NARA Goal One

3RD Cumulative Report

April 2014 - March 2015

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 roadmap 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 residue 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 residues to biojet:

1) Feedstock Logistics Team: Integrating feedstock collection, pre-processing, 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 ability for the Gevo fermentation/separation and WSU BioChemCat processes to produce aviation biofuels from representative pretreated forest residuals

Monitoring moisture in slash piles. NARA image

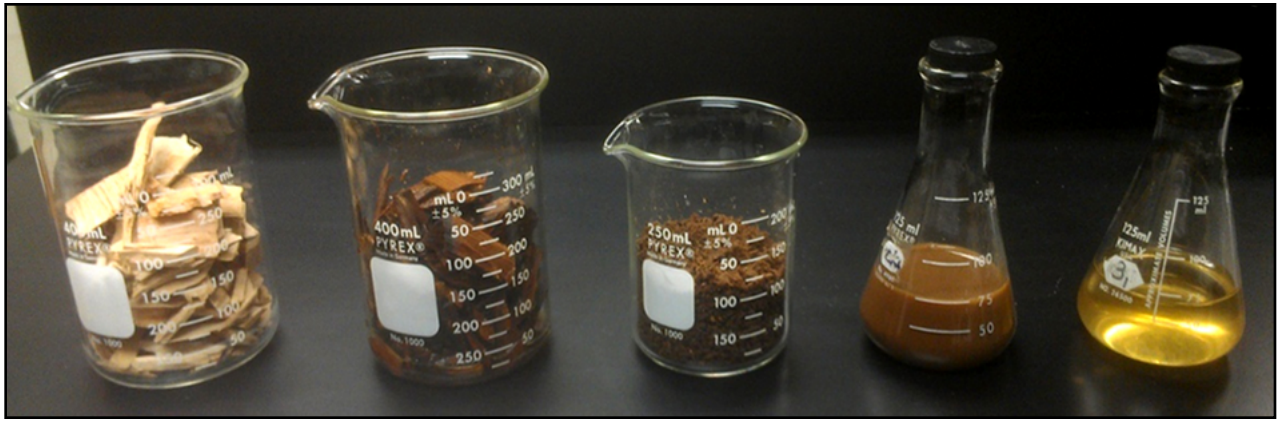
FEEDSTOCK LOGISTICS

The NARA Feedstock Team is divided into two efforts: feedstock logistics and feedstock development. The feedstock logistics efforts for this reporting period provided improved cost estimates associated with processing and transporting forest residual feedstock to a conversion facility. These data are being integrated into the NARA techno-economic analysis (TEA; Task SM-TEA-1), Life cycle assessment (LCA; SM-LCA-1.2) and biomass supply model (see task SM-SP-3).

Forest residual feedstock samples were analyzed at the Weyerhaeuser Analytical Laboratory using NREL protocols, which provided near 100% accounting for chemical components. These results will serve as a reliable chemical component benchmark for varied feedstock supplies in the Idaho, Washington, Montana and Oregon region. Ash content and “accepts” and “fines” distribution among the samples were determined (Task FL-1.1.2). The results show that all the samples tested are suitable for biojet conversion and comply with reasonable feedstock specifications. Live branch moisture content was recorded and compared between varied western softwood species, and a moisture and temperature model is being developed based on slash pile monitoring data (Task FL-2). Grinding trials were conducted on various feedstock classes to evaluate best practices for improved processing economics. These trials suggest that the use of best practices can improve logistics about $15/BDT, which improves the internal rate of return (IRR), calculated using the NARA

TEA model, for the conversion of forest residuals to biojet fuel and co-products by 0.8% (Task FL-1).

Using the improved NARA biomass supply model, cost curves were generated for feedstock costs to the mill gate at three putative conversion sites (one in Oregon and two in Washington). Average feedstock cost was $67.05 per bone-dried ton (BDT) (Task FL-1.1). The variation in feedstock cost, as determined in the three scenarios, had minimal impact (variation of 1.4%) towards the internal rate of return (IRR). The combined cost curve and grinding trial data suggest that improvements to feedstock costs can positively impact IRR, however, the impact will be relatively minimal.



Samples from wood-to-biojet conversion. NARA image

FEEDSTOCK DEVELOPMENT

For this reporting period, the feedstock development effort used technology at the WSU phenomics facility to screen ninety-eight Douglas-fir tree families with nine plants per family totaling 882 plants for drought response (Task FD-2). The screening revealed Douglas-fir candidates for further in-depth analysis by the gene chip approach developed in NARA Task FD-3. The aim is to identify differences in gene expression in the selected drought-sensitive and drought-tolerant families.

Narrow-sense heritabilities, genetic correlations between traits, and predicted genetic gains for pretreatment yield, pretreated holocellulose, enzymatic hydrolysis yield, and recalcitrance factor were predicted for 284 progeny trees, 30 woodsrun (unimproved) trees, 28 crosses (between 6 and 12 progeny per cross) and 46 parents. The estimates of heritability and predicted genetic gain indicate that Douglas-fir trees can likely be genetically selected for traits that favor their use to produce biojet fuel and co-products without sacrificing characteristics suitable for traditional timber use and justify efforts to identify genetic markers for improved Douglas-fir tree breeding (Task FD-5).

To develop the tools necessary to identify genetic markers, that contribute to favorable traits for biojet production and drought stress, a file of 221,674 targeted single nucleotide polymorphisms (SNPs) was assembled and submitted to GeneSeek/Affymetrix for construction of a genotyping array, which should be completed in April 2015. DNA samples from nearly 2000 selected Douglas-fir trees were collected and will be screened against the developing genotype array. In addition, 1,420 Douglas-fir seedlings were planted in southern Oregon to be used for genomic selection studies (Task FD-3).

SIGNIFICANT OUTPUTS REPORTED THIS PERIOD FOR THE FEEDSTOCKS TEAMS

- A peer-reviewed manuscript (Geleynse et al) was published titled “A multi-level analysis approach to measuring variations in biomass recalcitrance of Douglas fir tree samples” doi: 10.1007/s12155-014-9483-z (Task FD-5).
- A peer-reviewed manuscript (Kirchhoff, H) was published titled “Diffusion of molecules and macromolecules in thylakoid membranes” doi:10.1016/j.bbabio.2013.11.003 (Task FD-2).
- A peer-reviewed manuscript (Zamora-Cristales et al) was published titled “Effect of high speed blowing on bulk density of ground residues” doi: 10.13073/FPJ-D-14-00005 (Task FL-2).
- Four slash moisture monitoring stations were established, including in-pile sensors, weather stations, and data loggers (Task FL-2).
- Forest residuals were acquired from sites in western Oregon, western Washington and western Montana to be used to produce 1000 gallons of biojet fuel for demonstration purposes (Task FL-1)
- A peer-reviewed manuscript (Zamora-Cristales et al) was published titled “Economic Optimization of Forest Biomass Processing and Transport” doi: 10.5849/forsci.13-158 (Task FL-2).
- A peer-reviewed manuscript (Zamora-Cristales et al) was published titled “Ground-based thinning on steep slopes in Western Oregon: Soil exposure and strength effects” doi:10.5849/forsci.12-525 (Task FL-2).
- 1,420 Douglas-fir seedlings were planted in southern Oregon to be used for genomic selection studies (Task FD-3).
- 882 Douglas-fir seedlings were screened for drought resistance using Phenomics technology (Task FD-2).

SIGNIFICANT OUTCOMES

- None reported

PRETREATMENT

The Pretreatment Team's efforts this year focused on optimizing and evaluating the mild bisulfite pretreatment protocol under conditions suitable for a standard pulp mill. This effort will help integrate the pretreatment technology to existing industries and assist in the development of 1000 gallons of biojet fuel to be produced at the end of NARA Year-4. To do this, they bubbled SO_2 into a hydroxide solution to produce a sulfite solution rather than directly applying H_2SO_4 and sodium bisulfite as done in the laboratory. They also conducted enzymatic hydrolysis and fermentation directly to pretreated whole slurry at high solids without solids washing or slurry detoxification to simplify process integration. Under these conditions, substrate enzymatic digestibility reached 92% with a glucose titer of 97 g/L. A 284 L/tonne ethanol yield was obtained using Saccharomyces cerevisiae YRH-400. Additional modifications such as substituting $Mg(HSO_3)_2$, for $Ca(HSO_3)_2$, higher temperature or higher SO_2 loading for shorter pretreatment time, and eliminating solids milling were applied to accommodate specific pulp mill specifications in the Pacific Northwest. Enzymatic and fermentation experiments from the pretreated material based on these modifications are underway. The lignin product was also characterized under the modified mild bisulfite conditions and was determined to have comparable properties to commercial lignosulfonate (Task C-P-4).

In an effort to reduce the amounts of enzymatic and fermentation inhibitors present after sulfite-based pretreatment, a pH profiling process was evaluated and shown to significantly reduce inhibitor formation (Task C-P-1).

A final optimization was conducted for the wet oxidation pretreatment protocol that was under Gate review in NARA Year-3. Based on the optimization, a mass balance provided for the pretreatment of FS-10 forest residuals records a recovery rate of 99.9% glucan and 78.6% xylans. Initial lignin characterization indicated no structural modifications to the lignin occurred due to the wet oxidation pretreatment protocol (Task C-P-3).

SIGNIFICANT OUTPUTS REPORTED THIS PERIOD FOR THE CONVERSION TEAMS

- A peer-reviewed manuscript (Zhu et al) was published titled "Using sulfite chemistry for robust bioconversion of Douglas-fir forest residue to high titer bioethanol and lignosulfonate: A pilot-scale demonstration". doi:10.1016/j.biortech.2014.12.052 (Task CP-4).
- A peer-reviewed manuscript (Cheng et al) was published titled "High titer and yield ethanol production from undetoxified whole slurry of Douglas-fir forest residue using pH-profiling in SPORL". doi:10.1186/s13068-015-0205-3 (Task CP-4).
- A peer-reviewed manuscript (Zhang et al) was published titled "Using a combined hydrolysis factor to optimize high titer ethanol production from sulfite pretreated poplar without detoxification". doi:10.1016/j.biortech.2015.03.080 (Task CP-4).
- A peer-reviewed manuscript (Cheng et al) was published titled "High solids quasi-simultaneous enzymatic saccharification and fermentation of un-detoxified whole slurry of SPORL pretreated Douglas-fir forest residue" Link (Task CP-4).
- A peer-reviewed manuscript (Zhang et al) was published titled "Effect of hot-pressing temperature on the subsequent enzymatic saccharification and fermentation SPORL of pretreated forest biomass" doi: 10.1007/s12155-014-9530-9 (Task CP-4).
- A peer-reviewed manuscript (Lou et al) was published titled "Understanding the Effects of Lignosulfonate on Enzymatic Saccharification of Pure Cellulose" doi: 10.1007/s10570-014-0237-z (Task CP-4).
- A peer-reviewed manuscript (Zhang et al) was published titled "Using low temperature to balance enzymatic saccharification and furan formation during SPORL pretreatment of Douglas-fir" doi:10.1016/j.procbio.2013.12.017 (Task CP-4).
- A peer-reviewed manuscript (Zhang et al) was published titled "Comparison of dilute acid and sulfite pretreatment for enzymatic saccharification of earlywood and latewood of Douglas-fir" doi:10.1007/s12155-013-9376-6 (Task CP-4).
- A U.S Utililty Patent Application was submitted titled "Methods of Pretreating Lignocellulosic Biomass with Reduced Formation of Fermentation Inhibitors" (Task C-P-1)

SIGNIFICANT OUTCOMES

- None reported

AVIATION BIOFUELS

The NARA Aviation Biofuels Team is focused on two fermentation technologies: Gevo's fermentation, separation and upgrading process and WSU's BioChemCat process managed at WSU-BSEL.

Over NARA Year-4, Gevo continued to receive pretreated hydrolysate samples for sugar and inhibitor characterization and for fermentation experiments. The primary samples evaluated this year were hydrolysates derived from variations of the mild bisulfite protocol (also termed SPORL) adapted for specific pulp mill specifications (see task C-P-4) and hydrolysate from milled wood (MW) (Task E-8). Yeast biocatalyst growth rates were similar for the MW and FS-10 SPORL-Mg2+ hydrolysate samples; however, isobutanol production was higher (26.4%) with the FS-10 SPORL-Mg2+ hydrolysate.

Due to Gevo's extensive experimentation using hydrolysate produced through the mild bisulfite pretreatment protocol, selected in NARA Year-3 as the

preferred pretreatment protocol, Gevo has supplied the NARA LCA (SM-LCA-1.1) and TEA (SM-TEA-1) teams with the necessary CapEx and mass and energy balance information to complete their analyses involving Gevo's proprietary technology.

Two adapted biocatalysts (LB21 and LB23) are undergoing further adaptation. A nutrient package and pH specifications were established to maximize biocatalyst growth and isobutanol production; the isobutanol produced did not contain any impurities detrimental to the biojet conversion process. Gevo's biocatalyst LB23 is currently the superior biocatalyst as it produced 20% higher isobutanol concentrations that LB21 when directly pitched into FS-10 SPORL-Mg2+ hydrolysate. LB23 will be used in scaling the GIFT™ fermentation process to produce NARA's target of 1000 gallons of biojet fuel made from forest residuals.

Gevo will begin scale-up operations to produce 40,000 L of isobutanol once the hydrolysate from feedstock samples FS-17 (see Task FL-1) arrive (Task C-AF-1).

Research aligned with WSU's BioChemCat process compared a mesophilic fermentation approach to the thermophilic approach reported in previous years. The mesophilic fermentation approach is conducted at a significantly lower temperature (37°C) to the thermophilic approach (50°C) and produces some advantageous results; for instance, greater overall conversion was achieved to C-2 and C-3 volatile fatty acids plus C-4 and C-7 organic acids were produced. The process does not require sterile conditions, which significantly reduces production costs. Further studies are currently being done to optimize and characterize the catalyst for effective vapor-phase hydrogenation to produce alcohols (Task C-AF-2; concluded this reporting period).

SIGNIFICANT OUTPUTS REPORTED THIS PERIOD FOR THE AVIATION BIOFUEL TEAMS

- Yeast biocatalyst LB23 will be used to produce GIFT scale up for 1000-gallon biojet production (Task C-AF-1).
- Yeast nutrient package and growth conditions have been established for isobutanol scale-up production (Task C-AF-1).
- Isobutanol was recovered from 2L GIFT scale fermentations on various hydrolysate samples (Task C-AF-1).
- Optimal conditions were established for the wet-oxidation pretreatment protocol using FS-10 feedstock samples (Task C-AF-2).

SIGNIFICANT OUTCOMES

- None reported

TRAINING

Name	Affiliation	Role	Contribution
Matt Trappe	Oregon State University	Faculty Research Assistant	Sample collection, DNA extraction, seedling tagging, site layout, reporting
Rene Zamora	OSU	Post-Doc	Transportation model development and manuscript preparation
Francisca Belart	OSU	Graduate Student	Moisture Management --Field Research, Model development
Michael Berry	OSU	Graduate Student	GIS Model Development
Janna Loeppky	OSU	Graduate Student	Data Analysis, Report Preparation
Jorge Delgado	OSU	Graduate Student	Data Analysis, Report Preparation
Chet Miller	OSU	Undergraduate Student	Data collection, Data analysis, Report Preparation.
Subhash Chandra	USFS, FPL	Visiting Scholar	Ethanol fermentation
Feng Gu	USFS, FPL	Post-Doc	Separation of lignosulfonate
Yanlin Qin	USFS, FPL	Visiting PhD Student	Lignosulfonate property
Ricarda Hoehner	WSU	Post-Doc	Together with Magnus Wood, Dr. Hoehner organized, designed, and performed the Phenomics experiments. She analyzed the huge data sets from the Phenomics analysis

RESOURCE LEVERAGING

Resource Type	Resource Citation	Amount	Relationship or Importance to NARA
DOE BRDI	Awarded May, 2014. Humboldt State University, Lead	$300,000 sub-award to John Sessions as Co-PI.	Additional Funds for residue treatments including residue sorting and screening technologie
Salary	US Forest Service		Support of J.Y. Zhu & Roland Gleisner
Scholarship	China Scholarship Council		Support Yanlin QIN
Grant	USDA SBIR Phase II program to Biopulping International (Contract Number: 2010-33610-21589)		

FEEDSTOCK

FEEDSTOCK LOGISTICS TEAM

TASK FL-1: FEEDSTOCK SOURCING

Key Personnel
Gevan Marrs

Affiliation
Weyerhaeuser

Task Description

Gevan Marrs LLC will work collaboratively with Oregon State University (OSU) and NARA conversion staff to quantify costs and quantities of key Pacific Northwest (PNW) candidate feedstocks by region; determine feedstock key quality parameters, variation and impact on conversion processes; perform analyses to select optimum feedstock sourcing strategies, and distill the information and write full reports of all tasks for both Weyerhaeuser portions and NARA year 4 and 5 activities.

The main desired outcome for years 4 and 5 in this area of Feedstocks Logistics is to bring clarity to the economically optimum values for key specifications for the NARA bio-jet fuel production process. In the first 3 years the Logistics team has obtained and quantified a broad range of WA and OR softwood forest residuals feedstocks, demonstrating the extremely high variability in many properties. These results will be documented in final reports for the Weyerhaeuser portions. For years 4 and 5 some additional samples from elsewhere in the NARA region will be sampled, characterized, and reported.

Additional prior year's work has quantified the amount of economic leverage that can be obtained by altering key feedstock production factors in the field. These too will be documented in a written final report.

For year 4, a main task will be to obtain a representative sample of envisioned feedstocks (softwood forest residuals) from across the NARA region in sufficient quantity to prepare 1,000 gallons of bio-jet for a demonstration flight. To be assured of low conversion risk in a pilot facility and to buffer for unforeseen difficulties this means obtaining about 200 bone dried tons (BDT) of prepared feedstock.

Activities and Results

Task FL-1.1.1. Quantify costs and quantities of key PNW candidate feedstocks by region, scale, and year.

Certain feedstock parameters, judged likely to have significant impact on the overall NARA process economics, were entered into the techno-economic analysis (TEA) sensitivity analysis. Feedstock cost through the gate is considered by many to be a likely candidate for strong impact on overall economics. Upper (unfavorable) and lower (favorable) prices were determined by using newly-created sourcing curves developed by Adams and Latta at Oregon State University. Based on Oregon and Washington facility sites tentatively ranked as promising in Integrated Design Experience (IDX) course work, we had feedstock sourcing curves created for Cosmopolis and Longview, WA, and Springfield OR. When the delivered tons per year were chosen at the NARA base case of 850k BDST/yr, the low cost site was Cosmopolis at $61/BDST ($6/BDST less than base case). The high-cost site was Springfield, although it was marginally higher than the NARA base case (Figure FL-1.1). For a high sensitivity value, rather than using the marginally higher Springfield case, we instead assumed an increased market demand for forest residuals which causes the "stumpage" paid to the resource owner to rise from $7/BDST to $20/BDST (pretty arbitrary, but not implausible), leaving the average delivered cost at $81/BDST. This increase or decrease in feedstocks cost was then put in the model and IRR was calculated. The high and low had surprisingly little impact on overall project return, changing the base case V 6.42 IRR from 12.5% to 11.8% or 13.2% respectively—not a very powerful impact compared to many other elements. (Note that all sensitivity analysis elements are shown in more detail in the NARA TEA report; Task SM-TEA-1).

From this analysis, I conclude that, somewhat counter-intuitively, additional investigations at this project stage into feedstock cost reductions via improved logistics are not the key to breakthrough changes in project economics that are likely needed to induce investment in a yet unproven, large capital investment project like biofuels from wood.

Of course if a facility is actually operational, there are significant dollars to be made or lost each year depending upon every dollar per ton change in feedstock cost through the gate, yet this impact is not large enough to move the IRR needle up into the 20 to 30% range considered desirable to induce initial investment.

Task FL-1.1.2. Determine feedstock key quality parameters and variation and impact on conversion process

The chemical composition of feedstocks directly impacts the conversion efficiency and yield to different salable products and thus has a large impact on overall conversion economics. Because our forest residual feedstock consists of the "leftovers" after commercial softwood timber has been removed, the available material has varying tree parts (chunks, small logs, tops, branches) and tree species (main softwood timber crop, invasive non-commercial hardwoods) there is considerable variation in composition of potentially available feedstocks. Thus it follows that there will be measurable differences in overall economics, and selection of feedstock and control of "quality" will be important to process economic feasibility.

It is widely recognized that soil contamination in forest residuals can, if operators are not aware and careful, lead to high inorganic contents (ash) that have negative impacts on conversion. Accordingly all NARA samples were routinely screened in a mill-simulated process, removing the fines that passed a 1/8" wire mesh screen. These Fines were then analyzed

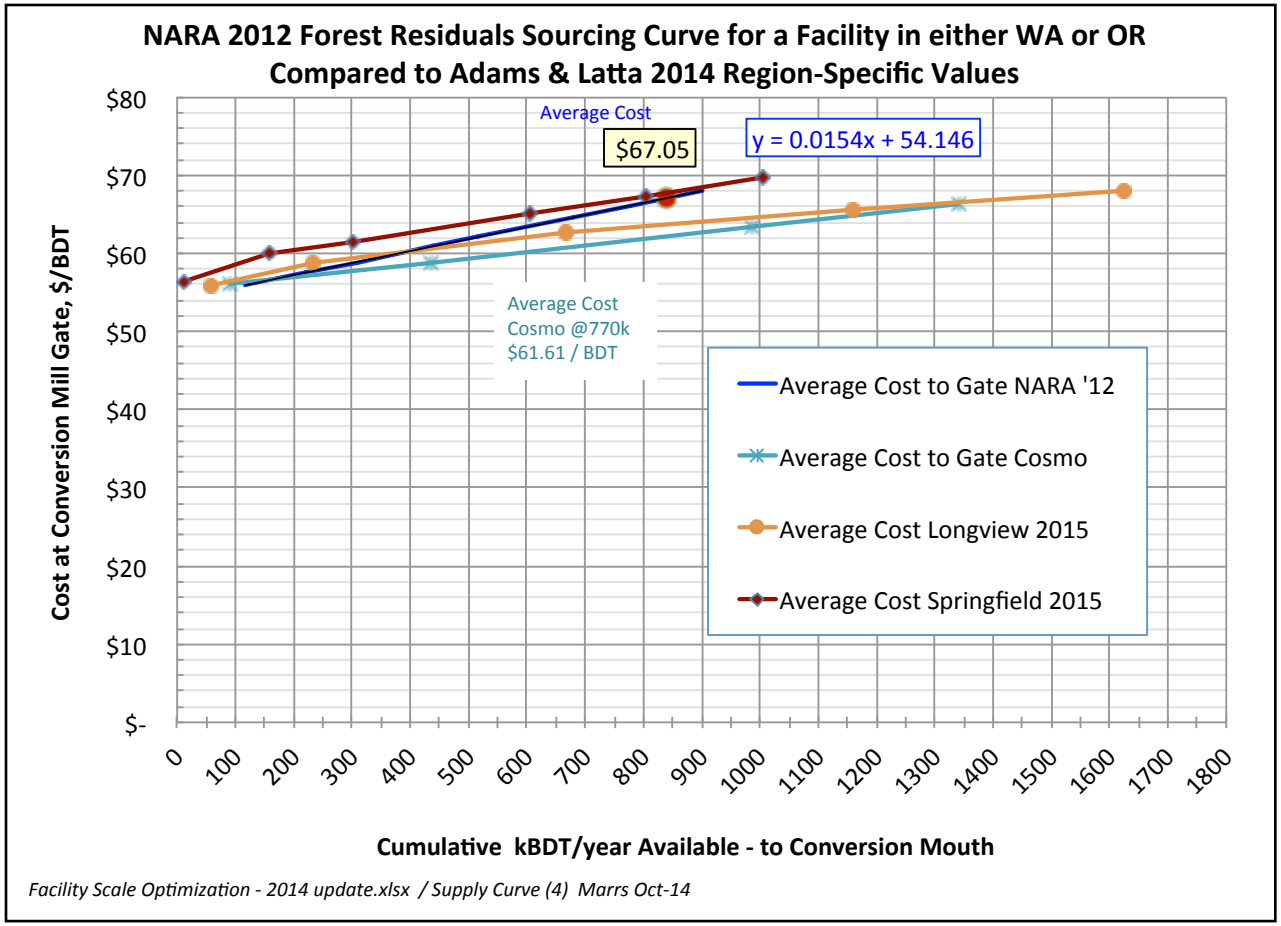


Figure FL-1.1. Comparison of 3 site-specific improved sourcing curves with historic NARA base case assumption. The historic values were very comparable to new improved values.

separately from the Accepts, and the difference in ash content is apparent, as shown in Figure FL-1.2. Although we cannot set firm specifications for our as yet un-built facility, current users of forest residuals (for biomass power) have specifications for ash in the range of 1.5 to 2.5%. It can be seen that screening out the -1/8" fines is very helpful in leaving the accept portion poised to meet this type of specification for ash. That is, operational fines screening of about this level is both necessary and (in most cases) sufficient for meeting biofuels feedstock ash specifications. Having accurate and precise measures of feedstock composition are not only important for the economics, they are required to perform a credible Life-Cycle Assessment (LCA), which is required to qualify for the renewability criteria of the Renewable Fuels Standards.

