevo for GIFT testing.
- In March 2014, 2250 lbs of FS-10 chips were shipped to [redacted] for tandem milling optimization studies in the improved HV-10 tandem milling machine.

- 1) Bowyer J. 2012, Life cycle impacts of heating with wood in scenarios ranging from home and institutional heating to community scale district heating systems, reported by Dovetail Partners, Inc. with funding provided by the Minnesota Environment and natural Resources Trust Fund.
- 2) da Silva AS, Inoue H, Endo T, Yano S, Bon EPS, 2009, Milling pretreatment of sugarcane bagasse and straw for enzymatic hydrolysis and ethanol fermentation. Bioresource Technology, 101, 7402–7409.
- 3) Hiden A, Inoue H, Tsukahara K, Fujimoto S, Minowa T, Inoue S, Endo T, Sawayama S. 2009. Wet disk milling pretreatment without sulfuric acid for enzymatic hydrolysis of rice straw. Bioresource Technology, 100, 2706–2711.
- 4) ICF International. 2012, Life cycle greenhouse gas emissions of natural gas: A literature review of key studies comparing emissions from natural gas and coal, prepared for The Canadian Natural Gas Initiative (CNGI).
- 5) Mori Y, Takahashi T, Ito k, Kobayashi J, Ito A, Gochi M. 2012. Examination of the energy efficiency of the continuation tandem ring mill. The Japan Society of Mechanical Engineers, Vol. 78, No. 787, 400-404.
- 6) Takahashi T, ItoA, Kobayashi J, Enda Y. 2008. Research and development of mechanical milling technology for bio-ethanol production. 16th European Biomass Conference & Exhibition, 2-6 June, 2008, Valencia, Spain.

TASK C-AF-1: PRODUCTION OF LIGNOCELLULOSIC ISOBUTANOL BY FERMENTATION AND CONVERSION TO BIOJET

<u>Key Personnel</u>	<u>Affiliation</u>
Andrew Hawkins	Gevo, Inc
Glenn Johnson	Gevo, Inc
Bob Wooley	Gevo, Inc

Task Description

Over the past year, Gevo received and tested several pretreated FS-03 and FS-10 derived Douglas fir biomass samples. Three batches of FS-10 wet oxidation (WO) pretreated biomass from Dr. Birgitte Ahring at Washington State University that are noted as Batch A, B, and C. One batch of FS-10 SPORL pretreated biomass was received from Dr. JY Zhu at the USDA Forest Products Lab. Two batches of FS-03 derived biomass (Clean and Combined) were received from Catchlight Energy. Two batches of FS-10 mild bisulfite (MB) pretreated biomass (a small batch separated into solids and SSL and a larger combined batch) that is a joint effort between Catchlight Energy and the Forest Products Lab. Lastly, two different batches (concentrated and unconcentrated) of FS-10 milled wood (MW) pretreated biomass were produced by a Japanese company but received through Dr. Johnway Gao at Catchlight Energy. All of the materials have been analyzed for sugars and inhibitors and characterized via fermentation using an isobutanol producing biocatalyst. Based on Pretreatment and Conversion Team meetings, three FS-10 biomass samples (WO, SPORL, and MB Solids) were tested in GIFT® fermentation systems in order to provide data for the pretreatment down-selection gate review. Following the gate review, the unconcentrated milled wood (UMW) and combined mild bisulfite (CMBS) biomass samples were tested in 1L GIFT® fermentation systems. Gevo also continued a biocatalyst adaptation program using previously received WO and SPORL pretreated hydrolyzates to generate biocatalysts with better performance. Isolates adapted to WO and/or

SPORL pretreated material with improved growth are continuously generated, selected, and characterized to verify improved growth and fermentation performance. To date, Gevo has produced isobutanol using multiple pretreatment types of Douglas-fir derived biomass in 1L batch fermentations and 1L GIFT® fermentation systems using the currently best hydrolyzate-adapted biocatalyst (LB4) producing isobutanol. Fermentation process parameters to improve biocatalyst performance in Douglas-fir hydrolyzates continue to be identified through numerous experiments. Over the last year, Gevo created a process box that includes material flows plus capital and operating costs, and that data was forwarded to the NARA Process Team.

Task C-AF-1.1. Characterize toxicity of a representative sample of pre-treated woody biomass (Douglas fir) for fermentation

Sugar and inhibitor concentrations were determined by high-pressure liquid chromatography (HPLC) analysis for each feedstock and pretreatment method received (Table C-AF-1.1). To date, Gevo has received and characterized the following: FS-01, FS-03, and FS-10 SPORL pretreated materials from Dr. JY Zhu at the USDA Forest Products Laboratory (Madison, WI), FS-01, FS-03, and FS-10 wet oxidation pretreated materials from Dr. Birgitte Ahring at Washington State University – Tricities (Richland, WA), FS-03 pretreated material (Clean and Combined) from Catchlight Energy (Federal Way, WA), FS-10 mild bisulfite pretreated materials (Combined, Solids, and Spent Sulfite Liquor (SSL)) that is a collaborative product from Catchlight Energy and the USDA Forest Products Lab, and FS-10 milled wood pretreated material (Concentrated and Unconcentrated) pretreated at a [redacted] company but received through Dr. Johnway Gao at Catchlight Energy.

After analytical analysis, characterization of new feedstock hydrolyzates occurs through benchmarking growth and fermentation using an isobutanol producing biocatalyst. A review of hydrolyzate samples tested in the shake flask benchmarking process is presented in Figure C-AF-1.1. Additional data can be found in the prior trimester reports from this year. A new hydrolyzate material received during the most recent trimester, FS-10 Concentrated Milled Wood, was characterized using shake flask fermentations. Growth performance was compared in different concentrations of the Concentrated Milled Wood pretreated hydrolyzates media (Figure C-AF-1.2). All of the pretreated hydrolyzate media was clarified and supplemented with a nutrient package prior to the experiments.

The growth and isobutanol production data using the Concentrated Milled Wood hydrolyzate media showed excellent results using the 100% hydrolyzate. Growth of LB4 in the 100% hydrolyzate media was only slightly lower than the mock (control) media, and isobutanol production was comparable with higher maximum titers (Figure C-AF-1.2). Based on these results, additional testing should be performed with Concentrated Milled Wood hydrolyzates if the process is economical and scalable.

Task C-AF-1.2. Adapt yeast biocatalyst to pretreated biomass hydrolyzates

Inhibitor concentrations in biomass pretreatments can vary widely depending on the pretreatment method. To generate robust biocatalysts, adaptation to a specific pretreated hydrolyzate is needed. A biocatalyst adaptation program is ongoing to generate better performing isobutanol producing biocatalysts to the different hydrolyzates. Hydrolyzate adapted strains with improved performance have been isolated from

all hydrolyzates used in the adaptation program. The parent isobutanol producing biocatalyst LB3, the current best performing biocatalyst (LB4 (FS-01 WO adapted LB3)), the current best corn starch biocatalyst (LB20), and additional engineered strains from Gevo's strain collection have all been used as starting biocatalysts for evolutionary engineering using both FS-03 WOX and SPORL pretreated hydrolyzates. As a review of the hydrolyzate adaptation program, data included in previous reports from this year have been included (Figures C-AF-1.3 and C-AF-1.4). Earlier this year, an improved SPORL hydrolyzate adapted isolate, LB19, showed improved growth rates in SPORL hydrolyzate media compared to the WO adapted LB4 strain (Figure C-AF-1.3). LB19 proved to be an unstable isolate after reviving from frozen culture stocks (data not shown).

In addition, previously reported data showed the growth improvement of hydrolyzate adapted strains over time in WO and SPORL hydrolyzates starting with an improved isobutanol producing biocatalyst (LB20) from Gevo's biocatalyst engineering group. Over a six week time-frame, cell densities of individual hydrolyzate adapted strains and the average cell density of all isolates were compared to the parent biocatalyst (LB20) using 40% (v/v) FS-03 WO or SPORL hydrolyzate media, Figure C-AF-1.4.

Previously unreported data using new FS-03 SPORL hydrolyzate adapted isolates shows an improvement in growth (Figure C-AF-1.5) and specific isobutanol productivity (Figure C-AF-1.6) compared to the parent strains. These new strains are very promising because they appear to be stable and not only show improved growth but also improved isobutanol productivity. They will continue to be characterized as well as other new isolates from the hydrolyzate adaptation program. New hydrolyzate adapted isolates are continuously being screened and selected through 1:10 serial dilutions of hydrolyzate medium approximately every week in 96 deep well plates. In addition, improved strains from Gevo's biocatalyst engineering group are

routinely tested for hydrolyzate tolerance.

Task C-AF-1.3. Produce isobutanol in a 1L batch fermentation from pretreated biomass sugars using the adapted yeast biocatalyst.

This task was completed in the previous year. In summary, growth and isobutanol production in a 1L batch fermentation were compared using FS-03 WO (Batch A) and SPORL with the current best hydrolyzate biocatalyst LB4. The fermentation was performed in two phases; growth using 20% (v/v) hydrolyzates and production using 60% (v/v) hydrolyzates. Comparatively, the total isobutanol titer increased rapidly to a maximum at 24 hours for the mock media but slowly reached higher maximum titers in both hydrolyzate types (Figure C-AF-1.7). Additional data can be found in previous reports from last year.

Additional learnings from fermentation process development include identifying impurities in the isobutanol and reducing or eliminating the impurity by using a different base for pH control. Previously, ammonium hydroxide was used for pH control, but changing to sodium hydroxide reduced the impurity significantly. In addition, sodium concentrations above 0.5 g/L (9 mM) are reported to inhibit yeast growth and fermentation, so a growth test with sodium chloride was used to measure the effects on the LB4 biocatalyst. At the lowest level tested of 11.7 g/L (0.2 M), the final cell density decreased by 35% and the growth rate decreased by 20%. For additional data related to sodium see previous NARA progress reports. Fermentation process development for growth and production is continually being improved using data from hydrolyzate experiments as well as data from corn starch fermentations.

Task C-AF-1.4. Economic assessment of wood to isobutanol, jet

Gevo performed various Aspen modeling work based on Douglas-fir Biomass hydrolyzate from the mild bisulfite process, supplied by the NARA project, to determine the mass and energy balance and CapEx

cost for that portion of the process involving Gevo Proprietary Technology. Gevo has supplied sufficient information to the NARA process team to enable them to complete a mass and energy balance for the portions of the process not included in the Gevo Process Box. Gevo also supplied the CapEx for the Gevo portion of the process. Figure C-AF-1.8 illustrates the information (streams 2 through 8 and CapEx) supplied to the NARA process team.

In summary, the saccharified biomass sugars are fermented and the isobutanol is recovered in a process essentially identical to the corn mash process being currently used at Gevo's plant in Luverne, MN. The process modeled here accommodates two feeds from the NARA mild bisulfite pretreatment, a liquid only stream separated from the mild bisulfite pretreatment by the NARA team and a solids containing stream where the cellulose has been enzymatically saccharified. Gevo discharges two whole stillage streams containing all the unreacted solids, insoluble and soluble, back to NARA for processing and recycling the water to pretreatment. Only a small amount of clean water, for vent scrubbers, is required by Gevo over what is present already in the hydrolyzate. Utility requirements include city water, steam, natural gas (for fired heaters and hydrogen production), cooling water, and electricity. No steam boilers or cooling towers have been assumed inside the Gevo box. Combined atmospheric vents (fermentation, fired heaters, etc.) were specified. Waste water was also specified as to flow and composition. Minor raw materials (other than biomass hydrolyzate) utilized in the process were specified as an operating cost amount. The material quantities are insignificant to the material balance. Hydrocarbon vents from the biojet (IPK) process are burned in the fired heaters and the combustion products included in the combined vent along with the hydrogen reformer vent and fermentation vent. No other hydrocarbon products besides biojet (IPK) are produced in the process. All lower molecular weight materials (e.g., isobutylene and isooctane) are recycled and incorporated in jet range molecules. Byproducts from isobutanol fermentation

are generally discharged in the whole stillage. Some lower molecular weight alcohols can be recycled to the fermentation.

Task C-AF-1.5. Produce isobutanol in 1L GIFT® fermentation from pretreated biomass sugars using the adapted yeast biocatalyst

The process parameters for producing isobutanol from pretreated biomass sugars in a 1L GIFT system were developed and identified using learnings from not only biomass based shake flask fermentations and 1L batch fermentations but also from fermentations using corn starch. Furthermore, process development will continue as the pretreated biomass process improves, strains adapt to hydrolyzates, and additional learnings are transferred from other internal Gevo teams.

Growth and isobutanol production of the current best hydrolyzate biocatalyst LB4 was compared in a 1L GIFT® fermentation systems using FS-10 derived wet oxidation (Batch C), SPORL, mild bisulfite solids, mild bisulfite spent sulfite liquor (batch, not GIFT®), combined mild bisulfite, and unconcentrated milled wood. The fermentation was performed in two phases; batch growth using 20% (v/v) hydrolyzates and production using 60% (v/v) hydrolyzates. The growth in all 20% (v/v) hydrolyzates was very similar considering they had ranging hexose concentrations (Figure C-AF-1.9). The growth during the production phase using 60% (v/v) hydrolyzates all had some growth except for the MB SSL, which may have been a result of not using a GIFT® system (Figure C-AF-1.10). The isobutanol titers had different maximum titers within a 24 hour timeframe with the highest coming from CMB, very similar titers from MB Solids, SPORL, and WO, and the two lowest values coming from UMW and MB SSL (Figure C-AF-1.11). The isobutanol titer differences are likely caused by one or two factors, inhibitors and hexose concentration.