NARA feedstock samples (PNW softwood forest harvest residuals – FHR) have so far been tested for chemical composition by Weyerhaeuser's analytical group for the major components of chemical composition and the results routinely fall significantly short of summation to 100% (totals range from 89 to 94%). While we are fairly confident that all of the important components of the analysis driving the economics (i.e., sugars and lignin residue) are reasonably accurate, the unidentified "missing pieces" cause difficulties for analyses like the ASPEN modeling, and the attendant LCA analysis (said to require accounting for 99% of the material). If we do not know what is missing, we cannot assign it a fate in the overall material balance for the NARA biofuel process. As a secondary objective, it has been found in the NARA project that analyses by different labs in the NARA project get different results for key components, leaving some uncertainty about the accuracy of WY Analytical and Testing ("A&T") results. Only with some known reference standard can we assess the accuracy and precision of analytical procedures.

Under the Weyerhaeuser (WY) portion of this task (prior to July, 2014) we implemented a series of tests of the WY Analytical lab implementations of the NREL protocols for summative analysis of lignocellulosic feedstocks (Figure FL-1.3). These exhaustive protocols were developed specifically to achieve accuracy and precision between different labs, as well as resulting in a near-100% accounting for chemical components.

The results of this test, judged by measurements of the two woody reference samples from NIST of certified composition (eastern cottonwood and radiata pine), showed that the WY analytical lab procedures agreed very well with NIST reference values for all measured components and thus the total material accounting. For the previously measured NARA samples, using the NREL protocols, the majority of the missing components in prior WY analytical tests were accounted for by the acetyl content (not measured previously). There was some indication of a bias in early years measurements of glucans for FS-01, and there were still some unexplained missing materials in FS-10, a key reference sample (95.5% accounted for).

It was concluded that using the WY analytical labs, running the NREL analysis protocols would give the most accurate and precise results where all measured NARA samples for the life of the project could be compared most directly. The summary of resulting measurements for samples measured through the end of the WY portion of the Logistics – Sourcing task are summarized in Table FL-1.1 and will be reported in detail in the written final report by mid-2015.

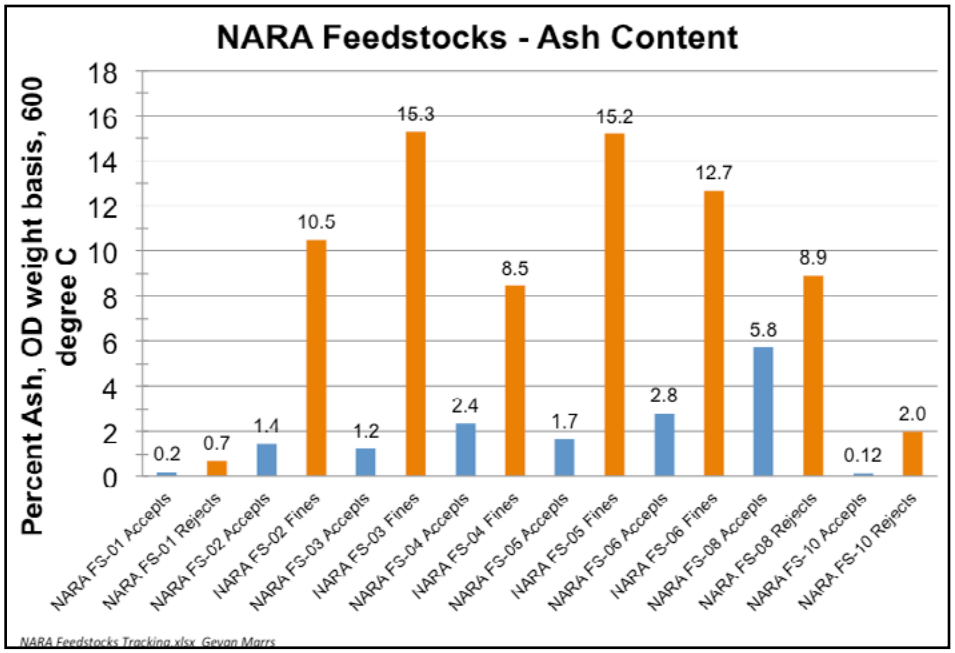


Figure FL-1.2. Ash content (600 degree C) of Accepts and Fines for NARA feedstocks.

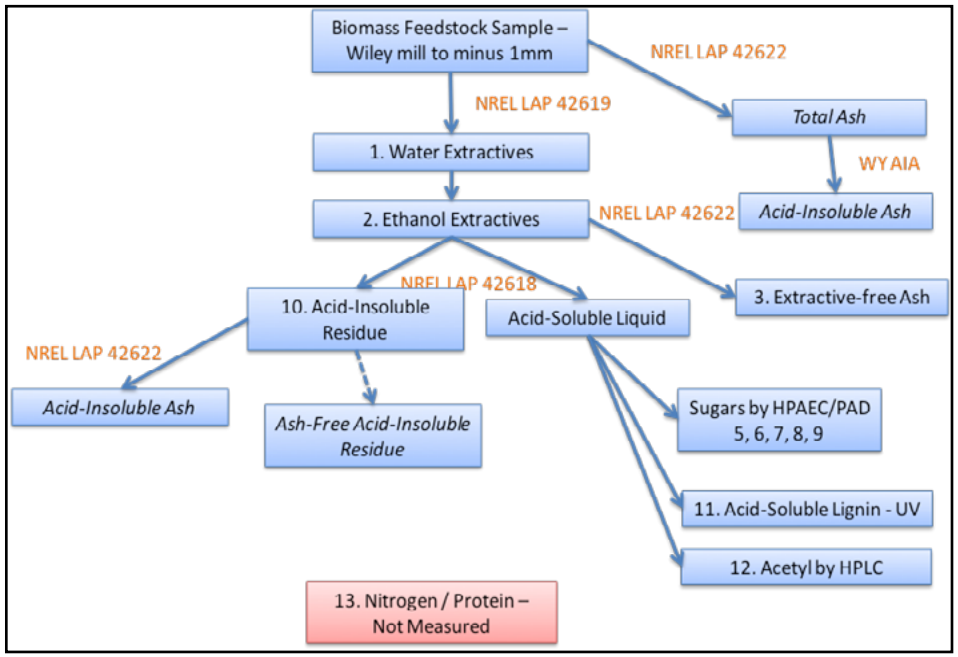


Figure FL-1.3. NREL summative analysis protocols implemented in Weyerhaeuser Analytical Lab.

Note that the common processing step was to include a mill-simulated size screening to remove the "fines", less than 1/8" from the materials since these have a large proportion of the inorganic material (ash, from soil contamination). For most materials the resulting "accepts" and "fines" were analyzed and reported separately in Table FL-1.1.

It should be noted that two of the NARA feedstocks tested and reported were NOT forest residuals. In order to compare to some other possible woody feedstock sources a sample of "clean" Douglas-fir pulp chips was sampled as FS-01. This material is quite pure in species content, very low in bark and fines, and low in ash. Of course it is also in demand and very high-priced—perhaps $120/BDST---compared to forest residuals. The other end of the quality spectrum was "hog fuel" – a mixture of bark, sawdust, and other woody debris that is only suitable for combustion. This material (FS-08) is accordingly very high in bark and fines and ash content. While far less costly than pulp chips—maybe $45/BDST—it is a marketable commodity which will have competing, existing demand, and it is very different in many aspects from forest residuals. It is not considered a viable NARA process feedstock contender and is shown only to demonstrate the extreme properties.

Task FL-1.1.3. Develop and test feedstocks value lift / cost reduction logistics improvements (harvesting, transport, etc.)

Under the WY portion of this task we collaborated with Sessions et.al. at OSU to characterize and process materials produced in their "grinding trials"—which investigated the impact of major controllable grinding variables on final economic impacts in the conversion process. The results (Figure FL-1.4) show that the range of economic impact from best to worst conditions is quite large ($30/BDT range) compared to the assumed base case of about $55/BDT harvest and hauling costs.

Since the potential gain over current practice is not actually worst case to best (since practice has already evolved to a mid-range conditions, shown as the base case above) we can only realistically capture the gains on the cost reduction side against current practice. Additionally, any sorting of materials into categories like those tested above (tops limbs, logs, chunks) will drive up feedstock acquisition costs as it reduces the amount available at each harvesting site if any exclusion of material is practiced. In summary, we might expect to improve logistics about $15/BDT by implementing the best practices as found in this study. This, however, only improves the project IRR by about 0.8%. A discussed in an earlier section, while this is very helpful to an on-going concern, it is far too little to bring a marginal return, unproven project into a desirable IRR range to induce the initial large, risky project investment.

Table FL-1.1. Key chemical composition results for NARA feedstocks tested through April, 2015.

Summary Chemical Analyses NARA Feedstocks										
Feedstock	Total Polysaccharides	Hexose Polysaccharides	Pentose Polysaccharides	Ash-free Lignin, Acid-Insoluble (Klason)	Acid-soluble Lignin	Hot Water Extractives	Ethanol Extractives	Ash	Acetyl Groups	Total
NARA-FS-01 SW WA Douglas-fir Reference Wood Chips	63.51	59.4	4.10	25.93	1.69	5.64	0.50	0.09	1.64	**99.0**
NARA-FS-02 SW WA Hem/Spruce Forest Residuals Accepts	51.63	45.8	5.82	36.18	0.50	5.22	2.81	1.45	NM	**97.8**
NARA-FS-02 SW WA Hem/Spruce Forest Residuals Fines	32.97	28.9	4.08	37.43	0.87	6.92	4.93	10.48	NM	**93.6**
NARA-FS-03 NW OR Dfir Forest Residuals Accepts	58.28	50.80	7.47	29.96	2.57	4.91	0.97	0.91	1.73	**99.3**
NARA-FS-03 NW OR Dfir Forest Residuals Fines	35.10	30.4	4.73	31.97	0.74	5.55	4.01	15.27	NM	**92.6**
NARA-FS-04 N OR Coast Forest Residuals Accepts	48.55	43.0	5.59	33.55	0.63	4.91	3.09	2.37	NM	**93.1**
NARA-FS-04 N OR Coast Forest Residuals Fines	29.38	24.9	4.50	39.29	1.10	6.07	6.47	8.47		**90.8**
NARA-FS-05 King/Horse Cr Doug-fir / Cedar Accepts	56.56	49.8	6.72	27.62	0.43	5.11	2.49	1.65	NM	**93.9**
NARA-FS-05 King/Horse Cr Doug-fir / Cedar Fines	35.10	29.8	5.30	30.71	0.83	7.49	6.16	15.20	NM	**95.5**
NARA-FS-06 Sisters OR Pine and Spruce Accepts	46.45	37.6	8.89	31.81	0.58	5.43	5.11	2.82	NM	**92.2**
NARA-FS-06 Sisters OR Pine and Spruce Fines	30.12	23.7	6.42	35.39	0.84	7.08	6.29	12.70	NM	**92.4**
NARA-FS-08 Longview Alder / DFir Hog Fuel Accepts	46.48	38.1	8.37	31.03	0.88	5.52	3.14	5.76	NM	**92.8**
NARA-FS-08 Longview Alder / DFir Hog Fuel Fines	44.10	32.8	11.28	31.95	1.20	5.79	2.48	8.92	NM	**94.4**
NARA FS-10 Douglas-fir Forest Residual - Accepts	57.89	52.80	5.10	27.04	1.96	6.10	0.63	0.12	1.76	**95.5**
NARA FS-10 Douglas-fir Forest Residual - Fines	50.60	44.9	5.66	30.66	0.56	4.34	4.33	1.97	NM	**92.5**
NARA FS-11 Douglas-fir Grinding Trials Composite as-received	57.99	51.7	6.31	26.93	0.35	4.81	3.96	0.31	NM	**94.4**
NARA FS-12 Douglas-fir Grinding Trials Tops & Limbs as-received	56.27	49.2	7.10	28.60	0.43	5.92	5.41	0.71	NM	**97.3**
NARA FS-13 Douglas-fir Grinding Trials Pulp Logs as-received	61.79	55.6	6.22	26.80	0.37	3.77	2.59	0.24	NM	**95.6**
NARA FS-14 Douglas-fir Grinding Trials Log Chunks as-received	61.74	57.4	4.30	27.23	0.27	3.89	3.71	0.16	NM	**97.0**
NARA FS-15 Fresh Douglas-fir Grinding Trials Accepts	44.64	37.8	6.88	34.47	0.64	6.60	3.35	10.73	NM	**100.4**

TASKS TO GEVAN MARRS LLC (SINCE 1-JUL-14)

Task FL-1.2.1. Determine feedstock key quality parameters and variation and impact on conversion process

Finding additional sources of forest residuals feedstocks for areas outside the western Oregon and Washington geography has been difficult as there are few, (or no) commercial operating production ventures. Since FHR are highly variable even with a single pile, in order to sample and characterize reliably requires production at realistic scale. We did obtain one east-side (Montana) sample and one far northwest sample (near Enumclaw, WA) in concert with feedstock acquisition for the 1,000 gaillon bio-jet production task. These will be processed and characterized in the same manner as prior NARA feedstocks and results compared in the final task report.

Task FL-1.2.2. Prepare final reports for WY portions of Feedstock Logisitcs - Sourcing

While various key pieces have been reported in a host of formats and forums over the early project years, pulling it all together in a formalized, full final written report has not yet begun.

Task FL-1.2.3. Identify, obtain, prepare, characterize and have delivered to conversion site sufficient feedstock across the NARA region to produce 1,000 gallons NARA bio-jet fuel

An assignment for a new task was created for supplying to a conversion partner sufficient quantity of a suitable feedstock to produce 1,000 gallons of bio-jet (IPK) for a demonstration flight before the NARA project ends in 2016. Considerable effort has been expended to quantify what constitutes “suitable”, as there are both technical and stakeholder criteria, and then identify candidate sources that can produce suitable materials at the needed scale, and a location and facility to process the material to conversion process needs, as well as store and ship to the conversion site.

Broadly speaking, we have narrowed the suitability criteria to be about 100 BDST of primarily Douglas-fir forest harvest residuals with fairly low bark and ash

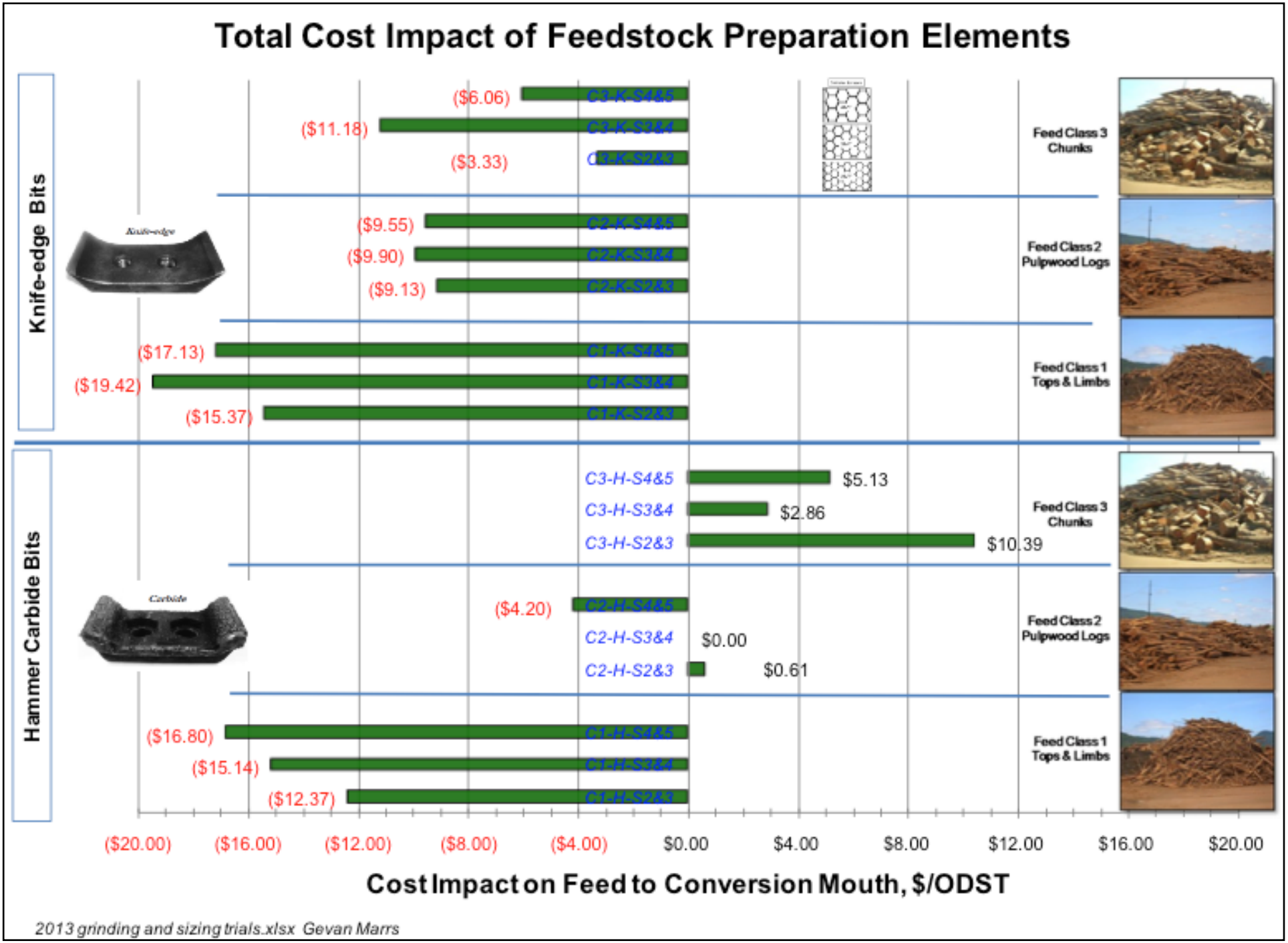


Figure FL-1.4. The net total impact range is about $30/ODST feedstock to conversion mouth.

content. In other words, the material would resemble the current NARA base case feedstock, FS-10. While it is likely that the material would be ground in the woods in a horizontal drum chipper, the resulting typical large particles, with a long aspect ratio (see Figure FL-2.1), may be too large for feeding a lab-to-pilot scale conversion reactor. We will have to identify the pretreatment conversion site before we can pin-point oversize removal and resizing criteria.

In mid-February a suitable amount and type of material was identified on a site belonging to a NARA (Weyerhaeuser) member property—dubbed the Siuslaw 900 site (Figure FL-1.5). This was located in Lane County, OR, west of Eugene. Thirteen truckloads were ground and delivered to a processing yard in Junction City, OR. The total delivered was 317 green tons, with a moisture of about 43%, yielding about 180 BDST starting material. Deliberations are underway to determine what size processing will meet the (as-yet selected) conversion facility equipment criteria.

Recommendations | Conclusions

The softwood forest harvest residuals tested to date, while quite variable, seem to have characteristics that make them suitable for conversion to bio-jet by the NARA process, after suitable woods production and mill-site screening. These steps are both necessary and sufficient to bring the key properties (size distribution and ash content) within reasonable feedstocks specifications.

In the early stages of the project, where iso-paraffinic kerosene (IPK) was the main product focus, much attention was given to factors that change the total polysaccharides content, as that principally determines IPK yield and thus impacts economics. However, as the need for significant revenue contribution from the co-products (derived mainly from the lignin fractions) became apparent and likely co-products were identified (activated carbon, ligno-sulfonates), the overall economic impact due to tradeoffs in polysaccharide and lignin content changed) mostly related to bark content differences.) That is to say, declining IPK production by reduced polysaccharides due to higher bark content are offset by increases in production and sales of activated carbon. Since the two products contribute roughly the same annual revenue in the current base case, this makes bark content a relatively unimportant quality specification—at least over the range of contents we have so-far encountered.

We have additionally demonstrated that there are important incremental cost reduction opportunities in feedstock production—mostly in increasing grinder utilization and decreasing transportation costs through higher bulk density. While these improvements will be important incrementally to an on-going operation, they are not sufficiently large to cause break-through cost reductions that dramatically improve the overall project IRR.

Figure FL-1.5. Main component of 1,000 gallon task feedstock – Siuslaw 900, aka FS-17.

TASK FL-2: LOGISTICS DECISION SUPPORT AND IMPROVEMENT

Key Personnel	Affiliation
John Sessions	Oregon State University
Kevin Boston	Oregon State University

Task Description

Our task is to 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 model to develop recoverable and residual biomass from TPO data. Draft field procedures to be developed by June 30, 2014. Data has been collected and is currently being analyzed. BEER groups are organizing their data to allow for combining the two approaches into biomass estimates.

Activities and Results

Task FL-2.1. Develop Biomass Recovery Coefficients for OR, WA, ID, MT

A manuscript is being prepared for submission and a white paper is being prepared for internal group use.

Task FL-2.2. Develop Moisture Management Strategies and Models

Live branch moisture data was completed in all four sites and four seasons by PhD candidate Francisca Belart. Data analysis is completed and a manuscript being written. Results show that average branch moisture content is not higher than 50% on any of the sites sampled, and there is statistically significant effect of season in branch average moisture content only in ponderosa pine and valley Douglas-fir. Both species have their lowest moisture content in summer. Western hemlock has almost no variation in moisture content through the year and ponderosa pine the highest variation (Table FL-2.1). This is consistent with the environment these species live in.

Additionally, good correlations were found between tree height, heartwood and average branch moisture content. These functions could be used to predict average branch moisture content with reduced data collection in the future.

The first Douglas-fir logging residue moisture monitoring trial located at the Oregon Coast was deconstructed after one year of monitoring. Most of the sensors were unrecoverable, including the weather station. As residue piles were deconstructed, samples were taken on each height level, corresponding to where sensors were located. Statistical tests show significant differences in moisture content by height in the pile. Moisture content can be as low as 20% in the outer shell and as high as 50% in the residue located in the bottom of the pile after one year of storage.

We installed four logging residue moisture monitoring trials with the new measuring protocol in a drier Willamette Valley Douglas-fir site, a wet Willamette Valley Douglas-fir site an Oregon Coast Western hemlock

Table FL-2.1. Mean seasonal branch moisture content by site

Site	Fall	Winter	Spring	Summer
Valley Douglas fir	0.46 ± 0.02	0.45 ± 0.02	0.46 ± 0.01	0.43 ± 0.02
Ponderosa pine	0.47 ± 0.02	0.50 ± 0.02	0.45 ± 0.02	0.43 ± 0.02
Western hemlock	0.48 ± 0.02	0.48 ± 0.02	0.48 ± 0.02	0.47 ± 0.02
Dry Douglas fir	0.46 ± 0.02	0.48 ± 0.02	0.46 ± 0.02	0.47 ± 0.02

site and a ponderosa pine site in eastern Oregon. Data collection and monitoring has been performed monthly for ten, seven, five and three months on each site respectively. The first pile site is planned to be deconstructed by late May and data collection to be completed by December 2015.

A finite element analysis model (FE) has been developed to simulate environmental conditions and determine the effect of different logging residue storage forms on drying patterns (Figure FL-2.1). The physics model has been implemented with fluid flow, heat transfer and moisture diffusion with the help of Professor Ben Leshchinsky, OSU. Temperature and wind functions were developed from data collected in the field and are already implemented in the model. This model is being calibrated and will be presented at a graduate student seminar in OSU in April by PhD candidate Francisca Belart. Pile and environmental variables such as relative humidity and temperature collected in the field will be used to validate the model.

Task FL-2.3. Refine Collection and Transport Models for regional modeling

A GIS model to determine the area of potential harvest areas by harvest method and distance to road is underway for the NARA region. These results will be used in the NARA feedstock supply model in combination with Kevin Boston's field data and a biomass collection model.

A model comparing double trailers to single trailers was prepared and submitted for review. At low moisture content single trailers are volume limited, but double trailers are not. The model combines simulation with field data to understand under what conditions double trailers are cost effective for transporting dry grindings under the different state regulations in the Pacific Northwest. These results will be integrated into the NARA feedstock supply model.

We worked with the LCA group to produce fuel consumption models for collection, processing and transport of forest residues.

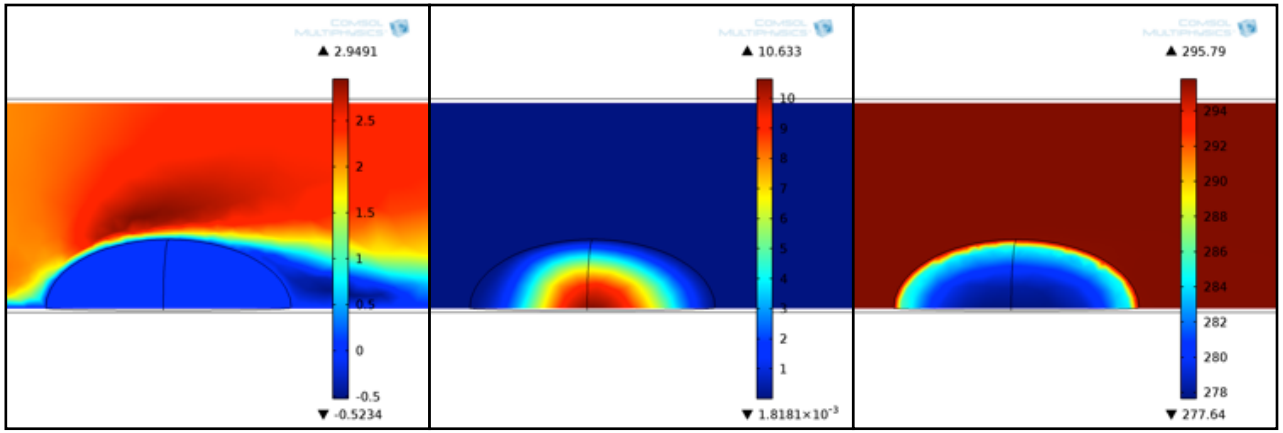


Figure FL-2.1. Wind, moisture content and temperature simulation at a hypothetical slash pile.