The average specific isobutanol productivity can be separated into three categories, those near 100%, those in the 60-80% range, and those below 20%

(Figure C-AF-1.12). The CMB hydrolyzate had the highest rate at 100% while WO, SPORL, UMW, and MB Solids were all in the 60%-80% range. The only material below 20% was the MB SSL. This trend was the same the average volumetric productivity (Figure C-AF-1.13). The differences in average specific productivity and average volumetric productivity results are likely caused by one or two factors, inhibitors and hexose concentration.

The average hexose consumption rate can be separated into three categories, those in the 80 to 100% range, those in the 30-50% range, and those below 30% (Figure C-AF-1.14). The CMB, UMW, and MB Solids hydrolyzate had the highest rates while WO and SPORL were in the 30%-50% range. The only material below 30% was the MB SSL. The percent of theoretical yields two categories, those above 50% and those below 50% (Figure C-AF-1.15). The WO, SPORL, CMB, UMW, and MB Solids were all similar with WO having slightly better yields than the rest. The only material below 30% was the MB SSL. The differences in hexose consumption rates and percent of theoretical yields are likely caused by one or two factors: inhibitors and total hexose available.

To summarize the performance results for the different hydrolyzate types in the 1L GIFT® fermentation systems, under the conditions tested, the CMB was the highest performing material overall, while the WO, SPORL, MB Solids, and UMW were very similar overall with one or the other having better results in one or two metrics. The lowest performing material overall was the MB SSL.

Production of isobutanol at a rate ≥ 0.3 g/L/h and $\geq 40\%$ theoretical yield using 60% (v/v) hydrolyzates of every pretreated material tested in the 1L GIFT® fermentation systems has been achieved.

Task C-AF-1.6. Analysis of isobutanol produced to close mass balance and determine potential low-level impurities

Analytical analysis and impurity tracking are being optimized through fermentation development to close the mass balance, decrease impurities, and increase yield. Production of isobutanol from 1L GIFT® fermentations using Douglas-fir biomass sugars has provided relevant samples to compare to corn starch derived isobutanol for analysis. Preliminary analysis of isobutanol quality from Douglas-fir biomass and corn starch are very similar. Analysis of isobutanol to produce an optimized product for conversion to biojet is ongoing and will continue at each scale-up.

Task C-AF-1.7. Produce isobutanol in 20L GIFT® SSF fermentation from pretreated biomass

A 20L GIFT® pilot scale system was designed and built. Development of fermentation process parameters for the 20L GIFT® system is underway with the help of data and process parameters identified in shake flask, 1L batch, and 1L GIFT® fermentations. Production of isobutanol using the 20L GIFT® system will begin in year 4.

Task C-AF-1.8. Produce ≥ 1000 gallons of isobutanol from GIFT® fermentations at 40,000L demonstration scale. Convert lignocellulosic isobutanol to ≥ 1000 gallons biojet for further testing

Initiation of the development and scale-up process for this task will begin in year 4.

Table CAF-1.1. Sugar and inhibitor concentrations in FS-01, FS-03, and FS-10 feedstocks from different pretreatment methods. Concentrations were determined using HPLC at Gevo. (n.d. = not detected) (n.a. = not available)

	Glucose (g/L)	Xylose (g/L)	Galactose (g/L)	Arabinose (g/L)	Mannose (g/L)	Acetate (g/L)	HMF (g/L)	Furfural (g/L)	Total Hexose (g/L)
FS-01 Wet Oxidation Hydrolyzate	57.20	6.67	5.12	1.58	20.87	7.27	3.90	0.99	83.19
FS-03 Wet Oxidation Hydrolyzate (Batch A)	87.54	4.67	5.14	0.76	10.06	12.46	3.66	0.81	102.74
FS-03 Wet Oxidation Hydrolyzate (Batch B)	89.58	5.25	3.00	n.d.	7.66	7.00	n.d.	n.d.	100.24
FS-10 Wet Oxidation Hydrolyzate (Batch A)	44.76	6.61	4.18	6.00	16.84	7.94	2.42	0.56	65.78
FS-10 Wet Oxidation Hydrolyzate (Batch B)	67.22	4.57	2.37	n.d.	9.04	3.90	n.d.	n.d.	78.63
FS-10 Wet Oxidation Hydrolyzate (Batch C)	54.79	12.01	5.38	4.43	9.59	7.99	3.53	0.26	69.76
FS-01 SPORL Hydrolyzate	93.65	6.89	4.94	1.24	23.01	4.56	0.79	0.10	121.60
FS-03 SPORL Hydrolyzate	81.81	5.79	3.82	0.40	7.02	5.78	1.84	0.63	92.65
FS-10 SPORL Hydrolyzate	62.74	6.84	5.07	n.d.	11.83	0.62	n.d.	n.d.	79.64
FS-03 Catchlight Combined Hydrolyzate	164.12	8.56	5.24	0.94	13.34	3.45	0.15	0.14	182.70
FS-03 Catchlight Clean Hydrolyzate	130.89	1.84	0.49	n.d.	1.34	0.26	0.14	0.01	132.72
FS-10 Mild Bisulfite SSL	7.83	7.32	5.29	2.21	18.06	3.61	n.d.	n.d.	31.18
FS-10 Mild Bisulfite Solids Hydrolyzate	63.88	3.77	2.07	0.68	7.52	1.65	n.d.	n.d.	73.47
FS-10 Combined Mild Bisulfite Hydrolyzate	84.22	10.14	5.26	2.02	20.93	3.44	n.d.	n.d.	110.41
FS-10 Unconcentrated Milled Wood Hydrolyzate	39.84	7.61	1.87	2.04	11.40	0.74	n.a.	n.a.	53.11
FS-10 Concentrated Milled Wood Hydrolyzate	61.84	9.21	0.46	1.36	12.76	0.77	n.d.	n.d.	75.06

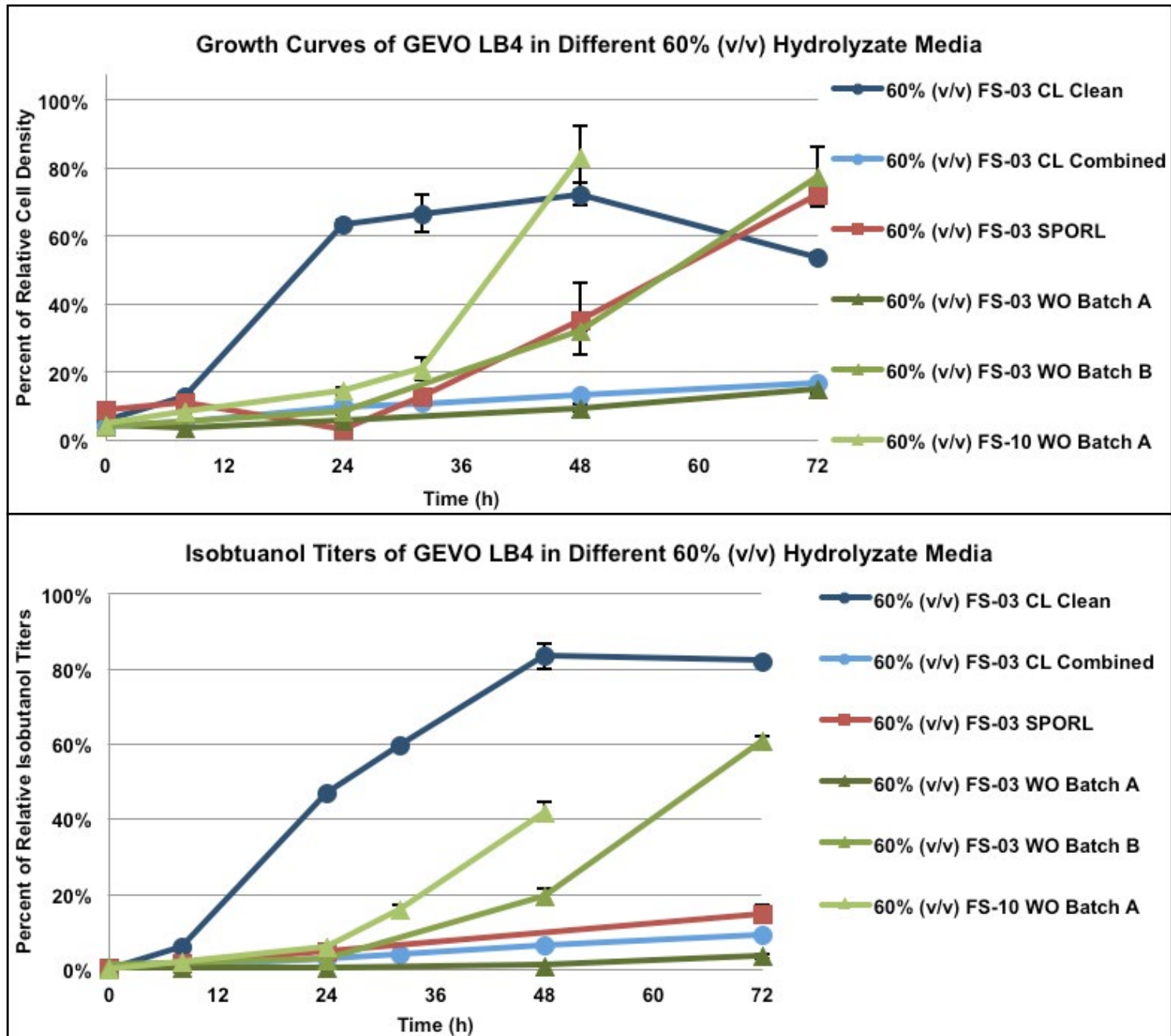


Figure C-AF-1.1. Relative growth (Top) and relative isobutanol titers (Bottom) using the current best hydrolyzate adapted biocatalyst (LB4) in 60% (v/v) hydrolyzates derived from WO, SPORL, and Catchlight Energy pretreated materials (FS-03 and FS-10) in shake flask fermentations. Data was combined from multiple experiments. All hydrolyzates were clarified to remove solids and were supplemented with a nutrient package, salts, and a buffering agent. The 60% (v/v) mixtures have sugars and acetate equivalent to 100% of the hydrolyzate. 100% hydrolyzate is equal to approximately 30-36% equivalent solids for all biomass materials. Fermentation was carried out at 33°C. Cell density was measured using a spectrophotometer and isobutanol levels were determined by GC analysis. Error bars represent the standard deviation. Abbreviations: WO, wet oxidation; SPORL, sulfite pretreatment to overcome recalcitrance of lignocellulose; CL, Catchlight Energy.

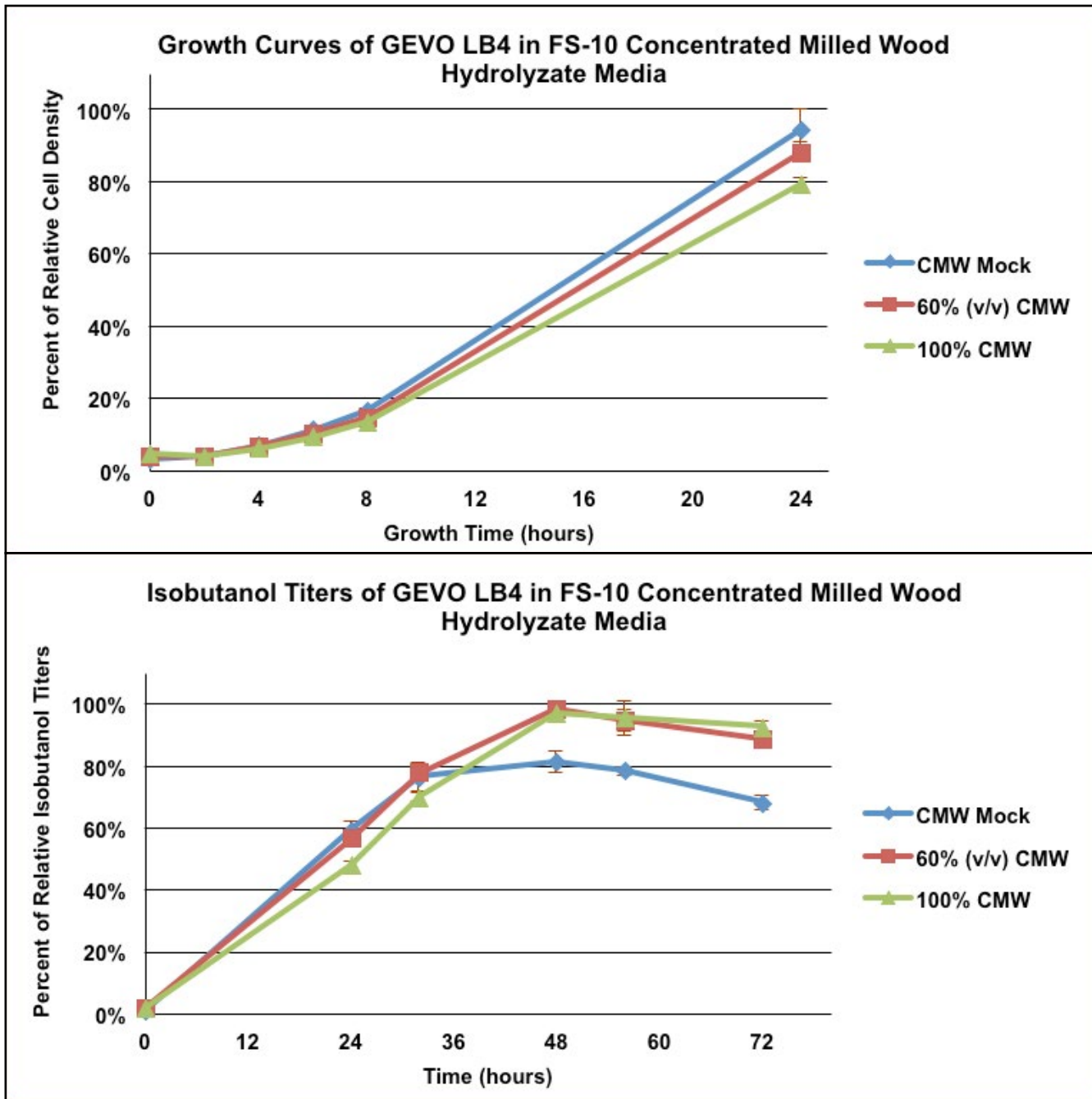


Figure C-AF-1.2. Relative growth (Top) and relative isobutanol titers (Bottom) of the current best hydrolyzate adapted biocatalyst (LB4) in mock, 60% (v/v), and 100% hydrolyzates derived from FS-10 Concentrated Milled Wood in shake flask fermentations. The material was received as a clarified hydrolyzate and all concentrations were supplemented with an equivalent nutrient package, salts, and a buffering agent. All media contained equal amounts of corresponding sugars and acetate. Fermentation was carried out at 33°C. Cell density was measured using a spectrophotometer and isobutanol levels were determined by GC analysis. Error bars represent the standard deviation. Abbreviations: CMW, Concentrated Milled Wood.