A manuscript is under review with the Journal of Forest Policy and Economics exploring biomass supply curves for western juniper in central Oregon under alternative business model and policy assumptions.

Task FL-2.4. Evaluation of chipping and grinding production to meet alternative feedstock specifications.

Chip flinger trials to increase bulk density in dry grindings were completed at the Lane Forest Products yard in Eugene, OR. Twenty trailer loads of dry primarily Douglas-fir grindings were first loaded by conventional gravity loading, then reloaded using a prototype chip flinger under a range of discharge velocities. Laboratory results of grinding samples have been completed. Manual methods of load volume estimation are being compared to aerial drone measurements carried out during the experiment. A manuscript is in preparation.

A manuscript summarizing the economics of grinder parameters on cost was submitted for the special issue on "Biomass-Based Materials and Technologies for Energy," sponsored by Advances in Materials Science and Engineering (Marrs lead author). A manuscript summarizing the high speed blowing tests conducted in Year 3 was reviewed and published. A manuscript comparing supply chain impacts of green versus dry residues is in final preparation and will be submitted in April, 2015. An abstract to the World Forestry Congress in Durban South Africa (7-11 September 2015) describing the economics of forest biomass feedstocks for aviation fuel production was accepted and a final manuscript is being prepared for the proceedings and to be considered for presentation.

Feedstock coordination for the 1000-gallon goal was completed, feedstocks were ground and delivered to Lane Forest Products from the Oregon Coast range, the Flathead reservation (western Montana), and the Muckleshoot tribe (western WA). The residues samples (600-800 lbs) of each will be shipped to Weyerhaeuser (WY). The rest will be mixed, screened, and stored at the Lane Forest Products yard until a pretreatment location is identified.

Task FL-2.5. Demonstrate and evaluate new trailer designs to improve transport efficiency

Preliminary investigation on methods/modifications to permit turning of chip trailers on dead-end landings using log loaders was started. Second generation self-steering trailer technology was reviewed for demonstration potential in year 5.

Task FL-2.6. Evaluate health and safety procedures for any new work processes

No work done this period.

Recommendations | Conclusions

Task FL-2.1. Develop Biomass Recovery Coefficients for OR, WA, ID, MT – The available volume of biomass being recovered is much less than the 70% used in standard analysis. The economically available biomass will be a further reduction with final stages of analysis being completed at the moment.

Task FL-2.2. Develop Moisture Management Strategies and Models – This task had early delays due to field installation redesign, but data collection is now going smoothly and an innovative finite element model is under development.

Task FL-2.3. Refine Collection and Transport Models for regional modeling – This task is well underway. Western Oregon and western Washington GIS analysis to support feedstock group (Adams/Latta) will be complete in June and extensions to remaining region will be done in Year 5.

Task FL-2.4. Evaluation of chipping and grinding production to meet alternative feedstock specifications – This task is about complete, manuscripts need to be followed through peer-review process.

Task FL-2.6. Evaluate health and safety procedures for any new work processes – Have not observed any new work processes where safety protocols are not covered in existing work procedures. The only exception might be steep slope ground-based operations, but these will need to follow any procedures developed for commercial tree operations.

Physical and Intellectual Outputs

PHYSICAL

• Four slash moisture monitoring stations were established, including in-pile sensors, weather stations, and data loggers. Stations are visited monthly.

• Three residue sites (western Oregon, western Washington, western Montana) were selected for 1000 gallon feedstock.

REFEREED PUBLICATIONS

Zamora-Cristales, R. and J. Sessions. A collection model for forest biomass residues. In preparation for Croatian J. of Forest Engineering.

Zamora-Cristales. R., J. Sessions, G. Marrs, and D. Smith. Impacts of green versus dry residues on the liquid fuel supply chain. In preparation for Canadian J. of Forest Res.

Zamora-Cristales, R. and J. Sessions. Economics and system dynamics of double trailers in transporting forest biomass on steep terrain. In review. California Agricultural J.

Lauer, C., J. McCaulau, J. Sessions and S. Capalbo. Biomass supply curves for Western Juniper in central Oregon, USA, under alternative business model and policy assumptions. In review. Journal of Forest Policy and Economics.

Marrs, G., R. Zamora and J. Sessions. Optimizing the Feedstock Value Chain for Bio-Jet Fuel from Pacific Northwest Softwood Harvest Residues. In review for the special issue on "Biomass-Based Materials and Technologies for Energy" sponsored by Advances in Materials Science and Engineering.

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

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

Zamora-Cristales R., J. Sessions, D. Smith, and G. Mars. 2014. Effect of high speed blowing on bulk density of ground residues. (Accepted) Forest Products Journal. http://dx.doi.org/10.13073/FPJ-D-14-00005

Zamora-Cristales, R., J. Sessions, K. Boston and G. Murphy 2014. Economic Optimization of Forest Biomass Processing and Transport. Forest Science. http://dx.doi.org/10.5849/forsci.13-158.

Zamora-Cristales, R., P. Adams, and J. Sessions. 2014. Ground-based thinning on steep slopes in Western Oregon: Soil exposure and strength effects. Forest Science 60(5):1014-1020. doi.org/10.5849/forsci.12-525.

CONFERENCE PROCEEDINGS AND ABSTRACTS FROM PROFESSIONAL MEETINGS (2014)

Zamora-Cristales, R. and J. Sessions. Economics of forest biomass feedstocks for aviation fuel production. In preparation for XIV World Forestry Congress, Durban, South Africa, 7-11 September 2015

J.Y. Zhu, M. Chandra, R. Gleisner, W Gilles, Johnway Gao, G. Marrs, D. Anderson, and J. Sessions. 2014. Reviving sulfite pulping for sugar production from woody biomass. Paper prepared for 2014 TAPPI PEERS conference, Sept 14-17, Tacoma, WA.

Zamora R., and J. Sessions. 2014. Forest harvest residue collection model. In Proceedings: 2014 Council on Forest Engineering (COFE) annual meeting, June 22 – 25, Moline, Illinois, USA.

RESEARCH PRESENTATIONS

Posters:

Zamora-Cristales R., and J. Sessions. 2014. Improving Efficiencies in forest biomass transportation. 2014 Annual NARA Meeting, Seattle, WA, September 15-17.

Belart, F., J. Sessions, G. Murphy, M. Jolly, B. Leshchinsky and K. Tuers. 2014. Moisture Management Model for Optimal Forest Biomass Delivery in the Pacific Northwest. 2014 Annual NARA Meeting, Seattle, WA, September 15-17.

Zamora-Cristales, R. and J. Sessions. 2014 Modeling Forest Harvest Residue Collection. Prepared for Biofuels and Co-products Conference. April 28-30, Red Lion, Seattle, WA.

Belart, F., J. Sessions, G. Murphy, M. Jolly and K. Tuers. 2014. Moisture Management Model for Optimal Forest Biomass Delivery in the Pacific Northwest. Prepared for Biofuels and Co-products Conference. April 28-30, Red Lion, Seattle, WA.

Presentations:

Sessions, J. and R. Zamora-Cristales. 2014. Decision Support Systems for Forest Harvest Residue Collection and Transport in PNW, USA. Norwegian University of Life Sciences, As, Norway, November 13, 2014.

Zamora-Cristales, Rene. 2014 International Union of Forest Research Organizations IUFRO World Congress 2014. "Economics of forest biomass processing and transport on steep terrain" Salt Lake City Utah, USA, October, 2014.

Zamora-Cristales, R. and J. Sessions. 2014. A solution procedure for forest harvest residue collection for bioenergy. Presented at 37th Council on Forest Engineering Annual Meeting. June 22-25 2014. Moline, Illinois, USA.

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

Zamora-Cristales, R. and J. Sessions. 2014. Washington Contract Loggers Association, Annual Meeting 2014. "Improving Efficiencies in Forest Biomass in Biomass Collection and Transportation" Spokane, Washington, USA, March 2014

VIDEOS AND WEBINARS

None, although we produced an internal use video on our chip flinger and drone trailer volume measurements

THESIS AND DISSERTATIONS

Loeppky, J. 2015, Energy consumption of grinding unbaled and baled forest harvest residues, Master of Forestry project, Oregon State University, Corvallis, Oregon.

FEEDSTOCK

FEEDSTOCK DEVELOPMENT TEAM

TASK FD-1: POPLAR AND ALDER PRODUCTION

Key Personnel	Affiliation
Norman Lewis	Washington State University

This task was terminated along with all other hard-wood activities in NARA project Year-2 to focus efforts on softwood forest residue challenges.

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 relies upon chlorophyll fluorescence analysis, a well-established and versatile tool for studying stress response in plants in situ. In addition to the numerous examples for annual plants, this technique has already been applied, for example, to study salt-stress responses in poplar trees. We will thus initially use chlorophyll fluorescence based phenomics as a second screening filter to identify individuals 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 a high fidelity of the data. The drought stress response of Douglas-fir trees will be analyzed with this approach.

Activities and Results

The aim was to identify Douglas-fir families out of about 900 tress from the collection of Dr. Jayawickrama (Oregon State) that are either more or less drought-tolerant. Therefore, we used non-invasive, automated optical screening realized by a new Phenomics facility at WSU, Pullman. Since this is the first time that Phenomics was used for Douglas-fir tress, we designed a pilot experiment with only 50 trees to gather information about the optimal design of the experiment (duration of drought stress, optical settings etc). This experiment was performed in the April-July 2014 report phase. Based on the experiences and preliminary results collected from the pilot experiment, we performed the main experiments with 882 plants (nine plants per family, 98 families total) in the second period (August 2014-November 2014). Due to space restrictions in the Phenomics facility, the experiment was divided in two separate runs. The overall arrangement of the plants is shown in Figure FD-2.1.

Measurements of physical conditions in the Phenomics facility reveal that light intensity and temperature fluctuations are below +/- 10% (light) and +/- 3% (temperature). Drought stress was established by stopping the watering of plants. A robust parameter that indicates stress in plants is the maximal photochemical efficiency of photosystem II (PSII) shown in Figure FD-2.2 (ΦII) as a function of days after the last plant watering. A decline in ΦII is indicative for a drought-induced impairment of plant metabolism, i.e. the lower ΦII the stronger the stress response of the plant. Figure FD-2.2 reveals that the drought-induced ΦII decrease is similar for most of the families but some families respond faster or slower to the stress. For better and easier comparison of the different Douglas-fir lines, we calculated the mean ΦII from Figure FD-2.2. A smaller mean ΦII corresponds to earlier drought-induced decline indicating that the plants are more drought-sensitive. The data in Figure FD-2.2 was measured for dark-adapted plants.

We did the same measurement for plants at the middle of the day. Both light and dark mean ΦII values are plotted against each other in Figure FD-2.3. Plants that are on the upper right of this plot have a lower ΦII in the night and in the day. In turn, plants on the lower left respond fast at both times of the day. We use Figure FD-2.3 for screening drought-sensitive and drought tolerant Douglas-fir families. The families given in colors are candidates that are based on ΦII screening are expected to respond differentially to drought stress. Plants indicated as green circles are more drought-tolerant whereas plans in red are more sensitive. Yellow circles represent plants that represent bulk behavior.

Recommendations | Conclusions

The Phenomics screening revealed candidates (Figure FD-2.3) for further in-depth analysis by the gene chip approach developed in the Feedstock development program of NARA. The aim would be identification of differences in gene expression in the selected drought-sensitive and drought-tolerant families. However, to validate the Phenomics data, it would be important to repeat the experiments on the selected lines and increase the number of trees per family to improve statistics. This experiment is planned for Fall 2015. After that, the data should be sufficient to write a summarizing manuscript of our analysis.

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.bbabio.2013.11.003

RESEARCH PRESENTATIONS

Hoehner, R. (2014) Poster at the Gordon conference on Photosynthesis (West Dover, VT)

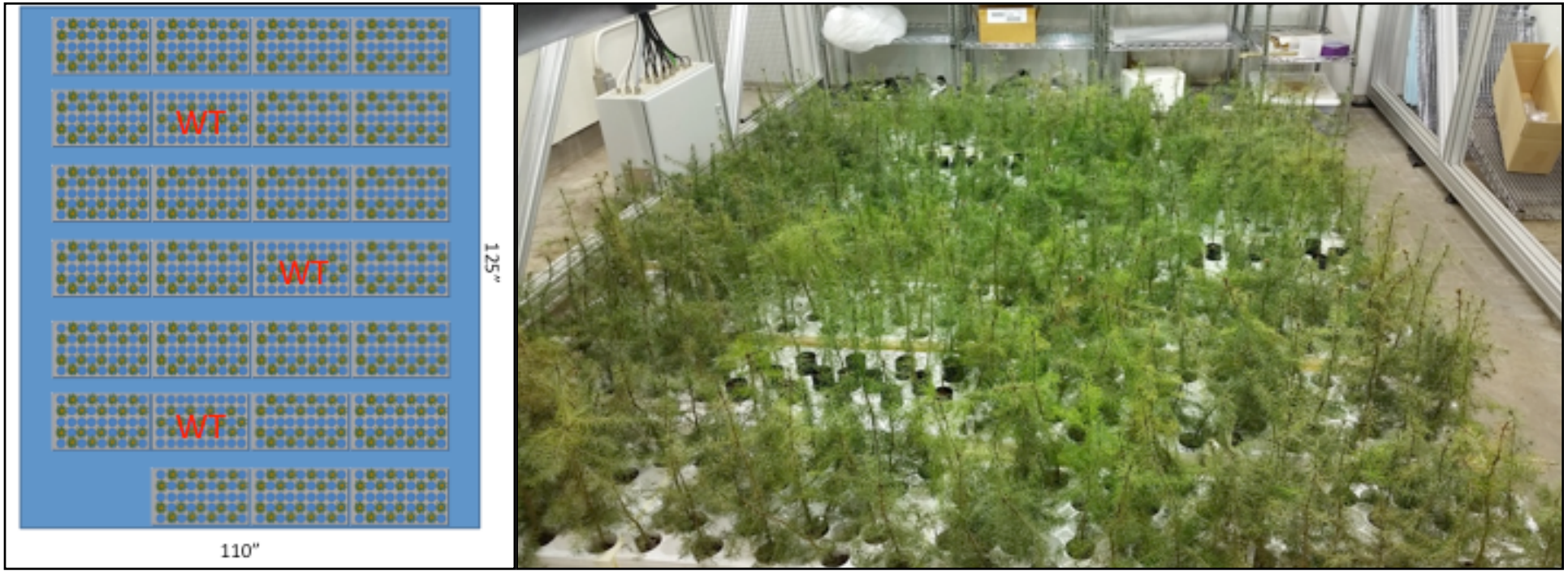


Figure FD-2.1. Left, cartoon showing the arrangement of Douglas-fir seeding's in the WSU Phenomics facility, WT are wildtype control plants. Right, picture of the seedlings in the facility before drought stress.

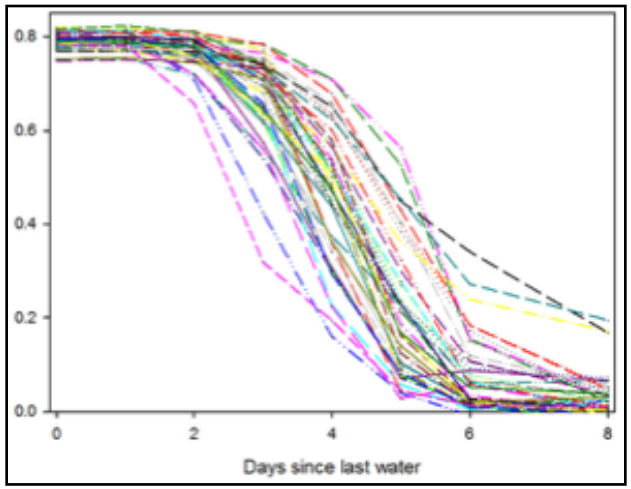


Figure FD-2.2. Photochemical efficiency of PSII (ΦII) as a function of days after the last watering to induce drought stress.

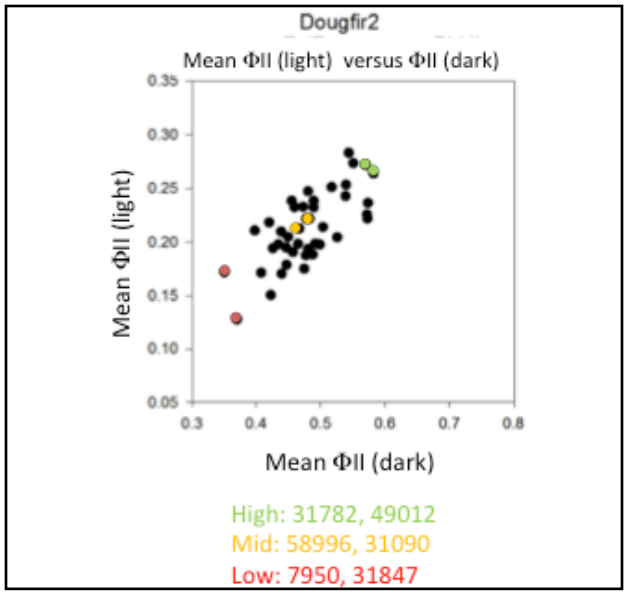


Figure FD-2.3. Mean ΦII (light) versus ΦII (light) to identify drought sensitive and drought insensitive families. Each data point represents the average of nine plants. High performers are located on the top right corner in these graphs.

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 we can expect variation and genetic control in traits pertaining to biofuel production. 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 U.S. conifers) can be put to use in this project. We propose as an expanded/strengthened Task 2 (Identify single nucleotide polymorphisms [SNP] genotypes) to use the power of both of these approaches in tandem, with a state-of-the art genotyping array based on SNP technology for marker-based selection of phenotypes conducive to production of biofuels from woody residuals as a value added trait of trees selected for 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

Measurement of specific gravity, pretreatment yield, pretreated holocellulose, enzymatic hydrolysis yield and recalcitrance factor were completed for the 2nd cycle CL98 population collected at the Moon Creek test site in SW Oregon. Narrow-sense heritabilities, genetic correlations between traits, and predicted genetic gains for pretreatment yield, pretreated holocellulose, enzymatic hydrolysis yield, and recalcitrance factor were predicted for 284 progeny trees, 30 woodsrun (unimproved) trees, 28 crosses (between 6 and 12 progeny per cross) and 46 parents. Table FD-3-1 shows heritabilities ranging from 0.18 to 0.7. These results compare well to many publications for other wood properties (jet-fuel related heritabilities have not be reported before). While specific gravity was favorably correlated with recalcitrance factor, the genetic correlation (Table FD-3-1) was not high enough to be a very reliable predictor (indirect selection trait).

One of the forward selections had a 40.6% predicted gain in holocellulose yield and 34.7% predicted gain in recalcitrance factor. If instead we were to select existing seed producing parents, one parent had a 27.0% predicted gain in holocellulose yield and 21.5% predicted gain in recalcitrance factor.

Table FD-3.1. Narrow-sense individual heritabilities and their standard errors for five wood traits in a Douglas-fir breeding population

	h^2i	s.e.
Density (SG)	0.315	0.219
Pretreatment Yield (PY)	0.767	0.180
Pretreated Holocellulose (PH)	0.185	0.190
Hydrolysis Yield (HY)	0.496	0.142
Recalcitrance Factor (RF)	0.443	0.136

Table FD-3.2. Genetic correlation coefficients (lower triangle) & their standard errors (upper triangle).

	SG	PY	PH	HY	RF
SG		0.251	0.241	0.219	0.212
PY	0.048		0.272	0.189	0.253
PH	0.343	-0.021		0.259	0.273
HY	0.325	-0.497	-0.246		0.015
RF	0.402	-0.111	-0.159	0.972	

Task FD-3.2. Design and Building of Genotyping Array

The contract for building the array (50K SNPs) using the Affymetrix platform, and for genotyping 1,920 samples, was awarded to GeneSeek Inc., part of NeoGen Corp (http://www.neogen.com/ Genomics/). Rich Cronn and Sanjuro Jogdeo (USDA-USFS Pacific Northwest Research Station) helped develop non-polymorphic sequences that Affymetrix used to design 'control' probes for the genotyping array. These

are sequences identical between the Douglas-fir transcriptome and the loblolly pine genome. The control probes were used to calculate a quality control metric (DQC) used to identify samples that have been processed correctly by Affymetrix. We also sent Affymetrix a file of unique transcriptome sequences consisting of all non-redundant contigs and singletons from the Howe et al and Muller et al sequencing projects. These were used to evaluate the uniqueness of the SNP designs that are being generated.

Selecting the SNPs for the array was a major project, involving Glenn Howe of OSU, the Bioinformatics Services team at Affymetrix Inc. (especially Lucy Reynolds and Lakshmi Radhakrishnan), and personnel at GeneSeek. We wrote bioinformatics programs to prepare our SNP database in the format needed by Affymetrix (Axiom myDesign Arrays Design Request Form for Agrigenomics Applications; Affymetrix Technical Note). This SNP database consists of SNPs identified by Muller et al. (2012) and Howe et al. (2013) (see previous NARA reports). The objective of this work was to increase the number of genes that could be assayed, thereby increasing genome coverage for genomic selection.

We originally sent an initial set of 197,200 target SNPs to Affymetrix, and subsequently worked with GeneSeek and Affymetrix to refine the design of the SNP array. This involved multiple rounds of SNP evaluation and prioritization. We sent what we expected to be our final list of high priority SNPs to GeneSeek on December 11, 2014. Just as we were about to finalize the design, a draft Douglas-fir genome sequence became available from Dave Neale, Jill Wegrzyn, and other members of the PineRefSeq Project. Further, we had access to other findings from a similar genotyping effort (ADAPTREE) underway using the Affymetrix platform on spruce and pine by a team based at the University of British Columbia. This gave additional data to improve our design. The team, therefore, ran through several more iterations. Although it postponed the final design, we feel it is now more likely to succeed and give desired results. Finally, we submitted a file of 221,674 target SNPs to GeneSeek/Affymetrix, and obtained information from GeneSeek/Affymetrix indicating the relative quality of the various SNPs (Table FD-3.3).

Table FD-3.3. SNP quality for 221,674 SNPs submitted to GeneSeek/Affymetrix.

15,384	recommended on both strands,
38,392	recommended on the forward strand only
39,388	recommended on the reverse strand only
31,251	neutral in both strands
26,947	neutral in forward strand only, (neutral best result)
27,128	neutral in reverse strand only, (neutral best result)
42,236	not-recommended in both strands
426	not-possible in forward and not-recommended in reverse
521	not-recommended in forward and not-possible in reverse
0	not possible in both strands (This sequence does not have enough non-ambiguous flanking sequence.)

On March 17, 2015, the array was finalized with 55,766 markers and 58,350 probe sets and sent to Singapore for manufacture. This would be most SNPs used for a genotyping array for coastal Douglas-fir. The expected date for completion of manufacture is April 29, 2015 after which genotyping can begin.

DNA Extraction

We collected 1,920 needle samples from selected Douglas-fir trees three progeny sites, four seed orchards, and one container nursery (Table FD-3.4). Each sample consisted of 5-10 green needles. Samples were placed in numbered 14 cm3 vials and 10 cm3 crystalline silicate desiccant added immediately to preserve DNA, and the vials were sealed. All samples were carefully tracked by spreadsheet. Subsamples of three needles were taken from each vial, manually minced to 2-3mm lengths, and each carefully loaded into a well in a 96-well DNA extraction plate (Qiagen DNeasy 96 Plant DNA kit). The location of each sample in each plate was carefully recorded. The loaded plates were transported to the USDA Forest Service National Forest Genetics Electrophoresis Laboratory (NFGEL) in Placerville, CA for extraction.

Table FD-3.4. Foliage samples have been collected from the following sets of trees to be processed through the SNP genotyping array

291	2nd-cycle CL98 progeny trees used in wood chemistry analysis or pilot genomic selection study
28	CL98 parents with wood chemistry data
46	other 1st generation parents or grandparents of 3rd cycle genomic selection crosses
26	2nd cycle parents of 3rd cycle genomic selection crosses
264	other 2nd cycle progeny, full-sibs of genomic selection study 2nd-cycle parents
1,141	3rd cycle progeny (genomic selection study selection population)
124	other parents of future 3rd cycle crosses

Recommendations | Conclusions

The estimates of heritability and predicted genetic gain show that it would be quite feasible to genetically select Douglas-fir for conversion to jet fuel. Given the sample sizes, these estimates should not be taken as the last word in genetic parameter estimates: we would typically want to sample from 100 families, 30 trees per family on at least three sites to increase our confidence in the estimates. However these results show a lot of promise.

From the CL98 test population, it would be possible to collect seed from a group of selected parents and start establishing high jet-fuel plantations in the near future. However for large-scale implementation into breeding programs in the Pacific Northwest it would essential to either (1) identify indirect selection traits that are less expensive to measure or (2) find ways to simplify and accelerate the measurement of the wood chemistry traits so that we could (3) screen many more populations and trees.