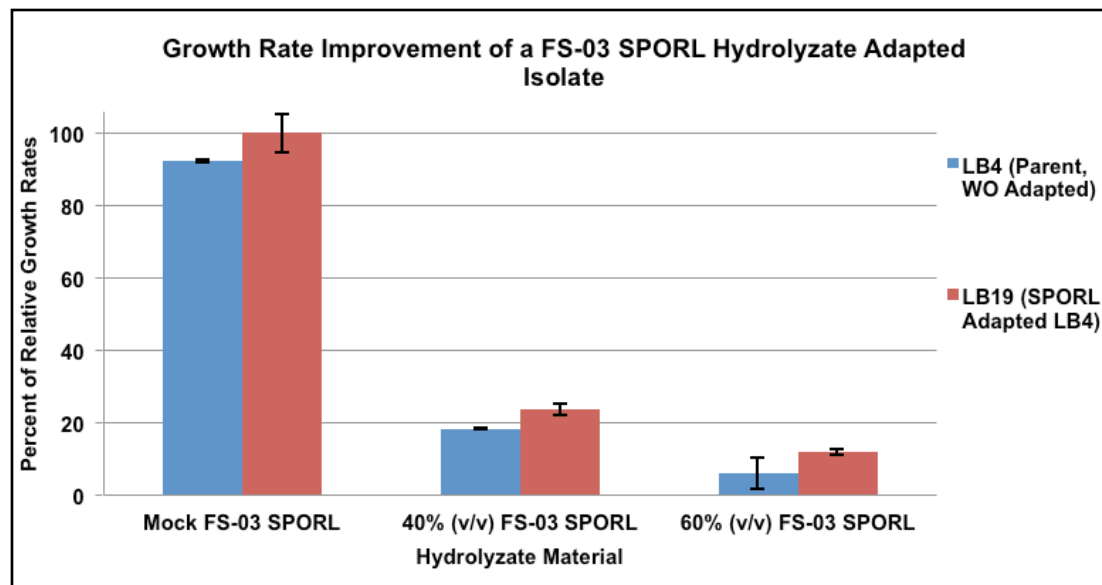


Figure C-AF-1.3. High throughput growth screening showing the percent of relative growth rates of the current best performing hydrolyzate adapted biocatalyst (LB4) and a new SPORL adapted biocatalyst derived from LB4 (LB19). Percent relative growth rates were obtained using a high-throughput microfermentation system (BioLector, m2p-labs). All hydrolyzates were clarified to remove solids and all were supplemented with a nutrient package, salts, and a buffering agent. The different percentages of hydrolyzate and mock media contained equal amounts of corresponding sugars, acetate, and supplements. 100% (v/v) hydrolyzate is equal to approximately 30-36% equivalent solids for all biomass materials. Error bars represent the standard deviation. Abbreviations: WO, wet oxidation; SPORL, sulfite pretreatment to overcome recalcitrance of lignocellulose.

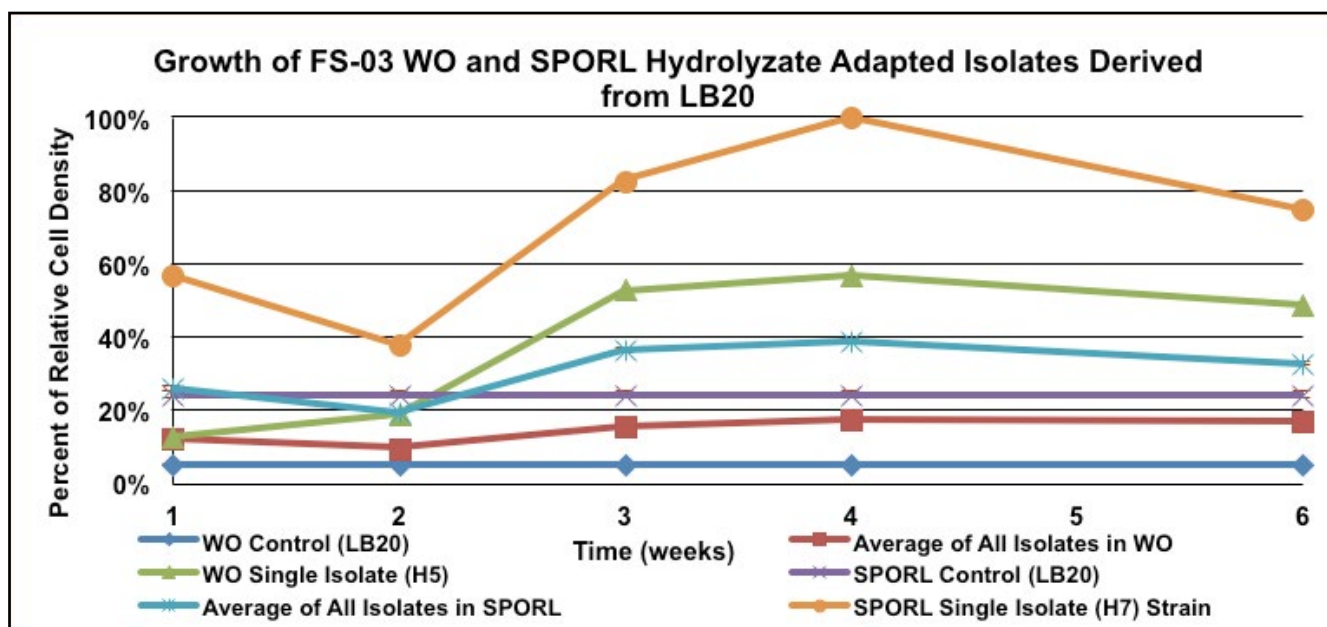


Figure C-AF-1.4. Relative maximum cell densities of hydrolyzate adapted LB20 biocatalysts over time in 40% (v/v) FS-03 WO and SPORL pretreated hydrolyzates. The maximum cell density of the parental strain, LB20 (control), was compared to newly adapted isolates by following the maximum cell density approximately each week. Cell transfer and cell density measurements were performed using a Tecan Freedom Evo robotic system and Infinite M1000 Pro microplate reader, respectively. Relative cell densities in the graphs were normalized to the maximum cell density. The clarified FS-03 WO and SPORL hydrolyzates were supplemented with a nutrient package, salts, and a buffering agent. Abbreviations: WO, wet oxidation; SPORL, sulfite pretreatment to overcome recalcitrance of lignocellulose.

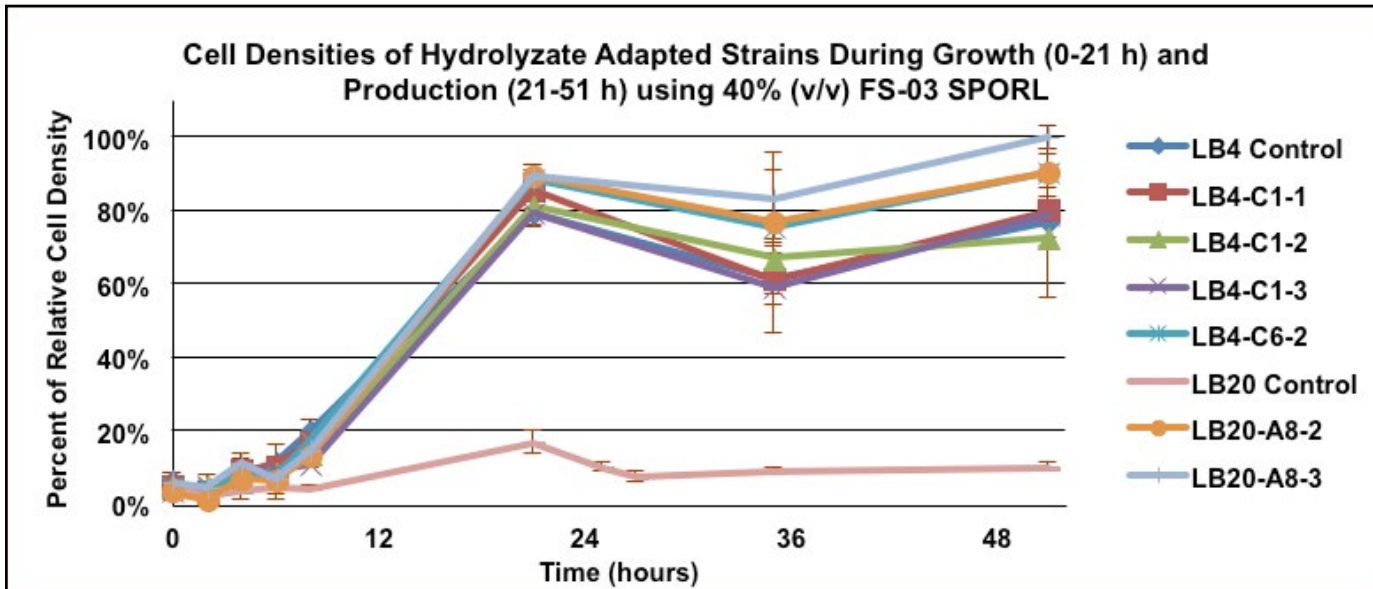


Figure C-AF-1.5. Relative cell densities of hydrolyzate adapted LB4 and LB20 derived biocatalysts using 40% (v/v) FS-03 SPORL pretreated hydrolyzate medium in shake flask fermentations. All hydrolyzates were clarified to remove solids and were supplemented with a nutrient package, salts, and a buffering agent. The 40% (v/v) mixtures have sugars and acetate equivalent to 100% of the hydrolyzate. 100% hydrolyzate is equal to approximately 30-36% equivalent solids. Fermentation was carried out at 33°C. Cell density was measured using a spectrophotometer. Error bars represent the standard deviation. Abbreviations: SPORL, sulfite pretreatment to overcome recalcitrance of lignocellulose.

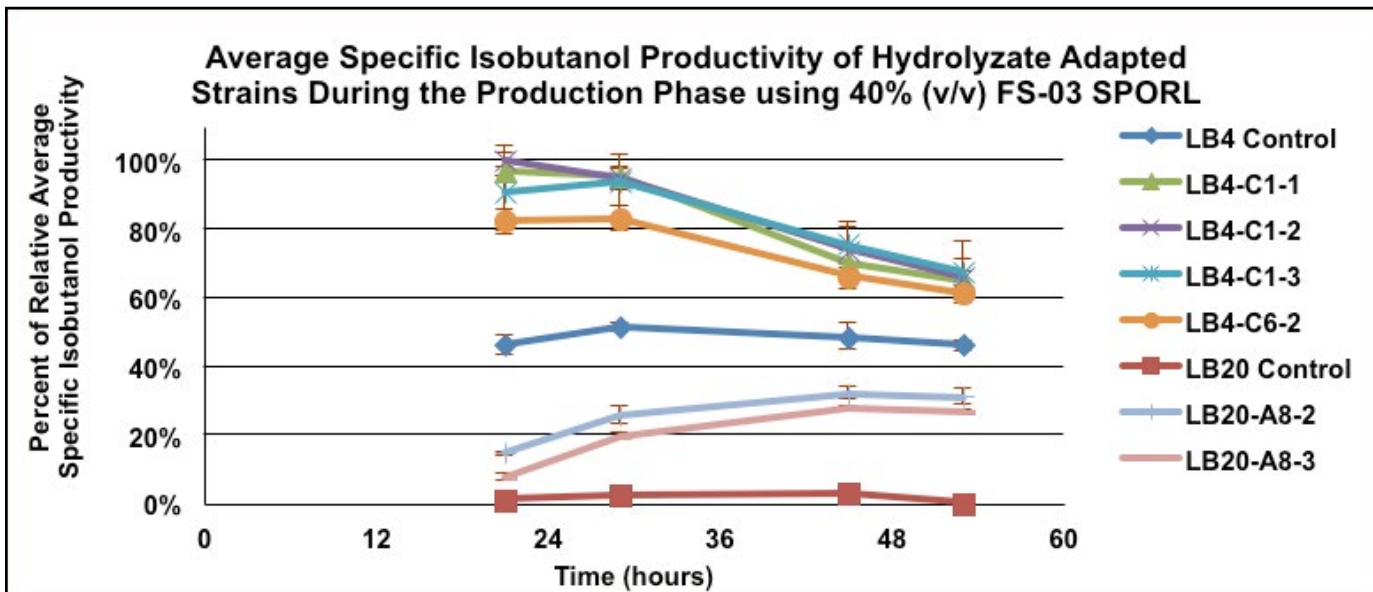


Figure C-AF-1.6. Relative average specific isobutanol productivities of hydrolyzate adapted LB4 and LB20 derived biocatalysts using 40% (v/v) FS-03 SPORL pretreated hydrolyzate medium in shake flask fermentations. All hydrolyzates were clarified to remove solids and were supplemented with a nutrient package, salts, and a buffering agent. The 40% (v/v) mixtures have sugars and acetate equivalent to 100% of the hydrolyzate. 100% hydrolyzate is equal to approximately 30-36% equivalent solids. Fermentation was carried out at 33°C. Cell density and isobutanol titers were measured using a spectrophotometer and GC, respectively. Average specific productivity is the g CDW / (g/L/h of isobutanol). Error bars represent the standard deviation. Abbreviations: SPORL, sulfite pretreatment to overcome recalcitrance of lignocellulose.