Physical and Intellectual Outputs

REFEREED PUBLICATIONS

Geleynse, S., Alvarez-Vasco, C., Garcia, K., Jayawickrama, K., Trappe, M., & Zhang, X. (2014). A multi-level analysis approach to measuring variations in biomass recalcitrance of Douglas fir tree samples. BioEnerg. Res., 7, 1411-1420. doi:10.1007/s12155-014-9483-z

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

Key Personnel	Affiliation
Callum Bell	National Center for Genome Resources

This task ended on schedule in NARA project Year-2. Portions of this task were 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	Affiliation
Xiao Zhang	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 not still 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 amount of sugars and low recalcitrance toward deconstruction to release 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 our research is to develop and implement a selective feedstock breeding methodology to identify and produce "ideal" softwood biomass to maximize sugar yield and reduce conversion (pretreatment and hydrolysis) cost.

Activities and Results

A method for screening woody biomass samples for biomass recalcitrance was developed in a previous period. This method focuses on a parameter we call the "recalcitrance factor", which summarizes the ease at which biomass polymers are converted into fermentable sugars. This method was applied to two separate populations of trees sampled in order to test the heritability and genetic correlations associated with the recalcitrance factor. The results from these analyses have been very good, showing high heritabilities and predict high genetic gains for the recalcitrance factor. These results indicated that biomass recalcitrance of woody biomass is influenced by genetic factors, and tree improvement techniques can be applied to improve it for future generations of Douglas-fir. Further investigations of these parameters has been applied to investigate the relative significance and correlations of the different parameters tested to optimize our recommendations for and understand the impact of the selection process for breeding.

The recalcitrance factor tested is composed of three factors: pretreatment yield, holocellulose, and hydrolysis yield. Together, these factors describe the effects of the entire biochemical deconstruction process used in a biorefinery to generate useful carbohydrates to be converted into fuels or chemicals. We have previously found that the hydrolysis yield in particular has high variability in the woody biomass and forms the most important contribution to variation in the recalcitrance factor.

Using the data gathered on the genetic correlations, variability, and heritability of the samples, we have further concluded that the hydrolysis yield is the main driving force behind the variation in sugar yields from different trees. This heritability and predicted gains are summarized in Table FD-5.1; note that positive improvements are desirable for all traits shown here. As is demonstrated by the large potential gains for hydrolysis yield (and thus, recalcitrance factor), the hydrolysis yield demonstrates the largest potential for improvement via genetic selection.

The genetic correlation coefficients generated for these traits are shown in Table FD-5.2. This data represents the amount that these traits share common genes, which control their variation.

Besides the expected relationship between hydrolysis yield and recalcitrance factor, the hydrolysis yield is also associated with density and pretreatment yield. The association with density is a useful (and interesting) result, as it indicates that improvement of these trees targeting biomass recalcitrance may also result in favorable gains in density. In addition, it provides valuable clues into the underlying structural and genetic factors responsible for variations in biomass recalcitrance of Douglas-fir; density is associated with several other physical traits that can be tested for. The association with pretreatment yield is unfortunate, in that trees bred for maximum hydrolysis yield will also return less biomass after pretreatment. However, we have concluded that this effect will result will be negated by the greater gains expected from hydrolysis yield (along with the positive gains predicted for the recalcitrance factor).

Further, these relationships have provided important insights into the underlying factors responsible for the variations we have been observing in biomass recalcitrance. Further investigations have begun into observing the structural differences between selected samples from these populations. This research is expected to provide more complete understanding of how these relationships function. This also serves to further develop a surrogate trait to be measured instead of the direct observation of hydrolysis yield (which is relatively costly and time-consuming). A potential surrogate trait, such as density or other traits associated with it (latewood/earlywood fraction is a main candidate), could pave the way to tree improvement of recalcitrance more economically attractive and feasible.

Table FD-5.1. Heritability and Predicted Genetic Gains for Parameters Tested in Moon Creek Population (second population tested)

Trait	Heritability	Largest Negative Gain	Largest Positive Gain
Density	0.3145166 ± 0.2188238	7.53%	10.49%
Pretreatment Yield	0.7667844 ± 0.1803037	6.14%	17.03%
Pretreated Holocellulose	0.1853049 ± 0.1903503	1.71%	2.10%
Hydrolysis Yield	0.4957738 ± 0.1420026	33.74%	40.63%
Recalcitrance Factor	0.4428915 ± 0.1356903	28.58%	34.72%

Table FD-5.2. Genetic Correlation Coefficients from Moon Creek Population

	Density	Pretreatment Yield	PT Holocellulose	Hydrolysis Yield
Recalcitrance Factor	0.4022 ± 0.2120	-0.1109 ± 0.2526	-0.1596 ± 0.2726	0.9720 ± 0.0147
Hydrolysis Yield	0.3255 ± 0.2186	-0.4972 ± 0.1890	-0.2462 ± 0.2588	
Pretreated Holocellulose	0.3429 ± 0.2415	-0.0207 ± 0.2723		
Pretreatment Yield	0.0478 ± 0.2512			

Recommendations | Conclusions

Along with the significant variation and high values for heritability of the recalcitrance traits of these trees, our analysis of the predicted gains and genetic correlations show strong potential benefits to the application of tree improvement targeting the recalcitrance of Douglas-fir feedstock. Improvement methods aimed to enhance the recalcitrance factor will result in trees demonstrating higher susceptibility to pretreatment processes, an unfortunate effect that will be counteracted by even greater improvements in the hydrolyzability of the feedstock. In addition, this selection may result in the improvement of the density of the wood. This research paves the way for tree improvement initiatives to apply these methods in order to improve the yield of biofuel products from forestry residues. These results also support the use of the genomic screening methods such as the "SNP chips" that are also to be applied by the Feedstock Development Team.

Data collected from this recent screening experiment also continues to lead to further research opportunities to further our understanding of biomass recalcitrance in softwoods. Deeper exploration of these data and the structure of selected samples from these populations are being applied to provide further insights into the underlying factors contributing the biomass recalcitrance. Further, these insights can be utilized to generate a more efficient screening method to better improve the selection of trees for breeding, making the improvement of biomass recalcitrance a more attractive prospect.

Physical and Intellectual Outputs

REFEREED PUBLICATIONS

Geleynse, S., Alvarez-Vasco, C., Garcia, K., Jayawickrama, K., Trappe, M., & Zhang, X. (2014). A multi-level analysis approach to measuring variations in biomass recalcitrance of Douglas fir tree samples. BioEnerg. Res., 7, 1411-1420. doi:10.1007/s12155-014-9483-z

RESEARCH PRESENTATIONS

Quantifying Variations in the Biomass Recalcitrance of Douglas Fir. American Institute of Chemical Engineers, 2014 Annual Meeting: Biomass Characterization, Pretreatment, and Fractionation Session. November 17, 2014

Investigations into the Chemical and Structural Factors Contributing to Large Differences in the Biomass Recalcitrance of Douglas Fir Trees. American Institute of Chemical Engineers, 2014 Annual Meeting: Recalcitrance of Woody Biomass Session. November 17, 2014

CONVERSION

PRETREATMENT TEAM

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

Key Personnel
Junyong (J.Y.) Zhu

Affiliation
USFS Forest Products Lab

Task Description

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 (Zhou et al., Ind. Eng. Chem. Res., 52:16057-16065, 2013). The major work for the proposed study is to demonstrate the performance of the SPORL using Douglas-fir softwood forest residue with relatively high lignin contents, and its scalability at two pilot plant facilities for 1000 gallon bio-jet fuel production. The specific objectives are: (1) to optimize SPORL pretreatment conditions for Douglas-fir and 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 FPL pilot scale pulping facility to realize first step scale-up study, 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 10 ton of wood, production of SPORL substrate at an Industrial scale facility potentially 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 bio-jet fuel production and lignin co-product development form SPORL hydrolysates and lignin fractions.

Activities and Results

Task C-P-1.1. Optimization SPORL and/or other pretreatments for Douglas-fir/Douglas-fir residues at lab bench scale (150 g /2kg)

This task has been completed. For additional related tasks, see Task C-P-4. The optimization study was partially supported by a USDA SBIR research project developed a combined hydrolysis factor (CHF, eq. (1)) that can optimize pretreatment duration for a given temperature at a given chemical loadings (see equation (2) below). Further study indicated that a low pretreatment temperature was favored to reduce sugar degradation to inhibitors (Eq. (3) and Figs. C-P-1.1a and C-P-1.1b).

$$(1)\quad CHF = e^{(a-\frac{E}{RT}+\beta C_A+\gamma C_B)}(C_A + C_B)\, t$$

$$(2)\quad t^{T145} = \exp\left[-\frac{E}{R}\left(\frac{1}{T_{145}} - \frac{1}{T_{180}}\right)\right]\cdot t^{T180}$$

$$(3)\quad \frac{D_{T1}}{D_{T2}} = \frac{k_d^{T1}}{k_d^{T2}}\cdot\frac{t^{T1}}{t^{T2}} = exp\left[\frac{E\text{-}E_d}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)\right]$$

Where C_A and C_B are the concentrations of chemical A (SO_2) and chemical B (hydroxide) used in pretreatment, respectively; α, β and γ are adjustable parameters, E = 100,000 J/mole is the apparent activation energy xylan dissolution for softwoods. Ed is the apparent activation energy of sugar degradation, R is universal gas constant of 8.314 J/mole/K, and T is absolute temperature (K). Defining D as the sum of the concentration of HMF and furfural.

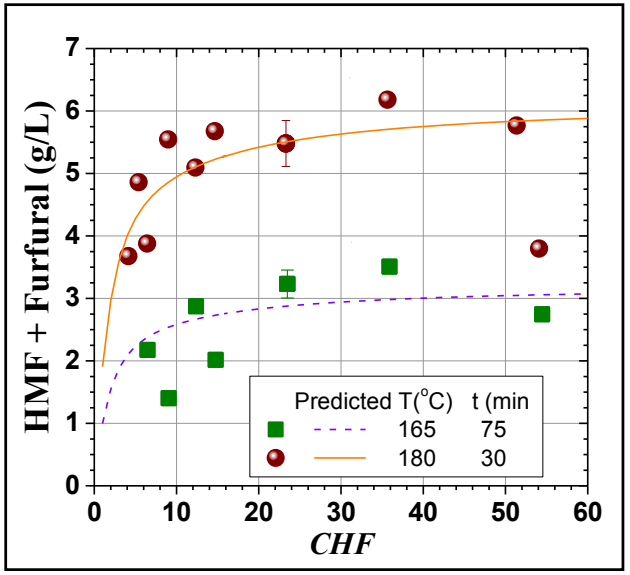


Figure C-P-1.1a. Correlation between the HMF and furfural concentration in the pretreatment hydrolysate with the combined hydrolysis factor (CHF) in comparisons with model predictions at 165°C and 180°C.

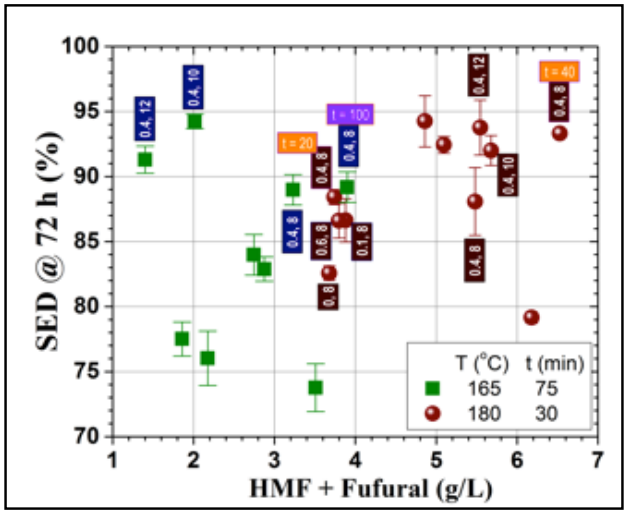


Figure C-P-1.1b. Comparisons of substrate cellulose enzymatic digestibility (SED) and furan (HMF + Furfural) production from SPORL pretreatments at 165°C and 180°C.

Table C-P-1.1. Calculated optimal pretreatment duration and relative inhibitor formation at SO_2 and hydroxide loadings of approximately 6.6 and 6.5 wt% on wood, respectively.

T (°C)	Time (min)	Relative Inhibitor
180	26	1.000
173	39	0.776
170	47	0.694
165	75	0.575
155	123	0.389
145	240	0.258

Table C-P-1.1 lists the optimal pretreatment duration at a given temperature and the corresponding relative inhibitor formation calculated based eq. (2) and (3). The advantage of low T pretreatment is obvious in terms of reducing sugar degradation to inhibitors. However a very long reaction is needed.

We also developed a pH Profiling process for SPORL pretreatment. The purpose of pH profiling run is to reduce sugar degradation to furans by delaying acid application in pretreatment. A U.S. patent has been filed. The basic concept is described in the below diagram (Figure C-P-1.2). Comparing with a control SPORL run, all pretreatment conditions (temperature, chemical loading, reaction time, etc.) were identical to a control run, except the same amount acid as those used in the control run were at a time ti rather at time 0 as in the control run. We did achieved equivalent enzymatic saccharification efficiency and glucose yield but at significantly reduced furan formation as shown in Figure C-P-1.3. This suggest that pH profiling technique can be applied to SPORL pretreatment to reduce furan formation to further facilitate fermentation along with low temperature pretreatment as reported previously. Pilot scale pH-profiling run were conducted. This is very desirable for high temperature pretreatment to reduce pretreatment duration as well as inhibitor formation.

To meet the requirement a sulfite mill operating condition for potential large scale trial run at low temperatures but substantially high SO_2 loading, for example, SO_2 on wood > 25 wt% on wood. Pilot-scale runs (50 kg) were also conducted using $Mg(OH)_2$ with SO_2 at 140C. It was found 60 min pretreatment was sufficient to produce substrate with excellent enzymatic digestibility and ultralow sugar degradation to inhibitor. This indicates increase SO_2 loading can substantially reduce pretreatment time at low temperatures with little sugar degradation to inhibitor. This condition is suitable for using existing pulping facility.

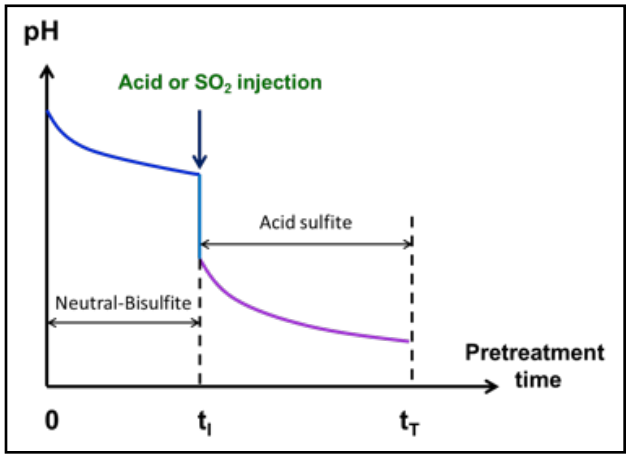


Figure C-P-1.2. Schematic of the SPORL-pH Profiling process

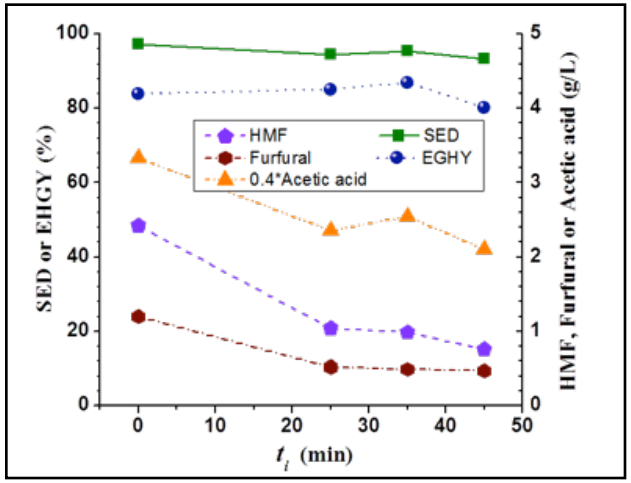


Figure C-P-1.3. Effect of delayed acid application in SPORL pretreatment on substrate enzymatic digestibility (SED), enzymatic hydrolysis glucose yield (EHGY), and furan and acids acid formation

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, we have prepared pretreated samples (up to 100 kg) from feedstocks supplied by Weyerhaeuser in accordance with their specific needs (Task C-P-3.1). The specific operational conditions for the different biomass materials will be determined initially including 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 doc fellow, along with a full time technician under supervision of the pilot plant research manager. This has allowed WSU BSEL to make full mass balances over feed stock materials and further to 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 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 the pretreatment process (Task C-P-3.2). In NARA Year 3, the results will be evaluated against other pretreatment methods used in the project. If our pretreatment method shows 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

Task C-P-3.2. Evaluate data from partners and adjust pretreatment systems

This task is completed. A final report for task C-P-3 will be issued in 2016. This report describes work done between April and July 2014. Most of the work done in this quarter was with FS-10 (Forest Slush material with Douglas-fir as the majority portion). The mass balance for the optimized wet explosion conditions for FS-10 treatment is shown in Figure C-P-3.1. The initial FS-10 was found to have around 33.79% glucan and 6.15% xylan with almost 46.47% present in the form of lignin. After optimization of the wet explosion pretreatment conditions, it was found that soluble solids in the biomass hydrolysate contained majority of the xylan sugars with 18% conversion of glucan to glucose monomer while most of the lignin and available cellulose was obtained in the insoluble solids, which were treated with Cellic Ctec2 and Cellic Htec2 enzymes. At optimum enzyme loading, it was found that we were able to recover close to 99.9% glucan and 78.6% xylans from FS-10 with minimal adverse effects from the high lignin content in the biomass feedstock. We are currently studying the biomass lignin portion left behind to understand its characteristics and have invested this biomass lignin after wet explosion pretreatment in co-products research. Initial characterization studies have indicated that the biomass lignin does not undergo any major structural transformation as is the case with traditional sulfuric acid or sulfite based pretreatments making the lignin ideal for co-products research. Literature studies have indicated that the biomass lignin structure opens up during traditional dilute acid pretreatment processes and re-condenses and re-distributes itself after the pretreatment over the outer cell wall making it difficult to activate it and convert into high value co-products. These literature studies also indicate that the re-condensation and re-polymerization of the lignin compounds over the cell wall adversely affects the cellulose enzyme activity, thereby, eventually affecting process kinetics and yields. However, such problems were not found with wet explosion pretreatment of the biomass lignin. These characterization studies have also revealed that hydroxyphenyl- or H-form of lignin was converted to syringyl- or S-form of lignin after wet explosion pretreatment making the process more amenable for co-product production from the biomass lignin.

From the discussions shown above, it is evident that wet explosion pretreatment at its optimized conditions can effectively breakdown lignocellulosic biomass (as dirty as forest slush) with effective C5 and C6 sugar conversions while providing a good and clean biomass lignin for valuable co-product production. We are currently working on further optimization of the lignocellulosic biomass and understanding the chemistry behind effective sugar conversion while characterizing the biomass lignin for co-products production. Further improvements in this area will be the main goal of the next quarter.

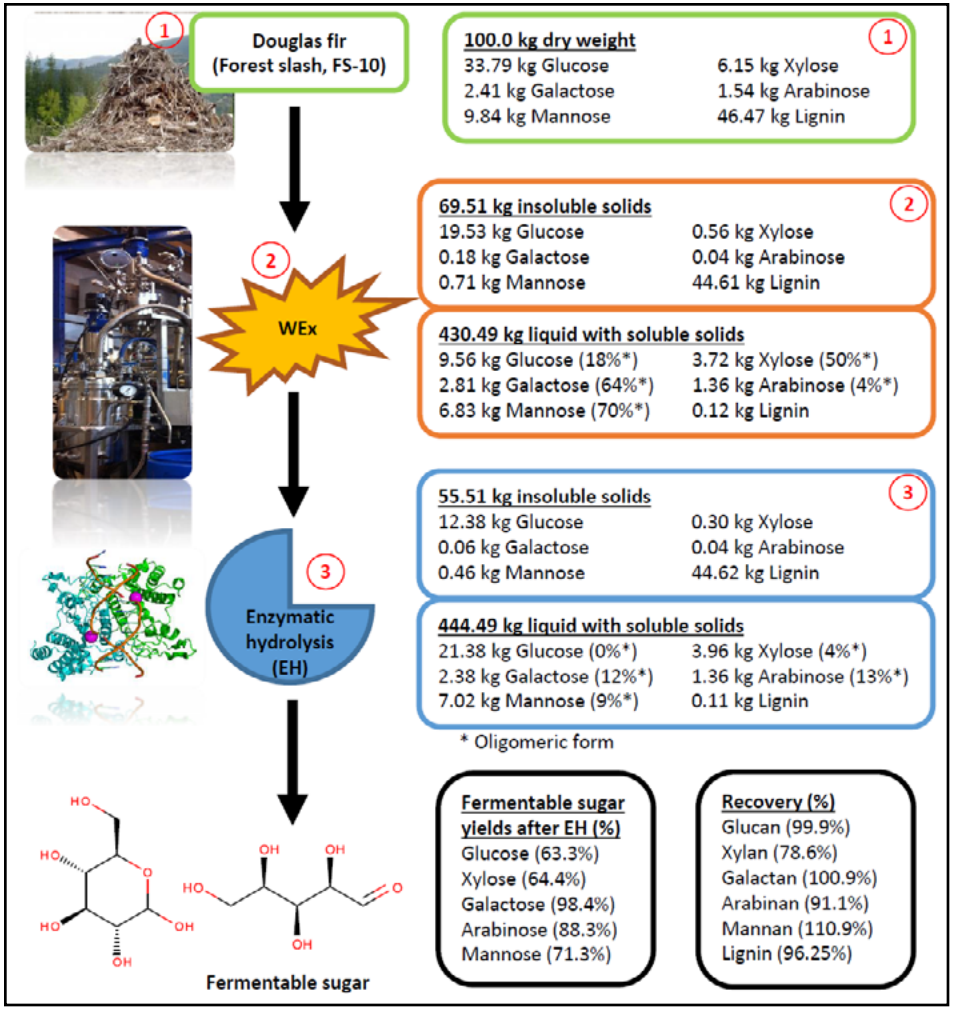


Figure C-P-3.1. Mass Balance for the optimized wet explosion of FS-10 to produce sugar monomers

Recommendations | Conclusions

The following parameters were found to give the highest digestibility of FS-10:

PRETREATMENT:
Dry content = 25% (not optimized)
Temp = 190C (optimized)
Time = 30min (optimized)
Oxygen loading = 7.5% of DM (optimized)

ENZYME HYDROLYSIS:
20% DM (not optimized)
40 mg EP/ g Cellulose (70% CTec2, 30% HTec2) (optimized)
50°C (optimized)
96h
pH = 5.3 (optimized)

A major study was done on FS-10 to determine the "sweet spot" where sugar yield and enzyme load is optimized for cost and biomass lignin is studied in detail for co-product production (papers in preparation/submitted).

Physical and Intellectual Outputs

REFEREED PUBLICATIONS

Biswas, R., Teller, P.J., and Ahring, B.K. 2014, Mass balance of pilot-scale pre-treatment of forest residues by wet explosion, In Preparation, To be Submitted to Biotechnology for biofuels.

Oliveira, F. D-C., Srinivas, K., Teller, P., Goncalves, A.R. and Ahring, B.K. 2014, Wet oxidation of pretreated biorefinery lignin for production of aromatic compounds, In Preparation, To be Submitted to Bioresource Technology.

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

Key Personnel
Junyong (J.Y.) Zhu

Affiliation
USFS Forest Products Lab

Task Description

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., Bioresour. Technol., 179:390-397, 2015). The major work for the proposed study is to demonstrate the performance of the SPORL using Douglas-fir softwood forest residue with relatively high lignin contents, and its scalability at pilot plant facilities for 1000 gallon bio-jet fuel production. The specific objectives are: (1) to optimize SPORL pretreatment conditions for Douglas-fir and 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 FPL pilot scale pulping facility to realize first step scale-up study, to determine optimal conditions based on total sugar yield after subsequent enzymatic saccharification under high solids loadings of approximately 20%; (3) Evaluate SPORL performance under different operating conditions required by potential industrial scale demonstration sites for large scale demonstration; (4) Conduct large scale, approximately 10 ton of wood, production of SPORL substrate at an Industrial scale facility potentially one-step production process under optimal conditions through preliminary large scale production study at FPL; (5) to work with Washington State University and Gevo for large scale bio-jet fuel production and lignin co-product development form SPORL hydrolysates and lignin fractions.

Activities and Results

Task C-P-4.4.1. Optimization SPORL and/or other pretreatments at FPL pilot plant facility (50 kg/run)

Pilot scale of 50 kg FS-10 Douglas-fir forest residue was conducted using Calcium bisulfite at 145°C using the FPL pilot scale digester. The technical issues addressed in this study are: (1) demonstrating SPORL process using commercial pulp mill chemistry i.e., bubbling SO_2 into a hydroxide solution to produce the sulfite solution, rather than using H_2SO_4 and sodium bisulfite as reported previously for ease of pretreatment experiments in the laboratory; (2) using a low pretreatment temperature of 145°C to accommodate facility limitations at pulp mills without reducing cellulose saccharification efficiency; (3) direct enzymatic saccharification and fermentation of the pretreated whole slurry at high solids without solids washing or slurry detoxification to simplify process integration. The pretreatment time was determined by maintaining the same pretreatment severity measured using Combined hydrolysis factor (CHF) reported previously, according to Eq. (2) described in Task C-P-1: Pretreatment to Overcome Recalcitrance of Lignocellulose.

$$(2) \quad t^{T145} = \exp\left[-\frac{E}{R}\left(\frac{1}{T_{145}} - \frac{1}{T_{180}}\right)\right] \cdot t^{T180}$$

Using the optimal pretreatment time of 25-30 min at 180ºC for softwood determined at the 0.15 kg lab scale reported previously, Eq. (2) indicates the required reaction time, tT145, for the pilot-scale pretreatment at 145°C to be 230 – 275 min.

A dilute sulfite solution, at approximately pH 2.0 and containing 2.28 wt% $Ca(HSO_3)_2$ and 0.87 wt% true free SO_2 was produced in a stirred barrel by bubbling SO_2 regulated at a gauge pressure of 34.5 kPa into a hydroxide solution of 139 L containing 1.25 kg (95% purity) of $Ca(OH)_2$. After 37 minutes bubbling, a solution weight gain of 3.3 kg was achieved while a small amount of calcium hydroxide remained at the outer edge of the barrel. Complete reaction of $Ca(OH)_2$ was achieved by manually stirring the solution 3 times. A cover was clamped and sealed with tape to the barrel and then stored at 4°C overnight.

As schematically show in Figure C-P-4.1, the digester was heated by a steam jacket and rotated at 2 rpm during pretreatment for mixing of chemicals with woody materials. The digester was first loaded with 61.75 kg FS-10 with solid content of 81.4% (50.26 kg in oven dry (OD) weight). At the current test condition, this required a total liquor volume of 150 L, total SO_2 mass concentration in the sulfite solution of 2.3 wt%, and free SO_2 and $Ca(HSO_3)_2$ charge on wood 2.48 and 6.46 wt%, respectively. This translates to a total SO_2 loading of 6.6 wt% on oven dry wood residue. Before calcium sulfite was added, the upper lid was lightly closed with the discharge valve open and low-pressure steam was injected into the top of the digester. This steaming was continued for 5 minutes after steam flow was observed at the discharge valve. The steam was stopped, the discharge valve closed and the lid quickly opened to obtain a sample. The amount of steam injected was determined to be 27.85 kg based on the moisture content of 44.18% of the steamed FS-10 in the digester. The actual liquor to wood ratio (L/W) for the pretreatment was therefore 3.55 (L/kg). The lid was then quickly sealed and a vacuum applied to the digester. The vacuum was applied for approximately 20 min at which time the vacuum valve was closed and a hose connection was made between the bottom of the digester and a centrifugal chemical transfer pump. The pump was used in case the vacuum was inadequate to pull all of the sulfite solution into the digester. After approximately 6 min all the sulfite solution was pulled into the digester. Two 50 mL samples of the liquor were collected just prior to injection for verification of sulfite concentration. The measured concentrations of $Ca(SHO_3)_2$ was

2.01 wt% and true free SO_2 was 0.43 wt% compared with calculated values of 1.93 wt% and 0.74 wt%, respectively, based on the amounts of $Ca(OH)_2$, SO_2, and steam applied. The lower measured SO_2 concentration could be due to losses during transit to Weyerhaeuser Company (Federal Way, WA). Rotation of the digester was started immediately. It took approximately 37 min to heat the digester from 30ºC to a terminal temperature of 145ºC. The temperature was maintained for 240 min. The digester contents were discharged into a blow tank through a stainless steel pipe. An additional air blow was applied to ensure all contents were discharged. Volatiles including SO_2 were vented to a wet scrubber (Figure C-P-4.1). The freely drainable portion of the pretreatment spent liquor of 42 kg was collected from the blow tank shortly after discharging from the digester. The remaining liquor stayed with the pretreated solids. After venting in the blow tank for two days to let the remaining small amount of SO_2 escape, the solids were collected and weighed.

The freely drainable spent liquor were neutralized and then proportionally fed with the pretreated solids to a laboratory disk refiner (Andritz Sprout-Bauer Atmospheric Refiner, Springfield, OH) to produce pretreated whole slurry of FS-10 (Fig. C-P-4.1). The whole slurry had a solids content of 24.49% (including the dissolved solids from pretreatment) and was directly used for subsequent saccharification and fermentation. A small sample of the whole slurry was separated into wet solids and liquor by pressing in a screen box. The moisture of the solid fraction was determined gravimetrically by oven drying the collected wet sample. An aliquot of the wet solid sample was thoroughly washed to remove soluble solids. Both the washed and unwashed solids were used to conduct enzymatic hydrolysis after neutralization using lime.

Enzymatic saccharification of the washed solids was conducted to evaluate the effectiveness of SPORL pretreatment in removing the recalcitrance of Douglas-fir forest residue FS-10. Substrate enzymatic digestibility (SED), defined as the percentage of washed solids glucan enzymatically saccharified to glucose,

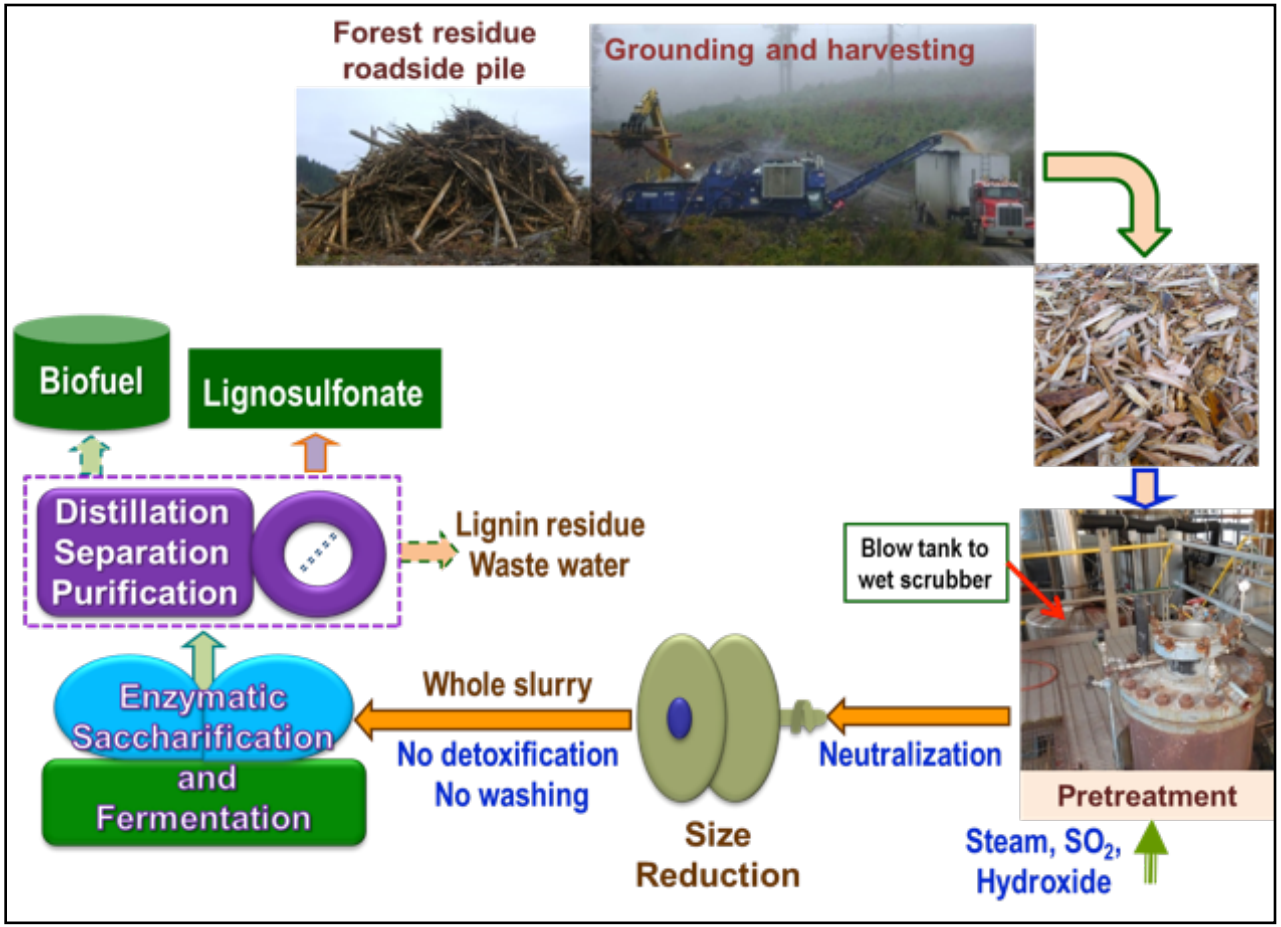


Figure C-P-4.1. A schematic process flow diagram shows Douglas-fir forest residue harvesting, transportation, pretreatment and downstream saccharification and fermentation.

reached 84% in 48 h with titer of 80 g/L at a washed solids loading of 15% (Fig. C-P-4.2). Saccharification efficiency increased to over 90% at 10% solids loading in 24 h. The dissolved lignin or lignosulfonate from sulfite spent liquor was found to have less affinity to cellulase and can act as a surfactant to enhance enzymatic saccharification. When enzymatic saccharification was conducted using unwashed solid substrates that containing dissolved lignin at 18.5% solids, SED reached 92% with a glucose titer of 97 g/L. This indicates more glucose will be available in Q-SSF of the SPORL pretreated whole slurry that contains lignosulfonate than using washed solids alone.

The pretreated liquor had very low fermentation inhibitors and was found it can be directly fermented at 16.7wt% total solids loading without detoxification using Saccharomyces cerevisiae YRH-400 (A strain developed by USDA-ARS). The ethanol yield of 284 L/tonne at 41.9 g/L were obtained. It is expected that the sample should be fermentable by GEVO strain without detoxification.

We have developed a pilot-scale membrane system for purify lignosulfonate. The spent liquor was first centrifuged to remove particulates. Any remaining particulates were further separated by passing through the 200 kDa membrane. The liquor was then sent to

a 4 kDa membrane to remove small molecular impurities such as sugars. Each fraction was collected and weighed to determine spent liquor mass distribution and for mass balance analysis. After UF through the two membranes, 69.6% of Ca-LS and 7.6% of total sugar were retained in the purified sample (fraction of 4 k-200 kDa), as shown in Table C-P-4.1. The lignin purity was only 44.5% in the original spent liquor and was increased to 86.8% after UF in the purified sample. Table 2 indicated that Ca-LS could be extracted with high recovery and purity by UF. The purified Ca-LS had a similar molecular weight and polydispersity to the commercial lignosulfonate D748 (Borregaard LignoTech, Rothschild, WI, USA) based on GPC MALS measurements. However, GPC UV measurements indicate that the purified Ca-LS had a smaller molecular weight with lower polydispersity than D-748 (Table C-P-4.1).

The Ca-LS is highly sulfonated with sulfur content of 69.2 ± 0.9 mg/g which is higher than the commercial LS D-748 of 60.1 ± 3.9 mg/g. The sulfur content along with the molecular weight information suggests that the Ca-LS from SPORL can be directly marketed with comparable properties of commercial lignosulfonate.

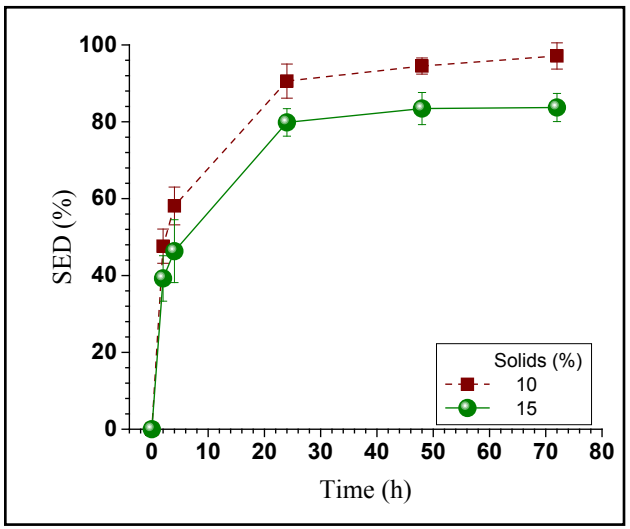


Figure C-P-4.2. Time-dependent enzymatic saccharification efficiency of washed SPORL pretreated FS-10 at pilot-scale at two solids loadings with CTec3 dosage of 15 FPU/g glucan.

Table C-P-4.1. Characterization of purified calcium lignosulfonate solution

Ultrafiltration experiment				
Sample	LS-Ca (%)	Sugar (%)	Mass (%)	Lignin Purity (%)
Original	100	100	100	44.5
> 200 kDa	3.9	n/a	2.4	89.1
4-200 kDa[a]	69.6	7.6	43.2	86.8
< 4 kDa	24.9	84.3	41.5	19.3
GPC (MALS) analysis				
Sample	Mw[b]	Mn[c]	Mw/Mn[d]	
4-200 kDa	23430	12910	1.8	
D748	24660	14190	1.7	
GPC (UV) analysis				
4-200 kDa	2704	1092	2.5	
D-748	13113	3293	4.0	

a) The fraction of 4-200 kDa is the purified calcium lignosulfonate sample
b) Number-average molecular weight
c) Weight-average molecular weight
d) Polydispersity

Task C-P-4.4.2. Identification and experimental verification of operating conditions that are suitable for industrial trial runs

Previous study $Ca(HSO_3)_2$ pretreatment of Douglas-fir harvest forest residue FS-10 at 145°C for 240 min with very low SO_2 loadings of approximately 18 g/L (or 6.6wt% on wood) in the pretreatment liquor. Excellent results were obtained when evaluated using ethanol and iso-buthanol production. Several industrial facilities were identified for potential 50 tonne FS-10 pretreatment. The first facility is a sulfite pulp mill using $Mg(HSO_3)_2$ with very high SO_2 loading of approximately 60-80 g/L (or >25 wt% on wood) in the pulping liquor at 140C. Mill stated that chemical formulation cannot be modified for pretreating 50 tonne FS-10. The second facility is has limited SO_2 handling capabilities, so a low SO_2 loading is desired, however, 240 min is too long for the facility to handle, a high temperature of 170°C is required, based on the scaling factor CHF reported in the previous quarter, to meet the 45 min longest residence time of the facility. The third facility has too low a capacity of 1 ton/day though has excellent capability for SO_2 handling and sugar concentration, therefore not serious consideration.

Our laboratory study in this quarter has been concentrated on using the operating conditions at the two potential industry sites to evaluate SPORL performance. Specifically, Mg was chosen for its substantially high iso-buthanol productivity based on a fermentation study at Gevo. The first set of conditions is to meet the requirements of sulfite mill, low temperature 140°C and high SO_2 loading of 60-80 g/L. We have identified that a pretreatment time of 60 min is sufficient to obtain good substrate enzymatic digestibility with very low inhibitors with this condition. We also conducted pretreatment at 170°C for 45 min using $Mg(HSO_3)_2$ plus sulfuric acid as the second industry facility has limited capability to handle SO_2. All pretreated samples have been shipped to Gevo

for iso-buthanol production evaluation. Mass balance data of these pretreatments are in progress.

We also evaluate the potential of eliminate size reduction for fermentation. We used a laboratory high shear mixing devise to conduct enzymatic saccharification of a $Mg(HSO_3)_2$ pretreated sample without size reduction. Different size fractions were used. The results will be reported in the next reporting period.

Task C-P-4.4.3. Industrial scale-up of SPORL technology

We have visited three industry sites and conducting laboratory evaluation of technical feasibilities as discussed above. A pretrial at Andritz spring field facility is scheduled in May 27.

Recommendations | Conclusions

The robust of the SPORL process lies in the fact that it can be carried out at several sets of conditions that can provide excellent sugar yield and low inhibitor formation. An industrial site can be chosen after the isobutanol fermentation evaluation completed using the SPORL pretreated samples produced in this quarter.

Physical and Intellectual Outputs

PHYSICAL

- Many pilot-scale (50 kg) SPORL pretreatments of FS-10 were conducted
- Sample analyses were in progress
- Shipped the lignosulfonate to the lignin-co-product team
- Three potential demonstration site were visited

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Zhu J.Y., M. Subhosh Chandra, Roland Gleisner, William Gilles, Johnway Gao, Gevan Marrs, Dwight Anderson, John Sessions, (2014) "Reviving Sulfite Pulping from Sugar Production from Woody Biomass" 2014 TAPPI PEERS Conference, Tacoma, WA, Sep, 2014

Zhu, J.Y., (2014), "Role of Lignin on Enzymatic Saccharification of Lignocellulose", 2014 TAPP IBBC, Tacoma, WA, Sep, 2014

Zhu, J.Y. (2014) "Bioconversion of Forest Residue using SPORL: Process Scale-up Design, Lignin-Coproducts, High Solids Fermentation without Detoxification", 36th Symposium on Biotechnology for Fuels and Chemicals, Clearwater Beach, FL, April 28 - May 1

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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

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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

OTHER PUBLICATIONS

Zhu, J.Y., (2015), "The pulp and paper industry in 2040" TAPPI Journal (TAPPI Centennial Editorial), 14(3):S19-S20

AWARDS AND RECOGNITION

1. J.Y. Zhu, US Forest Service Deputy Chief's Distinguished Science Award

2. J.Y. Zhu, TAPPI R&D International Technical Award and William Aiken Prize

3. J.Y. Zhu, Greater Madison Area Federal Agency Association Employee of the year - Technical

4. J.Y. Zhu, the Grantee of the Fulbright-Aalto University Distinguished Chair (scholarship) in Energy and Sustainable Use of Natural Resources, Helsinki, Finland, for the 2015-2016 Academic year.

5. J.Y. Zhu, Invited panel speaker and International Expert at the Second Generation Ethanol Workshop-2014, Brazilian National Center for Ethanol Science and Technology (CTBE), Campinas, Sao Paulo, Brazil

INTELLECTUAL PROPERTY

Zhu, J.Y., Gleisner, R., "Methods of Pretreating Lignocellulosic Biomass with Reduced Formation of Fermentation Inhibitors", U.S. Utility Patent Application No 14/299,926, 06/09/2014

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

Key Personnel	Affiliation
Johnway Gao	Weyerhauser

This task has been combined with the E-8 Distributed Sugar Depot task in NARA project Year-4.

CONVERSION

AVIATION TEAM

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

Key Personnel	Affiliation
Andrew Hawkins	Gevo, Inc
Glenn Johnson	Gevo, Inc
Bob Wooley	Gevo, Inc
Christopher Ryan	Gevo, Inc.
Joseph Ley	Gevo, Inc.

Task Description

Gevo has developed fermentation and process technology to convert biomass sugars to isobutanol and further into renewable jet fuel through chemical processing. Gevo will concurrently develop GIFT®, Gevo Integrated Fermentation Technology, to produce isobutanol at high productivity, titer, and yield using a yeast biocatalyst adapted to hydrolyzate. The goal of this project will be to produce isobutanol according to a specification developed by Gevo that ensures the isobutanol will be converted into renewable biojet using existing Gevo technology. Quantities of about 1,000 gallons of biojet will be prepared and validated as suitable jet fuel blend stock using ASTM's fit for purpose testing protocol and input from stakeholders. The specific tasks of this project are: (1) Characterize toxicity of a representative sample of pre-treated woody biomass (Douglas-fir) for fermentation; (2) Adapt yeast biocatalyst to pretreated biomass hydrolyzate; (3) Produce isobutanol in a 1L batch fermentation from pretreated biomass sugars using the adapted yeast biocatalyst; (4) Economic assessment of wood to isobutanol, jet; (5) Produce isobutanol in a 1L GIFT® fermentation from pretreated biomass sugars using the adapted yeast biocatalyst; (6) Analysis of isobutanol to close the mass balance and determine potential low-level impurities; (7) Produce isobutanol in a 20L GIFT® fermentation from pretreated biomass; (8) Produce ≥1,000 gallons isobutanol from GIFT® fermentations at a suitable demonstration scale. Convert lignocellulosic isobutanol to ≥1,000 gallons biojet for further testing.

Activities and Results

Over the past year, Gevo received hemlock reject fibers pretreated by Cosmo Specialty Fibers (Cosmo) and milled by Dr. Junyong Zhu (J.Y.) at the USDA Forest Products Laboratory (FPL). Preliminary growth and fermentation experiments were carried out with Gevo biocatalysts using the clarified Cosmo reject fiber hydrolyzate. FS-10 concentrated milled wood (CMW) hydrolyzate as well as FS-10 SPORL pretreated using magnesium was also received and tested in Gevo GIFT® fermentation systems. A small fraction of the FS-10 SPORL-Mg^{2+} pretreated material received from J.Y. was pressed on-site to separate as much of the liquor from the combined material as possible for further analysis. The new pressed material was hydrolyzed, at a similar solids percentage as the combined material, then clarified (FS-10 SPORL-Mg^{2+} pretreated solids hydrolyzate). The liquor stream was clarified (FS-10 SPORL-Mg^{2+} pretreated liquor) for further comparison to both FS-10 SPORL-Ca^{2+} and FS-10 SPORL-Mg^{2+} combined pretreated hydrolyzate. A sample of concentrated FS-10 SPORL-Mg^{2+} pretreated hydrolyzate (EHS$$FS10-SPO601401.05-M022615-01), processed using conditions similar to what a potential scale-up partner for pretreatment, American Process, Inc. (API) is capable of creating, was received from J.Y. and analyzed. Gevo also received FS-01 (PSU$$-FS01-DM04012000EW-J050114) and EW-01 (80 minute grind = PSU$$-EW01-DM08001050L-P102214 and 120 minute grind = PSU$$-EW01-DM12001050L-P102214) Milled Wood feedstocks from Dr. Jinwu Wang of Washington State University (WSU) to compare the effectiveness of a ball mill using a Japanese milling process to the SPORL pretreatment process. This material was analyzed on a high throughput scale and benchtop scale to further assess the optimal ball mill parameters and pretreatment method for scale-up purposes.

Work progressed to further define process conditions (operating pH, hydrolyzate loading during growth, and nutrient package development) now that a feedstock/pretreatment selection has been made. The Gevo evolutionary improvement project using first and second generation Gevo yeast biocatalysts continue to yield strains with improved growth in FS-10 SPORL-Mg^{2+} pretreated hydrolyzate material. Over the past year, Gevo has continued to refine the process box, which includes material flows and capital/operating costs. It still remains imperative that sufficient quantities of a single feedstock pretreated using a single method be provided to Gevo to continue process development work toward scale up and the ~1,000 gal biojet project.

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 performance 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. Characterization work of FS-10 SPORL has involved several varieties of SPORL pretreatment. Included in the characterization work was sodium pretreated FS-10 SPORL material, which was previously denoted as FS-10 mild bisulfite. Washed and unwashed solids variations of SPORL material have been characterized and the spent sulfite liquor has also been characterized. Calcium and

Table CAF-1.1. Sugar and inhibitor concentrations in FS-01, FS-03, FS-10, and Hemlock feedstocks from various pretreatment methods. Concentrations of sugars and inhibitors were determined using HPLC at Gevo. (n.d. = not detected, underlined samples indicate recent additions to this table)

	% solids in hydrolysis	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)	19.9	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)	23.19	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)	15.09	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)	24.99	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)	21.78	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	36.43	81.81	5.79	3.82	0.40	7.02	5.78	1.84	0.63	92.65
FS-10 SPORL-Na^+ Pretreated SSL		7.83	7.32	5.29	2.21	18.06	3.61	n.d.	n.d.	31.18
FS-10 SPORL-Na^+ Pretreated Washed Solids Hydrolyzate	24.48	63.88	3.77	2.07	0.68	7.52	1.65	n.d.	n.d.	73.47
FS-10 SPORL-Na^+ Pretreated Unwashed Solids Hydrolyzate	29.19	84.22	10.14	5.26	2.02	20.93	3.44	n.d.	n.d.	110.41
FS-10 SPORL-Ca^{2+} Pretreated Hydrolyzate	26.29	62.74	6.84	5.07	n.d.	11.83	0.62	n.d.	n.d.	79.64
FS-10 SPORL-Mg^{2+} Pretreated Hydrolyzate	24%	71.59	8.89	4.23	14.39	0.61	3.00	n.d.	n.d.	76.43
FS-10 SPORL-Mg^{2+} Pretreated Solids Hydrolyzate	27.29	88.56	8.39	3.47	12.68	0.66	2.71	n.d.	n.d.	92.70
FS-10 SPORL-Mg^{2+} Pretreated Liquor	As rec'd	7.11	12.38	7.82	24.09	0.00	4.57	n.d.	n.d.	14.94

Table CAF-1.1. continued

FS-03 Catchlight Combined Hydrolyzate	19.27	164.12	8.56	5.24	0.94	13.34	3.45	0.15	0.14	182.70
FS-03 Catchlight Clean Hydrolyzate	28.81	130.89	1.84	0.49	n.d.	1.34	0.26	0.14	0.01	132.72
FS-10 Unconcentrated Milled Wood Hydrolyzate	12.9%	39.84	7.61	1.87	2.04	11.40	0.74	n.d.	n.d.	53.11
FS-10 Concentrated Milled Wood Hydrolyzate	As rec'd	61.84	9.21	0.46	1.36	12.76	0.77	n.d.	n.d.	75.06
FS-01 Milled Wood (40 min. Grind)	~25%	90.34	9.77	0.61	2.12	10.67	1.58	n.d.	n.d	101.61
EW-01 Milled Wood (80 min. Grind)	~25%	28.83	3.54	0.63	1.22	6.09	0.99	0.05	n.d.	35.55
EW-01 Milled Wood (120 min. Grind)	~25%	92.30	10.86	1.25	2.60	15.01	2.27	0.06	n.d.	108.57
Cosmo Reject Solids Hydrolyzate	24.86	111.35	2.02	0.15	1.45	0.79	0.27	n.d.	n.d.	112.29
FS-10 SPORL-Mg^{2+} Pretreated Concentrate	As rec'd	282.63	44.05	18.25	7.18	75.28	10.39	n.d.	n.d.	376.2

magnesium pretreated SPORL material has also been included in the characterization work conducted by GEVO. SPORL pretreated materials were obtained from Dr. Junyong Zhu at the USDA Forest Products Laboratory (Madison, WI). FS-01, FS-03, and FS-10 wet oxidation pretreated materials were obtained from Dr. Birgitte Ahring at Washington State University – Tricities (Richland, WA). FS-03 pretreated material (Clean and Combined) was obtained from Catchlight Energy (Federal Way, WA). FS-10 milled wood pretreated material (Concentrated and Unconcentrated) was obtained from Dr. Johnway Gao at Catchlight Energy. Hemlock reject fibers that were washed solids produced by Cosmo and milled by Dr. Junyong Zhu and FS-01 (40 minute grind time) and EW-01 (80 and 120 minute grind time) Milled Wood (MW) samples from Jinwu Wang at Washington State University (Pullman, WA). Gevo has also worked to optimize the hydrolysis efficiency of the various pretreated feedstocks that have been received. Various solids loading and enzyme addition rates have been explored in order to optimize hydrolysis efficiency.