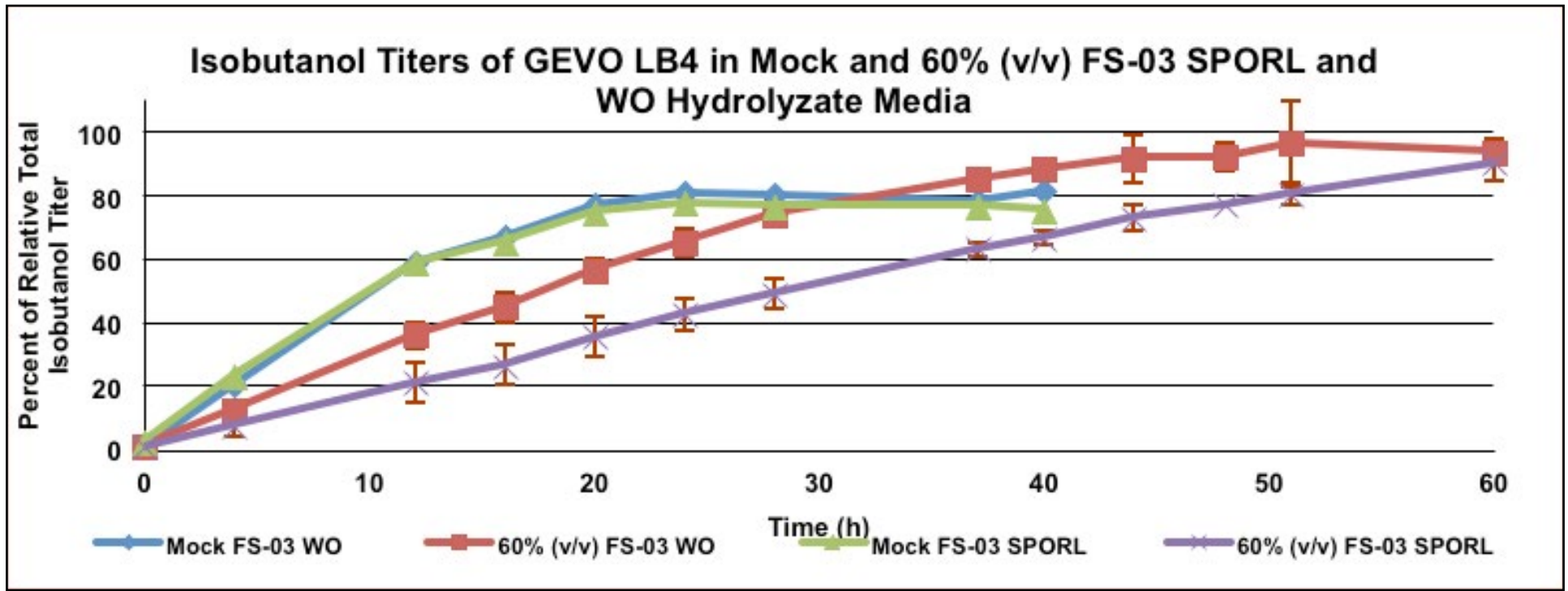


Figure C-AF-1.7. Relative average specific isobutanol productivities of hydrolyzate adapted LB4 and LB20 derived biocatalysts using 40% (v/v) FS-03 SPORL pretreated hydrolyzate medium in shake flask fermentations. All hydrolyzates were clarified to remove solids and were supplemented with a nutrient package, salts, and a buffering agent. The 40% (v/v) mixtures have sugars and acetate equivalent to 100% of the hydrolyzate. 100% hydrolyzate is equal to approximately 30-36% equivalent solids. Fermentation was carried out at 33°C. Cell density and isobutanol titers were measured using a spectrophotometer and GC, respectively. Average specific productivity is the g CDW / (g/L/h of isobutanol). Error bars represent the standard deviation. Abbreviations: SPORL, sulfite pretreatment to overcome recalcitrance of lignocellulose.

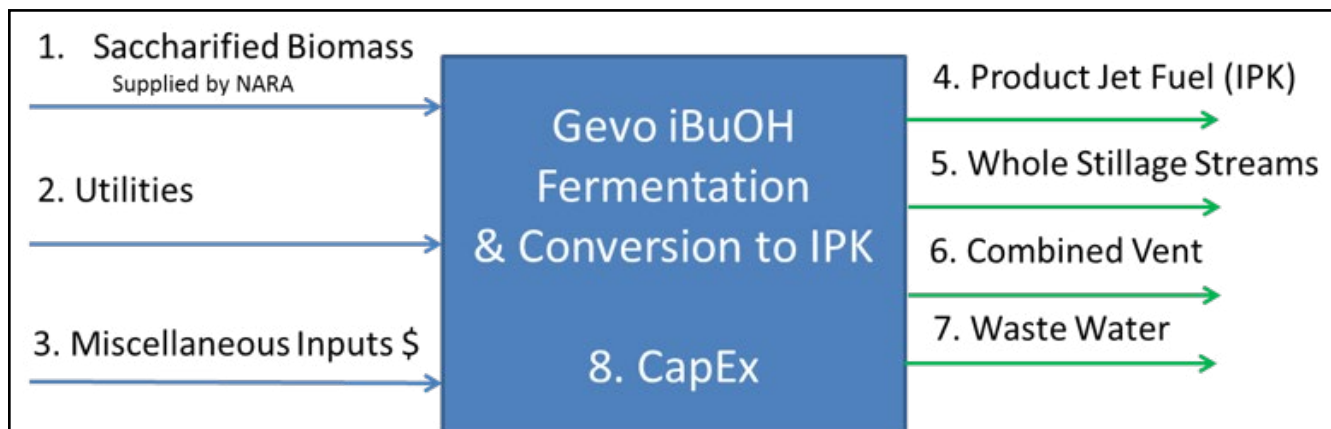


Figure C-AF-1.8. Gevo approach to modeling material flows and capital and operating costs

Growth Curves of GEVO LB4 in 20% (v/v) FS-10 Hydrolyzate Media During the Growth Phase

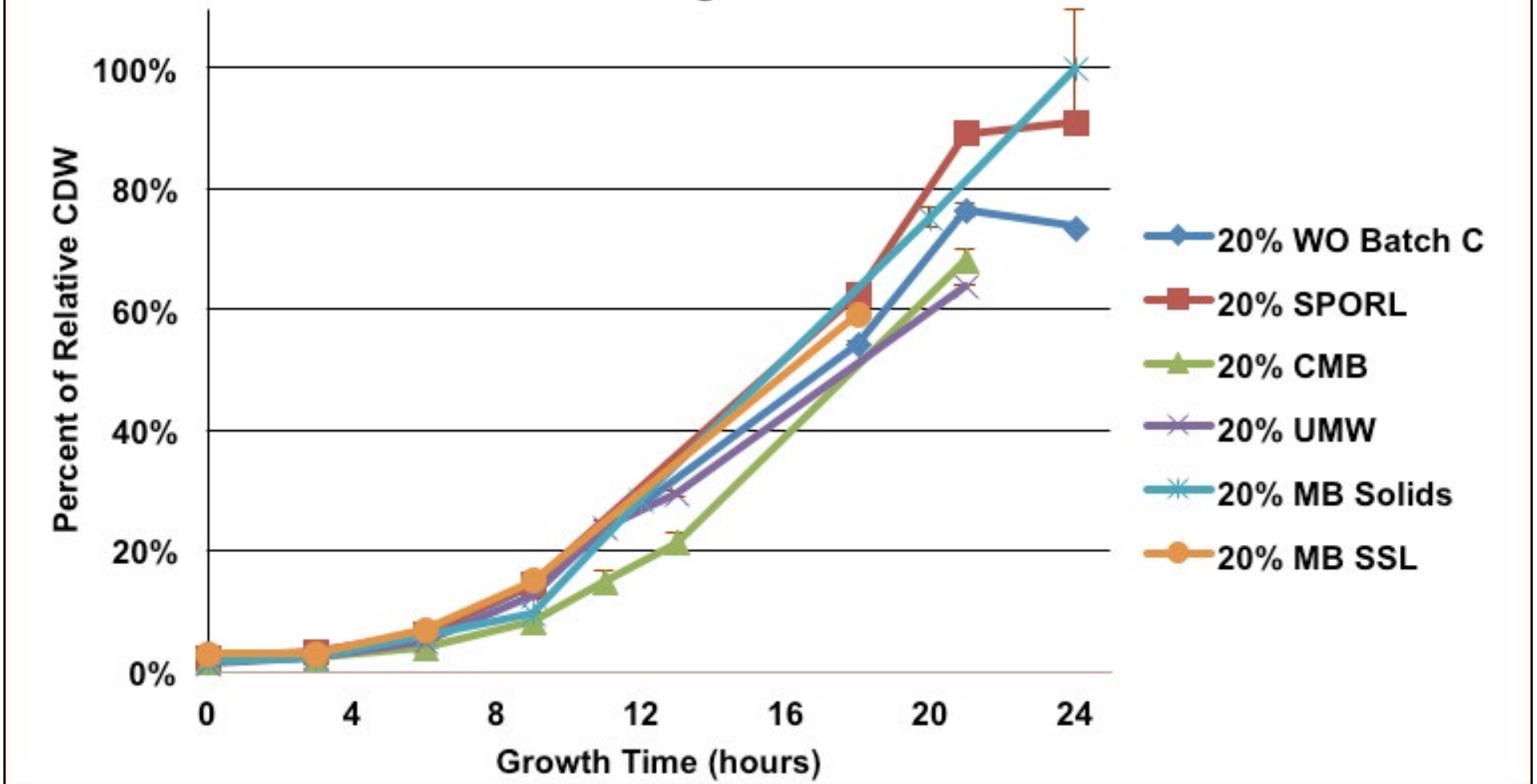


Figure C-AF-1.9. One liter GIFT® system fermentation data using the current best hydrolyzate adapted isobutanol producing biocatalyst (LB4) showing the percent relative dry cell weight during the growth phase in 20% (v/v) of each FS-10 hydrolyzate. The clarified hydrolyzates were supplemented with a nutrient package, salts, and pH controlled adjusted during the fermentation. The 20% (v/v) mixture of each hydrolyzate contained equal amounts of corresponding sugars to the 100% material and equivalent supplements were added to each hydrolyzate. Cell dry weight (CDW) was calculated using a conversion factor multiplied by the cell density measured on a spectrophotometer. All of the hydrolyzates were tested in GIFT® systems except the MB SSL. Error bars represent the standard deviation of duplicates. Abbreviations: WO, wet oxidation; SPORL, sulfite pretreatment to overcome recalcitrance of lignocellulose; CMB, combined mild bisulfite; UMW, unconcentrated milled wood; MB, mild bisulfite; SSL, spent sulfite liquor.