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

Inhibitor concentrations in SPORL pretreated material can vary widely depending on the pretreatment conditions. In order to generate a biocatalyst capable of maximized growth and production, adaptation to pretreated hydrolyzate is required. First generation hydrolyzate adapted biocatalysts with improved growth and isobutanol production performance have been isolated previously in both FS-03 (LB4) and FS-03 SPORL (LB21). A second generation biocatalyst and the current best corn starch biocatalyst (LB23), was also selected for hydrolyzate adaptation after it demonstrated maximum growth rates similar to LB21 in FS-10 SPORL-Ca^{2+} and Mg^{2+} pretreated hydrolyzate (Figure C-AF-1.1).

Both LB21 and LB23 adaptation in FS-10 SPORL-Mg^{2+} pretreated hydrolyzate is currently being conducted. Most recently, an LB23 evolution was examined using high throughput analysis to compare growth rates of the LB23 parent to various evolved LB23 isolates (Figure C-AF-1.2). Multiple LB23 evolved isolates had an improved maximum growth

rate in 20% (v/v) FS-10 SPORL-Ca^{2+} pretreated hydrolyzate compared to the parent strain. In the coming year LB23 evolved isolates will be examined further at the bench scale to investigate growth in FS-17 pretreated hydrolyzate as well as to establish the fermentation performance of each promising isolate.

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

No additional 1L batch fermentations were run from April 2014 to March 2015. The focus instead has shifted to 1L (baby) GIFT® runs.

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-1.3 illustrates the information (streams 2 through 8 and CapEx) supplied to the NARA process team.

In summary, the saccharified biomass sugars are fermented and isobutanol recovered in a process essentially identical to the corn mash process being used currently at Gevo's plant in Luverne, MN. The process modelled 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,

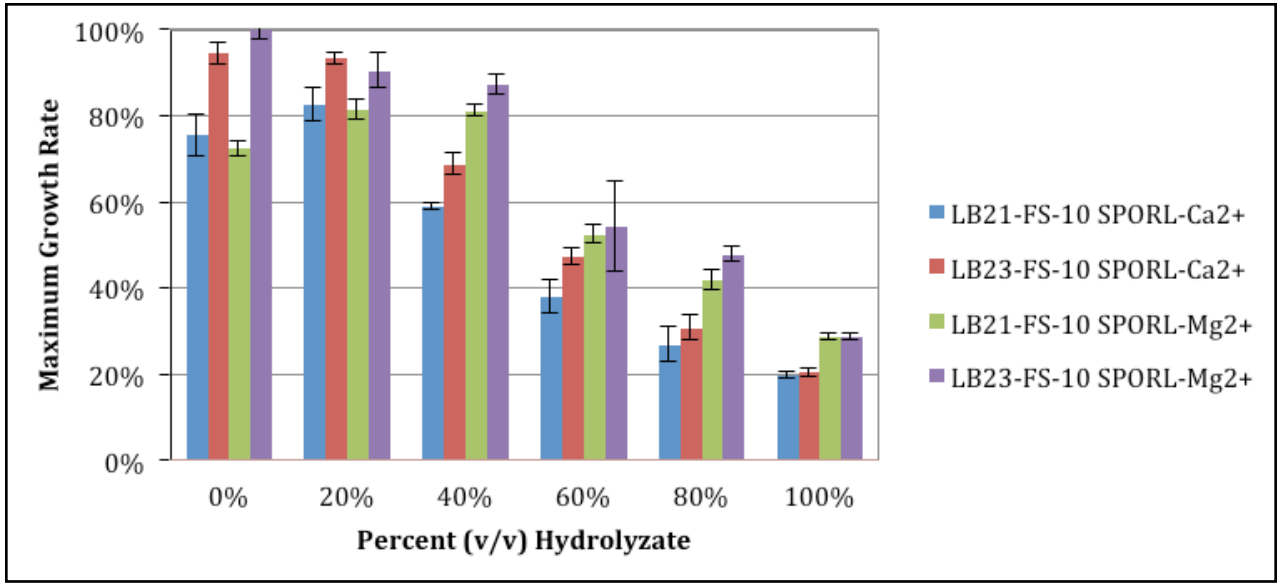

Figure C-AF-1.1. Maximum growth rate of LB21 and LB23 in FS-10 SPORL-Ca^{2+} and Mg^{2+} pretreated hydrolyzate with NP 2.0. Mock medium made for each hydrolyzate is a combination of hexoses, pentoses and acetate at the same concentrations outlined in Table C-AF-1.1 supplemented with NP 2.0. Dilutions (volume/volume) of hydrolyzate were created using mock media. Growth carried out at 33°C and maximum growth rate measured for each percent (v/v) hydrolyzate.

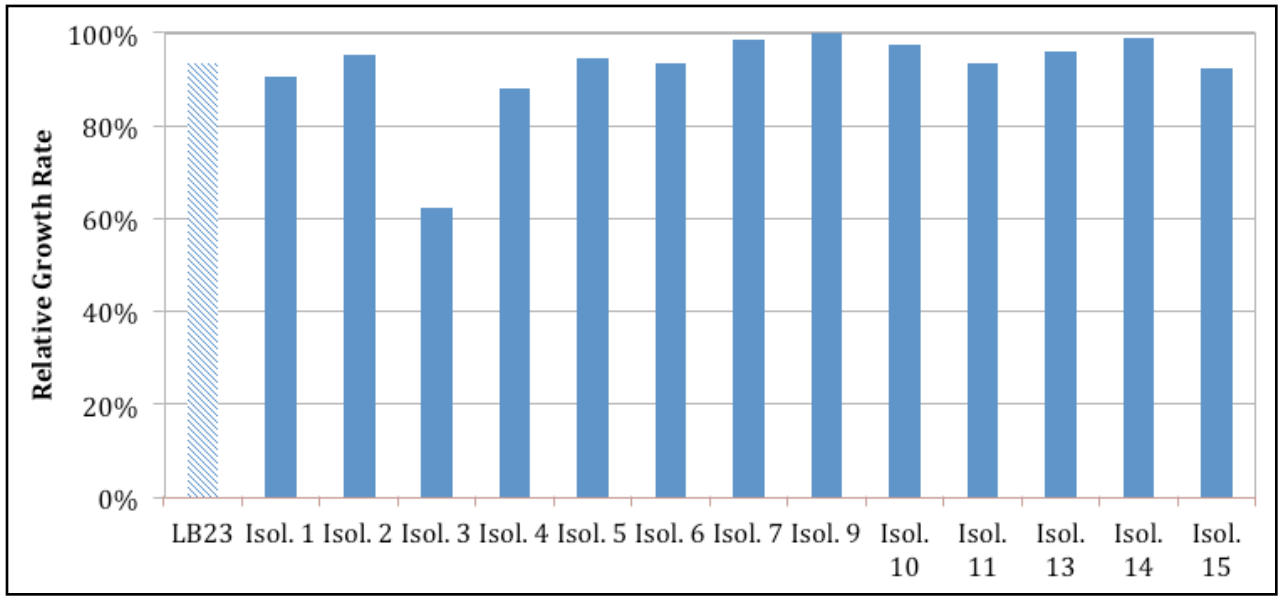

Figure C-AF-1.2. Maximum growth rate of LB23 parent strain compared to LB23 evolved isolates in 20% v/v FS-10 SPORL-Ca2+ pretreated hydrolyzate with NP 2.0. 20% v/v mixture created by diluting FS-10 SPORL-Ca2+ using buffered water. Growth carried out at 33°C under high aeration conditions and maximum growth rate measured for each isolate. Isolates 7,9, and 14 shown in figure 2 above warrant further characterization and potentially characterization of isobutanol production in 1L GIFT® fermentations in order to demonstrate improved performance compared to LB23

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.

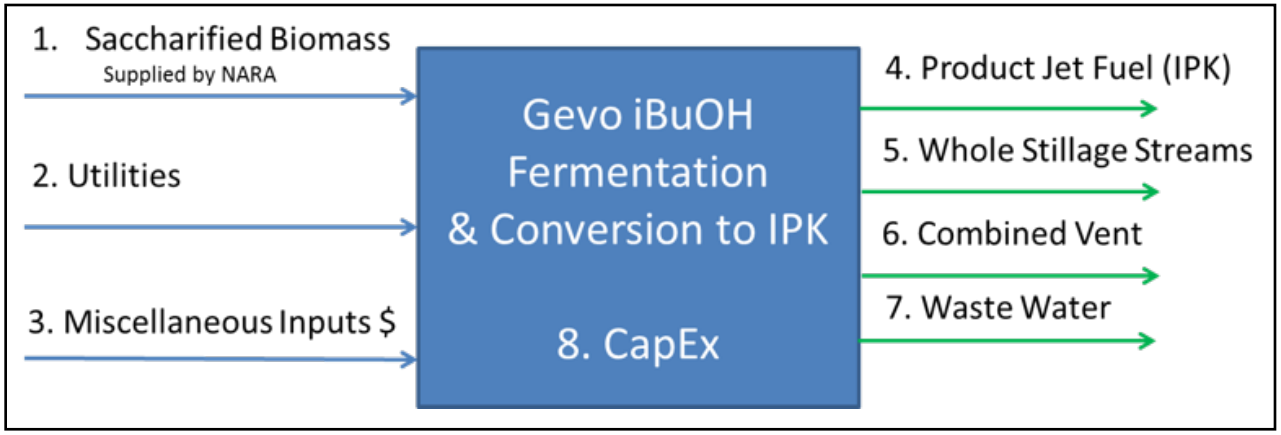

Figure C-AF-1.3. Gevo approach to modeling material flows and capital and operating costs.

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

A 1L GIFT® fermentation was performed using 100% (v/v) FS-10 concentrated milled wood (CMW) to further characterize the pretreatment and hydrolysis method used by Catchlight Energy. The volumetric rate of isobutanol production as well as the isobutanol titer achieved was comparable to 60% (v/v) FS-10 SPORL-Ca^{2+} pretreated hydrolyzate. In order to compare the milled wood process and SPORL pretreatment process, shipments of EW-01 and FS-01 MW samples were received from WSU and hydrolyzed at Gevo at similar solids percentages as previously hydrolyzed FS-10 SPORL-Mg^{2+} pretreated hydrolyzate. EW-01 MW (120 min. grind) hydrolyzed (23% solids, 108.6 g/L hexose sugars) under similar conditions as FS-10 SPORL-Mg^{2+} pretreated hydrolyzate (24% solids, 76.4 g/L hexose sugars) was the closest comparison in terms of hexose sugar concentration.

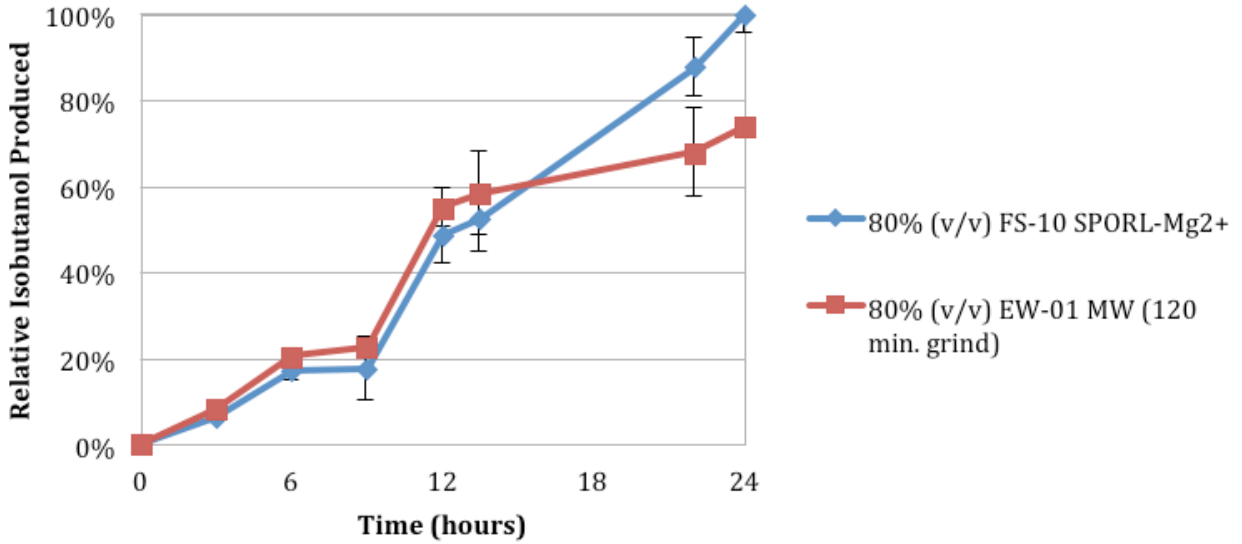

Figure C-AF-1.4. . Isobutanol produced by LB21 in 80% v/v FS-10 SPORL-Mg2+ pretreated and EW-01 MW (120 min. grind) hydrolyzate. Production of isobutanol was conducted in 1L fermentation vessels equipped with GIFT®, held at 33°C and pH controlled. Media was created using the corresponding mock media. Error bars represent the standard deviation.

Growth of LB21 was nearly identical in 20% (v/v) FS-10 SPORL-Mg^{2+} pretreated hydrolyzate and 20% (v/v) EW-01 MW (120 min. grind) hydrolyzate using their corresponding mock media. The seed culture of LB21 from each condition was then used to inoculate 80% (v/v) of corresponding hydrolyzate using mock medium as the balance and allowed to ferment for 24 hours when both hydrolyzates were depleted of hexose sugars. Volumetric isobutanol production of LB21 in 80% (v/v) FS-10 SPORL-Mg^{2+} pretreated hydrolyzate was an average of 26.4% higher than LB21 in 80% (v/v) EW-01 MW (120 min. grind) hydrolyzate after 24 hours. LB21 continued to produce isobutanol at similar rates in both hydrolyzates up to 12 hours before plateauing in EW-01 MW (120 min. grind) hydrolyzate (Figure C-AF-1.4). The theoret-

ical isobutanol yield of LB21 was 8.6% higher in 80% (v/v) FS-10 SPORL-Mg^{2+} pretreated hydrolyzate compared to the EW-10 MW (120 min. grind) hydrolyzate equivalent. Under the current pretreatment conditions, LB21 ferments better in FS-10 SPORL-Mg^{2+} pretreated hydrolyzate compared to EW-01 MW (120 min. grind) hydrolyzate under similar fermentation conditions.

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

Isobutanol collected from FS-10 (CMW) hydrolyzate lab-scale GIFT® fermentations were analyzed by gas chromatography for impurities. Table C-AF-1.2 shows very similar profiles for isobutanol produced from the mock hydrolyzate (pure sugars) medium and FS-10 CMW hydrolyzate. Consultation with the Gevo biojet conversion team did not identify any impurities that would be detrimental to the biojet conversion process. Work in this area will continue when the FS-17 feedstock using a single pretreatment method is fermented using GIFT® technology for process development work, scale-up, and production of ~1,000 gal biojet.

Task C-AF-1.7. Optimize process parameters for isobutanol fermentation from pretreated biomass

Over the past year, effort was put towards defining process conditions such as hydrolyzate loading during growth and production, and nutrient package development. This process information will be applicable to scale up from the 1L GIFT® scale and could be applied to producing isobutanol in the 20L GIFT® fermentation system as well as producing 1,000 gallons of biojet. Gevo, in consultation with NARA (Dr. Robert Wooley) no longer believes a step up to the 20L scale is required. Gevo has successfully scaled from 1L to hundreds of thousands of L directly. This, scaling from 1L GIFT® to ~23,000 L demonstration scale fermentations is directly feasible. Upon receiving a large pretreated FS-17 sample (15-20 kg) which resembles the conditions expected during the 1,000 gallon biojet production process, a 20L GIFT® fermentation experiment could be conducted if deemed necessary. Process development (including nutrient package development) will continue at the 1L scale.

Nitrogen supplementation using a variety of inorganic and organic (complex) nitrogen sources as well as supplemented with vitamins and minerals were tested using the LB21 biocatalyst. Based on the previously reported isobutanol productivity results, nutrient package (NP) 2.0 supplemented with a nitrogen source during propagation/growth and fermented in hydrolyzate containing NP 2.0 will create optimal conditions for isobutanol titer and volumetric isobutanol productivity (Figure C-AF-1.5).

In April/May 2014, three various industrially relevant operating pH ranges for growth of LB21 were also tested using 40% (v/v) FS-10 SPORL-Na^{+} pretreated hydrolyzate (previously labeled unwashed mild bisulfite solids, UMBS). Growth

Table CAF-1.2. Impurity profile of isobutanol produced in FS-10 CMW mock and 100% v/v FS-10 CMW hydrolyzate. Materials were analyzed by gas chromatography.

	FS-10 CMW Mock Media (Weight %)	FS-10 CMW Hydrolyzate (Weight %)
Methanol	0.0	0.0
Ethanol	3.0±0.2	3.9±0.7
Acetone	0.0	0.0
Isopropanol	0.0	0.0
1-Propanol	0.1	0.1
Isobutyraldehyde	0.2	0.1±0.1
2,3-Butanedione	0.0	0.0
2-Butanone	0.0	0.0
2-Butanol	0.0	0.0
Isobutanol	76.8±0.2	75.6±1.0
1-Butanol	0.0	0.0
Acetoin (3-Hydroxy-2-butanone)	0.0	0.0
3-Methyl-1-Butanol	1.1±0.1	1.0±0.1
2-Methyl-1-Butanol	0.6	0.5±0.1
Isobutyl Acetate	0.0	0.0
Isopentyl Acetate	0.0	0.0
Isobutyl Isobutyrate	0.0	0.0
2,3,5-Trimethylpyrazine	0.0	0.0
2,3,5,6-Tetramethylpyrazine	0.0	0.0
2-Phenylethanol	0.1	0.1
Phenethyl Acetate	0.0	0.0
All Unknown Peaks	0.1	0.2±0.1
Density	82.0±0.2	81.6±0.3
Water (weight %)	18.1±0.2	18.6±0.4
Water (Vol %)	21.4±0.5	21.8±0.3

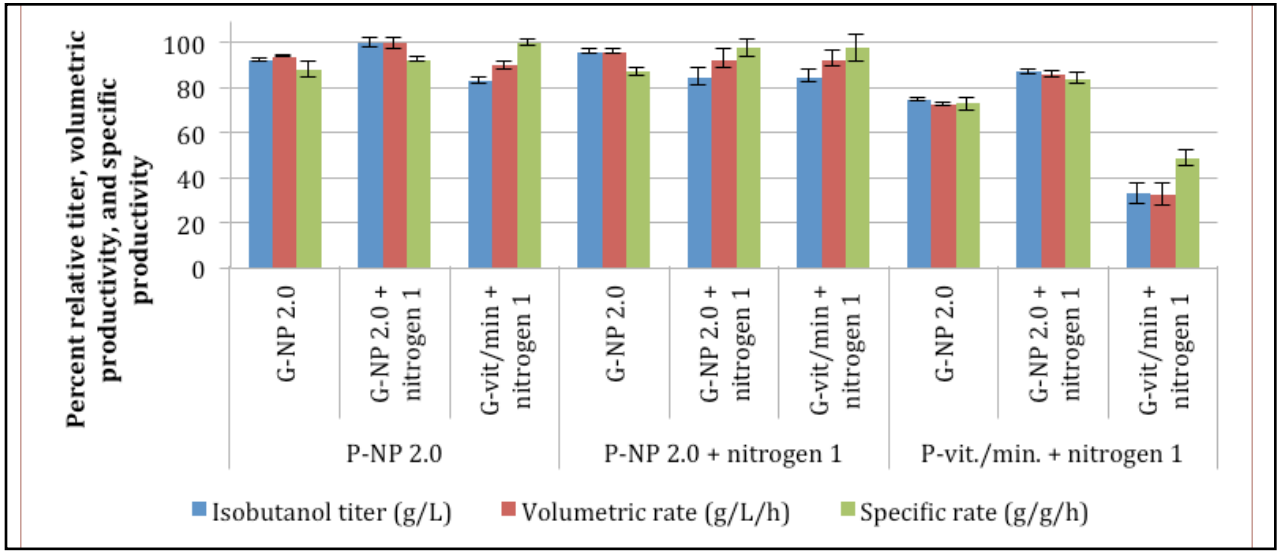

Figure C-AF-1.5. LB21 isobutanol metrics during nitrogen source optimization for isobutanol production. LB21 was grown (G) under high aeration conditions for 23 hours in shake flasks in either NP 2.0, NP 2.0 + nitrogen 1, or vit/min + nitrogen 1. After 23 hours of growth, production (P) was started with LB21 by transferring 1:4 volumetrically into shake flasks containing NP 2.0, NP 2.0 + nitrogen 1, or vit/min + nitrogen 1. All permutations of growth and production were tested for the different nitrogen media. The fermentation occurred under low aeration conditions for 24 hours. All media contained the same buffering agent Growth was carried out at 33ºC. Cell density was measured using a spectrophotometer. Error bars represent the standard deviation. Abbreviations: NP 2.0, yeast nutrient package containing complex nitrogen source; nitrogen 1, inorganic nitrogen source; vit/min, yeast vitamin and mineral mix essential for growth.

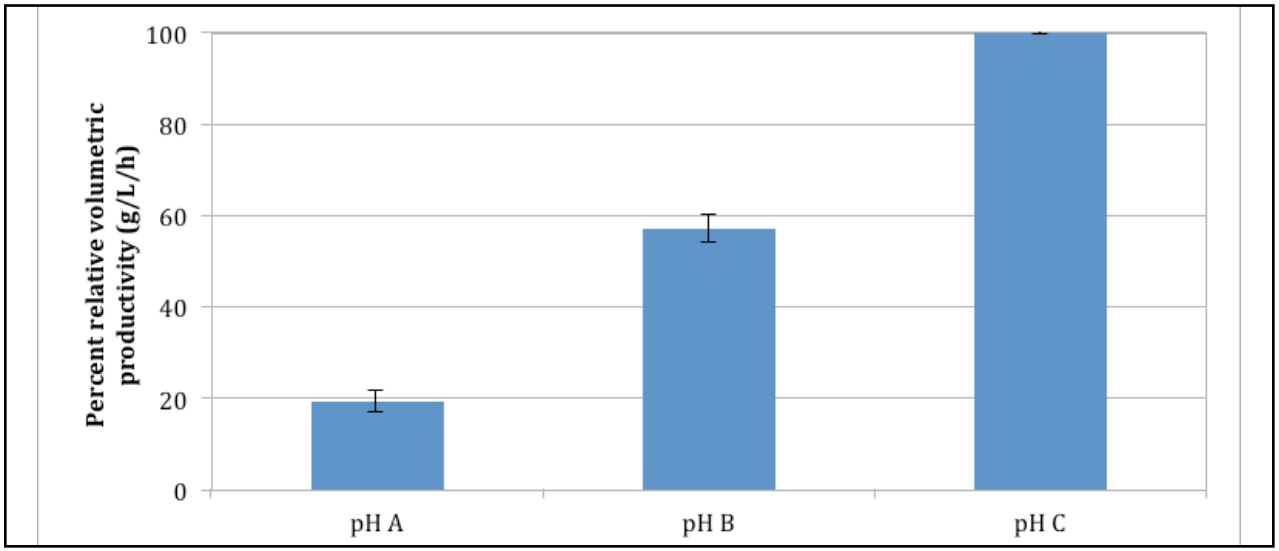

Figure C-AF-1.6. LB21 volumetric isobutanol productivity during fermentation at different industrially relevant pH conditions in 85% v/v FS-10 SPORL-Na+ pretreated unwashed solids hydrolyzate. To replicate potential commercial production conditions a 1:4 volumetric transfer from the 40% v/v FS-10 SPORL-Na+ pretreated unwashed solids hydrolyzate growth flask at pH A, B, and C into 100% v/v FS-10 SPORL-Na+ pretreated unwashed solids hydrolyzate fermentation flask at pH A, B, and C was carried out to yield an 85% v/v FS-10 SPORL-Na+ pretreated unwashed solids hydrolyzate medium. All media contained nutrient package 2.0 (NP 2.0). All conditions contained a buffering agent. Growth was carried out at 33ºC. Cell density was measured using a spectrophotometer. Error bars represent the standard deviation of 3replicates.

at pH C resulted in 40% and 22% higher biomass yield compared to pH A and B, respectively. After 23 hours of growth/propagation at the pH A/B/C the cultures were transferred into 100% (v/v) FS-10 SPORL-Na^{+} pretreated hydrolyzate held at the same pH. Volumetric isobutanol productivity was 80% and 40% higher at pH C compared to pH A and pH B, respectively (Figure C-AF-1.6).