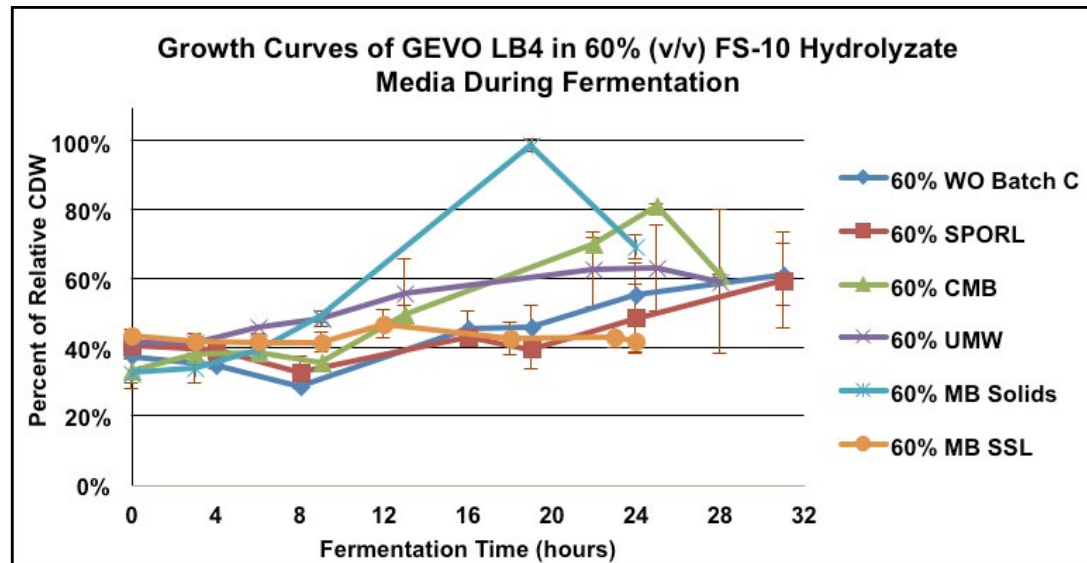


Figure C-AF-1.10. One liter GIFT® system fermentation data using the current best hydrolyzate adapted isobutanol producing biocatalyst (LB4) showing the percent relative dry cell weight during the fermentation phase in 60% (v/v) of each FS-10 hydrolyzate. The clarified hydrolyzates were supplemented with a nutrient package, salts, and the pH was controlled during the fermentation. The 60% (v/v) mixture of each hydrolyzate contained equal amounts of corresponding sugars to the 100% material and equivalent supplements were added to each hydrolyzate. Cell dry weight (CDW) was calculated using a conversion factor multiplied by the cell density measured on a spectrophotometer. All of the hydrolyzates were tested in GIFT® systems except the MB SSL. Error bars represent the standard deviation of duplicates. Abbreviations: WO, wet oxidation; SPORL, sulfite pretreatment to overcome recalcitrance of lignocellulose; CMB, combined mild bisulfite; UMW, unconcentrated milled wood; MB, mild bisulfite; SSL, spent sulfite liquor.

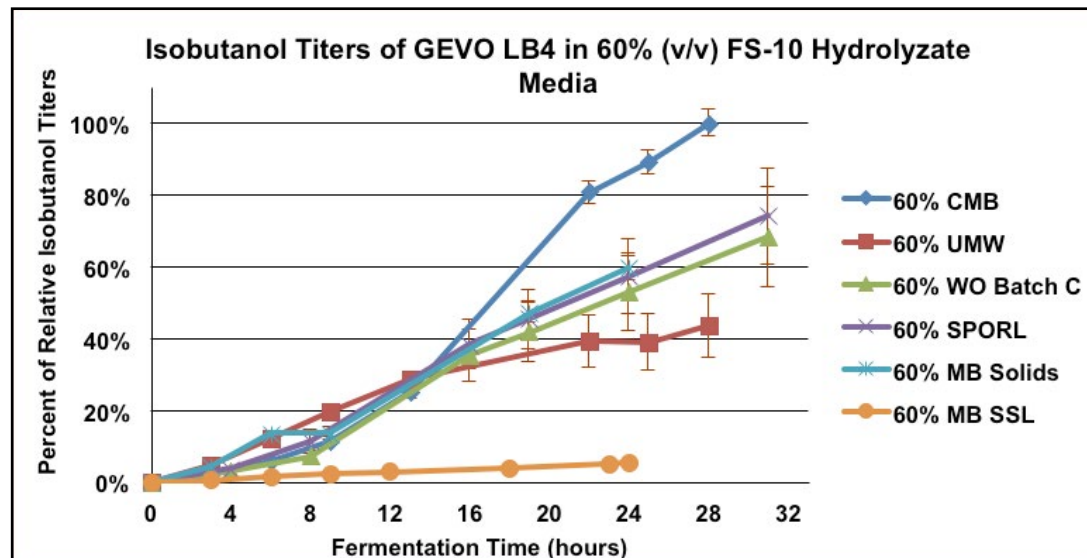


Figure C-AF-1.11. One liter GIFT® system fermentation data using the current best hydrolyzate adapted isobutanol producing biocatalyst (LB4) showing the percent of relative isobutanol titers in 60% (v/v) of each FS-10 hydrolyzate. The clarified hydrolyzates were supplemented with a nutrient package, salts, and the pH was controlled during the fermentation. The 60% (v/v) mixture of each hydrolyzate contained equal amounts of corresponding sugars to the 100% material and equivalent supplements were added to each hydrolyzate. Isobutanol titers were measure using a GC. All of the hydrolyzates were tested in GIFT® systems except the MB SSL. Error bars represent the standard deviation of duplicates. Abbreviations: WO, wet oxidation; SPORL, sulfite pretreatment to overcome recalcitrance of lignocellulose; CMB, combined mild bisulfite; UMW, unconcentrated milled wood; MB, mild bisulfite; SSL, spent sulfite liquor.

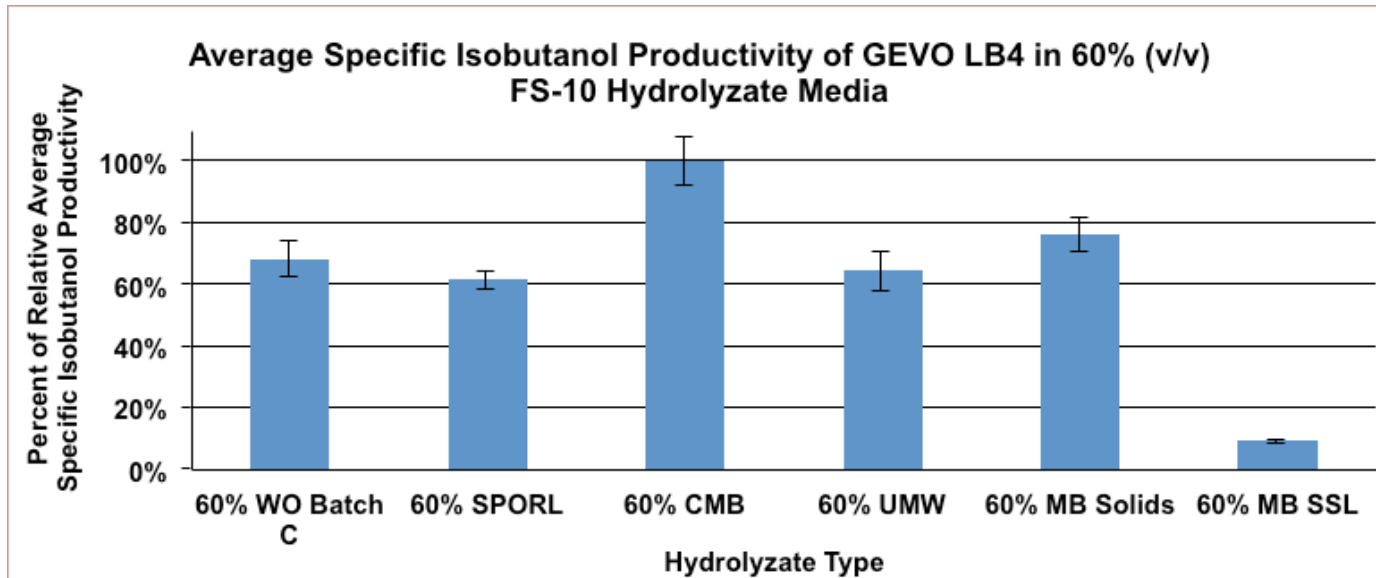


Figure C-AF-1.12. One liter GIFT® system fermentation data using the current best hydrolyzate adapted isobutanol producing biocatalyst (LB4) showing the average specific isobutanol productivity in 60% (v/v) of each FS-10 hydrolyzate. The clarified hydrolyzates were supplemented with a nutrient package, salts, and the pH was controlled during the fermentation. The 60% (v/v) mixture of each hydrolyzate contained equal amounts of corresponding sugars to the 100% material and equivalent supplements were added to each hydrolyzate. Cell dry weight (CDW) was calculated using a conversion factor multiplied by the cell density measured on a spectrophotometer and isobutanol was measured using a GC. All of the hydrolyzates were tested in GIFT® systems except the MB SSL. Error bars represent the standard deviation of duplicates. Abbreviations: WO, wet oxidation; SPORL, sulfite pretreatment to overcome recalcitrance of lignocellulose; CMB, combined mild bisulfite; UMW, unconcentrated milled wood; MB, mild bisulfite; SSL, spent sulfite liquor.

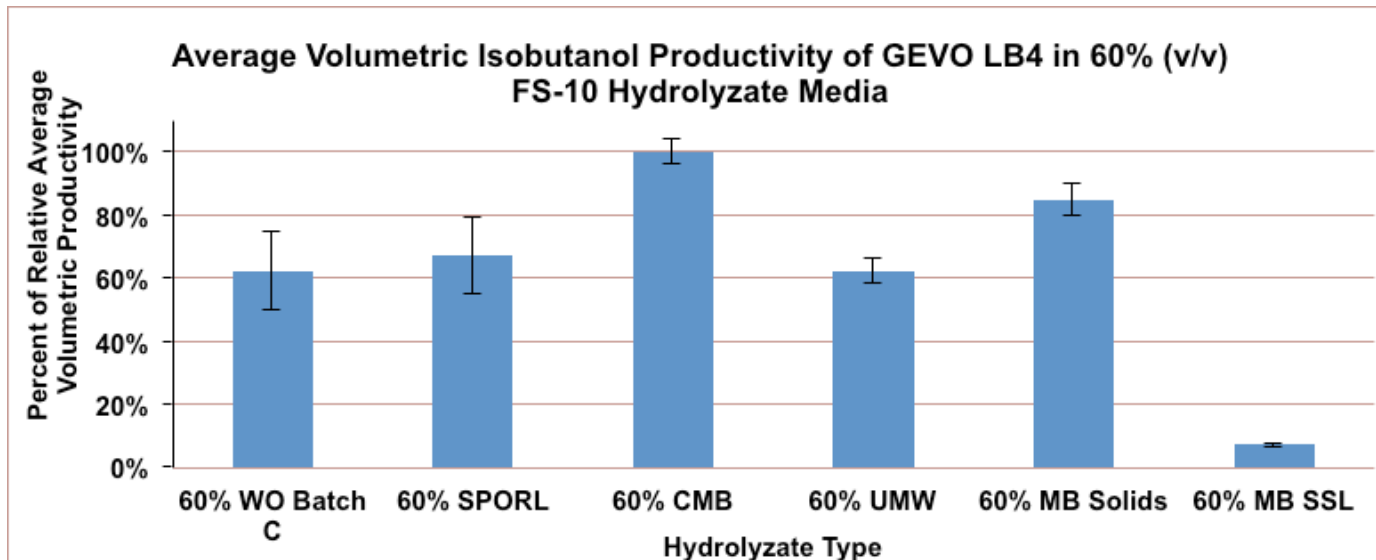


Figure C-AF-1.13. One liter GIFT® system fermentation data using the current best hydrolyzate adapted isobutanol producing biocatalyst (LB4) showing the percent of relative average volumetric isobutanol productivity in 60% (v/v) of each FS-10 hydrolyzate. The clarified hydrolyzates were supplemented with a nutrient package, salts, and the pH was controlled during the fermentation. The 60% (v/v) mixture of each hydrolyzate contained equal amounts of corresponding sugars to the 100% material and equivalent supplements were added to each hydrolyzate. Isobutanol was measured using a GC. All of the hydrolyzates were tested in GIFT® systems except the MB SSL. Error bars represent the standard deviation of duplicates. Abbreviations: WO, wet oxidation; SPORL, sulfite pretreatment to overcome recalcitrance of lignocellulose; CMB, combined mild bisulfite; UMW, unconcentrated milled wood; MB, mild bisulfite; SSL, spent sulfite liquor.

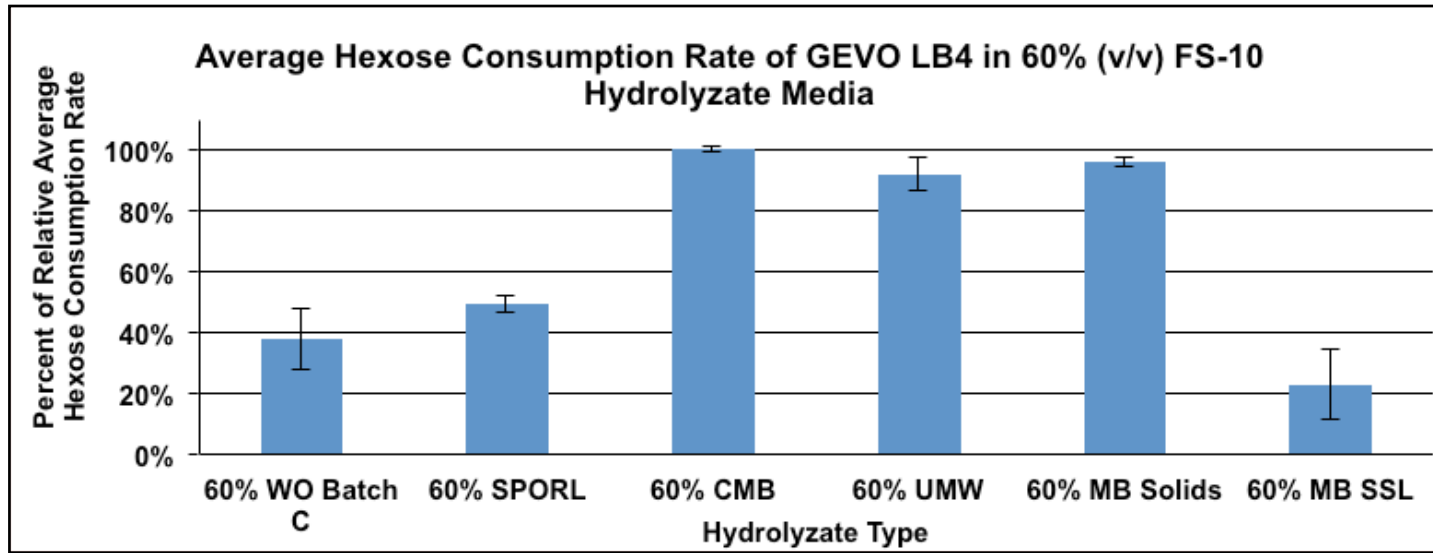


Figure C-AF-1.14. One liter GIFT® system fermentation data using the current best hydrolyzate adapted isobutanol producing biocatalyst (LB4) showing the percent of relative isobutanol titers in 60% (v/v) of each FS-10 hydrolyzate. The clarified hydrolyzates were supplemented with a nutrient package, salts, and the pH was controlled during the fermentation. The 60% (v/v) mixture of each hydrolyzate contained equal amounts of corresponding sugars to the 100% material and equivalent supplements were added to each hydrolyzate. Cell dry weight (CDW) was calculated using a conversion factor multiplied by the cell density measured on a spectrophotometer. All of the hydrolyzates were tested in GIFT® systems except the MB SSL. Error bars represent the standard deviation of duplicates. Abbreviations: WO, wet oxidation; SPORL, sulfite pretreatment to overcome recalcitrance of lignocellulose; CMB, combined mild bisulfite; UMW, unconcentrated milled wood; MB, mild bisulfite; SSL, spent sulfite liquor.

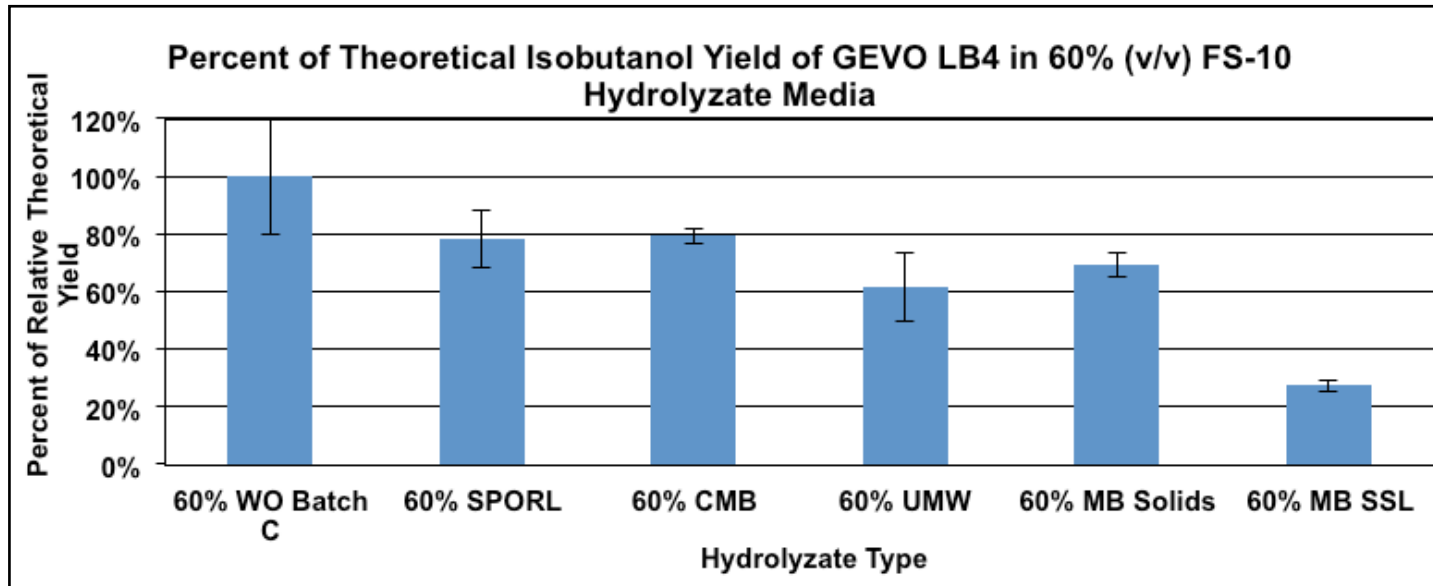


Figure C-AF-1.15. One liter GIFT® system fermentation data using the current best hydrolyzate adapted isobutanol producing biocatalyst (LB4) showing the percent of relative isobutanol titers in 60% (v/v) of each FS-10 hydrolyzate. The clarified hydrolyzates were supplemented with a nutrient package, salts, and pH controlled adjusted during the fermentation. The 60% (v/v) mixture of each hydrolyzate contained equal amounts of corresponding sugars to the 100% material and equivalent supplements were added to each hydrolyzate. Cell dry weight (CDW) was calculated using a conversion factor multiplied by the cell density measured on a spectrophotometer. All of the hydrolyzates were tested in GIFT® systems except the MB SSL. Error bars represent the standard deviation of duplicates. Abbreviations: WO, wet oxidation; SPORL, sulfite pretreatment to overcome recalcitrance of lignocellulose; CMB, combined mild bisulfite; UMW, unconcentrated milled wood; MB, mild bisulfite; SSL, spent sulfite liquor.

Recommendations | Conclusions Physical and Intellectual Outputs

Work is proceeding according to the project plan. Characterization of pretreated materials has been completed for the materials considered in the pretreatment downselection process. The adaptation program continues to provide biocatalysts with better performance in both growth and isobutanol production with new strains from Gevo's biocatalyst engineering group. In order to obtain the best biocatalyst possible and identify the best conditions for fermentations, narrowing down the types of pretreatments examined will allow a more focused adaptation program. Batch and GIFT® fermentations at the 1L scale showed that isobutanol production in hydrolyzates reach similar titers as the control media, under the conditions tested. One liter GIFT® fermentations will continue in order to find the optimum process parameters for isobutanol production. Gevo has completed a process model and will continue to refine it.

Physical

- Andrew Hawkins presented at the Joint USDA AFRI CAP/AAIC Meeting 10/12/13 in Washington, DC.
- Grant Balzer presented at the Harvesting Clean Energy Conference 2/4/14 in Helena, MT.

Conference Proceedings and Abstracts from Professional Meetings

Harvesting Clean Energy Abstract:

Fermentative Conversion of Hydrolyzed Douglas fir Biomass into Isobutanol and Biojet

The pretreatment and hydrolysis of lignocellulosic biomass to release nutrients and sugars can ultimately lead to the production of biofuels and biochemicals. Gevo has developed fermentation and process technology to convert biomass sugars to isobutanol, followed by chemical processing into renewable jet fuel. Gevo uses GIFT®, Gevo Integrated Fermentation Technology, to produce isobutanol at high productivity, titer, and yield using a biocatalyst. As part of the Northwest Advanced Renewables Alliance (NARA), Gevo's goal is to fermentatively convert hydrolyzed Douglas fir biomass into isobutanol at a specification developed by Gevo that ensures the isobutanol will be further converted into renewable biojet using existing Gevo technology. The specific tasks of Gevo's project for NARA include: (1) Characterize and evaluate representative samples of pretreated Douglas fir biomass; (2) Adapt yeast biocatalysts to pretreated biomass hydrolyzates; (3) Produce isobutanol at laboratory scale to optimize isobutanol production using the adapted yeast biocatalyst; (4) Economic assessment of wood to isobutanol, biojet; (5) Analyze isobutanol to close the mass balance and determine potential low-level impurities; (6) Produce isobutanol at demonstration scale using GIFT® fermentations and convert the lignocellulosic derived isobutanol to biojet for further testing.

TASK C-AF-2: PRODUCTION OF JET FUEL USING BIOCHEMCAT

Key Personnel

Birgitte Ahring

Affiliation

Washington State University

Task Description

Using the proprietary BioChemCat Process, the Washington State University's Bioproducts, Sciences and Engineering Laboratory (WSU BSEL) will, in parallel with Gevo, investigate the production of jet fuel from woody residues. In the project, WSU BSEL will concentrate its efforts on the following two areas: Task 1: Co-culture optimization for high production yield and productivity of volatile fatty acids (VFA) from pretreated biomass hydrolysate. Task 2: Catalysis of platform molecules into jet fuel. WSU BSEL will work with the Pacific Northwest National Laboratory (PNNL) using the PNNL combinatorial catalysis computational laboratory platform.

The BioChemCat process is a new way to produce more biofuels from biomass at a significantly lower cost. The cost reductions will come from: 1) the process does not use enzymes, a significant cost reduction; 2) the mixed culture fermentation used is non-sterile and robust; 3) no special seed culture needs to be grown, and the culture needs no expensive additives; 4) simple low-cost reactors as used for anaerobic digestion; 5) only a fraction of the biomass material needs to be pretreated and with low retention times which considerably lowers the cost of pretreatment (see below); 6) a large part of the lignin can be converted to biofuels along with the carbohydrates in the biomass material.

Activities and Results

[Redacted introduction]

Task 1

Fermentation was performed on both FS-01, FS-03 and FS-10 feedstock samples which were pretreated using the wet oxidation method and then further fermented using 2% Corn Steep Liquor as the only supplement. The fermentation was run in fed batch, and the productivity varied between 0.3 to 0.8 g/L/h. Compared to traditional processes operating from cellulosic sugars where enzymatic hydrolysis is needed before the fermentation, the overall process time is comparable.

Preliminary results have been done showing higher productivities when using continuous fermentation. [Redacted information]

Separation of the VFAs from the fermentation broth is a main unit operation of major importance for the economics of the overall process. Carboxylic acids are normally separated out by precipitation during the fermentation as a calcium salt using calcium hydroxide to stabilize pH. The salt will, however, interfere with the upgrading processes which is often performed in a gaseous phase, and gypsum will be formed when the acids are recovered with sulfuric acids. Furthermore, the acid salt will influence the fermentation in a negative way through product inhibition to the conversion process; therefore, different solutions have been investigated to remove the acids in the native form during their production.

Three different separation methods: (i) pressurized carbon dioxide extraction; (ii) ion exchange resin-mediated extraction; and (iii) esterification, have been investigated for the extraction of organic acids from fermentation broth.

The extraction of acetic acid from the model solution using pressurized carbon dioxide was optimized based on a response surface model as discussed in the NARA cumulative report for the year 2012-2013. In the first quarter of fiscal year 2013, the volatile fatty acids from the fermentation broth were extracted using pressurized carbon dioxide at the conditions optimized through the response surface model. The fermentation broth used as a model was spiked to contain approximately 15, 30, 50 and 75 g/L acetic acid, and carbon dioxide was used as the extraction solvent. The broth was acidified to pH at around 3.0 to facilitate the extraction process. The conditions for the extraction of acetic acid from the broth using carbon dioxide was at a temperature of 50°C, solvent flow rate of 991.4 standard cubic centimeters per second (sccm), pressure of 2500 pounds per square inch (psi) and extraction time of 1hr. The percent of acetic acid extracted, and the mass of acetic acid extracted per gram of CO₂, from cow rumen versus model acetic acid solution is shown in Figure C-AF-2.1. The data obtained in this study of pressurized carbon dioxide extraction of acetic acid from fermentation broth has been compiled into a manuscript which has been submitted to a peer-reviewed journal.

Unlike with supercritical carbon dioxide extraction, where negligible amount of studies were available in the literature, ion exchange extraction of volatile fatty acids has been studied for the last 20 years but has not been optimized for extraction from fermentation broth. A literature review revealed that the use of a weak-base ion exchange resin can help achieve greater extraction of volatile fatty acids while requiring lower amounts of a strong base such as sodium hydroxide or ammonium hydroxide to elute the acid from the resin. Amberlite™ IRA-67 and IRA-96 produced by DOW chemical was purchased to optimize the extraction of volatile fatty acids from fermentation broth.

Preliminary column experiments were done, using pure C-3 acid solution passing through a fixed bed of Amberlite™ IRA-67 (18 cm length x 1 cm i.d.) at different flow rates, to measure the breakthrough and saturation limits of the resin for C-3 acids. It should be noted that Amberlite™ IRA-67 has a micro-porous gel structure with ammonium free base cation that binds with the lactate ion from the solution to facilitate extraction.