Task C-AF-1.8. Produce ≥1,000 gallons of isobutanol from GIFT® fermentations at 40,000L demonstration scale. Convert lignocellulosic isobutanol to ≥1000 gallons biojet for further testing.

The scale-up process for this task will begin as soon as the FS-17 pretreated material arrives in sufficient quantities at Gevo in Year 4 or 5. In an effort to determine if a direct pitch of LB23 into a production vessel was feasible, a 1L GIFT® fermentation using 85% (v/v) FS-10 SPORL-Mg^{2+} pretreated hydrolyzate (32% solids) containing NP 2.0 was performed. The initial inoculation cell density of LB23 was identical to a similar LB21 1L GIFT® fermentation run but unlike the LB21 fermentation, the LB23 inoculum was not pre-conditioned to the hydrolyzate and a slight lag in growth and isobutanol production was noted. The amount of isobutanol produced by LB23 compared to LB21 in FS-10 SPORL-Mg^{2+} pretreated hydrolyzate (Figure C-AF-1.7) was greater in large part because of the nearly 50% higher hexose sugar concentration in the LB23 fermentation (89 g/L vs. 172 g/L). Also, LB23 has a 17% higher theoretical isobutanol production yield compared to LB21 meaning that more hexose sugar is being converted to isobutanol compared to other metabolic co-products. Finally, under the direct pitch conditions, LB23 had a 20% higher volumetric isobutanol productivity compared to LB21.

LB23 is the current best NARA and Gevo biocatalyst. Therefore, LB23 will be utilized going forward in the 1,000 gallon biojet production. Process optimization will be pursued aggressively in the time remaining in year 4 and into year 5 to maximize the performance of the LB23, or LB23 hydrolyzate evolved strains, yeast biocatalyst in FS-17 SPORL pretreated hydrolyzate.

Recommendations | Conclusions

Work continues to proceed according to the project plan. Optimization of fermentation conditions and characterization of fermentation performance has progressed now that pretreatment options have been narrowed down. Further optimization and characterization will be carried out as feedstock and pretreatment options continue to become representative of actual materials that will be used in process scale-up work. Currently, fermentation performance meets or exceeds target volumetric rates and yields. The adaptation program has continued to provide improved biocatalyst strains and has recently been used to adapt Gevo's second-generation biocatalyst. Gevo will continue to refine process model. Process development work has begun and will continue in order to support a demonstration scale-up trial targeted for later in the year.

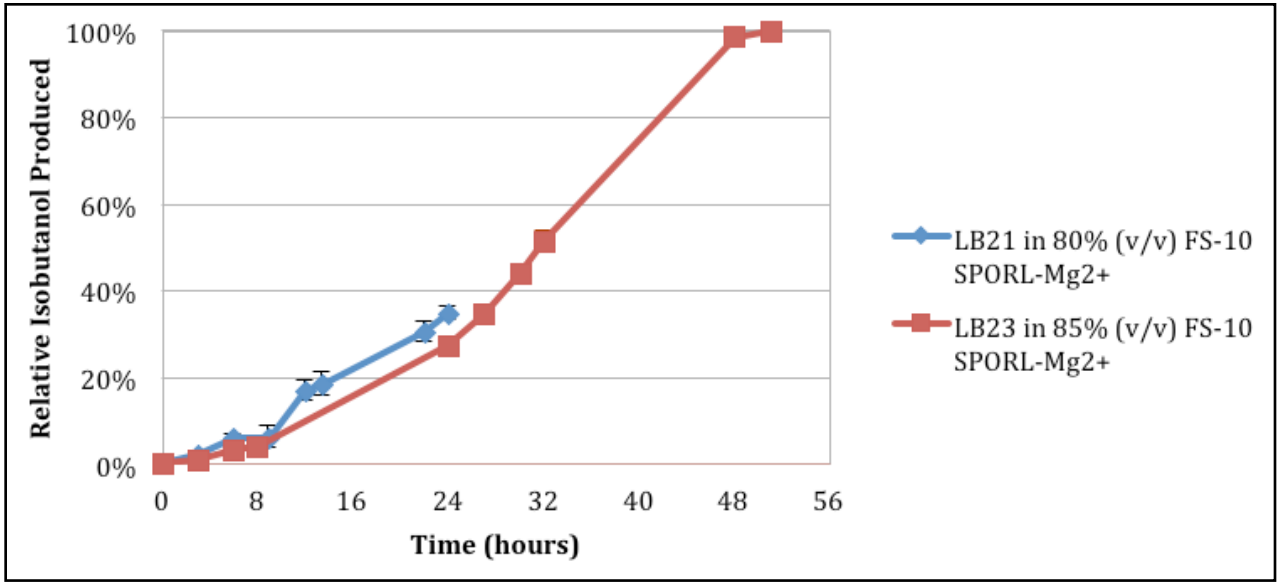

Figure C-AF-1.7. Isobutanol produced by LB21 in 80% v/v FS-10 SPORL-Mg2+ pretreated and LB23 in 85% v/v FS-10 SPORL-Mg2+ pretreated hydrolyzate. Production of isobutanol was conducted in 1L fermentation vessels equipped with GIFT®, held at 33°C and pH controlled. Medium was created using the corresponding mock media as the balance of 100%. LB21 was pre-conditioned in 20% v/v FS-10 SPORL-Mg2+ pretreated hydrolyzate before inoculating 80% v/v hydrolyzate compared to LB23 which was directly pitched into 85% v/v hydrolyzate. Error bars represent the standard deviation of two replicates. LB21 in 80% FS_10 SPORL-Mg2+ fermentation was run until all consumable sugars were exhausted.

Physical and Intellectual Outputs

PHYSICAL

- Demonstration site for hydrolysis and fermentation was established
- Demonstration site for conversion of isobutanol to iso-paraffinic kerosene was established
- Isobutanol recovered from 2L GIFT scale fermentations on various substrates

RESEARCH PRESENTATIONS

lHawkins, A.C. (2015). Development and commercialization of fermentative isobutanol production. American Chemical Society Division of Biochemical Technology. 249th ACS National Meeting. March 2015. Presentation number BIOT 144.

Parker, A.C., G.J. Balzer, L.T. Robinson, M.H. Schmalisch and A.C. Hawkins. 2014. Fermentative Conversion of Hydrolyzed Douglas fir Biomass into Isobutanol. 2014 Annual NARA Meeting, Seattle, WA, September 15-17.

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

Key Personnel	Affiliation
Birgitte Ahring	Washington State University

Task Description

Using the proprietary BioChemCat Process, WSU BSEL will in parallel with Gevo investigate the production of jet fuel from woody residues. In the project we will concentrate effort on the following two areas: Task 1: Co-culture optimization for high production yield and productivity of platform molecules (VFA) from pretreated biomass hydrolysate. Task 2: Catalysis of platform molecules into jet fuel. WSU BSEL will work with PNNL using the PNNL combinatorial catalysis computational laboratory platform.

The BioChemCat process is a new way to produce more biofuels from biomass for significant lower cost. The cost reductions will come from: 1) the process will have no need for enzymes, a significant cost reduction; 2) the mixed culture fermentations 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 can be used; 5) only a fraction of the biomass material needs to be pretreated and at low retention times which considerably lowers the cost of pretreatment; 6) a large part of the lignin can be converted to biofuels along with the carbohydrates in the biomass material.

Activities and Results

Task C-AF-2.1. Optimizing fermentation for making platform molecules

Based on results obtained from the previous quarter, the wet explosion pretreatment for woody biomass were made less severe to effectively obtain greater amount of cello-oligosaccharides available for the anaerobic fermentation. The mixed culture was used to ferment the pretreated biomass at 37°C and pH of 6.5. Under these mesophilic conditions, our maximum yield was found to be 72g of VFA as acetic acid equivalents per 100 g of sugar equivalents in the pretreated biomass. On an average, we obtained around 54g of VFA (acetic acid equivalents) per 100g of biomass sugar or 33g VFA per 100g of woody materials. The main VFAs obtained in the fermentation were C2-4 and C7 acids. It was found that greater amount of C7 acid was produced by pretreated biomass when compared with similar fermentation done using non-pretreated biomass as feed. It can be seen from Figure C-AF-2.1 that under optimized conditions, the anaerobic fermentation was capable of producing more than 0.5 wt% of VFA per gram of biomass sugars or 0.35 wt% VFA per gram of biomass. The VFAs produced in this process are currently being separated using different separation methodologies discussed in the previous quarterly reports such as supercritical carbon dioxide extraction and ion exchange resin. The mass balance and the techno-economic analysis for this optimized process are currently being done together CleanVantage to complete the work associated with this part of the project.

Task C-AF-2.2. Catalysis of Platform Molecules into Jet Fuel

Initial studies on the catalytic hydrogenation of VFAs were done using cobalt catalyst impregnated in silica as catalyst. Preliminary investigation indicates that in the conversion reaction scheme where dehydration mechanisms are important, silica may not be

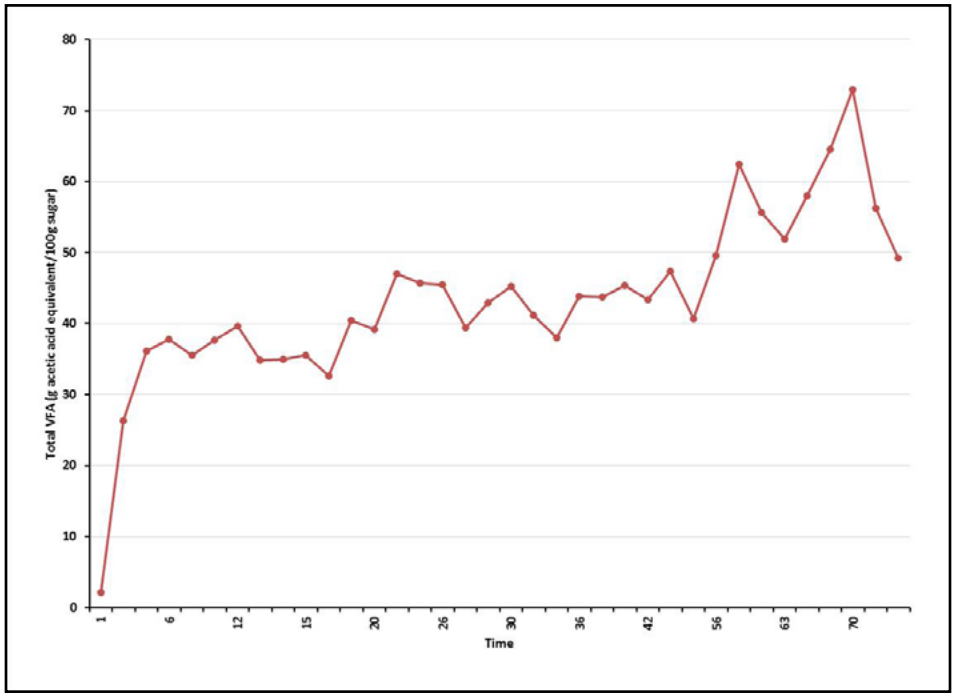

Figure C-AF-2.1. Total VFA as acetic acid equivalents as a function of time(days) obtained in a continuous mesophilic anaerobic fermentation of wet-exploded woody material using a mixed bacterial culture.

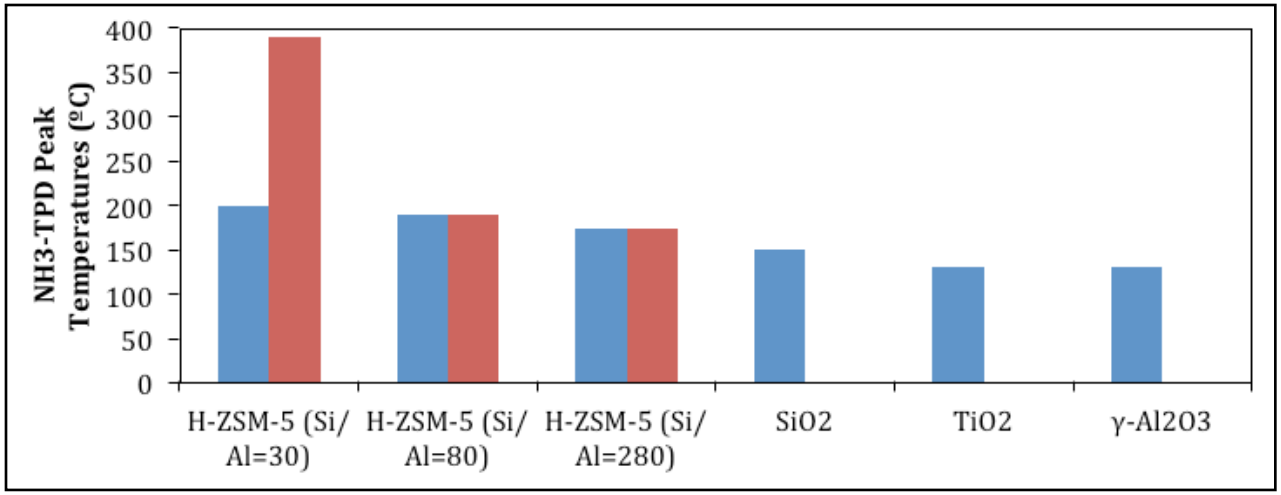

Figure C-AF-2.2. Average desorption temperatures for ammonia bound to various acidity catalysts supports.

	1	2	3	4	5	6	7	8	9	10	11
A	1Co/C	1Co/ SiO_2	1Co/ γ-Al_2O_3	1Co/ TiO_2	1Co/ HZSM-5	$Cu_2Cr_2O_5$	1Co/C	1Co/ SiO_2	1Co/ γ-Al_2O_3	1Co/ TiO_2	1Co/ HZSM-5
B	5Co/C	5Co/ SiO_2	5Co/ γ-Al_2O_3	5Co/ TiO_2	5Co/ HZSM-5	$Cu_2Cr_2O_5$	5Co/C	5Co/ SiO_2	5Co/ γ-Al_2O_3	5Co/ TiO_2	5Co/ HZSM-5
C	10Co/C	10Co/ SiO_2	10Co/ γ-Al_2O_3	10Co/ TiO_2	10Co/ HZSM-5	Pure Stock	10Co/C	10Co/ SiO_2	10Co/ γ-Al_2O_3	10Co/ TiO_2	10Co/ HZSM-5
D	15Co/C	15Co/ SiO_2	15Co/ γ-Al_2O_3	15Co/ TiO_2	15Co/ HZSM-5	Pure Stock	15Co/C	15Co/ SiO_2	15Co/ γ-Al_2O_3	15Co/ TiO_2	15Co/ HZSM-5
E	20Co/C	20Co/ SiO_2	20Co/ γ-Al_2O_3	20Co/ TiO_2	20Co/ HZSM-5	X	20Co/C	20Co/ SiO_2	20Co/ γ-Al_2O_3	20Co/ TiO_2	20Co/ HZSM-5

T (°C)

P (atm)	150	200	250
1	Run 1	Run 2	Run 3
34	Run 4	Run 5	Run 6
68	Run 7	Run 8	Run 9

Figure C-AF-2.3. Execution plan for high-throughput batch testing using CombiCat well plate reactor available at PNNL. This process will be run 9 different times to account for the desired changes in operating temperature and pressure. The value next to cobalt stands for metal loading percentage. Ex. 10Co/C denotes 10 wt% cobalt on carbon catalyst.

the most effective support, as there are many other much more acidic supports available with comparable surface area shown to be appropriate for propylene glycol dehydration. According to the ammonia temperature programmed desorption (TPD) results from investigation of various supports (Figure C-AF-2.2), a high Si/Al ratio HZSM-5 support is highly capable of dehydration reactions due to a strong affinity for protons (stronger acid-base bonds). An issue with selection of a support based solely on these values is that if a support is too acidic, coke will form, deactivating catalyst. Therefore, a combination of effective hydrogenation/hydrogenolysis metal must be combined with an appropriately acidic support in order to catalyze the one-pot synthesis of VFAs to alcohols. From these investigations, the main aim of our work is to investigate the production of mixed alcohols from VFAs by understanding the role of catalyst support acidity in the conversion reaction.

In order to gather data to understand and optimize dehydrations reactions in this conversion scheme, we plan to first study the conversion and yield of using VFA methyl and ethyl esters (specifically C2 and C3 acids which were predominantly produced in the mesophilic anaerobic fermentation discussed in the previous section) with a 10 wt% cobalt on fumed silica catalyst at the to-date reported best conditions for propanol production, as well as different weight loading of this catalyst on varying acidity of supports such as cobalt on carbon black, amorphous silica, γ-alumina, acidic zeolite (HZSM-5, varying Si/Al ratios), and titania to promote changes in catalyst dehydration ability. To screen all of these catalysts, small batch reactions will be used. The set of studies that are still planned to be executed to complete this study is listed in Figure C-AF-2.3. The best catalysts determined from batch studies will be characterized using a multitude of different physical and spectroscopy techniques to determine catalyst stability along with its effect on the conversion of alkyl VFA's to their corresponding alcohols at varying operating conditions.

Recommendations | Conclusions

The mesophilic fermentation was studied in this quarter and compared to the thermophilic fermentation previously discussed in the other quarterly reports. It was found that using the mesophilic fermentation at 37°C and pH 6.5, we obtained a greater amount of C-2 acids than C-3 acids, which indicated greater overall conversion when compared to thermophilic conditions of 50°C and pH 5.5 where a greater amount of C-3 acids were produced. Also, we found the presence of C-4 and C-7 organic acids in this fermentation process when compared to that previously discussed. This change in the type of VFA yield can be attributed to the different microbial organisms present in the mixed culture that are activated at the different conditions. This tuning of the fermentation conditions to obtained desirable VFA concentrations and yields at non-sterile conditions using the mixed bacterial culture adds to the significance of the discussed technique. With respect to VFA yields, comparison with literature studies has indicated that our process produces almost similar acid yields when compared to different pure bacterial culture. However, this process does not required high sterility conditions which significantly reduce process costs when compared to the current state-or-art.

The separation process for a mixed VFA concentration from the fermentation broth has been done previously and the work right now is being concentrated on the mesophilic culture. While ion exchange resins are better suited for C-3 and above acids, supercritical fluid extraction has been found to be better suited for C-2 acids. However, other stronger ion exchange resins are being tested with the mixed VFA feed. Esterification of the VFAs has been strongly recommended as indicated in the previous quarterly report. Preliminary experiments done using liquid phase hydrogenation of acetic acid using Pt-Rh catalyst on silica had indicated a catalyst conversion of not higher than 50% for a maximum selectivity greater than 90% towards ethanol production. This decreased conversion was found to be due to the conversion of acetic acid to ethyl acetate at higher concentrations of ethanol produced. This work was not a part of the USDA-NARA initiative and was primarily used to justify our decision to consider vapor phase hydrogenation of the methyl or ethyl ester of the VFAs to produce mixed alcohols. Further studies are currently being done to optimize and characterize the catalyst for effective vapor-phase hydrogenation to produce alcohols. Preliminary studies have indicated that temperatures above 200°C and pressures below 1 MPa are best conditions to test and optimized effective catalytic hydrogenation of VFAs to alcohols which can be further dehydrated and oligomerized in the presence of a zeolite catalyst to produce jet fuels.

Physical and Intellectual Outputs

REFEREED PUBLICATIONS

Fernandez, S., Murali, N. and Ahring, B. K., 2014, Effect of methanogens on VFA yield using anaerobic fermentation of biomass using mixed culture, Submitted to Biochemical Engineering Journal

Murali, N. and Ahring, B. K., 2014, Comparison between wet-exploded and non-pretreated corn stover as feedstocks for VFA production using anaerobic fermentation, Submitted to Bioresource Technology

Garrett, B.G., Srinivas, K., & Ahring, B.K. (2014). Design and optimization of a semi-continuous high pressure carbon dioxide extraction system for acetic acid. J.of Supercritical Fluids, 95, 243-251. doi:10.1016/j.supflu.2014.08.029

Garrett, G.B., Srinivas, K., & Ahring, B.K. (2015). Performance and stability of Amberlite TM IRA-67 ion exchange resin for product extraction and pH control during homolactic fermentation of corn stover sugars. Biochemical Engineering Journal, 94,1-8. doi:10.1016/j.bej.2014.11.004

FeedstockLogistics_Marrs

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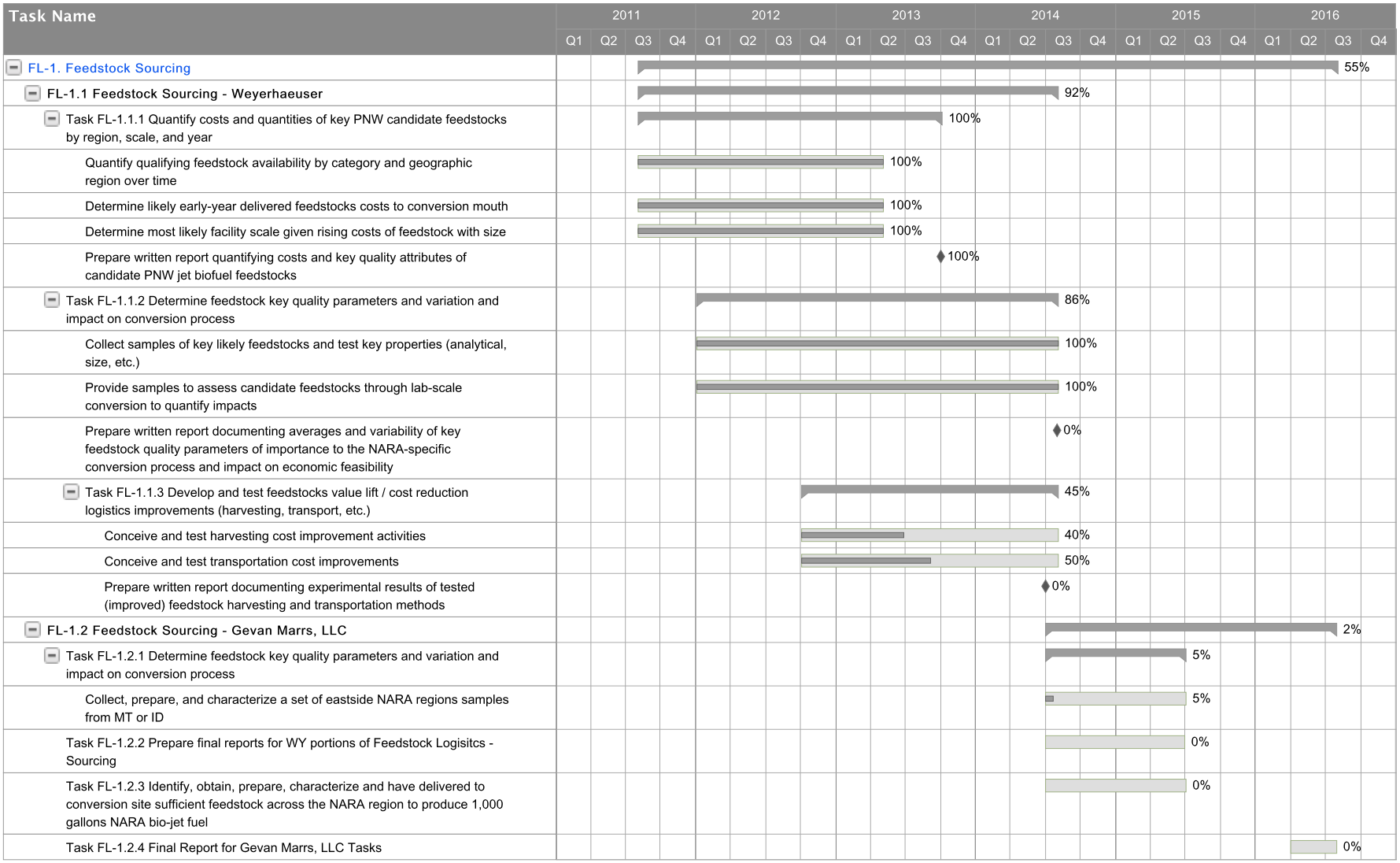