Further studies were done by using a mixed bacterial consortia to optimize the fermentation of pretreated FS-10 hydrolyzate to produce C-3 acids. Briefly, 120g of Amberlite™ IRA 67 free base resin was added to the resin flask after washing with approximately 2.5N NaOH, followed by washing with water, until the pH of the resin dropped to around 8.5-9.5. Varying amounts of deionized water (20-100mL) was added to the resin flask to maintain the resin in a semi-solid form. The initial pH of the solution in the fermentation flask was initially brought up to 5.5 using 5N NaOH solution. The pH of the fermentation was initially controlled using 5N NaOH solution until the concentration of C-3 acid-salt in the fermenter reached around 20 g/L. The pH control and C-3 acid separation was then changed to the resin flask usually after the first feeding (done when glucose concentration decreased below 10g/L in the broth) between 24 and 36 hours. Recirculation pumps were operated through a decrease in the pH of the fermenter allowing the broth to flow through the ion exchange resin bed to eventually regulate the pH inside the fermenter to the set point through adsorption of the C-3 acid onto the ion exchange resin. The fermenter was fed continuously until the recirculation pumps ran continuously due to the inability of the ion exchange resin to regulate pH of the fermenter due to saturation with C-3 acid. At this point, the ion exchange resin flask was swapped with a new or a regenerated resin bed and the C-3 acid was desorbed from the ion exchange resin using 2.5 N NaOH using three simultaneous elution's yielding concentrations of 60g/L, 25g/L and 3g/L C-3 acid-salt with each elution, respectively.

The fermentation using regenerated ion-exchange resin beds in the recirculation loop was run with minimal issues in repeated fed-batch form for around 65 days. It was found that during this time, the concentrations of sugar in the fermentation broth were around 10-12 g/L glucose and 8g/L xylose. The concentration of C-3 acid in the fermentation broth was found to be around 12g/L with the rest separated out using ion-exchange resin bed. The concentrations of the sugars, C-3 and C-2 acids in the fermentation broth, as a function of time, are shown in Figure C-AF-2.3.

The VFAs present in the fermentation broth, separated by either ion exchange resin bed or the salt precipitation process, form byproducts such as sodium sulphate or gypsum (based on the salt used for elution of the ion exchange resin or neutralization of fermentation broth) which have limited applications. The formation of such byproducts can be prevented through direct esterification of the carboxylic acid or carboxylic acid salt using an acid-catalyzed or reactive esterification method.

[Information and figures C-AF-2.4 and C-AF-2.5 are redacted.]

Recommendations | Conclusions

The acetic acid extracted from the fermentation broth and the model solution was not significantly different from each other especially at concentrations of acetic acid in the feed exceeding 50g/L. The difference in the total percent of acetic acid extracted using pressurized carbon dioxide between fermentation broth and model solution can be attributed to the presence of other volatile fatty acids in the broth. While the concentration of these other acids (propionic and butyric) does not change, it seems that the carbon dioxide preferentially extracted the higher fatty acids. It was found that as the concentration of the acetic acid in the fermentation broth increased, the ratio of the higher fatty acids decreased, thereby, significantly decreasing the difference between the experimental data and the prediction model. Further studies are

currently being done to study the effect of higher organic acids on the extraction of acetic acid from fermentation broth using supercritical carbon dioxide.

While the extraction of volatile fatty acids using pressurized carbon dioxide has shown close to 100% recovery of acetic acid at the optimized conditions, the technology at its current stage can be considered expensive due to the compression requirements for carbon dioxide. However, these costs can be mitigated by further optimizing the conditions and also through carbon dioxide serving as a carrier or solvent for these volatile fatty acids as it progresses further into catalytic upgrading to conversion to jet fuels. With carbon dioxide as a solvent for the acids in catalytic upgrading, the need for other solvents such as organic or hydroalcoholic solvents, and any acid-base reaction resulting in waste production, can be avoided.

Experiments done using ion exchange resin (Amberlite™ IRA-67) mediated acid separation from the fermentation broth indicated that the capacity of the resin bed was around 120 mg C-3 acid/g resin which varies with the presence of C-2 (20 mg/g resin). C-4 and other acids produced during fermentation also bind onto the resin bed and can be eluted using NaOH. The re-usability of the ion exchange resin after washing with 2.5 N NaOH to desorb the separated C-3 acid was also studied using two ion exchange resin beds, used interchangeably with the fermentation. These two ion exchange beds were regenerated twice, and the resin loading (mg acid/g initial resin) for C-3 and C-2 acids was plotted as a function of regeneration (or elution) cycles. It was found that the ion exchange resins can be sufficiently regenerated, and the regeneration process does not affect the ability of the resin to separate the acids from fermentation broth.

While the ion exchange resin mediated separation process showed high capacity for selective removal of VFA from the fermentation broth, while subsequently maintaining pH in the fermentation broth, the capital costs associated with using ion exchange resins in a scaled-up process could be prohibitive. Also, the acid

needs to be eluted from the ion exchange resin after saturation using a salt. However, the further conversion of this acid to alcohols through catalytic hydrogenation is more efficient when done in vapor phase versus liquid phase since the water content in the acid feed, which is toxic to several common hydrogenation catalysts, is used. Hence, it would significantly reduce process costs and increase process efficiency if the acid was selectively converted to its ester and further taken in the vapor phase to be hydrogenated, in the presence of a catalyst, to produce alcohols that can then be eventually converted to jet fuel components. Studies published in the literature indicate VFA purity as high as 98% through esterification, and several methods show greater conversion efficiency of esters to alcohols through vapor phase hydrogenation when compared to the VFAs in their acid forms.

[Information redacted]

Physical and Intellectual Outputs

None for this reporting period

Future Work

Task 1

[Information redacted]

Task 2

Work to covert VFAs into mixed alcohols has been initiated. [Information redacted]

After next year, all elements will be in place to move the BioChemCat process to large scale pilot and further commercialization scenarios. (Figure C-AF-2.6 redacted)

As can be seen from Figure C-AF-2.7, a lab-scale catalytic system has been designed and is currently in process of being set-up in the laboratory at BSEL. There is also significant impetus towards financing the new equipment through different sources. The parts required for constructing the catalytic hydrogenation system have been purchased through funding from other sources, and an experimental procedure has been designed to optimize and study the conversion of the methyl ester of carboxylic acids into alcohols. Improvements have been made in this task from those reported in previous NARA progress reports. The hydrogenation catalysts have been produced and are being characterized using various equipment available through our collaboration with Pacific Northwest National Laboratory (service facility).

Table CFA-2.1. The yield of VFA and the calculated yield of mixed alcohols and jet fuel from the fermentations performed under NARA.

Biomass	Yield of VFA	Yield of Mixed Alcohols	Yield of Jet Fuel
FS 01	510 kg/ton	126 gal/ton	74.8 gal/ton
FS 03	410 kg/ton	101 gal/ton	60.1 gal/ton
FS 10	440 kg/ton	109 gal/ton	64.5 gal/ton

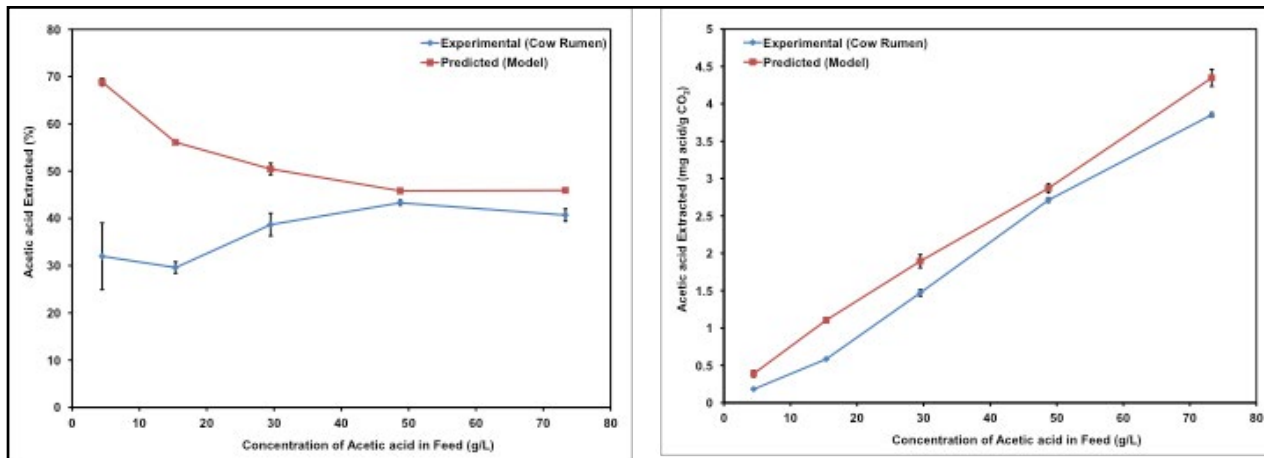


Figure C-AF-2.1. % acetic acid extracted; and (b) mg of acetic acid extracted per gram CO₂ from fermentation broth versus model solution as a function of the concentration of acetic acid in the feed.

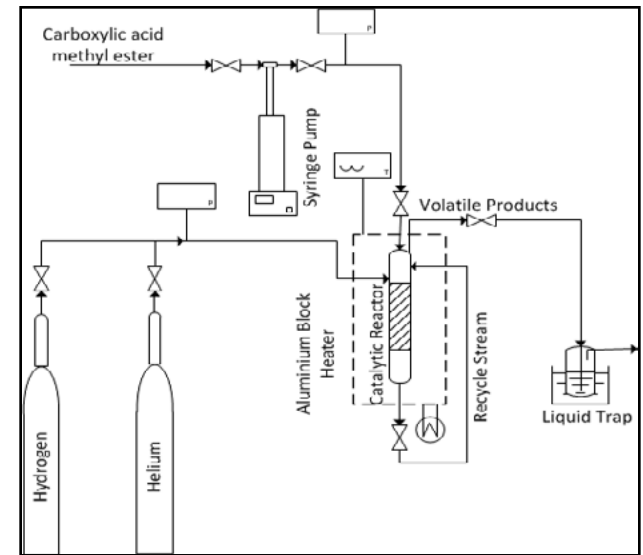


Figure C-AF-2.7. Process schematic for the catalytic vapor phase hydrogenation system for converting carboxylic acid methyl ester to alcohols.

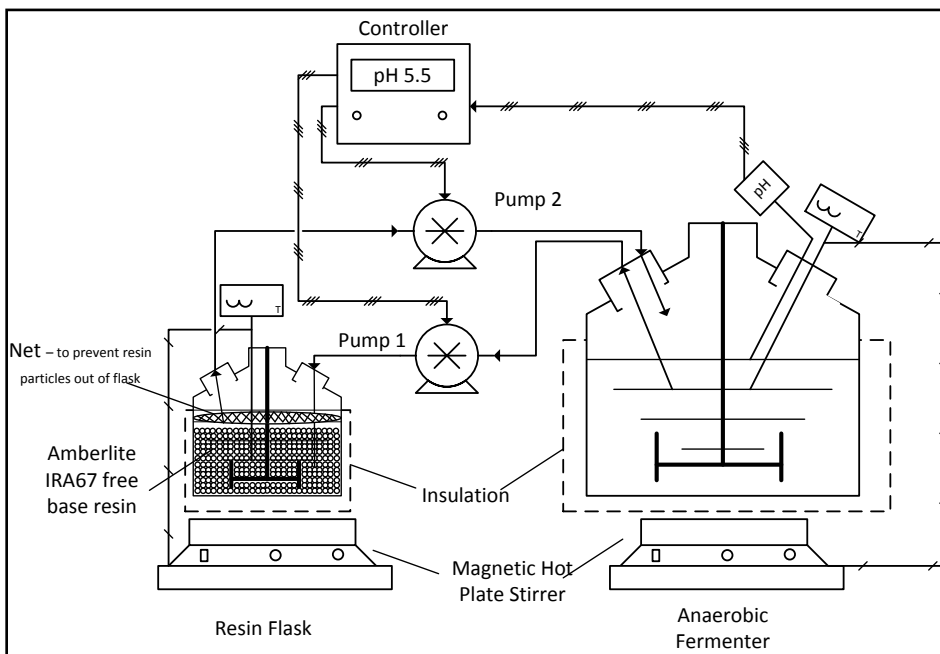


Figure C-AF-2.2. Process Schematic of Anaerobic Fermentation of Biomass Hydrolyzate with Separation Using Ion Exchange Resin in Recirculation Loop

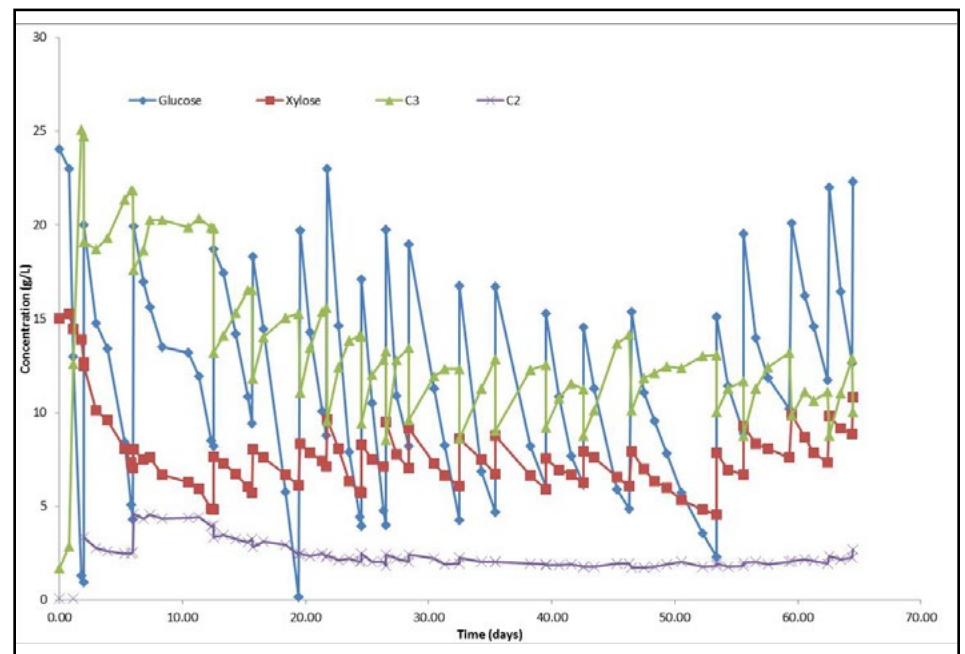


Figure C-AF-2.3. Variation of the concentrations of sugars (glucose, xylose), C-3 and C-2 acids in the fermentation broth as a function of time

FeedstockLogistics_Marrs



Task Name	2011				2012				2013				2014				2015				2016				
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	
1 FL-1. Feedstock Sourcing																									45%
2 <input type="checkbox"/> Task FL-1.1. Quantify costs and quantities of key PNW candidate feedstocks by region, scale, and year																									100%
3 Quantify qualifying feedstock availability by category and geographic region over time																									100%
4 Determine likely early-year delivered feedstocks costs to conversion mouth																									100%
5 Determine most likely facility scale given rising costs of feedstock with size																									100%
6 Prepare written report quantifying costs and key quality attributes of candidate PNW jet biofuel feedstocks																									100%
7 <input type="checkbox"/> Task FL-1.2. Determine feedstock key quality parameters and variation and impact on conversion process																									57%
8 Collect samples of key likely feedstocks and test key properties (analytical, size, etc.)																									100%
9 Collect, Prepare, and characterize a set of eastside NARA regions samples from MT or ID																									5%
10 Identify, collect, prepare and disseminate one or two "reference feedstocks" of 5-10 ton scale from MT or ID																									0%
11 Provide samples to assess candidate feedstocks through lab-scale conversion to quantify impacts																									100%
12 Determine how mixtures of feedstock types can best be dealt with in conversion process																									0%
13 Prepare written report documenting averages and variability of key feedstock quality parameters of importance to the NARA-specific conversion process and impact on economic feasibility																									0%
14 <input type="checkbox"/> Task FL-1.3. Develop and test feedstocks value lift / cost reduction logistics improvements (harvesting, processing, transport, etc.)																									3%
15 Conceive and test harvesting cost improvement activities																									5%
16 Conceive and test transportation cost improvements																									5%
17 Conceive and test processing improvements																									0%
18 Develop integrated overall improved logistics system																									0%
19 Prepare written report documenting experimental results of tested (improved) feedstock harvesting and preparation methods																									0%
20 Task FL-1.4. Final Report																									0%

FeedstockLogistics_Sessions



Task Name	2011				2012				2013				2014				2015				2016				
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	
1 FL-2. Logistics Decision Support and Improvement																									52%
2 Task FL-2.1. Develop Biomass Recovery Coefficients for OR, WA, ID, MT																									80%
3 Draft field procedures developed and statistical model to develop recoverable biomass from TPO data																									0%
4 Regression model for biomass recovery as a function of timber strata, silvicultural prescription, harvesting system, feedstock specifications, merchantability specifications for species in NARA region. Field procedure and statistical model to predict recoverable biomass from TPO data.																									0%
5 Task FL-2.2. Develop Moisture Management Strategies and Models																									65%
6 Physical and economic model for moisture management for species in NARA region																									0%
7 Task FL-2.3. Refine Collection and Transport Models for regional modeling																									90%
8 Decision support model for identifying most efficient collection and transport system based on residue location, specifications, and facility location																									0%
9 Task FL-2.4. Evaluate grinding and chipping production and costs to meet alternative feedstock specifications																									50%
10 Report describing comminution costs to meet alternative feedstock specifications																									0%
11 Task FL-2.5. Demonstrate and evaluate new trailer designs to improve transport efficiency																									0%
12 Public demonstration of new trailer technology																									0%
13 Evaluation report on trailer efficiency and improved forest access																									0%
14 Task FL-2.6. Evaluate health and safety procedures for any new work processes																									0%
15 Report on identification of need for new health and safety procedures and recommendation for new procedures																									0%
16 Task FL-2.7. Synthesis of Logistics Work and Final Report																									0%
17 Final report describing results of work																									0%

FeedstockDevelopment_Kirchhoff



Task Name	2011				2012				2013				2014				2015							
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4				
1 <input type="checkbox"/> FD-2. Phenomics Analyses																					25%			
2 Reduced lignin lines testing																					100%			
3 <input type="checkbox"/> Task FD-2.2. Douglas-fir/western hemlock																					11%			
4 Effect of drought/salinity																					30%			
5 Influence of soil composition (e.g. N-deprived soil)																					0%			
6 Use of fertilizers																					0%			
7 Task FD-2.5. Final Report																					0%			

FeedstockDevelopment_Jayawickrama



Task Name	2011				2012				2013				2014				2015			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1 <input checked="" type="checkbox"/> FD-3. Combining Genomic and Field-based Breeding and Testing Methods to Improve Woody Feedstock Production																				61%
2 <input checked="" type="checkbox"/> Task FD-3.1. Collect wood samples and combine with phenotypic data																				66%
3 Select optimal populations of trees to obtain samples of woody tissue																				80%
4 Obtain wood samples																				80%
5 Measure variation in chemical properties																				60%
6 Combine with existing growth rate data to estimate variation in biofuel potential																				0%
7 Report																				0%
8 <input checked="" type="checkbox"/> Task FD-3.2. Collect tree tissue samples and obtain marker data																				53%
9 Write a plant breeding plan																				50%
10 Write a marker data plan																				80%
11 Summarize available NWTIC materials																				60%
12 Select optimal populations of trees to obtain needle samples																				75%
13 Collect, store and manage tree tissue samples																				0%
14 Extract DNA																				0%
15 Initiate and manage contract for building genotyping array																				50%
16 Genotype samples																				0%
17 Report																				0%
18 <input checked="" type="checkbox"/> Task FD-3.3. Develop relationships between multiple data sources																				0%
19 Develop relationships between phenotypic and marker data																				0%
20 Develop improved methods for detecting marker-trait associations																				0%
21 Make selections for increased biofuel production using phenotypic and marker data																				0%
22 Task FD-3.4. Provide Douglas-fir seedlings for phenomics study																				85%
23 Task FD-3.5. Final Report																				0%

FeedstockDevelopment_XZhang



Task Name	2011				2012				2013				2014			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1 <input type="checkbox"/> FD-5. Screen and Identify Suitable Plant Feedstocks for Large Scale Pretreatments to Produce High Yield Sugar and High Quality Lignin.																60%
2 <input type="checkbox"/> Task FD-5.1. Determine the chemical compositions of Douglas-fir, western hemlock, hybrid/transgenic poplar and red alder feedstocks																75%
3 Sample collection and preparation																60%
4 Chemical composition analyses																85%
5 Report on chemical composition analysis																0%
6 <input type="checkbox"/> Task FD-5.2. High throughput pretreatment screening to identify most efficacious plant feedstocks for large scale conversion processes for fuel and lignin production																53%
7 High throughput pretreatment screening in reactor tubes																80%
8 Report summarizing the screening results																5%
9 Lignin extraction and characterization																0%
10 Report on lignin extraction and characterization																0%
11 Task FD-5.3. Write Final Report																0%
12 Final Report																0%

Pretreatment_Zhu



Task Name	2011		2012				2013				2014				2015				2016			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4		
1 <input type="checkbox"/> C-P-1. Pretreatment to Overcome Recalcitrance of Lignocelluloses																					62%	
2 <input type="checkbox"/> Task C-P-1.1. Optimization SPORL and/or other pretreatments for Douglas-fir/Douglas-fir residues at lab bench scale (150 g/2kg)																						100%
3 Log chipping, experiments design, pretreatment																						100%
4 Sample analysis for chemical composition, enzymatic saccharification																						100%
5 Material and energy balance, determining optimal SPORL condition																						100%
6 <input type="checkbox"/> Task C-P-1.2. Optimization SPORL and/or other pretreatments at FPL pilot plant facility (50 kg/run)																						96%
7 Experiment design based on Task 1, pretreatment																						100%
8 Sample analysis, high solids (~20%) enzymatic saccharification																						90%
9 Material and energy balance, correlate data between Task 1 and Task 2																						100%
10 <input type="checkbox"/> Task C-P-1.3. Scale-up of SPORL technology																						22%
11 Identification and experimental verification of operating conditions that are most cost-effectively implemented into a first commercial operation																						25%
12 Scale up from 2-3 to 50 kg feedstock. Integrate downstream processes of defibering, hydrolysis, and concentration of sugar																						25%
13 Hydrolyze and concentrate a sample of waste fiber from a commercial sulfite mill and compare the resulting sugar to the sugar produced in the Mild Bisulfite Pretreatment																						0%
14 Work with Washington State University, Weyerhaeuser and Gevo for jet fuel/lignin products production, data analysis, reporting																						25%
15 Task C-P-1.4. Final Report																						0%

Pretreatment_Ahring



Task Name	2011				2012				2013				
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	
1 <input type="checkbox"/> C-P-3. Preparation of Pretreated Biomass													79%
2 <input type="checkbox"/> Task C-P-3.1. Samples of Pretreated Biomass													100%
3 Receive biomass samples from Catchlight Energy													100%
4 Analyze biomass samples to determine characteristics and compositions													100%
5 Pretreat biomass and deliver samples back to partners													100%
6 <input type="checkbox"/> Task C-P-3.2. Evaluate data from partners and adjust pretreatment system													50%
7 Evaluate data from initial pretreatment results													50%
8 Modify pretreatment process to improve outputs													50%
9 Task C-P-3.3. Final Report													50%

Pretreatment_Catchlight



Task Name	2012				2013			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1 <input type="checkbox"/> C-P-4. Mild Bisulfite Pretreatment of Forest Residuals							65%	
2 Task C-P-4.1. Identify effect of pretreatment process variables on total sugar yield							60%	
3 Task C-P-4.2. Produce lignosulfonate and lignin sample for study by Co-Products team							80%	
4 Task C-P-4.3. Final Report							0%	

Pretreatment_Gao



Task Name	2013				2014				2015				2016			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1 <input type="checkbox"/> C-P-5. Clean Sugar and Lignin Pretreatment Technology																6%
2 Task C-P-5.1. Comprehensive literature study of wood milling technologies																15%
3 Wood milling technology review report																◆ 15%
4 Task C-P-5.2. Conduct life cycle analysis LCA of wood milling																0%
5 Life cycle analysis report on wood milling																◆ 0%
6 Task C-P-5.3. Verify energy consumption in tandem mill at Akita University																100%
7 Energy consumption verification report on tandem milling																◆ 100%
8 Task C-P-5.4. Small scale milling screening																10%
9 Small scale milling screening report																◆ 10%
10 <input type="checkbox"/> Task C-P-5.5. Hydrolysis Optimization																5%
11 Milling equipment (small unit for optimization), order and installation																0%
12 Milling equipment (small unit for optimization) installed																◆ 0%
13 Optimize hydrolysis																10%
14 Hydrolysis optimization report																◆ 10%
15 Task C-P-5.6. Small-scale clean sugar and lignin production for Gevo fermentation and lignin co-product evaluation																30%
16 Small scale clean sugar to Gevo and lignin sample to co-product																◆ 100%
17 Small scale clean sugar to Gevo and lignin sample to co-product																◆ 0%
18 Gate Analysis and Review - Decision to proceed with Large Scale																◆ 0%
19 Task C-P-5.7. Large scale production of clean sugar																0%
20 Clean sugar sample delivered to Gevo																◆ 0%
21 Task C-P-5.8. Large scale production of residual lignin																0%
22 Lignin residuals production completion																◆ 0%
23 <input type="checkbox"/> Task C-P-5.9. Large scale production optimization and engineering analysis																0%
24 Further optimize large scale clean sugar production																0%
25 Provide more samples to Gevo and co-product team																0%
26 Engineering assessment for pilot demonstration																0%

Task Name	2013				2014				2015				2016			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
27 Task C-P-5.10. Industrial pilot demonstration for clean sugar production worth of 1000 gallon jet fuel.																0%
28 Task C-P-5.11. Industrial pilot demonstration for residual lignin production from sugar production																0%
29 <input type="checkbox"/> Task C-P-5.12. Techno-economics analysis and report																0%
30 <input type="checkbox"/> Task C-P-5.12.1. Small Scale																
31 Techno-economics analysis report (initial)																0%
32 Techno-economics analysis report (mid)																0%
33 <input type="checkbox"/> Task C-P-5.12.2 Large Scale																
34 Techno-economics analysis report (final)																0%
35 Techno-economics analysis report (updates w/ new information)																0%
36 Final Techno-economics analysis and report																0%

Conversion-AF_Ahring



Task Name	2011				2012				2013				2014			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1 <input checked="" type="checkbox"/> C-AF-2. Production of Jet fuel using BioChemCat																30%
2 <input checked="" type="checkbox"/> Task C-AF-2.1. Optimizing fermentation for making platform molecules																38%
3 Investigate the fermentation method																50%
4 Optimize the fermentation process																50%
5 Optimize the separation and recovery of platform molecules																40%
6 Integrate and optimize production and extraction of platform molecules																0%
7 <input checked="" type="checkbox"/> Task C-AF-2.2. Optimize the catalysis of platform molecules into jet fuel																0%
8 Screen and selection optimal catalysts																0%
9 Optimize the catalysis process																0%
10 Task C-AF-2.3. Final Report																0%
11																