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FeedstockLogistics_Sessions

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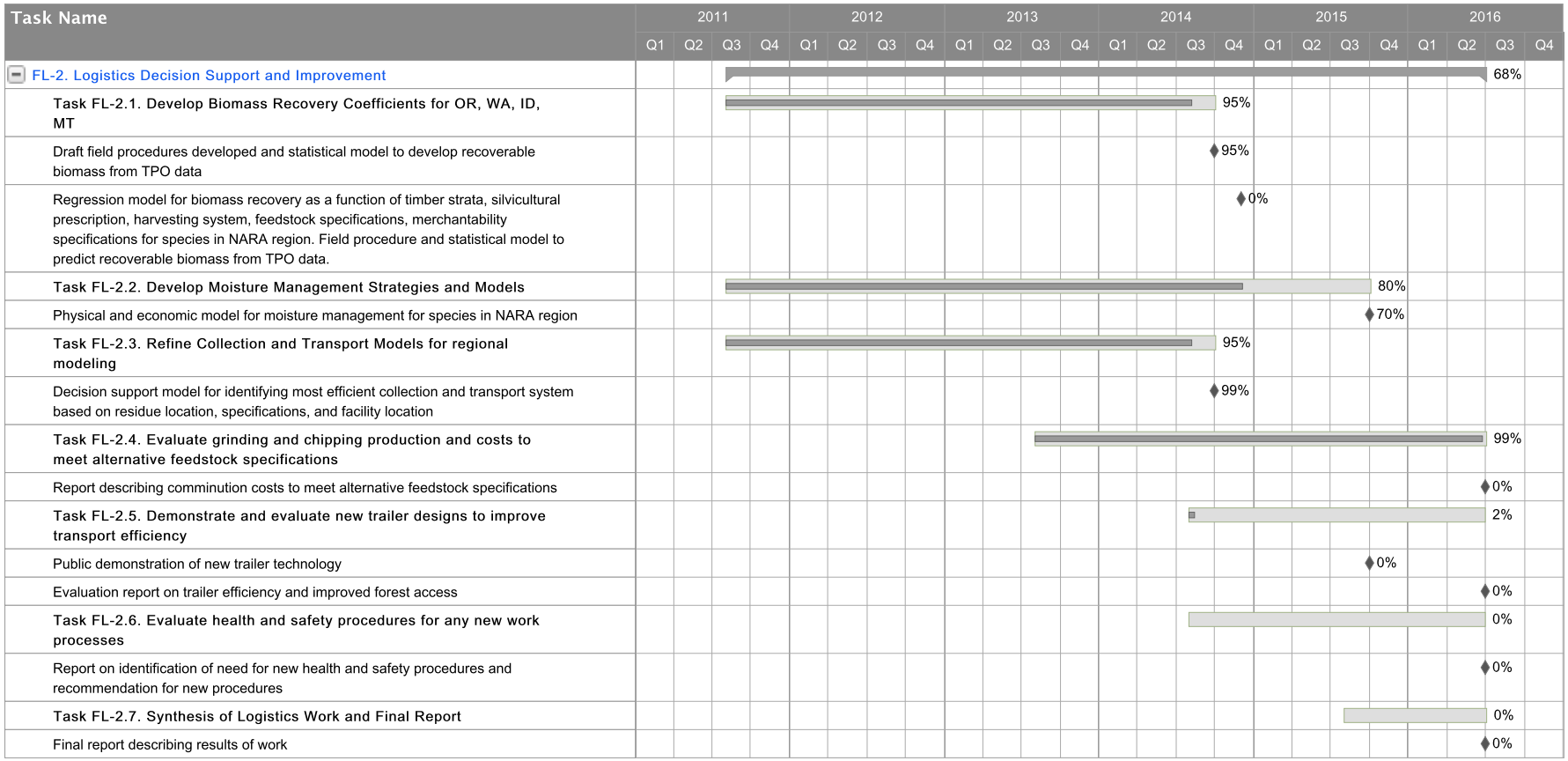

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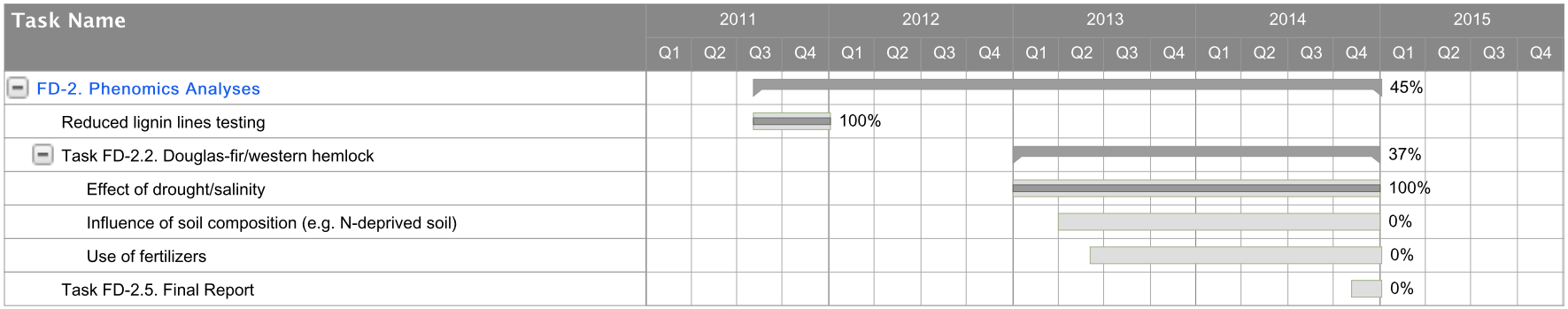

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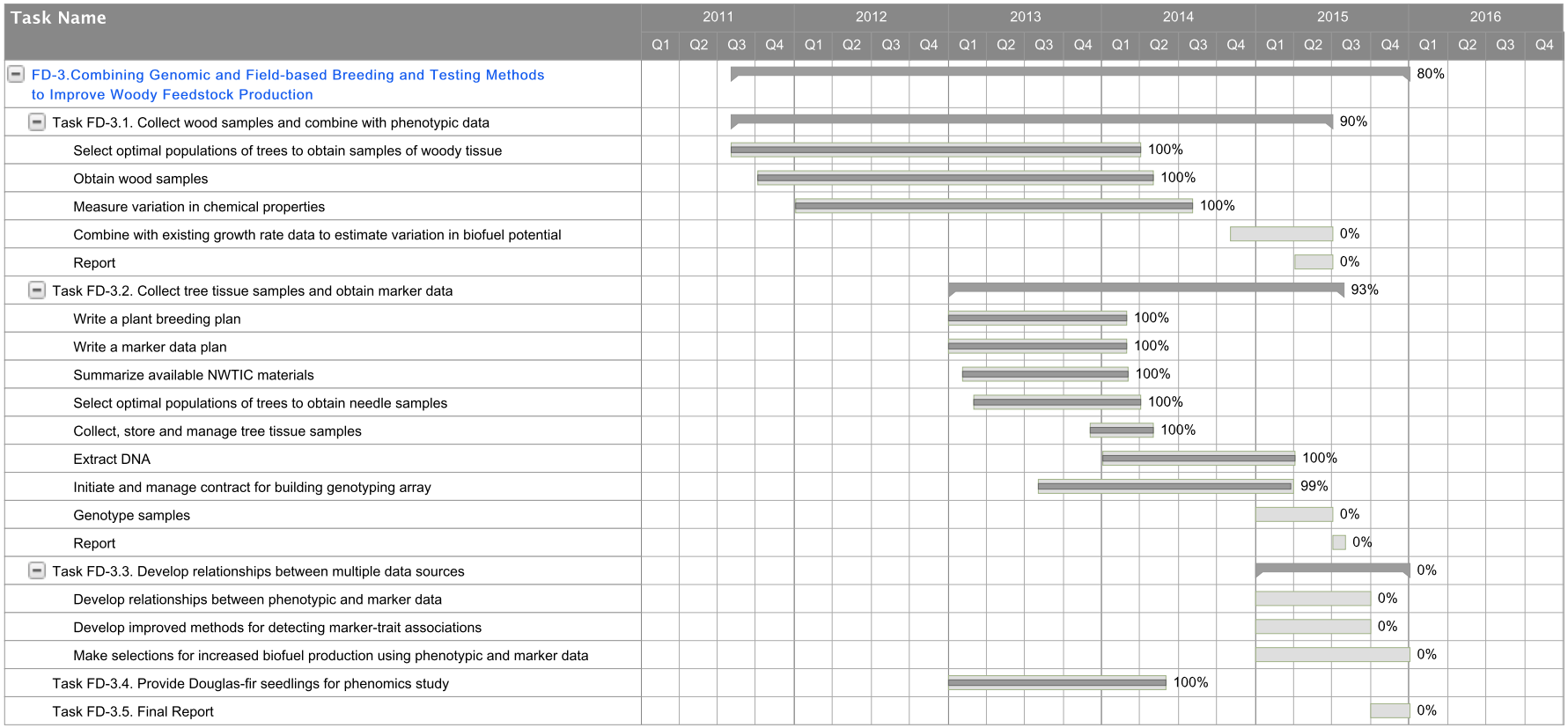

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FeedstockDevelopment_XZhang

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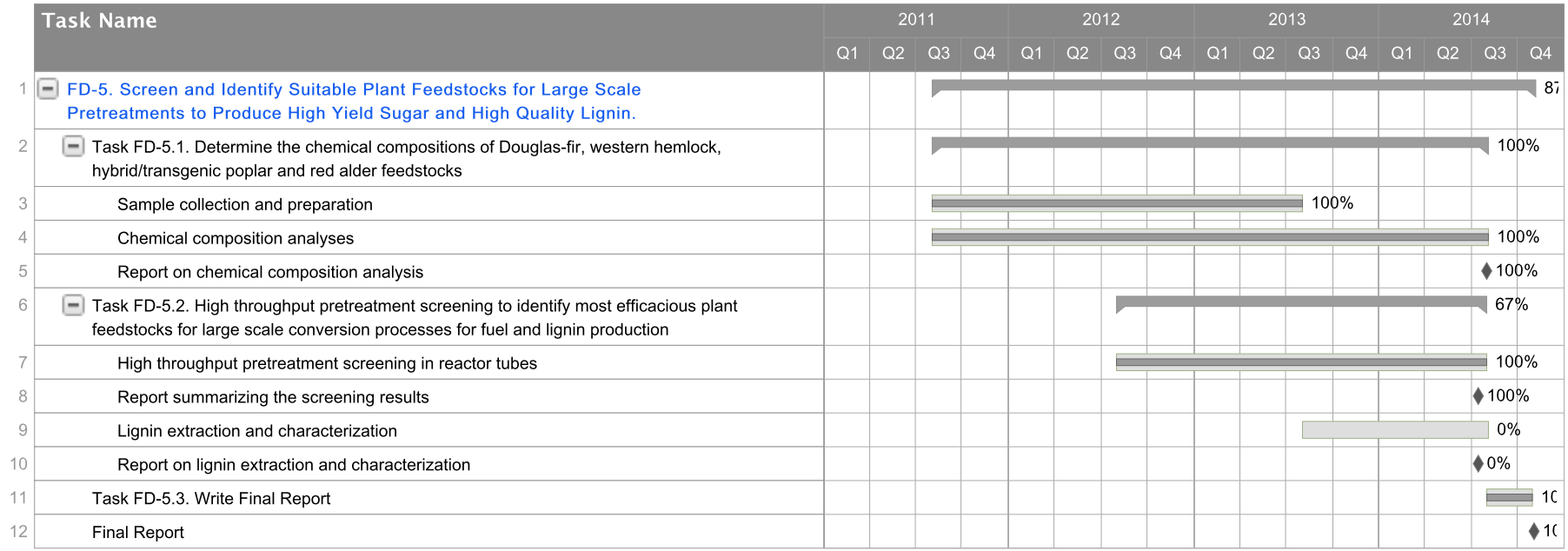

	Task Name	2011				2012				2013				2014			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1	FD-5. Screen and Identify Suitable Plant Feedstocks for Large Scale Pretreatments to Produce High Yield Sugar and High Quality Lignin.																87
2	Task FD-5.1. Determine the chemical compositions of Douglas-fir, western hemlock, hybrid/transgenic poplar and red alder feedstocks																100%
3	Sample collection and preparation												100%				
4	Chemical composition analyses																100%
5	Report on chemical composition analysis																100%
6	Task FD-5.2. High throughput pretreatment screening to identify most efficacious plant feedstocks for large scale conversion processes for fuel and lignin production																67%
7	High throughput pretreatment screening in reactor tubes																100%
8	Report summarizing the screening results																100%
9	Lignin extraction and characterization																0%
10	Report on lignin extraction and characterization																0%
11	Task FD-5.3. Write Final Report																10
12	Final Report																10

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Pretreatment_Zhu

NARA

	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	C-P-4. Mild Bisulfite Pretreatment of Forest Residuals																								69%
2	Task C-P-4.4.1. Optimization SPORL and/or other pretreatments at FPL pilot plant facility (50 kg/run)																				92%				
3	Experiment design based on Task 1, pretreatment																100%								
4	Sample analysis, high solids (~20%) enzymatic saccharification																				80%				
5	Material and energy balance, correlate data between Task 1 and Task 2																				100%				
6	Task C-P-4.4.2. Scale-up of SPORL technology																								66%
7	Identification and experimental verification of operating conditions that are suitable for industrial trial runs																								50%
8	Scale up from 2-3 to 50 kg feedstock. Integrate downstream processes of defibering, hydrolysis, and concentration of sugar																100%								
9	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																100%								
10	Work with Washington State University, Weyerhaeuser and Gevo for jet fuel/lignin products production, data analysis, reporting																								60%
11	Task C-P-4.4.3. Industrial scale-up of SPORL technology																								18%
12	Identify operating conditions for industrial scale trial run																				50%				
13	Evaluation runs at an industry facility for designing industrial trail runs																				0%				
14	Industrial scale trial run																				0%				
15	Sample analyses and data analyses																								0%
16	Task C-P-4.4.4. Final Report																								0%

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Pretreatment_Ahring

	Task Name	2011				2012				2013			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1	C-P-3. Preparation of Pretreated Biomass												92%
2	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	Task C-P-3.2. Evaluate data from partners and adjust pretreatment system												100%
7	Evaluate data from initial pretreatment results												100%
8	Modify pretreatment process to improve outputs												100%
9	Task C-P-3.3. Final Report												25%

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Pretreatment_Catchlight

NARA

	Task Name	2012				2013				2014			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1	C-P-4. Mild Bisulfite Pretreatment of Forest Residuals										100%		
2	Task C-P-4.1. Identify effect of pretreatment process variables on total sugar yield										100%		
3	Task C-P-4.2. Produce lignosulfonate and lignin sample for study by Co-Products team										100%		
4	Task C-P-4.3. Final Report										100%		

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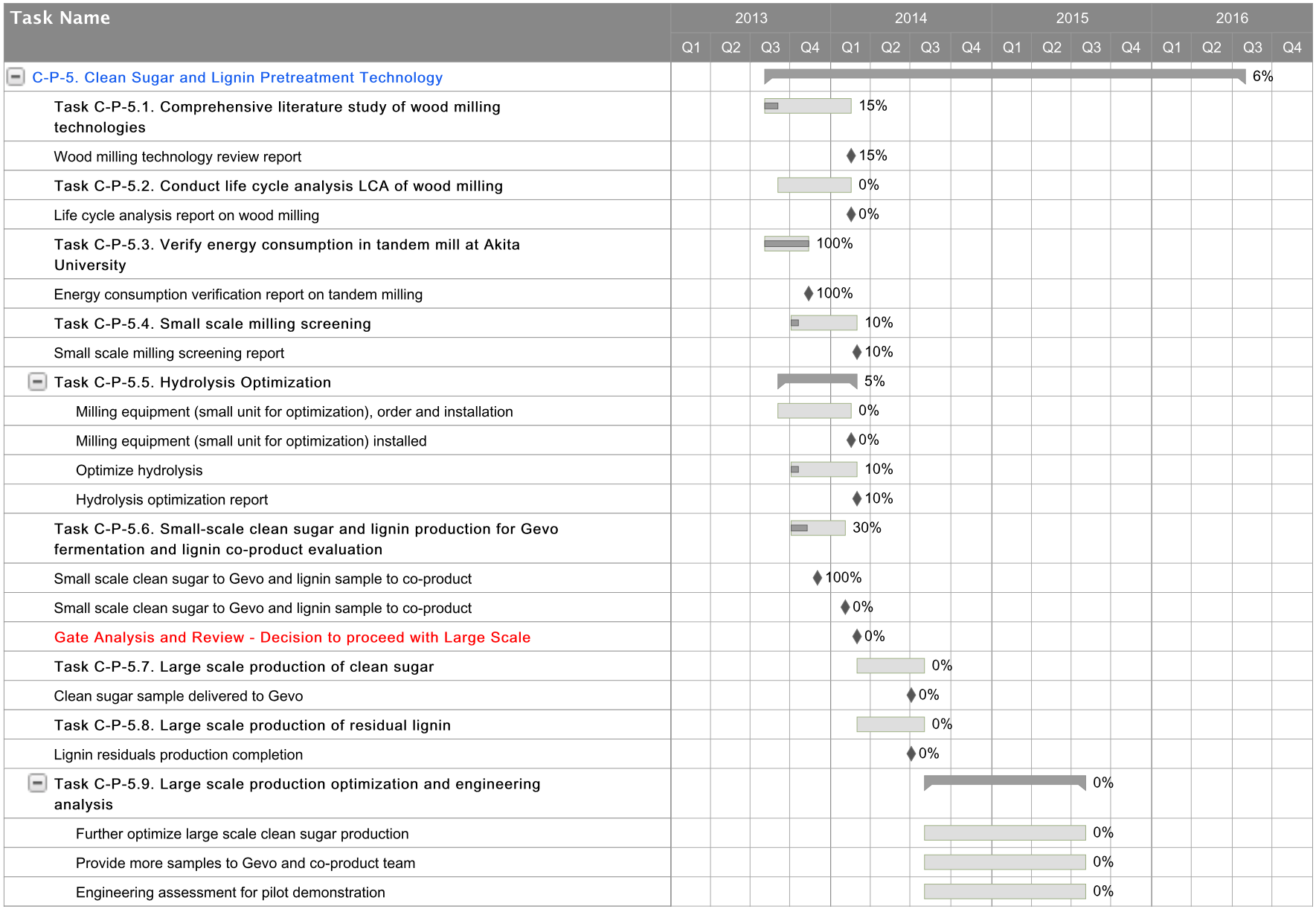
Pretreatment_Gao

NARA



	Task Name	2013				2014				2015				2016			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1	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	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	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%								

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	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	Task C-P-5.12. Techno-economics analysis and report															0%	
30	Task C-P-5.12.1. Small Scale																
31	Techno-economics analysis report (initial)				0%												
32	Techno-economics analysis report (mid)					0%											
33	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%					

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Conversion-AF_Gevo

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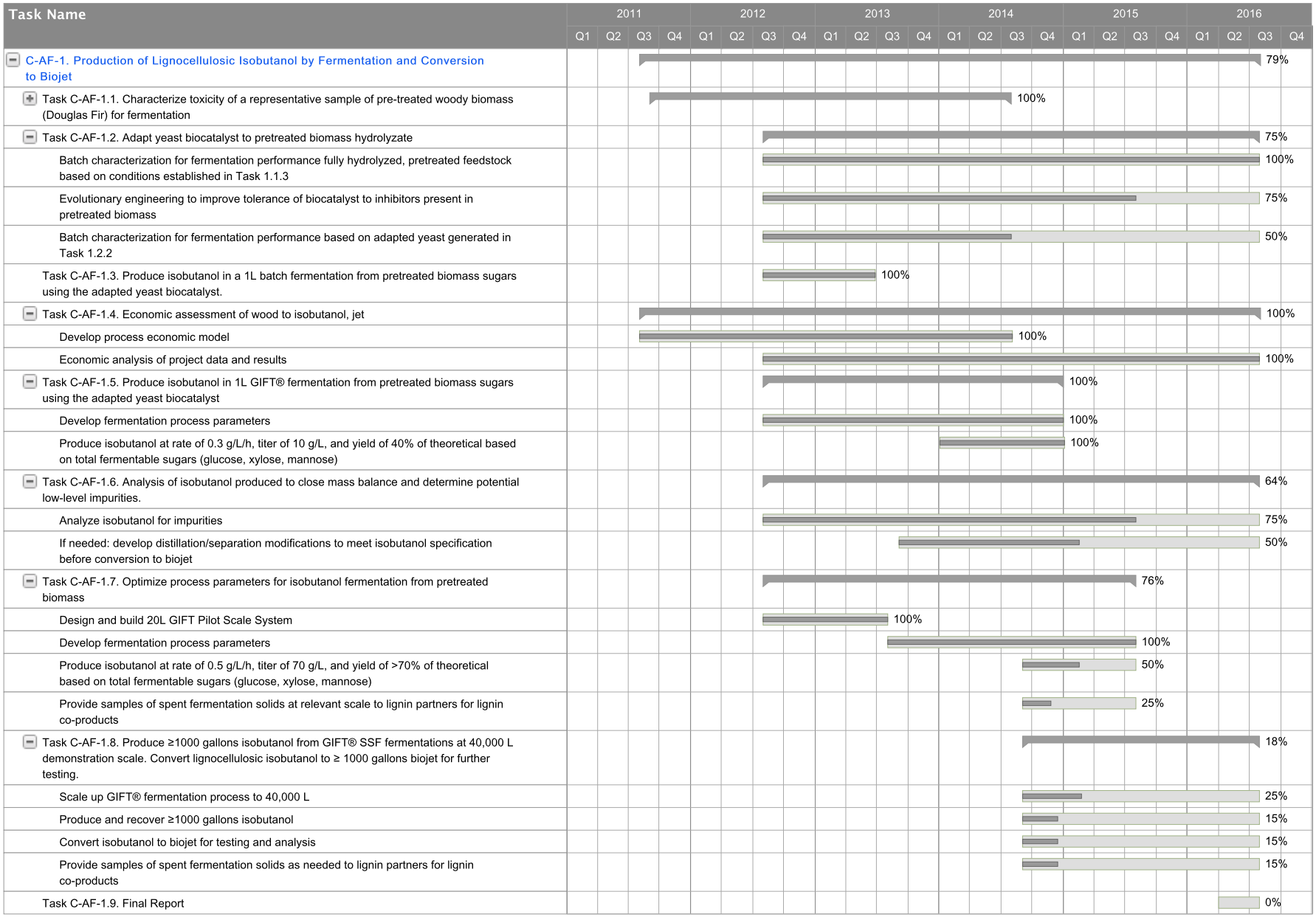

	Task Name	2011				2012				2013				2014				2015				2016			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1	C-AF-1. Production of Lignocellulosic Isobutanol by Fermentation and Conversion to Biojet																								79%
2	Task C-AF-1.1. Characterize toxicity of a representative sample of pre-treated woody biomass (Douglas Fir) for fermentation																100%								
6	Task C-AF-1.2. Adapt yeast biocatalyst to pretreated biomass hydrolyzate																								75%
7	Batch characterization for fermentation performance fully hydrolyzed, pretreated feedstock based on conditions established in Task 1.1.3																								100%
8	Evolutionary engineering to improve tolerance of biocatalyst to inhibitors present in pretreated biomass																								75%
9	Batch characterization for fermentation performance based on adapted yeast generated in Task 1.2.2																								50%
10	Task C-AF-1.3. Produce isobutanol in a 1L batch fermentation from pretreated biomass sugars using the adapted yeast biocatalyst.																								
11	Task C-AF-1.4. Economic assessment of wood to isobutanol, jet																								100%
12	Develop process economic model																100%								
13	Economic analysis of project data and results																								100%
14	Task C-AF-1.5. Produce isobutanol in 1L GIFT® fermentation from pretreated biomass sugars using the adapted yeast biocatalyst																								
15	Develop fermentation process parameters																								
16	Produce isobutanol at rate of 0.3 g/L/h, titer of 10 g/L, and yield of 40% of theoretical based on total fermentable sugars (glucose, xylose, mannose)																								
17	Task C-AF-1.6. Analysis of isobutanol produced to close mass balance and determine potential low-level impurities.																								64%
18	Analyze isobutanol for impurities																								75%
19	If needed: develop distillation/separation modifications to meet isobutanol specification before conversion to biojet																								50%
20	Task C-AF-1.7. Optimize process parameters for isobutanol fermentation from pretreated biomass																								
21	Design and build 20L GIFT Pilot Scale System																								
22	Develop fermentation process parameters																								
23	Produce isobutanol at rate of 0.5 g/L/h, titer of 70 g/L, and yield of >70% of theoretical based on total fermentable sugars (glucose, xylose, mannose)																								
24	Provide samples of spent fermentation solids at relevant scale to lignin partners for lignin co-products																								
25	Task C-AF-1.8. Produce ≥1000 gallons isobutanol from GIFT® SSF fermentations at 40,000 L demonstration scale. Convert lignocellulosic isobutanol to ≥ 1000 gallons biojet for further testing.																								
26	Scale up GIFT® fermentation process to 40,000 L																								
27	Produce and recover ≥1000 gallons isobutanol																								
28	Convert isobutanol to biojet for testing and analysis																								
29	Provide samples of spent fermentation solids as needed to lignin partners for lignin co-products																								
30	Task C-AF-1.9. Final Report																								

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Conversion-AF_Ahring

NARA

	Task Name	2011				2012				2013				2014			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1	C-AF-2. Production of Jet fuel using BioChemCat																69%
2	Task C-AF-2.1. Optimizing fermentation for making platform molecules																83%
3	Investigate the fermentation method																90%
4	Optimize the fermentation process																90%
5	Optimize the separation and recovery of platform molecules																75%
6	Integrate and optimize production and extraction of platform molecules																75%
7	Task C-AF-2.2. Optimize the catalysis of platform molecules into jet fuel																28%
8	Screen and selection optimal catalysts																30%
9	Optimize the catalysis process																25%
10	Task C-AF-2.3. Final Report																0%
11																	

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